OLIGOMERIZATION REACTIONS OF DEOXYRIBONUCLEOTIDES ON MONTMORILLONITE CLAY: THE EFFECT OF MONONUCLEOTIDE STRUCTURE, PHOSPHATE ACTIVATION AND MONTMORILLONITE COMPOSITION ON PHOSPHODIESTER BOND FORMATION

JAMES P. FERRIS, KAMALUDDIN, and GÖZEN ERTEM
Rensselaer Polytechnic Institute, Troy, NY 12180-3590, U.S.A.

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Abstract. 2′-d-5′-GMP and2′-d-5′-AMP bind 2 times more strongly to montmorillonite 22A than do 2′-d-5′-CMP and 5′-TMP. The dinucleotide $d(pG)_2$ forms in 9.2% yield and the cyclic dinucleotide $c(dpG)_2$ in 5.4% yield in the reaction of 2′-d-5′-GMP with EDAC in the presence of montmorillonite 22A. The yield of $d(pC)_2$ (2.0%) is significantly lower but comparable to that obtained from 5′-TMP. The yield of dimers which contain the phosphodiester bond decreases as the reaction medium is changed from 0.2 M NaCl to a mixture of 0.2 M NaCl and 0.075 M $MgCl_2$. A low yield of $d(pA)_2$ was observed in the condensation reaction of 5′-ImdpA on montmorillonite 22A. The cyclic nucleotide (3′,5′-cdAMP) was obtained in 14% yield from 3′-ImdpA. The yield of $d(pA)_2$ obtained when EDAC is used as the condensing agent increases with increasing iron content of the Na^+-montmorillonite used as catalyst. Evidence is presented which shows that the acidity of the Na^+-montmorillonite is a necessary but not sufficient factor for the montmorillonite catalysis of phosphodiester bond formation.

1. Introduction

The observation of the catalytic activity of RNA suggested that it may have had a central role in primitive life (Been and Cech, 1988). The non-enzymatic template directed synthesis of RNA has been reported (Weimann *et al.*, 1968) but so far there has been few reports of the prebiotic synthesis of the requisite RNA template (Sawai *et al.*, 1989: Schwartz and Orgel, 1985; Schwartz, 1986). In previous papers the montmorillonite-catalyzed formation of RNA oligomers on clays was reported (Ferris *et al.*, 1989a, b). This research was extended to adenine and thymidine deoxynucleotides to gain insight into the factors that are important in phosphodiester bond formation (Ferris and Kamaluddin, 1989). It is recognized that contemporary deoxyribonucleotides are probably too complex to have been utilized in the first life (Ferris, 1987; Ferris and Usher, 1988) but they do provide an understanding of the chemical processes which may have occurred if simple nucleotide precursors (e.g., with only one phosphate and hydroxyl group) were used (Joyce *et al.*, 1987).

In the present research the condensation reactions of deoxyribonucleotides on montmorillonite are extended to 2′-d-5′-GMP and 2′-d-5′-CMP and to the reaction of the 3′- and 5′-phosphorimidazolides of 2′-deoxyadenosine. The variation in the reactivity of montmorillonite clays for the binding and catalysis of these condensation reactions is also reported.

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2. Experimental*

The materials and methods used in this research were described previously (Ferris *et al.*, 1988, 1989a, b; Ferris and Kamaluddin, 1990). Montmorillonite 22A was obtained from Wards Natural Science Establishment, and Volclay SPV-200 was a gift from American Colloid Compound, Arlington Heights, Illinois. Japan montmorillonite (montmorillonite – extra pure grade) was a gift from Dr. Seiji Yuasa of Osaka University who obtained it from Nakarai Tesque Ltd. The remaining montmorillonites were obtained from the Clay Minerals Society, Source Clay Minerals, Department of Geology, University of Missouri, Columbia, Missouri 65211. The analysis of these clays are listed in Table I. EDAC was obtained from Aldrich and nucleotides and enzymes from Sigma with the exception of venom phosphodiesterase low in 5′-nucleotidase activity which was obtained from Boehringer Mannheim and d(pC)$_2$ was obtained from Pharmacia.

The procedures used for the reaction of nucleotides with EDAC and the HPLC and enzymatic characterization of the reaction products were the same as described previously (Ferris and Kamaluddin, 1989). The imidazolides 5′-ImdpA and 3′-ImdpA were prepared by the procedure of Joyce *et al.* (1984). The 5′-ImdpA was shown to be 90% pure and 3′-ImdpA 65% pure by HPLC analysis. The major impurity in the latter was 2′-d-3′-AMP (27%). The hyperchromicity of d(pG)$_2$ and 3′,5′-c(dpG)$_2$ was determined to be 1.34 from the ratio of the UV absorption of the ribonuclease T$_2$ hydrolysis products of GpG and the UV absorption of GpG.

Condensation reactions of imidazolides

A 0.0175 M solution (1.0 mL) of the imidazolide was prepared in 0.2 M NaCl and 0.075 M MgCl$_2$ and 50 mg of Na$^+$-montmorillonite 22A was added. The pH was adjusted to 8 and the reaction was allowed to proceed at room temperature. The reaction was complete after 3–4 days when the proportion of starting material was less than 5% of the total reaction products as measured by HPLC analysis.

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* Abbreviations: EDAC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; 2′d-5′-AMP, 2′-deoxy-5′-adenosine monophosphate; 2′d-3′-AMP, 2′-deoxy-3′-adenosine monophosphate; 3′d-5′-AMP, 3′-deoxy-5′-adenosine monophosphate; 2′dA, 2′-deoxyadenosine; 5′-AMP, 5′-adenosine monophosphate; 5′-TMP, 5′-thymidine monophosphate; 3′-TMP, 3′-thymidine monophosphate; 3′,5′-cdpA, 3′,5′-cyclic deoxyadenylic acid; d(pA)$_2$, deoxyadenyl-3′,5′-deoxyadenosine-5′-phosphate; d(Ap)$_2$, deoxyadenyl-3′,5′-deoxyadenosine-3′-phosphate; 3′,5′-c(dpA)$_2$, cyclic derivative of (pA)$_2$; dAppA, P$_1$,P$_2$-dideoxyadenosine-5′,5′-pyrophosphate; dA$^{3'}$pp$^{3'}$A, P$_1$,P$_2$-dideoxyadenosine-3′,3′-pyrophosphate; AppApA, P$_1$,P$_2$-diadenosine-5′-diphosphate derivative of adenyl adenosine; TppT, P$_1$,P$_2$-dithymidine-5′,5′-pyrophosphate; T$^{3'}$pp$^{3'}$T, P$_1$,P$_2$-dithymidine-3′,3′-pyriphosphate; (pT)$_2$, thymidyl-3′,5′-thymidine-5′-phosphate; 3′,5′-c(pT)$_2$, cyclic derivative of (pT)$_2$; 3′,5′-cpT, 3′,5′-cyclic thymidyclic acid; 2′-d-5′-GMP, 2′-deoxy-5′-guanosine monophosphate; 2′-d-5′-CMP, 2′-deoxy-5′-cytidine monophosphate; d(pG)$_2$, deoxyguanyl-3′,5′-deoxyguanosine-5′-phosphate; c(dpG)$_2$, cyclic derivative of d(pG)$_2$; d(pC)$_2$, deoxycytidyl-3′,5′-deoxycytidine-5′-phosphate; 5′-ImdpA, 5′-imidazolide of 2-deoxyadenosine-5′-phosphate; 3′-ImdpA, 3′-imidazolide of 2′-deoxyadenosine-5′-phosphate; 5′-ImpA, 5′-imidazolide of 5′-adenosine monophosphate; dG$^{5'}$pp$^{5'}$G, P$_1$.P$_2$-dideoxyguanosine-5′,5′-pyrophosphate; dC$^{5'}$pp$^{5'}$G, P$_1$,P$_2$-dideoxycytidine-5′,5′-pyrophosphate; d(pN)$_2$, deoxynucleoside-3′,5′-deoxynucleoside-5′-phosphate; c(dpN)$_2$, the cyclic nucleotide derivative of d(pN)$_2$; NppN, P$_2$,P$_2$-dinucleoside-5′,5′-or 3′,3′-pyrophosphate.

TABLE I

Na^+-montmorillonites

Origin	Abbreviation	Composition
		Tetrahedral Octahedral
Amory, Miss., U.S.A. API 22A	22 A	$(Si_{3.88}Al_{0.12})(Al_{1.40}Fe_{0.32}Mg_{0.31})\ 0_{10}(OH)_2{}^{a}$
Volclay SPV-200 American Colloid Co.	Vol	$(Si_{3.89}Al_{0.11})(Al_{1.57}Fe_{0.17}Fe_{0.02}Mg_{0.27})\ 0_{10}(OH)_2{}^{b}$
Crook County, Wy., U.S.A. Clay Mineral Sources SWy-1	Wy	$(Si_{3.84}Al_{0.16})(Al_{1.53}Fe_{0.20}Mg_{0.32})\ 0_{10}(OH)_2{}^{c}$
Japan Nakarai Tesque Ltd.	Jpn	Composition not available
Apache County, Az., U.S.A. Clay Mineral Sources SAz-1	Az	$(Si_{3.83}Al_{0.17})(Al_{1.39}Fe_{0.08}Mg_{0.72})\ 0_{10}(OH)_2{}^{c}$
Gonzales County, Tx., U.S.A. Clay Mineral Sources STx-1	Tx	$(Si_{3.74}Al_{0.26})(Al_{1.56}Fe_{0.09}Mg_{0.50})\ 0_{10}(OH)_2{}^{c}$
Otay, San Diego County, Ca., U.S.A. Clay Mineral Sources SCa-3	Otay	$(Si_{3.99}Al_{0.01})(Al_{1.43}Fe_{0.03}Mg_{0.64})\ 0_{10}(OH)_2{}^{a}$
Grant County, Wash., U.S.A. Clay Mineral Sources SWa-1 (Ferruginous Smectite)	Nont	$(Si_{3.65}Al_{0.35})(Al_{0.53}Fe_{1.37}Mg_{0.13})\ 0_{10}(OH)_2{}^{d}$

[a] Composition from Kerr *et al.*, 1951.

[b] Brindley and Ertem, 1971.

[c] Composition is calculated (Ross and Hendricks, 1945) from the published analytical data (van Olphen and Fripiat, 1979). The SiO_2 analyses are too high because of the presence of quartz and silicate so the silicon content used is that sufficient to complete the tetrahedral layer. The same approach was used in deriving the composition of the API clays (Kerr *et al.*, 1951).

[d] Goodman *et al.*, 1976.

3. Results and Discussion

A. NUCLEOTIDE BINDING TO Na^+-MONTMORILLONITE

The purine nucleotide 2′-d-5′-GMP binds two times more strongly to Na^+-montmorillonite than does the pyrimidine nucleotide 2′-d-5′-CMP (Figure 1). The extent of binding of 2′-d-5′-GMP to montmorillonite 22A is comparable to that of 2′-d-5′-AMP while that of 2′-d-5′-CMP is comparable to 5′-TMP (Ferris and Kamaluddin, 1989). The binding trends of the different 2′-deoxynucleotides to Na^+-montmorillonite 22A parallel those observed for the corresponding ribonucleotides (Ferris *et al.*, 1989) but with up to three times stronger binding with the deoxy derivatives.

The binding of 2′-d-5′-AMP to all the montmorillonites was investigated to assess the role of binding in catalysis. The clays fell into two binding classes as a result of this study. Those which bind the nucleotide almost as strongly to 22A include Vol, Wy and Jpn montmorillonites and those where the binding was 2–3 times less include Az, Tx and Otay montmorillonites and the high iron smectite Nont (Table I). Comparable trends were observed in the binding of the deoxynucleotide

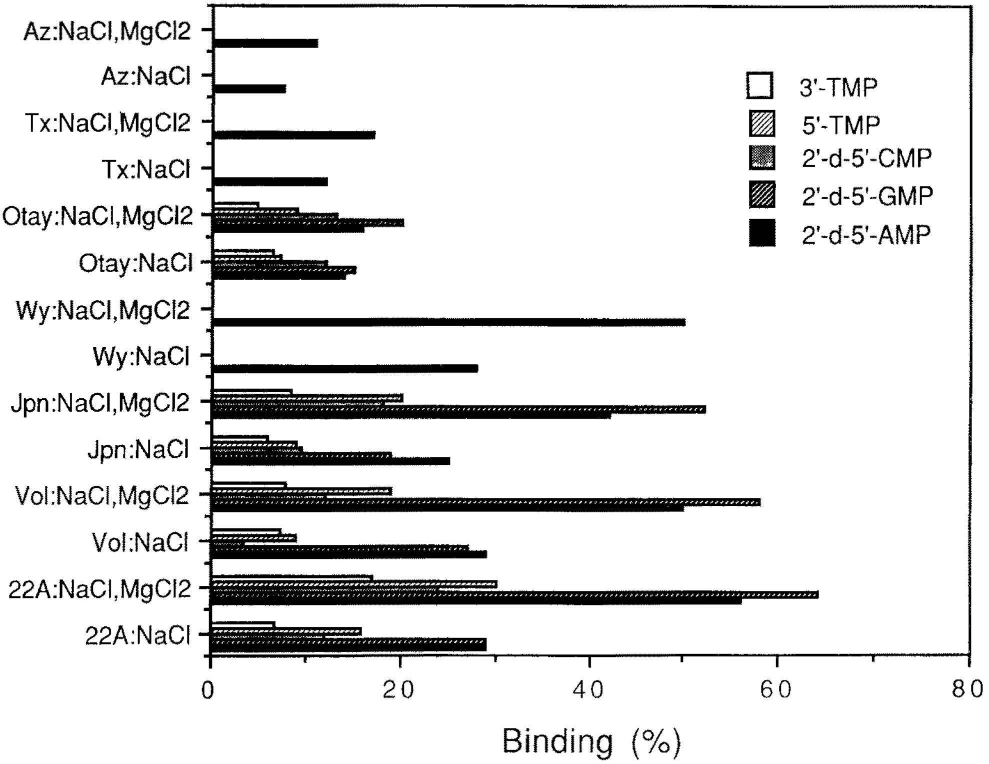


Fig. 1. Nucleotide binding to montmorillonite. The percent bound at 25 °C and pH 6.5 in 0.2 M NaCl or 0.2 M NaCl and 0.075 M $MgCl_2$. See Ferris and Kamaluddin (1989) for experimental procedures.

derivatives of T and C to a smaller group of the same montmorillonites (Figure 1).

The differences observed in binding may be due to the differences in the negative charge density on the lattice of each montmorillonite clay. Those montmorillonites with the greater substitution of Al^{3+} for Si^{4+} and Mg^{2+} for Al^{3+} and consequently greater negative charge on the clay lattice (Az, Tx and Otay – Table I) bind nucleotides less strongly than those with lower lattice charge (22A, Vol, Wy - Table I). This suggests that repulsive interactions between the negative phosphate group of nucleotide and the negative charge on the clay lattice results in diminished binding. This explanation is also consistent with the observation that the montmorillonites fall into two groups with respect to their ability to bind mononucleotides. The lattice charge on Az, Tx and Otay are of about the same (0.65–0.89) and those of 22A, Vol and Wy are also comparable (0.4–0.48) (Table I).

B. REACTIONS OF EDAC WITH 5′-DEOXYNUCLEOTIDES

The dinucleotide $(dpG)_2$ is formed in 9.2% and 3.6% yields in the reaction with 2′-d-5′-GMP with EDAC in the presence of 0.2 M NaCl and a mixture of 0.2 M NaCl and 0.075 M $MgCl_2$ respectively at pH 6.5 (equation 1, table II) (Figure 2). These yields are comparable to the yields of $(pA)_2$ obtained when 2′-d-5′-AMP

TABLE II

Product yield in the reaction of nucleotides with EDAC[a]

	No Clay		22 A		Vol		Jpn		Otay	
	NaCl	NaCl MgCl$_2$	NaCl	NaCl MgCl$_2$	NaCl	NaCl MgCl$_2$	NaCl	NaCl MgCl$_2$	NaCl	NaCl MgCl$_2$
A 2′-d-5′-GMP	91	71	74	69	82	72	85	79	86	80
3′,5′-cdpG	0.0	0.0	0.9^{c,d,c}	0.8	0.3	0.2	0.6^{c}	0.2	0.3	0.1
3′,5′-c(dpG)$_2$	0.0	1.0	5.4^{e}	2.7	0.5	0.0	0.0	0.0	0.2	1.2
$dG^{5'}pp^{5'}G$	2.9	12	8.1^{d,e}	18	8.0	11	3.9^{d}	12	7.2	12
d(pG)$_2$	0.2	0.2	9.2^{d}	3.6	5.6	0.2	1.1^{d}	0.2	0.2	0.2
B 5′-TMP	92^{b}	92^{b}	74^{b}	79^{b}	87	88	91	89	90	89
3′,5′-cpT	0.3	1.1	0.0	0.0	0.3	0.2^{b}	0.2	0.2	0.1	0.0
3′,5′-c(pT)$_2$	1.1	0.0	6.1	3.1	1.2	0.5	0.4	0.3	0.1	0.0
$T^{5'}pp^{5'}T$	0.7	2.8	11	7.1	3.1^{c}	4.0	1.1	4.1	3.1	3.1
(pT)$_2$	0.0	0.0	2.3	2.3	2.3^{c}	1.1	2.0	1.5	0.5	0.3
C 2′-d-5′-CMP	89	82	68	70	82	84	87	85	81	86
$dC^{5'}pp^{5'}C$	2.4	8.4	15^{c,e}	20	8.3	9.2	4.5	7.1	12	10
d(pC)$_2$	0.0	0.0	2.0^{c,d}	1.8	1.2	0.8	1.2	0.8	0.7	0.4
D 3′-TMP	91^{b}	88^{b}	61^{b}	51^{b}	81	85	87	86	85	84
3′,5′-cTMP	0.1	0.2	25	34	7.8	6.2^{c}	4.8	5.0	6.2^{c}	5.4
$T^{3'}pp^{3'}T$	0.9	3.1	7.9	6.8	2.2	1.8^{d}	1.5	3.4	1.8	2.6
(Tp)$_2$	0.4	0.3	1.5	1.4	1.1	0.5^{d}	0.8	0.8	0.5	^{f}

[a] The percent yield after reaction at pH 6.5 for 7 days in either 0.2 M NaCl or 0.2 M NaCl and 0.075 MgCl$_2$. See Ferris and Kamaluddin (1989) for experimental procedures.

[b] Data in these columns from Ferris and Kamaluddin, 1989.

[c] HPLC identification confirmed by coinjection with an authentic sample.

[d] Reaction mixture subjected to enzymatic hydrolysis and products identified by HPLC analysis and coinjection of authentic samples of the hydrolysis products.

[e] Reaction products peak purified by preparative HPLC and subjected to enzymatic hydrolysis. The hydrolysis product was identified by coinjection with an authentic sample.

[f] HPLC peak not resolved from others present.

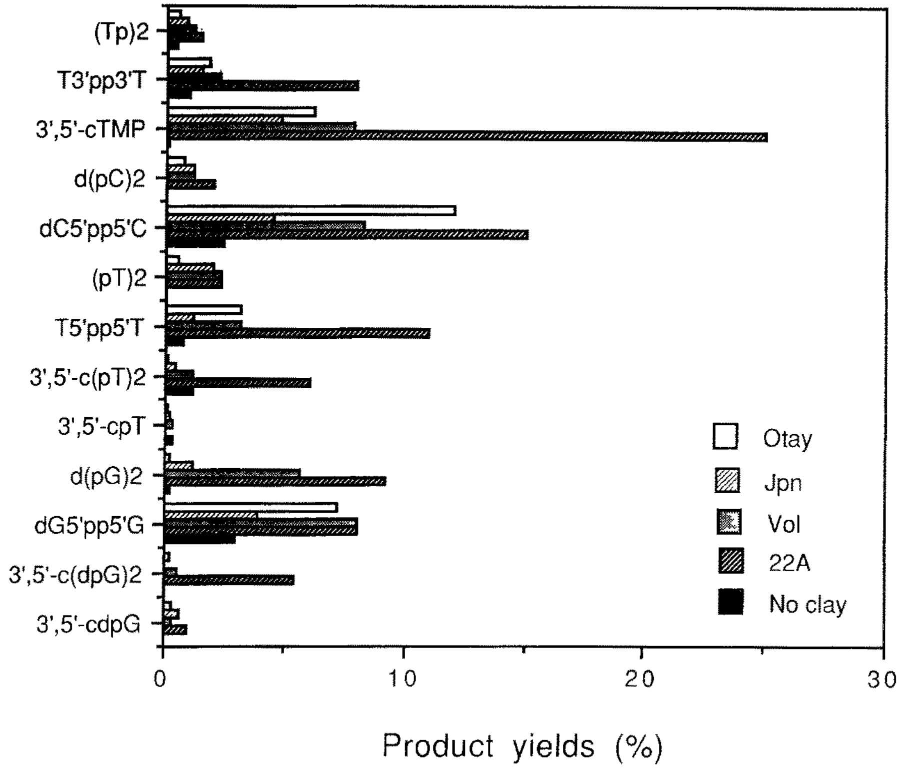
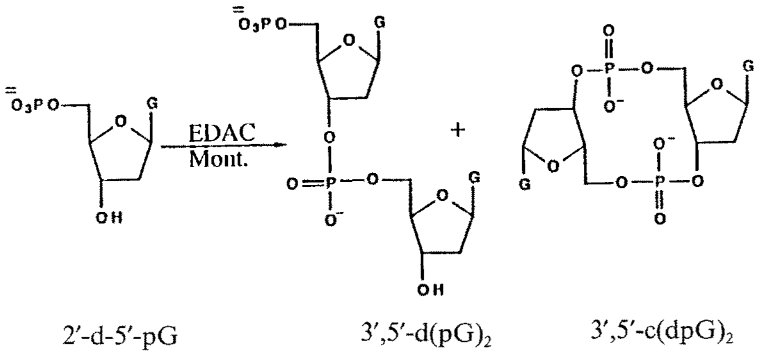


Fig. 2. Products yield in the reaction of nucleotides with EDAC at pH 6.5 in 0.2 M NaCl for 7 days. See Ferris and Kamaluddin (1989) for experimental procedures.

and EDAC react in the presence of 22A (Ferris and Kamaluddin, 1990). A significant part of the $d(pG)_2$ (5.4%) is converted to $c(dpG)_2$, a reaction that was not observed with $d(pA)_2$.



Significantly lower yields of $(pC)_2$ (2.0% and 1.8%) were obtained in the reaction of 2'-d-5'-CMP with EDAC in the presence of 0.2 M NaCl and a mixture of 0.2 M NaCl and 0.075 M $MgCl_2$ respectively (Table II) (Figure 2) than were observed with the purine nucleotides. These $(pC)_2$ yields are comparable to those obtained in the reaction of pT and EDAC in the presence of 22A (Ferris and Kamaluddin, 1990).

The yields of dimers $(pN)_2$ which contain the phosphodiester bond decrease as the reaction medium is changed from 0.2 M NaCl to a mixture of 0.2 M NaCl and 0.075 M $MgCl_2$ (Table II). In some cases the lower yield of dimer containing the phosphodiester bond reflects the more efficient formation of the pyrophosphate bond in the presence of Mg^{2+}. Pyrophosphate is the principal reaction product formed in the absence of montmorillonite and the yields of NppN are substantially higher in the presence of Mg^{2+}.

The extensive investigation of the template directed reactions of the 5′-imidazolides of nucleotides (Weimann *et al.*, 1968; Joyce *et al.*, 1984) prompted a study of their reactivity on clays. Initial studies performed on ribonucleotides resulted in efficient formation of the internucleotide bond (Kebbekus, 1988; Ertem, unpublished results). The rate of reaction of 5′-ImpdA is much faster in the presence of clay as shown by the recovery of 5′-ImdpA when no clay is used (Table III). This observation is consistent with catalysis by the acidic surface of the clay (Ferris *et al.*, 1989). Protonation of the imidazole group of the imidazolide enhances its rate of reaction (Kanavarioti *et al.*, 1989).

A low (1.2%) yield of $d(pA)_2$ was obtained in the reaction 5′-ImdpA on 22A (Table III). Clay catalysis was involved in its formation as indicated by the absence of $d(pA)_2$ in the absence of clay, the much greater yield of $dA^{5'}pp^{5'}A$ in the absence of clay and the presence of unreacted starting material in the absence of clay. A small increase in the yield of $d(pA)_2$ was observed when 4 times the initial amount of 5′-ImdpA was added over a 4-day period to the montmorillonite (Table III). The yield of $d(pA)_2$ is 7-fold greater using EDAC instead of the imidazolide to activate the phosphate group of 2′-d-5′-AMP. The yields based on moles of

TABLE III

Reaction of 5′-ImpdA on Na^+-montmorillonites 22A

	No clay[a]	Na^+-mont. 22A[a]	Na^+-mont. 22A[b]
2′-d-5′-AMP	63	85	44
3′,5′-cdpA	0.1	0.3	0.4[c]
5′-ImdpA	13	0.0	12
$dA^{5'}pp^{5'}A$	22	11	38[d]
$d(pA)_2$	0.0	1.2	1.7[d]

[a] Addition of one portion of 5′-ImpdA at the beginning of the reaction. Other experimental details given in Materials and Methods. All HPLC analysis were performed after a 6 day reaction time. See Experimental for reaction conditions.

[b] Addition of the same amount of 5′-ImpdA at the beginning of the reaction and after 24, 48, and 78 hr. Thus the total amount 5′-ImpdA used is 4 times that used in the other reactions in the Table.

[c] HPLC peak identity confirmed by coinjection with an authentic sample.

[d] HPLC peak identity confirmed by enzymatic hydrolysis and comparison of the retention time of the hydrolysis product with that of an authentic sample.

condensing agent are approximately 3-fold greater with EDAC since a 4-6-fold excess of EDAC is used while two molar equivalents of the imidazolide are reacted per molar equivalent of dimer formed.

The lower yields of d(pA)$_2$ reflect the diminished reactivity of the 3′-OH group of deoxynucleotides as compared to the 2′- and 3′-hydroxyl groups of the ribonucleotides (Lohrmann and Orgel, 1977; Ferris and Kamaluddin, 1990). The low efficiency of d(pA)$_2$ formation and the enhanced rate of ImdpA reaction in the presence of montmorillonite indicates that the montmorillonite serves as a catalyst mainly by enhancing the reactivity of the activated 5′-phosphate grouping. The acidic surface of the montmorillonite serves to protonate the imidazole group thus making the phosphate grouping more electrophilic. A similar acid catalysis would also serve to enhance the rate of reaction of the adduct between EDAC and the phosphate group of nucleotides. There is no evidence for enhancement of the reactivity of the 3′-hydroxyl groups of these nucleotides.

3′-ImdpA underwent an efficient cyclization to 3′,5′-cdpA in the presence of montmorillonte (Equation 2) and no d(pA)$_2$ was formed (Table IV). Very little 3′,5′-cdpA is formed in the absence of montmorillonite. 3′,5′-cdpA is also the main product in the reaction of 2′-d-3′-AMP with EDAC (Ferris and Kamaluddin, 1990). The yields based on nucleotide are almost identical but the yields based on activated nucleotide (imidazolide or EDAC) are much higher with the imidazolide because of 4–6-fold excess of EDAC was used in the investigation of the reaction of EDAC and 2′-d-5′-AMP (Ferris and Kamaluddin, 1990). The enhanced reactivity of the imidazolide may reflect a nucleotide conformation which favors cyclization resulting from the binding of the protonated imidazole grouping of the imidazolide to the surface of the clay.

TABLE IV

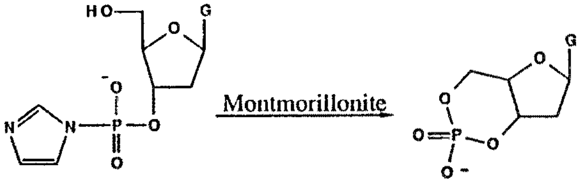
Reaction of 3′-ImpdA on Na$^+$-montmorillonites 22A[a]

	No clay	Na$^+$-mont. 22A
2′-d-3′-AMP	83	79[b]
3′,5′-cdpA	0.6	14[b]
3′-ImdpA	2.5	0.5
$dA^{3'}pp^{3'}A$	14	8.9[c]
d(pA)$_2$	0.0	0.0

[a] The preparation of 3′-ImpdA contained 65% 3′-ImpdA, 27% 2′-d-3′-AMP and 2.6% $dA^{3'}pp^{3'}A$. The yield of 3′,5′-cdpA, and $dA^{3'}pp^{3'}A$ were corrected to reflect the composition of the starting material. The HPLC analysis of the reaction was performed after a 3 day reaction time. See Experimental for reaction conditions.

[b] HPLC peak identity confirmed by coinjection with an authentic sample.

[c] HPLC peak identity confirmed by enzymatic hydrolysis and comparison of the retention time of the hydrolysis product with that of an authentic sample.



2'-d-3'-pG 3',5'-cdpG

C. VARIATION OF PRODUCT YIELD WITH Na^+-MONTMORILLONITE

The yield of $d(pA)_2$ and other products formed in the reaction of 2′-d-5′-AMP and EDAC was investigated to assay the catalytic activity of montmorillonites from a variety of sources. Significant differences were observed in the binding of 2′-d-5′-AMP to montmorillonites (Table I) and these differences were reflected in the yields of $d(pA)_2$ (Tables V and VI). It is expected that the extent of a surface catalyzed reaction has some relationship to the binding of 2′-d-5′-AMP to the surface of the catalyst. Those montmorillonites which catalyze the formation of $d(pA)_2$ (22A, Vol, Wy and Jpn) also bind 2′-d-5′-AMP more strongly. Those clays which exhibit significantly lower binding of 2′-d-5′-AMP (Az, Tx, Otay and Nont) (Figure 1) do not effect a significant enhancement of the $d(pA)_2$ yield over that observed in the absence of montmorillonite. The correlation between binding and $(dpA)_2$yield is not linear. For example, Na^+-montmorillonite 22A gives significantly higher yields than do Vol, Wy and Jpn yet all four montmorillonites exhibit similar binding of 2′-d-5′-AMP.

The trends in montmorillonite catalysis observed with 2′-d-5′-AMP were also apparent with the deoxynucleotides of G, C, and T (Table II). The most effective catalysis was observed with 22A, somewhat less with Vol and Jpn and no catalysis with Otay. The binding trend observed with 2′-d-5′-AMP was also observed with

TABLE V

Reaction of 2′-d-5′-AMP with EDAC in 0.2 M NaCl in the presence of different Na^+-montmorillonites[a]

	No Clay[b]	22 A[b]	Vol	Wy	Jpn	Az	Tx	Otay	Nont
2′-d-5′-AMP	82	62	75	77[c]	81[c]	79	79	71	82
3′,5′-cdpA	0.0	3.4	0.0	0.9	0.1[c]	0.3	0.0	0.1	0.0
$dA^{5'}pp^{5'}A$	15	15	16	14	9.4[c]	13	14	13	11
2′dA	1.1	0.2	0.0	0.2[c]	0.0	0.7	0.7	5.2	0.4
$d(pA)_2$	0.2	8.7	3.6	4.4	3.3	0.3	0.4	0.4	1.0
$dA^{5'}pp^{5'}ApA$	0.0	3.4	0.6	0.8	0.6	0.0	0.0	0.0	0.0
$d(pA)_3$	0.0	2.1	0.0	0.2	0.0	0.0	0.0	0.0	0.0

[a] The percent yield after 7 days reaction at pH 6.5 and 25 °C. See Ferris and Kamaluddin (1989) for experimental procedures.

[b] Ferris and Kamaluddin, (1989).

[c] HPLC peak identity confirmed by coinjection with an authentic sample.

TABLE VI

Reaction of 2′-d-5′-AMP with EDAC in 0.2 M NaCl and 0.075 M $MgCl_2$ at pH 6.5 in the presence of different Na$^+$-montmorillonites

	No Clay[a]	22 A[a]	Vol	Wy	Jpn	Az	Tx	Otay	Nont
2′-d-5′-AMP	68	62	70	75	66	74	70	62	75
3′,5′-cdpA	0.0	2.0	0.1	0.4	0.1	0.4	0.5	0.0	0.0
$dA^{5'}pp^{5'}A$	32	17	26	18	29	18	22	27	17
2′-dA	1.1	0.0	0.0	0.4	0.0	0.8	0.9	2.7	0.5
$d(pA)_2$	0.3	4.8	1.0	1.6	1.4	0.1	0.2	0.4	0.3
$dA^{5'}pp^{5'}ApA$	0.0	4.7	0.6	0.0	0.8	0.0	0.0	0.0	0.0
$d(pA)_3$	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a] The percent yield after 7 days reaction at pH 6.5 and 25 °C. See Ferris and Kamaluddin (1989) for experimental procedures.

[b] Ferris and Kamaluddin, (1989).

the corresponding guanosine nucleotide (Figure 1) but the binding differences between pyrimidine nucleotides were less pronounced. The combined yield of $d(pN)_2$ and $c(dpN)_2$ were also much lower with the pyrimidine nucleotides than with the purine nucleotides.

D. THE ROLE OF MONTMORILLONITE

Studies on the extent of catalysis were initiated using different montmorillonites with the objective of uncovering particular structural characteristics present in those clays which are effective catalysts and absent in those which are not.

(1) Acid Catalyst – As noted previously, Na$^+$-montmorillonite acts as an acid catalyst in the reaction of 5′-ImpA and presumably also in the reaction of EDAC with 2′-d-5′-AMP. Data has also been presented to show that acidity is important for the binding of purine nucleotides to montmorillonite (Ferris *et al.*, 1989a). The role of surface acidity in the reactions of organic compounds on montmorillonite is well documented (Theng, 1974). This surface acidity is due principally to the polarized water molecules in the solvation layer of the exchangeable cations on the clay surface (Sposito, 1984). Since Na$^+$-montmorillonites were used in these studies there should be little difference in the surface acidities of these montmorillonites. The Ca^{2+}- and Mg^{2+}-forms of the Otay montmorillonite, the Na$^+$-form of which exhibited little catalysis of phosphodiester bond formation, were shown to be strong acids (Swoboda and Kunze, 1968). Thus it appears that acidity is a necessary but not sufficient property for catalysis of phosphodiester bond formation.

(2) Binding – Monomers must bind to the clay in the proper orientation for oligomerization to occur. Zn^{2+}- and Cu^{2+}-montmorillonite 22A bind 2′-d-5′-AMP more strongly than does Na$^+$-montmorillonite but these transition metal derivatives do not promote oligomer formation using EDAC as the condensing agent (Ferris *et al.*, 1980, 1989a, b). The absence of reactivity is ascribed to complexation between

the transition metal ion and the phosphate grouping (Clayden and Waugh, 1983) so that the phosphate is blocked from reaction with an alcohol grouping to form a phosphodiester bond. Catalysis is observed when alkali or alkaline earth metal ions are the exchangeable cations. The basic purine nucleotides are protonated by the clay and then they bind electrostatically with the negative sites on the surfaces of the clay platelets. This adsorption leaves the phosphate and hydroxyl groups available for chemical reaction (Ferris *et al.*, 1989a, b). The extent of catalysis increases non-linearly with binding to Na^+-montmorillonite. This suggests that binding is but one of several factors essential for phosphodiester bond formation on montmorillonite.

(3) Clay Lattice Charge – Isomorphous substitution of Mg^{2+} for Al^{3+} or Al^{3+} for Si^{4+} results in a negatively charged aluminosilicate lattice in the montmorillonites. In some clays there is a predominance of isomorphous substitution in either the tetrahedral silicate layer or the octahedral aluminate layer which results in a concentration of charge in a particular region of the lattice. For example, there is extensive isomorphous substitution in the octahedral layers of Az, Tx and Otay montmorillonites (Table I). There is a report of a correlation of the catalysis of hydrocarbon cracking with the total charge on the montmorillonite where the clays

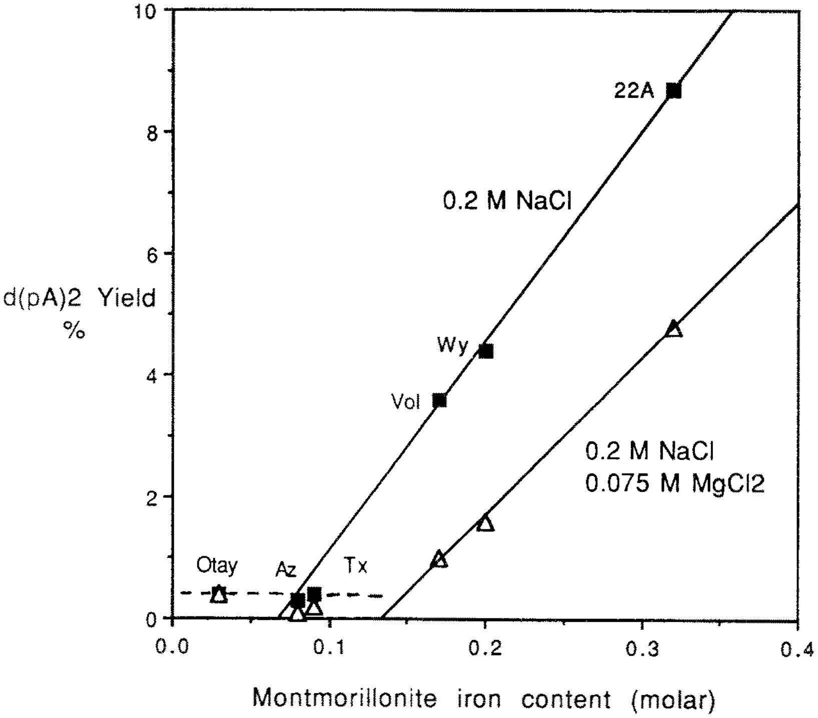


Fig. 3. Variation in the yield of $d(pA)_2$ with the iron content of the montmorillonite. The yield decreases in the order 22a > Wyoming > Volclay. No catalytic activity is shown by ---------. This represents a yield of $<0.5\%$ and is observed with Texas, Arizona and Otay montmorillonites and without added montmorillonite.

had most of their charge in the octahedral layer (Panaiotov *et al.*, 1977). In the present study low catalytic activity is associated with those clays with extensive isomorphous substitution in the octahedral layer (Table I). The clays with the greatest activity, 22A, Vol and Wy, have a low total charge with slightly more in the octahedral than tetrahedral layers. The mechanistic implications of this correlation (if any) are not clear.

(4) Iron Content – A linear correlation was observed between the iron content of the montmorillonite and its catalytic activity for the formation of $d(pA)_2$ in 0.2 M NaCl (Figure 3). Those clays with low iron content (Az, Tx, Otay) do not facilitate the formation of more $d(pA)_2$ than does the control reaction in which no clay is used. The clays with intermediate iron content (Wy, Vol and Jpn) give about a 3% yield of $d(pA)_2$ while use of 22A results in an 8.7% yield. A similar correlation was observed for the formation of $d(pA)_2$ in a mixture of 0.2 M NaCl and 0.075 M $MgCl_2$ (Figure 3). The two lines of Figure 3 have almost the same slopes but have different X intercepts which reflect the differences in the yields of $d(pA)_2$ as the salt content of the medium is changed. These correlations predict that the catalytic activity of the montmorillonite will direct the reaction of EDAC and 2′-d-3′-AMP towards $d(pA)_2$ if the molar proportion of iron in the clay is greater than 0.1 when 0.2 M NaCl is the reaction medium or 0.16 if a mixture of 0.2 M NaCl and 0.075 M $MgCl_2$ in the reaction medium.

The redox properties of clays are usually dependent on their iron content. The extent of oxidation of benzidine or the polymerization of styrene or hydroxymethylmethacrylate is proportional to the Fe^{3+} or Fe^{2+} content, respectively, of montmorillonite clays (Theng, 1974). These reactions are initiated by a one-electron transfer, a process which is not likely in $d(pA)_2$ formation.

We can only speculate on the possible role of lattice iron on catalysis. Iron substitution in the octahedral layer deforms the aluminosilicate lattice because the radius of Fe^{3+} (0.64 Å) is greater than that of Al^{3+} (0.51 Å) (Grim, 1968). Deformation of the clay may generate a site with the appropriate geometry at the face or edge of the lattice to catalyze the formation of the phosphodiester bond. Nontronite may not exhibit such catalysis because the defects are so great that the proper geometry is not present or because the substitution of iron is so extensive that the defects are not present. Mössbauer spectra demonstrate that the environments of iron in nontronite and montmorillonite are different (Cardille and Johnston, 1985, 1986; Cardille, 1988). The Fe^{3+} in montmorillonites are in octahedral sites with trans-hydroxyl groups while the Fe^{3+} in nontronites has cis-substitution on the lattice hydroxyl groups. Since the specific basis for the correlation between catalysis and iron content is unclear it is not possible to speculate on the possible effects of these structural differences on catalysis.

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