Properties of RNA Containing Bacteriophage f4

Bacteriophage f4 is one of the 7 male specific coli phages, which were isolated by LOEB and ZINDER[1]. Because of their specificity for the strains carrying fertility factor f, they were labelled f1 through f7, the numbers designating the isolation from different batches of sewage. Of the original set, only f1 does not contain RNA but is unique in possessing a single stranded DNA[2]. The properties of the second number in the series, (f2) have been extensively studied by ZINDER et al.[3–6]. These include the peptide mapping of the coat protein along with their amino acid composition and sequence of amino acids in peptides, the site of mutation in the peptide and the cistron expression. RNA of f2 has also been shown to possess the messenger activity[3]. A detailed discussion of the properties of RNA containing phages has been made by ZINDER[2].

Bacteriophage f4 differs from that of f2 in having cross reaction with f2 antisera and hence is selected for the present study to compare some of the properties of this phage with those of f2.

Material and methods. Stock culture of f4 was prepared by infecting the host strain of *E. coli* (K 37) on the agar plates. Number of the infective particles per milliliter of the stock was determined by serial dilution and the number of plaques produced. For the large scale preparation of the phage, *E. coli* was grown in tryptone broth using baffled flasks on a rotary shaker till it reached log phase (2×10^8 organisms/ml). The cultures were then infected with stock phage preparation at the multiplicity of 5 particles/cell with addition of calcium chloride ($CaCl_2$ is essential for the adsorption of the phage). The infection was continued for 1 h. Pure phage was then isolated by adopting the procedure of COOPER and ZINDER[7]. The phage yield was 100 mg/10 l culture. Amino acid composition was studied after the acid and alkaline hydrolysis using the commercial columns supplied by Technicon Auto analyser. The protein was extracted by shaking the phage with 88% buffer saturated phenol. The aqueous layer containing RNA was aspirated off and RNA was precipitated with 2 volumes of ethanol. The proteins, after removal of phenol, were precipitated by 6 volumes of ethanol-ether(1:1). The protein constitutes about 75% of the phage particle.

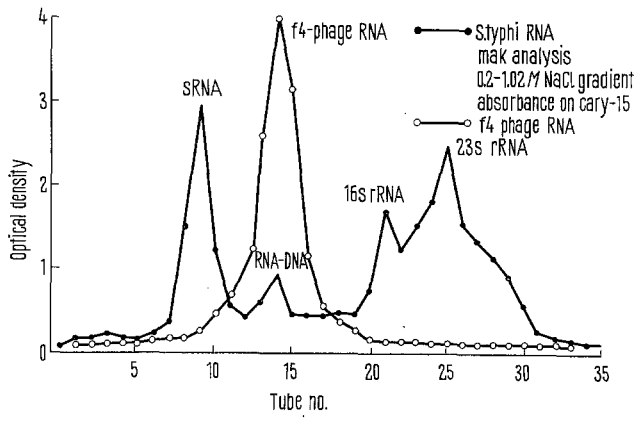
Finger printing of the tryptic digest of the coat protein was carried out after oxidizing the protein with performic acid followed by the trypsin digestion and separating the tryptic peptides by high voltage electrophoresis[3]. RNA was hydrolyzed with KOH and nucleotides separated by electrophoresis using the procedure of MARKHAM and SMITH[8]. MAK column analysis of RNA was performed using 0.2–1.2*M* sodium chloride gradient[9].

Amino acid composition of f4 coat protein

Amino acid	Residue/ mol.	Amino acid	Residue/ mol.
Lysine	6	Arginine	4
Aspartic acid	14	Threonine	9
Serine	13	Glutamic acid	11
Proline	6	Glycine	9
Alanine	14	$^1/_2$ Cystine	2
Valine	14	*Methionine*	2
Isoleucine	8	Leucine	8
Tyrosine	4	Phenylalanine	4
Tryptophan	2		

f2 coat-protein has only 1 methionine residue as against 2 residues in f4 coat-protein. The figures are rounded of to nearest integer. Rest of the figures are identical for both f2 and f4 coat protein.

Results and discussion. F4 coat protein gives similar amino acid composition as that of f2 (Table). Histidine is absent in both. F4 coat protein differs from that of f2 in having an additional methionine residue per molecule (and possibly that is why it cross-reacts with f2 antisera).



Finger prints of f4 are identical with those of f2 tryptic peptides. It revealed 9 peptides. The base composition of RNA shows more or less equimolar proportions of the 4 bases. RNA, when subjected to MAK analysis, gave only one peak (Figure 1). This peak corresponds to RNA: DNA hybrid position of *E. coli* or *S. typhi* RNA preparations. Both *E. coli* and *S. typhi* RNA give identical pattern and hence *S. typhi* RNA, which is readily available at this laboratory, was mixed with that of f4 RNA and run on MAK.

Résumé. Les propriétés du coliphage f4 ont été étudiées. L'hydrolysat acide révèle une composition en acides aminées semblable à celle de f2. L'histidine est absente, et, en comparaison avec le phage f2, il y a un résidu additionnel de méthionine par molécule. L'analyse de la digestion trypsique a montré neuf peptides, comme celle de f2. Le RNA a des proportions équimolaires de bases. Par analyse sur colonne MAK ce RNA présente seulement un pic correspondant à la position de l'hybride RNA:DNA bactérien.

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