THE COAT PROTEIN SUBUNITS OF N4 COLIPHAGE

G.C. Schito, A.M. Molina and A. Pesce
Institute of Microbiology, University of Genoa Medical School
Genoa, Italy

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N4, a virulent bacteriophage active on Escherichia coli K12S strains, is a deoxyribonucleoprotein virus containing 52% protein and 48% DNA (Schito, Rialdi and Pesce, 1966a). In mass culture N4-infected cells become lysis-inhibited (Doermann, 1948) and, while unable to divide, increase in volume due to the continued synthesis of host components. N4 maturation in these complexes proceeds at a linear rate of about 21 phage per cell-minute for at least 180 minutes; consequently, the final yield of virus exceeds 3000 infectious units per bacterium. Condensation of N4 progeny into large paracrystalline aggregates under these conditions has been recently reported (Schito, 1967). Physico-chemical and electron microscope studies on purified N4 preparations (Schito, 1966a) have shown that the phage has a particle molecular weight of 83×10^6, and that it consists of a single, double stranded DNA molecule of 40.5 million daltons (Schito, Rialdi and Pesce, 1966b) surrounded by an icosahedral shell measuring 700 Å in diameter. The protein coat is rendered asymmetric by a small adsorption apparatus composed of a base plate and 4-6 prongs or spikes. This report describes some of the properties of the protein monomer which forms the structural subunit of the phage.

EXPERIMENTAL

The protein moiety of the phage was dissociated from the DNA by using the phenolic extraction method of Anderer (1959). 500 mg of the phage in 50 ml of 0.1 M ammonium acetate, pH 7.0, was mixed with an equal volume of phenol. After 20 minutes at 25°, the protein was precipitated from the phenol phase by adding 10 volumes of methanol, washed twice with the same solvent, then three more times with diethyl ether, and finally lyophylized. The recovery of protein, estimated from dry weight, was 75 to 80%.

The amino acid composition of the virus protein was determined as described by Spackman, Stein and Moore (1958). Weighed samples were hydrolyzed in evacuated sealed tubes in 6 N HCl at 110° for 24, 48 and 72 h, respectively. The hydrolysates were analyzed for constituent amino acids by means of a Beckman amino acid analyzer. Total cystine plus cysteine was measured by oxidation to cysteic acid (Hirs, 1956). Tryptophan was estimated by the method of Spies and Chambers (1949).

RESULTS AND DISCUSSION

The homogeneity of N4 protein preparation was checked by ultracentrifugation and chromatography on DEAE cellulose. Since the phenolic subunits proved to be insoluble in acqueous buffers, the protein was dissolved in 0.1 M Tris, pH 7.2, containing 6 M guanidine (Reithel, 1963). Only one symmetrical peak was observed in sedimentation velocity runs (Fig. 1).

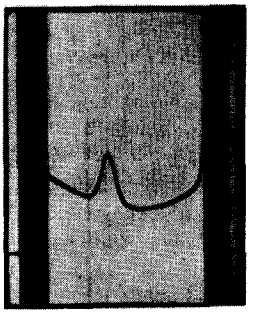

Fig. 1. Sedimentation velocity pattern of N4 coliphage protein dissolved, at a concentration of 11.5 mg per ml, in 0.1 M Tris buffer, pH 7.2, containing 6 M guanidine. Synthetic boundary cell, rotor speed 59,780 rpm, bar angle 65°; the photograph was taken 96 minutes after reaching speed. The S value observed was 0.84.

The sedimentation coefficient of this material, extrapolated to infinite dilution and corrected to standard conditions, was 1.92 S. Chromatography of the coat protein on DEAE cellulose at pH 7.2 in 8 M urea with a gradient of increasing concentration of NaCl also resolved a single broad band. Although these results cannot be used to establish unequivocally the purity of N4 protein, they indicate the absence of gross contamination from other molecules of differing size and surface charge.

The results of amino acid analyses made on N4 coat protein are shown in

Table I. The values were obtained by averaging two sets of duplicate 72 h hydrolysates.

TABLE I

AMINO ACID COMPOSITION OF N4 COLIPHAGE PROTEIN

Amino acid	Moles per 100 moles amino acid	Relative molar ratio°	Number per subunit°°
ALA	8.63	5.9	12
ARG	3.56	2.4	5
ASP	12.11	8.4	17
CYS	-	-	-
GLU	10.70	7.4	15
GLY	8.94	6.1	12
HIS	1.45	1.0	2
ILEU	6.45	4.4	9
LEU	7.70	5.3	11
LYS	5.71	3.9	8
MET	3.31	2.3	5
PHE	3.50	2.4	5
PRO	3.52	2.4	5
SER+	5.29	4.2	8
THR+	8.47	6.4	13
TRY	2.05	1.1	2
TYR+	3.65	2.5	5
VAL	6.98	4.9	10
		Total	144

° Based on histidine as 1.
°° Relative molar ratios x 2. See text for derivation of this factor.
+ Extrapolated to zero hydrolysis time.

N4 structural subunits contain 17 of the commonly occurring amino acids and no unusual ones. Although no cystine-cysteine was detected, the high level of methionine renders this protein unusually rich in sulfur, particularly when compared to the findings for most other viruses (Fraenkel-Conrat, 1965). The coat protein contains over 1.9 times as many acidic as basic amino acid residues and the content of aspartic and glutamic acid residues alone is of about 22%. From the data of Table I, on the basis of one histidine per subunit, a minimum molecular weight of about 9.2×10^3 can be calculated for N4 protein. However, it was found

that 0.214 μmoles of histidine, the amino acid present in least quantity, was obtained from the hydrolysis of 3.96 mg of protein, thus giving a minimum estimate for the molecular weight of 19×10^3. This indicates that a factor of 2 should be used to compute the number of each residue in the protein from total amino acid analysis. As shown in Table I, the results of such calculations indicate a total of about 145 amino acid residues in the coat protein, from which a molecular weight of 18.5×10^3 may be inferred. The subunit molecular weight obtained from these amino acid analyses is consistent with that previously proposed, on the basis of hydrodynamic studies, for the water-soluble N4 protein monomer (Schito, 1966b).

Considering the available chemical evidence, it may be calculated that N4 coliphage is coated by approximately 2300 protein subunits. However, since this virus is known to be morphologically complex, these results, pointing to the existence of a single polypeptide chain of about 145 amino acids in the phenolic preparation, seem difficult to interpret. Indeed, besides those forming the outer coat, several other protein species, including those composing the various substructures of the phage adsorption apparatus, may be present in the virus particle. Whether these minor components were preferentially lost during the extraction procedure or were present in amounts too small to be detected by the analytical methods adopted cannot as yet be decided.

Studies on the primary structure of N4 coat protein subunits are being continued and will be reported in due course.

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