Purification of the Sabin Strain of Poliovirus Type I Through Treatment with Sarkozyl

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Sodium dodecyl sulfate was found to aggregate virions from the Sabin Lsc2ab strain of poliovirus type 1. Aggregation was prevented by high ionic strength buffers. A procedure is described for the rapid purification of the virus through the use of sarkozyl.

Rapid and simple purification procedures exist for the Mahoney strain of poliovirus. None has been reported, however, for the Sabin strain. The Mahoney strain of poliovirus is known to resist treatment with sodium dodecyl sulfate (SDS) at neutral pH (8). Taking advantage of this property, a procedure was developed for the rapid purification of the virus (5). Cytoplasmic extracts from infected cells were treated with 1% SDS and then centrifuged through a gradient of sucrose in SDS buffer [0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, pH 7.4, 0.1 M NaCl, 0.005 M ethylenediaminetetraacetic acid (EDTA), and 0.05% SDS]. It was found, however, in the course of purifying other strains of poliovirus, that such a procedure could not be used for the purification of the Sabin Lsc2ab strain of poliovirus type 1 since it led to aggregation and partial decapsidation of the virions. Use of sarkozyl in high ionic strength buffers prevented these effects and allowed us to devise a procedure for the rapid purification of the virus.

HeLa cells growing in suspension were infected with poliovirus strain Lsc2ab at an input multiplicity of 30 to 50 plaque-forming units (PFU)/cell. One hour after infection they were labeled with 5 μ Ci of tritiated uridine per ml (20 Ci/mole, Commissariat à l'Energie Atomique, Saclay, France) in the presence of 5 μ g of actinomycin D per ml. Four and a half hours later, the cells were washed with Earle's saline, and a cytoplasmic extract was prepared by Dounce homogenization in hypotonic buffer (9). The extract was made 1% with respect to deoxycholate and 0.02 M with respect to EDTA. It was layered on top of a 5 to 20% sucrose gradient in 0.01 M Tris-hydrochloride (pH 7.4), 0.01 M NaCl, and 0.02 M EDTA and centrifuged for 3 hr at 25,000 rev/min at 4 C in the Spinco model SW 25.1 rotor. Fractions of the gradient corresponding to

the peak of virus (approximately 150S) were pooled and used as source of labeled Sabin strain virions.

Sabin strain virions were treated with 1% SDS and centrifuged through a gradient of 15 to 30% sucrose in SDS buffer. Labeled Mahoney strain virions were prepared from cells previously infected as described (5). The two preparations were centrifuged in parallel in the same rotor through similar gradients in SDS buffer. Fig. 1A shows that less than 5% of the SDS-treated Sabin virus was recovered in the peak of virions. Part of the virus was uncoated, liberating 33S virus ribonucleic acid which sedimented at the top of the gradient, whereas another part was found as a pellet at the bottom of the gradient. Centrifugation of the SDS-treated virions through a 15 to 30% sucrose gradient, layered above a 60% sucrose cushion, yielded the results shown in Fig. 1B. Again, from 50 to 70% of the Sabin strain virus sedimented faster than virions. It was recovered as a heterogeneous peak at the bottom of the gradient, above the 60% sucrose cushion. This heavy material was infectious. It had the same density in CsCl as purified virus. It was therefore assumed to represent aggregates of virions.

No dissociation of the aggregates could be obtained whether by dialysis against a variety of low ionic strength buffers or through removal of SDS by exclusion chromatography in 4% agarose (Sephacrose 4 B, Pharmacia, Sweden) or by precipitation with 0.1 M KCl in the cold. However, it was found that increasing the NaCl concentration to 0.3 M resulted in almost complete dissociation of the aggregates with concomitant release of labeled material sedimenting as virions. Since SDS precipitates at such high ionic strength, the detergent sarkozyl (Geigy, Switzerland) was chosen instead. Fig. 2 shows that treatment of Sabin virions with 1% sarkozyl gave the same

FIG. 1. Sedimentation pattern of SDS-treated virions. Virions from the Sabin strain (●) or the Mahoney strain (○) of poliovirus type 1, labeled with tritiated uridine and partially purified as described in the text, were treated with 1% SDS and centrifuged in parallel through various sucrose gradients. (A) Sucrose (15 to 30%) in SDS buffer, for 3.5 hr at 25,000 rev/min at 22 C, in the Spinco model SW 25.1 rotor. (B) Same as A, except for the presence of a 2-ml cushion of 60% sucrose in SDS buffer at the bottom of the gradient. Arrow in B refers to the position of the Mahoney virions.

results as with SDS. In 0.01 M NaCl virions were partially aggregated, whereas 0.3 M NaCl allowed the recovery of a homogeneous peak of intact virions. A successful procedure for the routine purification of the Sabin strain of poliovirus type 1 was therefore developed, by which cytoplasmic extracts from infected cells were made up to $2\times$ SSC (SSC: 0.15 M NaCl and 0.015 M Na citrate) by addition of an appropriate volume of $10\times$ SSC, then treated with 1% sarkozyl, and centrifuged through a gradient of sucrose in $2\times$ SSC as for Fig. 2.

The sensitivity of the Lsc2ab strain toward SDS treatment at neutral pH is not shared by the Bruneilde or the Mahoney strain. On the other hand, virus strain VS 41, which has been derived from Lsc2ab by repeated passages at 41 C and differs from it by a higher *rt* (3), showed the same

sensitivity as its parents. Sensitivity is therefore inherent to the Sabin strain and its derivatives. It was thought that such property might reflect instability of the capsid. Kinetics of inactivation of infectivity by heat and by pH were therefore

FIG. 2. Effect of ionic strength on the sedimentation pattern of sarkozyl-treated virions. Labeled virions from the Sabin strain were treated with 1% sarkozyl either in 0.01 M Tris-hydrochloride (pH 7.4), 0.01 M NaCl, and 0.02 M ethylenediaminetetraacetic acid (EDTA) (●) or in the same buffer made up to 0.3 M NaCl and 0.03 M Na citrate ($2\times$ SSC) (○). They were centrifuged for 3.5 hr at 25,000 rev/min at 4 C in the Spinco model SW 25.1 rotor through 15 to 30% sucrose gradients layered above 2-ml 60% sucrose cushions. The buffers used for making the gradients were 0.01 M Tris-hydrochloride (pH 7.4), 0.1 M NaCl, 0.005 M EDTA, and $2\times$ SSC, respectively.

determined. These studies failed, however, to disclose any major difference between the Sabin and Mahoney strains (M. Fiszman, D. Buchini, and M. Girard, *unpublished data*). Yet, analysis of the capsid proteins of these two strains by electrophoresis through polyacrylamide gels in the presence of SDS (6, 7, 10) showed a profound difference in their composition. The main component of the Sabin strain capsid was VP₂, and capsid protein VP₁ was barely detectable

(Fig. 3). This result is similar to that reported for Mahoney ts mutants (1, 2). It is not known whether sensitivity to SDS is related to such an altered capsid protein composition. Work is in progress to investigate the protein composition of other poliovirus strains and to determine the components of the Sabin strain procapsid.

The use of sarkozyl in 2× SSC could afford generally applicable means for the purification of nonenveloped viruses which have been reported to be sensitive to SDS. Preliminary results with SV40 show that this virus resists treatment with 0.25% sarkozyl, provided that the ionic strength of the buffer is adjusted to that of 2× SSC.

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LITERATURE CITED

- Cooper, P. D. 1969. Genetic analysis of poliovirus. In H. B. Levy (ed.), *The biochemistry of viruses*. Marcel Dekker, New York-London.
- Cooper, P. D., J. V. Maizel, and D. F. Summers. 1970. Evidence for ambiguity in the posttranslational cleavage of poliovirus proteins. *Virology* 41:408-418.
- Fiszman, M. Y., D. Buchini, M. Girard, and A. Lwoff. 1970. Inhibition of poliovirus RNA synthesis by supraoptimal temperatures. *J. Gen. Virol.* 6:293-304.
- Girard, M., L. Marty, and F. Suarez. 1970. Capsid proteins of simian virus 40. *Biochem. Biophys. Res. Commun.* 40:97-102.
- Granboulan, N., and M. Girard. 1969. Molecular weight of poliovirus ribonucleic acid. *J. Virol.* 4:475-479.
- Maizel, J. V. 1969. Acrylamide gel electrophoresis of proteins and nucleic acids. In K. Habel and N. P. Salzman (ed.), *Fundamental techniques in virology*. Academic Press Inc., New York.
- Maizel, J. V., and D. F. Summers. 1968. Evidence for difference in size and composition of the poliovirus specific polypeptide in infected HeLa cells. *Virology* 36:48-54.
- Mandel, B. 1964. The extraction of ribonucleic acid from poliovirus by treatment with sodium dodecyl sulfate. *Virology* 22:360-367.
- Penman, S., Y. Becker, and J. E. Darnell. 1964. A cytoplasmic structure involved in the synthesis and assembly of poliovirus components. *J. Mol. Biol.* 8:541-555.
- Shapiro, A. L., E. Vinuela, and J. V. Maizel. 1969. Molecular weight of polypeptide chains by electrophoresis in SDS polyacrylamide gels. *Biochem. Biophys. Res. Commun.* 28:815-820.

FIG. 3. Electrophoresis in polyacrylamide gels of the capsid proteins of the Sabin and Mahoney strains of poliovirus type 1. Mahoney and Sabin viruses were grown in HeLa cells in the presence of a mixture of ¹⁴C-amino acids (Commissariat à l'Energie Atomique, Saclay, France) and purified from cytoplasmic extracts of infected cells as described in the text. Treatment of the virions and conditions for electrophoresis through 7.5% polyacrylamide gels in the presence of SDS were as described previously (4). Only the fractions corresponding to the three major capsid proteins are shown.