CHAPTER 5

Chemical Modification of Viruses

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1. INTRODUCTION

Viruses were first subjected to chemical modification at a time when this was an approach generally used to characterize biologically active substances and when the nature and the complexity of viruses were not yet understood. After it had been established that viruses were composed of proteins, nucleic acids, and often also lipids, and that they had definite and different architectures and topographies, it became evident that the results of many modification reactions would be difficult to interpret, and such techniques were used less frequently with entire viruses. However, with focus on the results of modification of a specific virus component within the particle, such as the protein or the nucleic acid of the simple viruses, and disregarding "side reactions" of the other component(s), such reactions continued to be of some usefulness. This chapter will summarize the methods, purposes, and conclusions arrived at by chemical (and a few enzymatic) modification reactions of viruses. Photochemical modifications will be dealt with in the following two chapters. We will not discuss the much larger topic of reactions of the separated components or that of agents that bind by other than covalent linkage.

The rationale for most virus modification studies, particularly in

H. Fraenkel-Conrat et al. (eds.), *Comprehensive Virology*

the early days of experimental virology, were either to inactivate them, frequently for the purpose of then using such inactivated viruses to immunize animals or man, or to produce mutants. More recently, studies of the chemical reactivity of viruses have frequently been aimed at the localization of various proteins and other components in determining the fine structure and mode of assembly of virus particles. Whatever the purpose, virus modifications must obviously be performed under conditions where the viruses are stable. Thus all reactions to be discussed are, unless otherwise specified, carried out in at least 90% aqueous media of pH 6–7.5 at 0–37° C, and usually at low reagent concentrations.

This chapter first will deal with the reagents that have been used to modify viruses, and then will focus on the purposes of carrying out virus modifications.

2. REAGENTS USED FOR VIRUS MODIFICATION

2.1. Acylating Agents

Early experiments with typical acylating agents (e.g., acid anhydrides, ketenes, toluene sulfonylchloride, carbobenzoxychloride, and phenylisocyanate; Table 1, 1–5)* showed that these compounds usually caused virus inactivation and often solubility changes; such results were interpreted as evidence for the protein nature of viruses. Although we are now inclined to attribute most virus-inactivating reactions to nucleic acid modifications, this is probably least the case with acylating agents since acylation of nucleic acids does not occur readily and reverses easily. Thus the search for the production of mutants by acylating agents has generally given negative results. Acylation reactions, however, have been successfully used for the structural characterization of virus particles. The action of monofunctional reagents has given data concerning the steric availability of specific groups in viral coat proteins of known amino acid sequence. Thus the finding that in TMV only one of the four tyrosines, residue 139 near the *C* terminus, is acylated by acetic anhydride, as well as one of the two amino groups (residue 68, not 53), has supplied data of importance in establishing the conformation of that protein in the virus particle (Fraenkel-Conrat and Colloms, 1967; Durham and Butler, 1975).

* All numbers in parentheses after the names of reagents refer to the chemical formulas presented in Fig. 1.

Acylating agents have also been used to determine which of several coat or envelope proteins of the more complex viruses are more available for reaction, and thus more external. This type of study does not require a knowledge of the amino acid sequences of the proteins involved. Thus it was established that in the polio and rhino virions VP1 is most reactive, and that VP4 is quite unreactive to acetic anhydride (Lonberg-Holm and Butterworth, 1976). The conclusions concerning the different reactivities in terms of locations of these proteins were borne out by iodination studies to be discussed in Section 2.3. In contrast to the picornaviridae, all proteins and lipids in retroviridae became acetylated to similar extents by acetic anhydride, suggesting a looser structure for such enveloped viruses (Montelaro and Rueckert, 1975). One may surmise that the hydrophobic nature of both the viral envelope and the reagent plays a role in the outcome of these experiments.

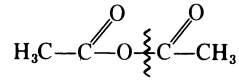
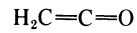
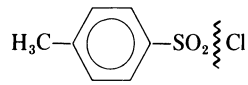
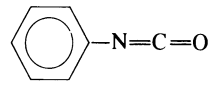
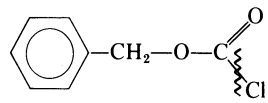
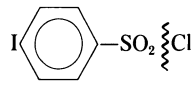
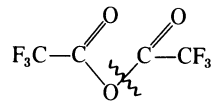
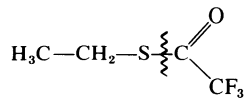
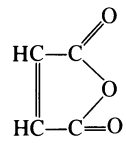
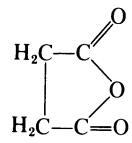
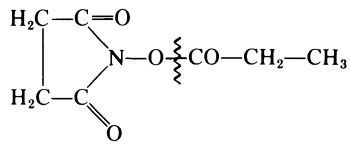
Another important use of these, as of other types of modifications, is to ascertain by means of bifunctional reagents the relative position and proximity of several different proteins occurring in complex virions. Such cross-linking through acylation is achieved by diisocyanates, bisanhydrides, tartryldiazides, etc. These reactions will be discussed in Section 3.1.2.

As far as reaction specificity is concerned, thiol and imidazole groups are the most readily acylated, but are usually also the most labile, so that brief exposure to alkali may hydrolyze such acyl bonds. The same is true to a lesser extent with the phenolic groups, which react similarly to the amino groups (Fraenkel-Conrat and Colloms, 1967). The latter groups, however, because of their usually external location, represent the main acceptors of stably bound acyl groups. Yet there are several well-established exceptions to this ranking. Thus, in TMV protein, two of four tyrosines are readily acetylated, while, in the virus rod, as previously noted, only the tyrosine near the *C* terminus reacts; in both instances, the rates of acetylation of available tyrosines and lysines are similar. Of the two amino groups, that in position 53 is much less reactive than that in position 68 (Fraenkel-Conrat and Colloms, 1967; Perham and Richards, 1968; King and Perham, 1971; Perham, 1973). *p*-Iodobenzenesulfonyl chloride (pipsyl chloride) (6) reacts only with tyrosine residue 139 (Perham, 1973).

Trifluoroacetic anhydride and *S*-ethyltrifluorothioacetate (7, 8) have the advantage of reversibility over acetylation of amino groups, and the latter shows considerable amino group specificity. That reagent has given the same results as acetic anhydride in regard to the role of

TABLE 1

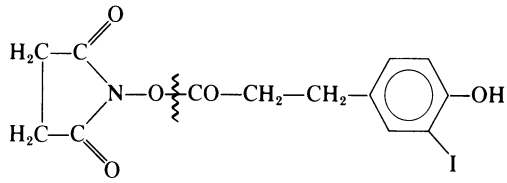
Chemicals Used for Virus Modifications[a]

1. Acetic anhydride	
2. Ketene	
3. Toluene sulfonylchloride	
4. Phenylisocyanate	
5. Carbobenzoxychloride	
6. Pipsyl chloride	
7. Trifluoroacetic anhydride	
8. *S*-Ethyltrifluoroacetate	
9. Maleic anhydride	
10. Succinic anhydride	
11. *N*-Succinimidylpropionate	

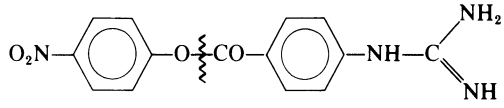
(*Continued*)

TABLE 1. (*Continued*)

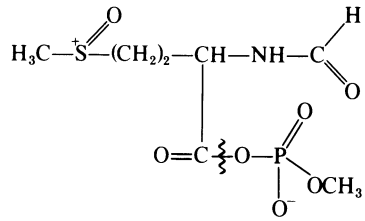
12. *N*-Succinimidyl-3(4-hydroxy-5-iodophenylpropionate) (Bolton-Hunter reagent)



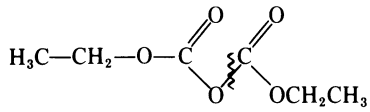
13. *p*-Nitrophenyl-*p'*-guanidino-benzoate



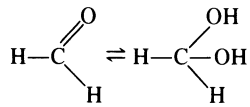
14. Formylmethionylsulfone methyl-phosphate



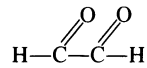
15. Ethoxyformic anhydride (or diethylpyrocarbonate)



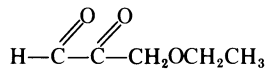
16. Formaldehyde



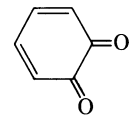
17. Glyoxal



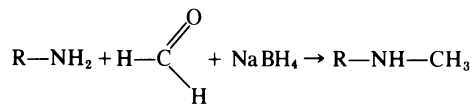
18. Kethoxal



19. *o*-Quinones



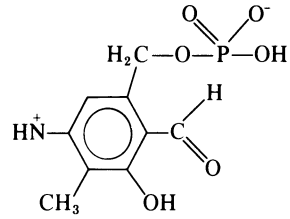
20. Reductive alkylation



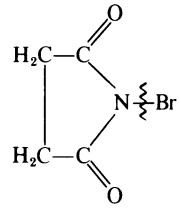
(*Continued*)

TABLE 1. (*Continued*)

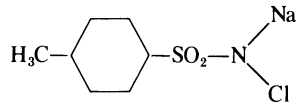
21. Pyridoxal phosphate



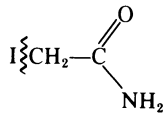
22. *N*-Bromosuccinimide



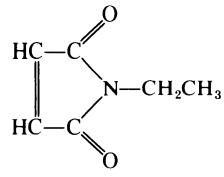
23. Chloramine T



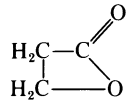
24. Iodoacetamide



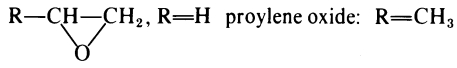
25. *N*-Ethylmaleimide



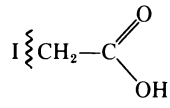
26. β-Propiolactone



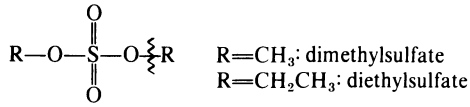
27. Ethylene oxide



28. Iodoacetate

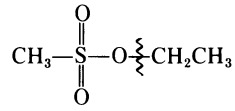
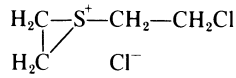
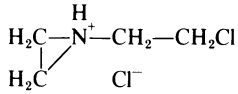
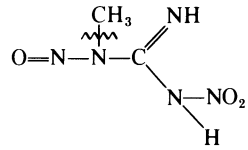
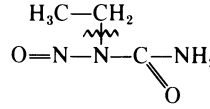
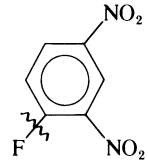
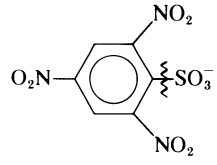
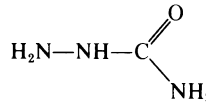


29. Alkylsufate



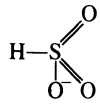
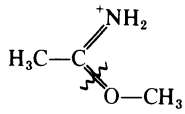
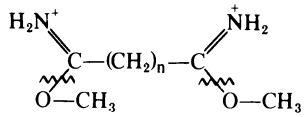
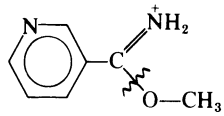
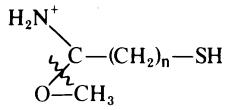
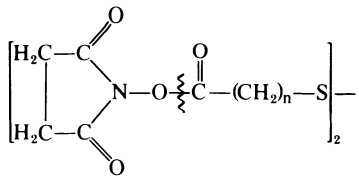
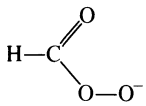
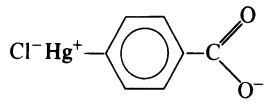
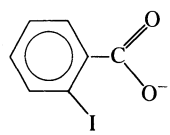
(*Continued*)

TABLE 1. (*Continued*)

30. Ethylmethane sulfonate	
31. *S*-Mustard	
32. *N*-Mustard	
33. *N*-Methyl-*N*-nitroso-*N'*nitro-guanidine	
34. *N*-Ethyl-*N*-nitrosourea	
35. 2,4-Dinitrofluorobenzene (DNFB)	
36. 2,4,6-Trinitrobenzene sulfonic acid (TNBS)	
37. Hydroxylamine	$H_2N—OH$
38. Hydrazine	$H_2N—NH_2$
39. Semicarbazide	
40. *O*-Methoxyamine	$H_2N—O—CH_3$

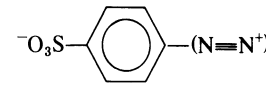
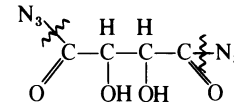
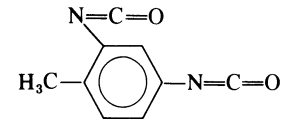
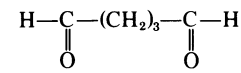
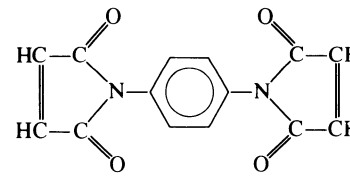
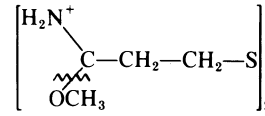
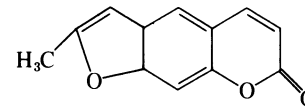
(*Continued*)

TABLE 1. (*Continued*)

41.	Bisulfite		
42.	Methylacetimidate		
43.	Dimethyladipimidate		n=4
44.	Dimethylsuberimidate	Same	n=6
45.	Methylpicolinimidate		
46.	Methyl-3-mercaptopropionimidate		n=2
47.	Methyl-4-mercaptobutyrimidate	Same	n=3
48.	Dithiobissuccinimidyl propionate		n=2
49.	Dithiobissuccinimidyl butyrate	Same	n=3
50.	Performic acid		
51.	Methyl mercuric salts	H_3C-Hg^+ NO_3^- or Cl^-	
52.	*p*-Chloromercuribenzoate		
53.	*o*-Iodosobenzoate		

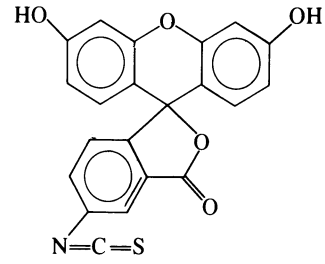
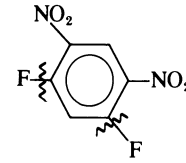
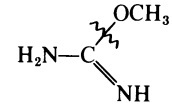
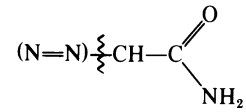
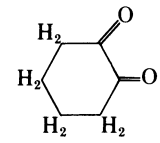
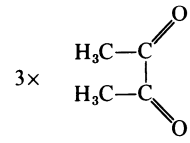
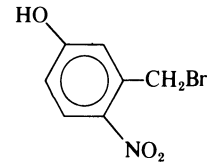
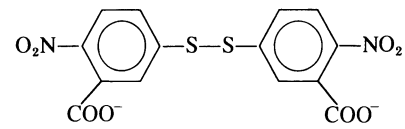
(*Continued*)

TABLE 1. (*Continued*)

54.	Diazosulfanilic acid	
55.	Water-soluble carbodiimide (an example)	$H_3C—CH_2—N=C=N—(CH_2)_3—N(CH_3)_2$
56.	Mercaptoethanol	$HO—CH_2—CH_2—SH$
57.	Dithiothreitol	$HSCH_2—CHOH—CHOH—CH_2SH$
58.	Tartryldiazide	
59.	Toluene-2,4-diisocyanate	
60.	Hexamethylenediisocyanate	$O=C=N—(CH_2)_6—N=C=O$
61.	Glutaraldyhyde	
62.	*N,N'-p*-Phenylenedimaleimides	
63.	*N,N'*-Alkylenedimaleimides	Same $—(CH_2)_n—$ Same n=1—5
64.	Dimethyl-3,3'-dithiobispropionimidate	
65.	Trioxsalen (psoralene derivative)	

(*Continued*)

TABLE 1. (*Continued*)

66. Fluorescein isothiocyanate	
67. 1,5-Difluoro-2,4-dinitrobenzene	
68. *O*-Methylisourea	
69. Diazoacetamide	
70. Cyclohexanedione	
71. Diacetyltrimer	
72. 2-Hydroxy-5-nitrobenzyl bromide	
73. Tetranitromethane	$C(NO_2)_4$
74. 5,5′-Dithiobis-(2-nitrobenzoic acid)	

[a] The wavy line crossing bonds is to indicate which part of the molecule is released in the binding of the reagent to a viral component.

the two amino groups of TMV coat protein in virus assembly (Perham and Richards, 1968). The action of maleic and succinic anhydride (9, 10) on viruses has also been studied (King and Perham, 1971; Rushizky and Mozejko, 1973; Singh and Terzaghi, 1974; Sehgal and Hsu, 1977). A particular advantage of maleic anhydride is the reversibility of the reaction in weak acid (Butler *et al.*, 1969). At high levels of acylation, these reagents cause extensive degradation, but levels can be found where they acylate only one to three groups; then they show the same selectivity for available amino groups as acetic anhydride. However, acylation of sterically available tyrosine, serine, and threonine groups may also occasionally occur (King and Perham, 1971).

A new type of acylating agents frequently used with radioactive substituents on the acyl group are the succinimidyl compounds, such as succinimidylpropionate. These compounds react with great affinity with available groups, usually the amino groups (11, 12) (see Wetz and Habermehl, 1979).

Among rather untypical and specialized acylating agents that have been used with viruses is the protease inhibitor, *p*-nitrophenyl-*p'*-guanidinobenzoate (NPGB) (13) (Chase and Shaw, 1969). The conclusion concerning its inactivating action on enveloped viruses, as determined for Sindbis virus, is that it is unable to attack the viral proteins but is able to penetrate the lipid bilayer, and it then acylates and inactivates the viral RNA (Bracha *et al.*, 1977*a*,*b*). An acylating agent that is specifically designed to be unable to pass through membranes is [^{35}S]-formylmethionyl sulfone methylphosphate (14); it acylates at pH 10 primarily the ϵ-amino groups, and only those at the surface of Semliki Forest virus (Gahmberg *et al.*, 1972).

Another acylating agent that is of interest, even though it is as nonspecific as the above agent was believed to be specific, is ethoxyformic anhydride (diethylpyrocarbonate) (15). This chemical is highly reactive, and its virus-inactivating action may also be due to its reaction with the nucleic acid, particularly if the acid is single stranded (Ehrenfeld, 1974). TMV virions are not readily inactivated by ethoxyformic anhydride (Oxelfelt and Arstrand, 1970).

2.2. Aldehydes, Ketones, and Reductive Alkylation

Formaldehyde (16) is a classical protein reagent, and its affinity for the amino groups has long been used as a means of titrating these. In a series of papers by the author and various co-workers, it was

shown that, besides the reversible addition reaction of formaldehyde to the amino groups, there occurred slower and more stable cross-linking reactions of the resultant amino-methylols through condensation with many other protein side chains, yielding methylene bridges (e.g., —NH—CH_2—NH—CO—; —NH—CH_2—(C_6H_3OH)—) (Fraenkel-Conrat and Olcott, 1946; Fraenkel-Conrat and Olcott, 1948*a*,*b*). The study of the effect of formaldehyde on TMV actually initiated the author's collaboration with W. M. Stanley's laboratory at Princeton and led to his subsequent association with the U. C. Berkeley Virus Laboratory.

He later showed that formaldehyde reacts in readily reversible fashion with the available amino groups of nucleic acids, and thus much more with single-stranded than with double-stranded nucleic acids (Fraenkel-Conrat, 1954). Methylene bridges resulting in stable cross-linking were subsequently demonstrated by Feldman (1973) to occur between guanine residues.

It appears probable but not proven that the long-known inactivating effects of formalin on microorganisms and viruses are primarily due to the affinity of this compound for the amino groups of the RNA which are unprotonated at pH 6–7. Cross-linking between such —NH—CH_2OH groups and reactive residues on vicinal protein and polynucleotide chains is also a good possibility and would surely be very destructive to viral function (Heicken and Spicher, 1959). A particularly important aspect of formaldehyde modification is that, because of the smallness of the reagent and its lack of hydrophobic groups, it causes minimal disturbance of protein conformation. Its cross-linking action is confined to sterically close groups and is thus likely to stabilize the original conformation by new primary linkages. Thus the antigenic properties of formaldehyde-treated proteins or viruses appear not to differ greatly from those of the original material. It is for this reason that formaldehyde treatment has been and remains the preferred method of producing "killed vaccines," which are biological materials the toxicity of which has been abolished without loss or change of their serological properties. The presence of methylene bridges between lysine and tyrosine side chains has actually been demonstrated in formalin-treated bacterial toxins (Blass *et al.*, 1965).

Formalin treatment is what Salk (1955) used in producing the first polio vaccine, a landmark in the history of medicine. Certain small-molecular alkylating agents (β-propiolactone, ethylene oxide, etc.; see section 2.4), as well as UV light have also been used at times to prepare viral vaccines. The question whether "killed" vaccines are to be pre-

ferred over live, attenuated virus strains as means of producing immunity (Salk vs. Sabin in regard to polio) has been argued at length over many years. At present, the ever-increasing evidence for possible contamination of all biological materials with cryptic tumor viruses seems to favor the chemical methods of inactivating viruses for the purposes of vaccine preparation. Theoretically the best method, although unfortunately not often practical, is surely that advocated by Fraenkel-Conrat *et al.* (1959) of irreversibly inactivating the isolated viral RNA by reagents such as formaldehyde or β-propiolactone, and subsequently reconstituting it with unmodified viral coat protein to serologically pristine virions. This was actually accomplished successfully with TMV (Staehelin, 1960). Theoretically even better would appear to be the use of only the viral coat protein as the antigen (see Chapter 4). However, unless the protein can be assembled into viruslike particles, its antigenicity is neither qualitatively nor quantitatively equivalent to that of the original virus, and such nucleic-acid-lacking protein shells are generally not very stable.

While monofunctional aldehydes can only make short (—CH_2—) cross-links, dialdehydes, e.g., glutaraldehyde, have been used to produce intermolecular cross-links (Section 3.1.2) and thus establish relative proximity of protein molecules (Schäfer *et al.*, 1975; Sehgal and Hsu, 1977). In these, as in almost all reactions of viruses, inactivation is more probably attributable to the interaction of the reagent with the nucleic acid rather than with the protein. Glutaraldehyde has proven particularly useful in preparing specimens for electron microscopy. This "tanning" action of aldehydes has obviously been of considerable usefulness for a long time.

Glyoxal (Thomas and Hannoun, 1957; Thomas *et al.*, 1957) (17) and its more stable homologue, Kethoxal (18), have a particular affinity for guanine residues (Staehelin, 1960), where the cross-linking action results in transforming into a five-membered ring the 1- and 2-amino positions of the base, a reaction that occurs much more readily in single-stranded than in double-stranded nucleic acids. While Kethoxal is, on TMV-RNA, more inactivating than glyoxal and formaldehyde in the virus the order of efficiency of these three reagents is inversely related to their molecular weights (Staehlin, 1960). The *o*-quinones (19) resulting from oxidation of plant phenolic compounds were also found to react with viral proteins, but these reactions have not been studied in detail (Pierpoint, 1973).

Reductive alkylation (20) represents a useful extension of the use of aldehydes in protein modification (Means and Feeny, 1968). This is

achieved by reducing the primary aldehyde–amine addition or condensation produce with sodium borohydride. With formaldehyde, this leads to ϵ-*N*-methyl lysine residues with high specificity, a particularly useful modification because it allows the easy introduction of a radioactive label into proteins (either as [^{14}C]formaldehyde or as [^{3}H]borohydride) (Rice and Means, 1971). Such modification has the advantage over other labeling procedures, such as iodination or acylation, of having no marked effect on the physicochemical properties of the modified protein. This reaction has not been used with viruses as much as one might expect.

The corresponding reaction with pyridoxal phosphate (21) has been used to test the availability of certain amino groups to this large, charged, and UV-absorbing aldehyde (Pierpoint, 1974; Pierpoint and Carpenter, 1978). By means of ^{32}P-labeled reagent, high specific activities can be introduced into viruses (Eger and Rifkin, 1977).

A variation on this theme is to generate the aldehyde in the envelope of viruses by treatment with galactose oxidase, and then to label the available site by reduction with [^{3}H]-$NaBH_4$ (Luukkonen *et al.*, 1977).

2.3. Halogenating Agents

Molecular halogens, as well as *N*-bonded halogens such as *N*-bromo-succinimide (22) are quite reactive and usually harmful to viruses, probably largely because of their reaction with the nucleic acids (Young *et al.*, 1977). Thus chlorine, forming H-O-Cl in water, is frequently used as a nonspecific inactivating agent (e.g., Vorob'eva *et al.*, 1978). However, under carefully controlled conditions, iodine and bromine compounds can be used for specific modification purposes.

2.3.1. Iodination

Iodine is possibly the most useful and versatile tool of virus modification, again with particular reference to the proteins of virions. Iodine, usually in the form of I_3 or ICl, rapidly oxidizes available SH groups, and allowance must always be made for this reaction in —SH-containing proteins unless the —SH group is in some way protected. The first step in the reaction of —SH with iodine is the formation of a sulfenyl iodide group (—SI), which rapidly decomposes to —S—OH and then becomes oxidized further. It was thus a surprising finding that the

single —SH group of the TMV protein in the virion yields a stable sulfenyliodide group, giving the virus a yellow color (Fraenkel-Conrat, 1955). This represented an early illustration of the power of the quaternary structure of a protein in affecting the reactivity of specific groups through creation of particular microenvironments. Recently, the poliovirus was found to bind much more iodine than was found, on dissociation, to be bound by its components, and it was suggested that sulfenyliodide formation, stable only in the virion, might be the explanation for this observation (Lonberg-Holm and Butterworth, 1976).

The iodination of tyrosine and at times histidine residues in proteins represents the main objective in the use of iodine for a variety of purposes and under different conditions. Different tyrosine residues may show different reactivities in the virion as contrasted to its isolated native or denatured proteins, and these differences again give information about the conformation of such proteins under different conditions (Fraenkel-Conrat and Sherwood, 1967). Iodine treatment of crystals of TMV protein disks (33 molecules) have shown that, here also, the tyrosines near the *C* terminus and to a lesser extent the *N* terminus are the most reactive (Graham and Butler, 1978). Even the relative reactivity of the two orthopositions of a given tyrosine residue may differ under different conditions, resulting in varying proportions of mono- and diiodotyrosine being formed.

A far more trivial use of iodine in virology lies in its availability in high-specific-activity radioactive forms (^{125}I, ^{131}I) which makes it a convenient tool for labeling and detecting very small amounts of viral material.

The use of iodination that has found greatest interest in recent years is as a tool in determining the location of specific proteins in the particles of many different viruses. The grounds for this application is that iodine can be generated not only by oxidation of iodide with small molecules such as H_2O_2 or chloramine T (23) (Hunter and Ludwig, 1962), but also enzymatically by peroxidases in conjuction with very low peroxide concentrations (Marchalonis, 1969; Phillips, and Morrison, 1970). The use of lactoperoxidase appears most effective. Its molecular weight of 78,000 restricts its action to iodination only of proteins located at or near the surface of a virus particle. Thus the approximate position of virus proteins within a particle can be deduced from the extent to which they become enzymatically iodinated, as compared to their reactivity after degradation of the particle, or with chemically produced iodine. Such methodology has been successfully applied to members of most virus families (see Table 2). Through coupling of the lactoperoxidase to sepharose, the generation of iodine can be rendered

TABLE 2

Tests for Surface or External Location of Viral Proteins

Method, Reagent	Virus	References
Acetic anhydride	Poliomyelitis	Lonberg-Holm and Butterworth (1976)
Fluorescein isothiocyanate (66)	Vaccinia	Sarov and Joklik (1972)
Formylmethionylsulfone methylphosphate (14)	Semliki Forest	Gahmberg *et al.* (1972) Garoff and Simmons (1974)
Iodine—lactoperoxidase	Phage ϕ6	Van Etten *et al.* (1976) Sehgal and Hsu (1977)
	PM2	Brewer and Singer (1974)
	Southern bean mosaic	Van Etten *et al.* (1976)
	Adeno	Everitt *et al.* (1975)
	Adeno-associated	Lubeck and Johnson (1977)
	Bovine entero	Carthew and Martin (1974)
	Influenza	Stanley and Haslam (1971)
	Mengo	Lund *et al.* (1977)
	Mouse mammary tumor	Witte *et al.* (1973)
	Poliomyelitis	Lonberg-Holm and Butterworth (1976) Beneke *et al.* (1977)
	Sindbis	Sefton *et al.* (1973)
	Vesicular stomatitis	McSharry (1977)
	Vaccinia	Sarov and Joklik (1972) Katz and Margalith (1973)
Iodine—solid state	Adeno-associated	Lubeck and Johnson (1977) David and Reisfeld (1974)
Iodine—chloroglycoluril	Sendai	Markwell and Fox (1978)
	Newcastle disease	Markwell and Fox (1978)
Iodoacetate (28)	Poliomyelitis	Lonberg-Holm and Butterworth (1976)
Phospholipase C	Vesicular stomatitis	Cartwright *et al.* (1969)
	Influenza	Rothman *et al.* (1976)
Trypsin	Vaccinia	Sarov (1972)
	PM2	Brewer and Singer (1974)
	Vesicular stomatitis	Cartwright *et al.* (1969)
Chymotrypsin	Bovine entero	Carthew and Martin (1974)
	Foot and mouth disease	Cavanagh *et al.* (1977)
Transglutaminase	PM2	Brewer and Singer (1974)
Bromelain	PM2	Hinnen *et al.* (1974)
Pronase	Vesicular stomatitis	McSharry *et al.* (1971)
	Corona	Sturman (1977)
Galactose oxidase + [^{3}H]-$NaBH_4$	Semliki Forest	Luukkonen *et al.* (1977)

yet more superficial, as was done in showing that all three proteins of adeno-associated virus are at the surface (David and Reisfield, 1974; Lubeck and Johnson, 1977).

To achieve high radioactive iodine labeling without addition of iodine and oxidants to sensitive viruses, the use of the Bolton-Hunter reagent in particular can be recommended. Another recently advocated method for gentle labeling of surface proteins utilizes a water-insoluble film of an oxidant, chloroglycoluril, in conjunction with carrier-free Na ^{125}I (Markwell and Fox, 1978).

2.3.2. Bromination

The reaction of bromine-water with viruses appear not to have been studied in detail. It readily reacts with all nucleic acid bases except adenine in RNA and DNA (Brammer, 1963). The primary product with the pyrimidines is the 5,6 addition of BrOH; the reaction with guanosine is more complex. Thus any inactivating effects of brominating agents on viruses are to be attributed to these reactions, although the primary reaction products do not appear to account for the mutagenicity of these reactions (Means and Fraenkel-Conrat, 1971).

N-Bromosuccinimide (22) has been advocated as a specific peptide-chain-cleaving agent in attacking the carbonyl bond of tryptophane. However, it was always recognized that, under the rough conditions necessary to achieve this, other groups would also be oxidized or brominated, with particular reference to the tyrosine residues. Under carefully controlled conditions near neutrality, reaction can be confined to the first step of the tryptophan modification, hydroxybromination of the double bond of the pyrole ring. That the reaction of *N*-bromosuccinimide with proteins is again quite conformation dependent is illustrated by the report that only two of the three tryptophans of TMV protein become oxidized, and that, in the virion and in protein rod aggregates, tryptophan does not react at all (Fairhead *et al.*, 1969). More recently, it was found that actually tyrosine 139 of the TMV protein is the first site of interaction, becoming dibrominated (Okada *et al.*, 1972); it is this same tyrosine that is the most readily iodinated and acetylated (Fraenkel-Conrat and Sherwood, 1967; Fraenkel-Conrat and Colloms, 1967). In the light of these findings, it is particularly surprising that the tryptophan near this tyrosine and the exposed *C* terminus, residue 152, is the only one to be completely resistant to *N*-bromo-succinimide oxidation in the virus protein. Bromination of the reactive

tyrosine did not prevent TMV protein from reconstituting stable and infective virus (Ohno *et al.*, 1972).

2.4. Alkylating and Arylating Agents

The relative reactivity of protein groups to alkylation is generally quite pH dependent; at neutrality, it is thiol>imidazole>amino>phenol groups. However, in acid, methionine is the most reactive, and in alkali, the amino groups become extensively alkylated. Apart from these general rules, the actual reactivities in each protein are much more a consequence of its specific conformation than of any such order of affinities. The —SH groups in viral proteins are in general conformationally masked, but when, even after dissociation of the virus particle, a protein does not readily react with a reagent such as iodoacetamide (24) or *N*-ethylmaleimide (25) which also acts as an alkylating agent, this is taken as evidence that the protein lacks —SH groups.

Alkylating agents had been used with viruses for a long time before it was realized that these agents had a great affinity for nucleic acids and that their virus-inactivating effects were largely due to these reactions. Agents such as β-propiolactone (26) (Brown *et al.*, 1974; Brusick, 1977), ethylene or propylene oxide (27) (Hoff-Jørgensen and Lund, 1972), and iodoacetate or its amide (28, 24) (Singh and Terzaghi, 1974; Lonberg-Holm and Butterworth, 1976) have been used frequently in recent years to achieve virus genome inactivation. The main target for these reagents, as well as for dimethylsulfate (29), is the 7-position of the guanosine residues (Lawley *et al.*, 1969). The lability of the glycosidic bond of this and other alkylated purines which leads to spontaneous depurination in DNA appears to contribute to the virus inactivating action of such alkylations (Shooter *et al.*, 1974*a*,*b*; Shooter, 1975; Karska-Wysocki *et al.*, 1976).

Many comparative studies of the action of alkylating agents such as β-propiolactone, dialkyl sulfates, alkyl alkanesulfonates (30), and *S*- and *N*-mustards (31, 32) on bacteriophages have been reported, with particular concern for their inactivating and mutagenic action (Corbett *et al.*, 1970; Ray *et al.*, 1972). There are no detailed reports, to our knowledge, concerning the extensive modification of the phage proteins by these various alkylating agents. There are, however, several reports concerning their effects on the biological capabilities of treated phages. Thus it is reported that bifunctional alkylating agents (e.g., *S*-mustards and *N*-mustards), while not interfering with adsorption of the phage,

frequently prevent the transfer of the DNA into the bacterium (Lawley *et al.*, 1969; Shooter *et al.*, 1971–1975; Karska-Wysocki *et al.*, 1976). This probably signifies a cross-linking between phage coat proteins and DNA, and as such is of interest from the phage structural point of view.

A particularly interesting result of methylnitrosonitroguanidine (MNNG) (33) treatment of TMV was that this reagent alkylated the RNA and was highly mutagenic when acting on the virus, but not when acting on the viral RNA (Singer *et al.*, 1968; Singer and Fraenkel-Conrat, 1969*a*,*b*). The interpretation that this was due to a proportionately higher alkylation of C than G in the virus is now recognized not to explain the observed and confirmed high mutagenesis of methyl- and ethylnitrosoguanidines (MNNG, ENNG) on TMV. Although 3-MeC has been firmly established to represent a mutagenic base (Ludlum and Wilhelm, 1968; Singer and Fraenkel-Conrat, 1970; Singer *et a.*, 1979; Kröger and Singer, 1979), these reagents, like all *N*-alkyl-*N*-nitroso compounds, have since been found to alkylate all oxygens of nucleic acids much more than the nitrogens (Singer, 1976; Singer *et al.*, 1978). Thus methylnitrosonitroguanidine yields more of the also mutagenic *O*-methyl U and C derivatives than of 3-MeC, and produces these in similar proportions in the TMV virion as in the isolated RNA. The particularly high mutagenicity of alkyl nitrosonitroguanidines when acting on the virion, as contrasted to the RNA, thus remains unexplained, particularly in view of the fact that the other nitroso-alkylating agents show low mutagenicity with TMV and its RNA, resembling the simple alkyl sulfates and alkyl alkanesulfonates in this regard. That the mutagenicity of MNNG is a very complex phenomenon is also indicated by a recent study using phage λ (Yamamoto and Kondo, 1978).

It has become evident in recent years that all *N*-nitrosoalkylating agents, whether formed in the body or produced environmentally, are highly carcinogenic, either directly or after metabolic activation. The nitroso-alkyl ureas (34) are directly carcinogenic; thus their action on viruses and their nucleic acids has been intensely studied in recent years. As stated above, these agents also alkylate with great preference all oxygens in nucleic acids, including those on ribose and phosphate, the ethyl derivative being less reactive but more oxygen specific and carcinogenic than methylnitrosourea (Singer, 1976; Smith, 1976; Singer *et al.*, 1978). Their effects on proteins, including viral proteins, remain to be determined. That the mutagenizing action of ethylnitrosourea on TMV and its RNA is similarly low as that of the simple alkylating agents, quite in contrast to the action of MNNG and ENNG on TMV, discussed above, also remains a puzzle to be resolved in the future. Most of the actions of alkylnitrosourea on nucleic acid bases are mutagenic,

and all compounds of this class are definitely highly carcinogenic. The many detailed studies of the correlations between the chemical action of the alkylnitroso compounds on nucleic acids and their lethality, mutagenicity, and carcinogenicity is beyond the scope of this chapter dealing with virus modifications. The reader is referred to several excellent reviews on this subject (Singer, 1975, 1977).

It has been recognized in recent years that carcinogenic polycyclic hydrocarbons (e.g., 7-bromomethylbenz[a]anthracenes), while not becoming covalently bound by viruses, also yield alkylating agents in the course of metabolic "detoxification," namely, epoxides. A few studies deal with the effect of these "activated" compounds on viruses (e.g., Lotlikar *et al.*, 1972; Dipple and Shooter, 1974; Singer *et al.*, 1980); many more studies use only viral nucleic acids, which in any case are the more reactive and more interesting target compounds. We refer the reader to a recent review of this topic (Grunberger and Weinstein, 1979).

Certain large arylating compounds such as 5-fluoro-2,4-dinitrobenzene (FDNB) (35) and 2,4,6-trinitrobenzene sulfonate (TNBS) (36) have also been used occasionally to ascertain the relative availability of the amino groups of viral proteins (e.g., Scheele and Lauffer, 1969; Perham, 1973; Singh and Terzaghi, 1974; Kaper, 1976). None of these reagents are any more amino-specific than typical alkylating agents, but their color and/or UV absorbance represents an analytical advantage over simple alkylating agents.

2.5. Pyrimidine Modifications (Amines, Bisulfite, etc.)

Various modifications of viruses result secondarily from the primary addition of nucleophilic compounds such as hydroxylamine, hydrazine, semicarbazide, etc. (37–39) to the C5, C6 double bond of the pyrimidines, reactions which are generally quite reversible.* These additions lead to activation of the exocyclic amino group of cytosine, which then tends to become replaced by a substituted nitrogen, or deaminated. The mutagenicity of hydroxylamine and *O*-methoxyamine (40) is due to such replacement reactions and consequent tautomeric shift (Maes and Mesquita, 1970; Fraenkel-Conrat and Singer, 1972; Budowsky *et al.*, 1974; Newlin and Bussell, 1975; Khromov *et al.*, 1977). The lability of the double-bond addition products may account for phenomena such as the ability of MS2 phage to spontaneously

* The addition of water to the double bond under the influence of UV light is discussed in Chapter 6.

recover some of its lost infectivity (Budowsky and Pashneva, 1971; Budowsky *et al.*, 1974).

Bisulfite (41) deaminates cytosine to uracil by the same mechanism, but, in the presence of amino compounds, it greatly potentiates transamination and mutagenicity. Partial recovery of infectivity can also be observed, particularly with semicarbazide (Hayatsu, 1977; Hayatsu and Shiragami, 1979). If protein amino groups are in the vicinity, they can replace the amino group of cytosine; primary nucleic acid–protein cross-linking is the consequence, a reaction that is also potentiated by bisulfite (Krivisky *et al.*, 1973; Turchinsky *et al.*, 1974; Khromov *et al.*, 1977; Sklyadneva *et al.*, 1978). These cross-linking reactions (Tikchonenko *et al.*, 1971, 1973; Andronikov *et al.*, 1974) with particular reference to bacteriophages, of both RNA and single- and double-stranded DNA type, have been discussed by Tikchonenko in Volume 5 of this series. Poliovirus, on a single lethal hit by hydroxylamine, loses all early intracellular functions (Borgert *et al.*, 1971). It must be noted, however, that the inactivation of ϕX174 and that of its naked DNA proceed at similar rates, which suggests that there is little or no chance of such cross-linking in this phage (Krivisky *et al.*, 1973). It also appears of interest to note that the intraphage double-stranded DNA of S_D is similarly available to these reagents as single-stranded nucleic acids, while that isolated DNA (Sklyadneva *et al.*, 1970; Tikchonenko *et al.*, 1971) and T2 DNA (Hayatsu, 1977) are very unreactive. This is in agreement with other indications that the tight packaging of DNA leads to much loss of complementary base interaction.

Several authors have noted that different families of enveloped viruses show great differences in their sensitivity to hydroxylamine (2 M) and its derivatives at pH 7.0 (Franklin and Wecker, 1959; Newlin and Bussell, 1975). It appears likely that the structure of the different envelope components and their reactivity toward these agents accounts for the sensitivity of the orthomyxoviridae and the relative insensitivity of the paramyxoviridae.

Another indication that reagents of this type, under certain circumstances, can affect proteins rather than nucleic acids was reported by Kudo *et al.* (1978).

2.6. Imidoesters

Compounds of the type of methylacetimidates (42) have come into increasing use because of their specificity for substituting the amino groups of proteins (Hunter and Ludwig, 1962; Davies and Stark, 1970).

(Reaction with the amino groups of nucleic acids does not seem to have been studied, but appears possible). The main drawback of these reagents is that they usually require somewhat basic media, e.g., pH 8.5. The presence of $MgCl_2$ appears favorable in the modification of viruses labile at that pH, such as the bromoviridae (Bancroft and Smith, 1975). Of particular advantage are the bifunctional members of this group, e.g., dimethyladipimidate and dimethylsuberimidate (43, 44), because of their ability to form cross-links across definite distances (Davies and Stark, 1970; Lomant and Fairbanks, 1976) (see Section 3.1.2). However, the recent finding that these reagents can act in polymeric fashion throws some doubt on the quantitative aspects of such data (Siezen, 1979).

Methylpicolinimidate (45) has been used on various instances in the hope that the metal-chelating property of the derivative would prove useful for X-ray diffraction studies (see Section 3.2). Its UV absorbance makes it easy to determine the extent of reaction, which appears to be rather specifically only with the amino groups, probably for reasons discussed in Section 2.1. Several mutants of TMV with additional lysine residues have been modified in that manner and for this purpose. These residues were found to show different reactivity depending on their location in the molecule (Perham and Richards, 1968; Perham, 1973).

Another attempt to facilitate X-ray diffraction studies that makes use of an imido ester is the use of methyl-3-mercaptopropionimidate (46), which gives the single reactive lysine of TMV a mercury-binding capability (Perham and Thomas, 1971). This reagent or its butyl analogue (47) (Traut *et al.*, 1973) can also be used for the creation of reversible cross-links by oxidizing the —SH group with hydrogen peroxide either before or after the coupling to protein amino groups (Dubovi and Wagner, 1977) (see Section 3.1.2). The same purpose is served by dithiobis-succinimidyl propionate or butyrate (48, 49), acylating agents of the type discussed previously (Lomant and Fairbanks, 1976).

2.7. Nitrous Acid (HNO_2)

Nitrous acid is the classical reagent for amino nitrogen analysis, causing gaseous nitrogen to be formed in acid solution. The amino group thus become replaced by a hydroxyl group. Since the reaction proceeds at a significant rate only below pH 4.5 and also is not particu-

larly specific, it has found little use for virus protein modification. Its action on nucleic acid, in contrast, has made it the chemical mutagen par excellence, since it transforms cytosine to uracil; deamination of adenine to hypoxathine is also quite straightforward and of biological interest (Schuster and Schramm, 1958; Mundry and Gierer, 1958; Gierer and Mundry, 1958; Boeye, 1959; von Vielmetter and Weider, 1959; Vielmetter and Schuster, 1960; Robinson, 1973). Its action on guanine is more complex, yielding other products besides the expected xanthine, and leading to cross-linking (Shapiro, 1964; Shapiro *et al.*, 1977).

When viruses are treated with nitrous acid, the reactivity of the three amino bases of their nucleic acid differs at times in characteristic manner from their intrinsic reactivity, which must be interpreted as a consequence of intraviral nucleic acid conformation (e.g., Schuster and Wilhelm, 1963). The inactivation of different viruses was found to be both much faster (Sehgal, 1973) and much slower (Sehgal and Krause, 1968) than that of their isolated nucleic acid under the same conditions. When it is slower, this is attributed to protection of the RNA by the coat. When it is faster, there is usually evidence for protein–nucleic acid covalent linkage. This is probably also the explanation for progressive inactivation of nitrous-acid-treated ϕX174, which is not observed on storage of the DNA (Lytle and Ginoza, 1970). Cross-linking of intraphage double-stranded DNA has also been observed and explained (Shapiro, 1964; Shapiro *et al.*, 1977). It appears probable that these various side reactions are due to formation of the intermediate diazo compounds which complicate the nitrous acid reaction more than is usually assumed.

2.8. Sulfhydryl Reagents

Thiol groups, being the most reactive amino acid sidechain groups, have been mentioned in almost every one of the preceding sections. A coordinated critical evaluation of means of modifying these groups appears all the more needed. Since thiol groups are susceptible even to atmospheric oxygen, they are, in most proteins, at least partly masked by conformational restriction. When the —SH groups are sterically available, they yield with acylating agents usually labile acyl derivatives, and with alkylating agents quite stable products. They become oxidized by agents such as halogens, performic acid (50), and hydrogen peroxide. Thus when selective removal or substitution of groups other

than SH are the objective, these must usually first be blocked, or special reagents, conditions, or conformational situations must be exploited.

The substitution by iodine of the single —SH group of TMV coat protein in the virion (Fraenkel-Conrat, 1955) represents an example of conformational control. Control by means of specificity often utilizes the affinity of thiols for mercury. Mercuric chloride ($HgCl_2$), methyl mercuric salts (51), and *p*-chloromercuribenzoate (52) are convenient for such purposes (Fraenkel-Conrat, 1958). As expected, the first two can react with —SH groups which may not be accessible to the larger reagent in the intact virus. Mercury compounds are often useful as a means of disrupting virions in controlled and monitorable fashion (Dorne and Hirth, 1971; Kaper and Jenifer, 1968; Philipson, 1964). Other relatively specific —SH reagents, when used under gentle conditions, are *N*-ethylmaleimide (25) and *o*-iodosobenzoate (53). [The latter was recently reported as splitting peptide chains after all tryptophan residues selectivity in 80% acetic acid at ambient temperature (Mahoney and Hermodson, 1979).] Since many enzymes, including the reverse transcriptase of the retroviridae, have essential thiol groups, this type of reagent can be utilized to pinpoint the role of such enzymes in viral infection (Hung, 1973).

2.9. Other Reagents

Diazotized sulfanilic acid (54) and other diazo compounds have occasionally been used, although they are of low specificity. Diazosulfanilic acid tends to react with imidazole and amino groups, but seems to prefer the amino groups of phospholipids in the case of PM2 phage (Hinnen *et al.*, 1974).

Water-soluble carbodiimides (55) couple amines or amino acids to carboxyl groups, groups that are not readily modifiable under conditions where viruses are stable. In TMV, three carboxyls near the *C* terminus were found to accept cystamine under the influence of this reagent (King and Leberman, 1973).

When it is necessary to modify disulfide groups, two approaches can be used. Oxidation by reagents such as performic acid requires rough conditions and is not very specific for disulfide groups; it has, however, proven very useful in sequence analysis.

Complete reduction by great excess of mercaptan, e.g., mercaptoethanol (56) or dithiothreitol (57), proceeds quickly at neutrality and is thus applicable to biological materials. The great autoxidizability of the

many resulting —SH groups, however, represents a serious drawback. A methodology to overcome this by alkylation immediately following the reduction, and preferably in the presence of the reagent thiol, was developed by Fraenkel-Conrat *et al.* (1951). This approach assures quantitative and stable abolishment of all disulfide bonds. Iodoacetate and iodoacetamide have proven most useful for this purpose. The sensitivity of viral infectivity to gentle treatment with reducing agents has also been used to determine the role of available disulfide bonds in the virion's structure (Carver and Seto, 1968).

An enzymatic method has been used for the purpose of modifying glutamine groups. By means of transglutaminase isolated from guinea pig liver, about 1 mole of radioactive glycine ester was introduced into several of the protein chains of PM-2 phage (Brewer and Singer, 1974).

Degradative enzymes have frequently been used to study the availability of certain proteins, glycoproteins, and lipids, and thus obtain evidence about their location relative to the virion's surface. Some of these applications are listed in Table 2.

3. MODIFICATIONS FOR SPECIFIC PURPOSES

3.1. Location of Viral Components

3.1.1. In Regard to Surface of Virion (Table 2)

By far the most useful reaction to ascertain the location of virus proteins in relation to the virion's surface is iodination. The advantage of this modification is the fact that the reactive iodine species can be generated at different levels of the virion through the use of the easily diffusable oxidants such as H_2O_2 or chloramine T, or of enzymes of various molecular weights and dimensions such as lactoperoxidase. By binding the enzyme to a solid support, its action can be made even more surface specific, and the need to separate the iodinated particle and the enzyme can be avoided. Much of our information about the architecture of virions comes from this type of study (Table 2). Other reagents used for this purpose are also listed in that table.

3.1.2. In Regard to Other Components (Table 3)

Cross-linking between intra- and particularly intermolecular protein groups has become a powerful tool in the study of virion structure,

TABLE 3

Relative Location of Virion Proteins by Cross-Linking

Method, reagent	Virus	Reference
1,5-Difluro-2,4-dinitro-benzene (67)	Murine and feline leukemia	Pinter and Fleissner (1979)
Dimethyladipimidate (43)	Cowpea chlorotic mottle	Bancroft and Smith (1975)
	Mengo	Hordern *et al.* (1979)
	Polio	Wetz and Habermehl (1979)
Dimethylsuberimidate (44)	Influenza	Wiley *et al.* (1977)
	Mengo	Hordern *et al.* (1979)
	PM2	Schäfer *et al.* (1975)
	Polio	Wetz and Habermehl (1979)
	Semliki Forest	Garoff (1974)
	Southern bean mosaic	Sehgal and Hsu (1977)
Dimethyl-3,3′-dithiobis-propionimidate (64)	Influenza	Wiley *et al.* (1977)
	Newcastle disease	Nagai *et al.* (1978)
	Semliki Forest	Richardson and Vance (1978)
	Vesicular stomatitis	Mudd and Swanson (1978)
Dithiobissuccinimidyl propionate (48)	Murine and feline leukemia	Pinter and Fleissner (1979)
	Mengo	Hordern *et al.* (1979)
	Semliki Forest	Richardson and Vance (1978)
	Vesicular stomatitis	Dubovi and Wagner (1977)
Formaldehyde (16)	Mengo	Hordern *et al.* (1979)
	Southern bean mosaic	Sehgal and Hsu (1977)
	Vesicular stomatitis	Dubovi and Wagner (1977)
		Brown *et al.* (1974)
		Mudd and Swanson (1978)
Glutaraldehyde (61)	PM2	Schäfer *et al.* (1975)
	Sindbis	Brown *et al.* (1974)
	Vesicular stomatitis	Brown *et al.* (1974)
Hydrogen peroxide	Vesicular stomatitis	Dubovi and Wagner (1977)
	T7	Hartman *et al.* (1979)
Methylmercaptobutyrimidate (47)	Vesicular stomatis	Dubovi and Wagner (1977)
N,N′-p-Phenylenedimale-imide (62)	PM2	Schäfer *et al.* (1975)
Tartryldiazide (58)	Adeno	Everitt *et al.* (1975)
	Vesicular stomatitis	Dubovi and Wagner (1977)
Toluene 2,4-diisocyanate (59)	PM2	Schäfer *et al.* (1975)

function, and assembly. The simplest reagent with such capability is formaldehyde, which can create methylene cross-links. Wider gaps can be bridged by a great number of bifunctional agents, such as tartryldiazide and higher homologues (58); diisocyanates (59, 60); difunctional alkylating agents (*N* or *S*-mustard gas (31, 32); dialdehydes such as glutaraldehyde (61); bismethylimidates (43, 44); phenylene or alkylene

dimaleimides (62, 63). The last-named one is —SH specific. The others will react, as their monomeric equivalents, mainly with available —SH and —NH_2 groups.

Of particular advantage are agents that cross-link in reversible manner. Hydrogen peroxide has been used to cross-link —SH groups to disulfide bonds. Other reagents introduce thiol groups or disulfide bonds (64, 48, 49), but all of these cross-links present problems due to the possibility of disulfide exchange. Results obtained with tartryl-type cross-linking agents (58), which are available as several homologues of different molecular length and which are split by gentle periodate treatment, thus appear of greater reliability. Applications of these types of studies are listed in Table 3.

In combination with near-UV irradiation, H_2O_2 has also recently been reported to cross-link T7 DNA and protein (Hartman *et al.*, 1979). Other light-catalyzed inactivating agents which may cause cross-linking are 8-methoxy psoralene and chlorpromazine, both used in conjunction with long-wavelength UV light (Esipova *et al.*, 1978; Hanson, 1979). Various psoralene derivatives are beginning to come into use for intraviral localizations, e.g., Trioxsalen (65) (Shen *et al.*, 1977) and certain other derivatives (Hearst and Thiry, 1977; Hallick *et al.*, 1978). Since these are much more active on viruses containing double-stranded than single-stranded nucleic acids, it appears probable that nucleic acid cross-linking is the predominant reaction. In general, these agents and in particular the four substituted psoralenes are inactivating all kinds of viruses (e.g., EMC, reo, herpes) at low concentrations and cross-link at higher concentrations. The actions of psoralene-type compounds are also discussed in Chapter 6.

3.2. Isomorphic Replacement for X-Ray Diffraction Strains

Since SH groups, because of their affinity for mercury, have proven considerably useful for X-ray diffraction studies of viruses, introduction of additional —SH groups at clearly defined sites is of interest. Methyl-3-mercaptopropionimidate (46) was used with TMV for this purpose (Perham and Thomas, 1971). Maleic anhydride modification of TMV was performed for the same purpose, since it was known that mercuric acetate could be subsequently added to the double bond. Methylpicolinimidate has also been used because of the metal-chelating ability of this group.

3.3. For Inactivation and Mutagenesis

Reagents which have particular affinity for nucleic acid groups are most effective for inactivation and mutagenesis. Reactions involved are alkylation and pyrimidine modification (Sections 2.4 and 2.5). Nitrous acid, because of its action on nucleic acid bases, even though this occurs less frequently than its reaction with proteins, is biochemically dramatic. Nitrous acid is the most active point mutagen known; it is followed by hydroxyl- and methoxylamine, because these render cytidine ambiguous, making it act with high frequency like uridine. The simple alkylating agents (alkyl sulfates) have low mutagenic activity, but the more complex ones and particularly the nitrosoalkyl compunds show, under biological conditions, considerable mutagenesis. With RNA *in vitro*, none of these appears to be of comparable mutagenicity to nitrous acid, with the particular exception of the alkyl nitrosoguanidines, when acting on TMV-RNA in the virus rod. All mutagenic reagents are also potentially lethal due to side reactions and the fact that many mutations are *per se* lethal. However, all reagents that attack nucleic acids, whether mutagenic or not, are potentially lethal and can be used for virus inactivation.

4. CONCLUSIONS

Virus modification reactions may be applied for a variety of reasons. Presently, the most frequent use of these procedures is to determine the location of structural components of a virion in regard to the virion surface or to one another. Tables 2 and 3 summarize much of the data now available for these purposes, and experimental details can be found in the cited papers. Such studies have contributed greatly to our understanding of viral architecture and fine structure.

When specific groups in viral proteins are to be modified, reagents of high selectivity are required. Such reagents and their limitations are listed in Table 4. Specific details of methodology can be found in Means and Feeney (1971). These methods are of particular usefulness when specific biological functions, such as enzymatic actions of viral components, are under study.

It should be reinterated that most reactions that cause rapid loss of viral infectivity involve viral nucleic acids. In terms of classes of reagents, those of greatest affinity for nucleic acids are the alkylating agents, the nucleophilic compounds, and nitrous acid and formaldehyde.

TABLE 4

Preferred Reagents for Modification of Specific Groups in Virus Proteins[a]

Groups (amino acid)	
Amino (lys)	Imido esters (42–44), *N*-succinimidylpropionate (11), *O*-methylisourea (68)[a]
Carboxyl (asp,glu)	Diazoacetates (69)[a] (also cySH), carbodiimides (55)
Disulfide (cys)	Sodium borohydride (20), thiols (56, 57)
Guanidyl (arg)	1,2-Cyclohexanedione (70)[a], diacetyl trimer (71)[a]
Imidazole (his)	Photo-oxidation (also cySH, trp), ethoxyformic anydride (15) (also amino)
Indole (trp)	*N*-Bromosuccinimide (22) (also cySH, tyr), 2-hydroxy-5-nitrobenzyl bromide (72)[a] (also cySH)
Phenolic (tyr)	Tetranitromethane (73) (also cySH), iodine (also his, cySH)
Sulfhydryl (cySH)	*N*-Ethylmaleimide (25), 5,5′-dithiobis (2-nitrobenzoic acid) (74)[a], *o*-iodosobenzoate (53), *p*-mercuribenzoate (52), methyl mercuric salts (51), mercuric chloride
Thioether (met)	Cyanogen bromide (CNBr)

[a] Reagents that seem not to have been used with virions.

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