EVIDENCE FOR MULTIPLE COMPONENTS IN THE STRUCTURAL PROTEIN OF TYPE 1 POLIOVIRUS*

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Poliovirus has often served as a model for the structure of the simple or minimal viruses.** Schaffer and Schwerdt (see review, 1959) found the virus particle, or virion, to have a molecular weight of 6.8×10^6 and to consist of 25% RNA and 75% protein. X-ray diffraction studies have found poliovirions to have icosahedral symmetry largely determined by their protein (Finch and Klug, 1959). This property is best explained by a viral capsid with either sixty, or a multiple of sixty, repeating crystallographic structural subunits, each containing a maximum of one-sixtieth of the approximately 5×10^6 Daltons of protein per virion, or about 80,000 Daltons (Crick and Watson, 1957; Finch and Klug, 1959). Evidence is here presented that the polypeptide chains of the structural units of poliovirus have a size of about 27,000, and more unexpectedly, that the virions probably contain several types of proteins.

Cells used for the production of virus were a clonal isolate derived from Hela strain S3 by Dr. N. P. Salzman of the National Institutes of Health. Fifteen liter batches of suspension cultures were grown in 20

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**See the Cold Spring Harbor Symposium on Basic Mechanisms in Animal Virus Biology, 1962, pages 49 and 51-55 for a discussion of the classification of viruses and the terminology of virus structure.

liter carboys in Eagle's medium (Eagle, 1959) supplemented with 4mM glutamine and 5% by volume of either horse or calf serum. Growth characteristics were similar to those of small cultures.

Cells were harvested in a Servall continuous flow centrifuge at 2000 rpm with a flow rate of 200 to 400 ml/min. and infected with a type 1 Mahoney strain of poliovirus. Infection and purification were carried out by a scaled-up modification of the method of Levintow and Darnell (1960).

Protein was separated from the RNA of purified virus by either of two methods. In the first, protein was extracted and RNA precipitated by adding two volumes of cold glacial acetic acid to one volume of cold 0.1 to 1% virus in 2.6M cesium chloride solution. After 20 minutes at 0°C the mixture was centrifuged in the Spinco SW 39 rotor for 20 minutes at 10,000 rpm. Up to 95% of the protein remained in solution. In the second procedure virus was exposed to 5% trichloroacetic acid at 90°C for 10 minutes. Trichloroacetic acid was washed from the protein with alcohol and ether.

Neither of the above protein preparations was soluble in dilute buffers suitable for analytical ultracentrifugation. Suitable preparations could be obtained by oxidation with performic acid using the modification described by Nathans et al (1962) followed by dialysis against 0.05 or 0.1M formic or acetic acid.

Sedimentation velocity experiments on 0.5% solutions of protein in 0.05M formic acid showed heterogeneity but with the same concentration of protein in 0.1% sodium dodecyl sulfate (SDS), a single peak with sedimentation rate of 2S was observed (Maizel, 1963).

Sedimentation equlibrium of oxidized protein in 0.1M acetic acid by the method of Yphantis (1962) gave a molecular weight of 28,400. Although a plot of the natural logarithm of concentration versus radius squared, as shown in lower curve of Figure 1, was straight, indicating that the protein in solution was homogeneous in size, material accumulated at the cell bottom. When dialized against 0.1% SDS in the same buffer (upper line, Figure 1) all of the protein remained in solution while homogeneity and size of the protein were unaffected. Nine determinations on various preparations of protein gave an average molecular weight of 27,000 ± 4,000. Hersch and Schachman (1958) previously used SDS in studies of bushy stunt virus and similarly found negligible effect on protein size.

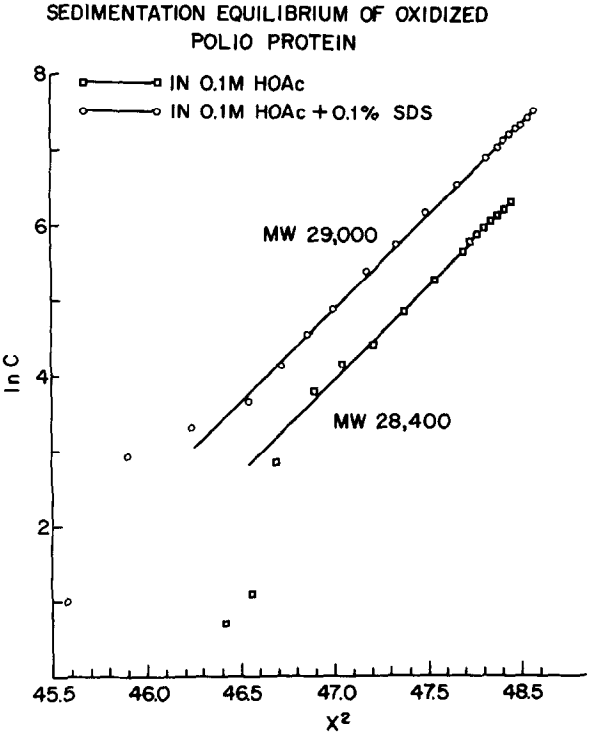

Figure 1 Sedimentation equilibrium of oxidized poliovirus protein at 39,460 rpm for 20 hrs.

Further support for the uniform size of the protein subunits of poliovirus was obtained from zone centrifugation in sucrose gradients of C^{14} amino acid-labeled virus dissociated at 0°C with 0.1N sodium hydroxide. All of the counts could be recovered in the region corresponding to a sedimentation rate of about 2S.

All of the above mentioned evidence is consistent with protein subunits of uniform size. When techniques were used which would reveal differences in composition or sequence the protein was found to be heterogeneous.

Acrylamide gel electrophoresis is a powerful tool for resolving mixtures of proteins. When acetic acid-extracted protein in 10M urea, 0.1M acetic acid and 1% (v/v) 2-mercaptoethanol was subjected to electrophoresis in the pH 4.3 system of Reisfeld et al (1962) modified by the addition of 8M urea to the gel at least four components could be resolved as shown in Figure 2. With C^{14} lysine-labeled protein, up to 90% of the protein could be recovered in the stained bands. The two strong bands were nearly equally labeled and were about twice as radioactive as the weaker bands. Estimation of the molar ratios of the components await further analysis on the size and composition of the individual fractions. Protein prepared by the trichloroacetic acid method gave similar results as did acetic acid-extracted protein from samples of type 1 Parker strain virus purified by another procedure (Charney, et al, 1961). Protein heterogeneity is thus not restricted to a single laboratory strain of virus. When whole virus was dissociated in other ways including direct mixture with 10M urea, 10M urea plus 0.1 to 1% mercaptoethanol, 70-80% dimethylformamide or formamide, or when performic acid oxidized protein was employed, multi-component patterns were also obtained.

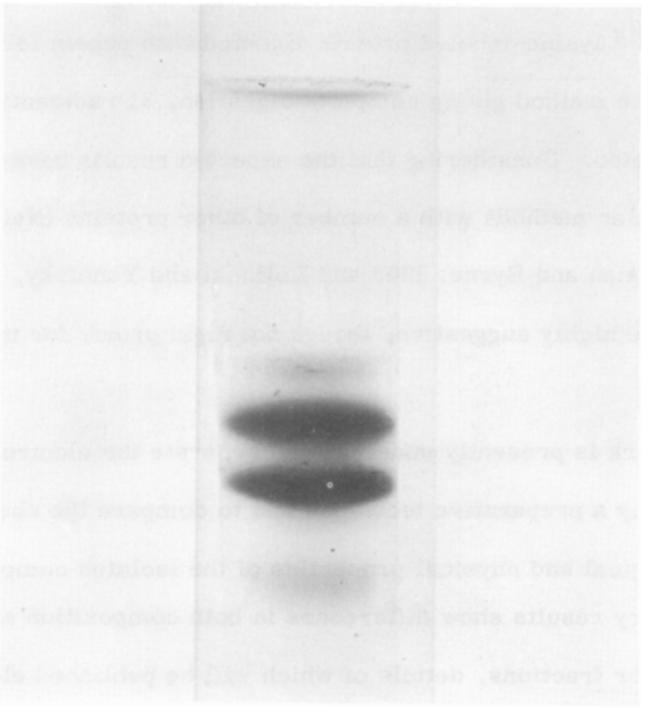

Figure 2 Electrophoresis of acetic acid extracted protein in 8M urea, pH 4.3 acrylamid gel. Cathode at the bottom. The origin was at the faint line near the top. 4 hr. at 16 v/cm.

Results of peptide mapping studies of poliovirus protein using the system of Katz et al (1959) are also more complex than would be expected for a single protein of molecular weight 27,000. The amino acid analyses of this strain of virus (Levintow and Darnell, 1960; Munyon and Salzman, 1962) show that there is about one residue of lysine per 2500 Daltons of protein. Therefore in protein labeled with C^{14} lysine about 10 or 11 radioactive peptides would be expected in a reaction ultimately digested with trypsin. In a variety of digestions of radioactive protein with trypsin or trypsin in combination with other enzymes radioautograms showed over twice the expected number of radioactive peptides. In

maps of C^{14} lysine-labeled protein digested with pepsin followed with trypsin, the method giving complete digestion, 31 radioactive peptides were detected. Considering that the expected results have been obtained using similar methods with a number of other proteins (Nathans, et al, 1962; Rothman and Byrne, 1963 and Helinski and Yanofsky, 1962) these results are highly suggestive, though not rigid proof, for multiple proteins.

Work is presently under way to separate the electrophoretic fractions by a preparative technique and to compare the chemical, immunological and physical properties of the isolated components. Preliminary results show differences in both composition and sequence of the major fractions, details of which will be published elsewhere.

This is the first case of a minimal virus with more than one protein component. Studies on the nature of the differences and the genetic control of these proteins may be expected to give further insight into the structure of viruses.

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