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Single Major Polypeptide of a Calicivirus: Characterization by Polyacrylamide Gel Electrophoresis and Stabilization of Virions by Cross-Linking with Dimethyl Suberimidate

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A calicivirus, San Miguel sea lion virus serotype 4, isolate 15FT, externally labeled with ^{125}I, was shown by gel electrophoresis to possess a single major polypeptide. The polypeptide migrated anomalously upon electrophoresis in two sodium dodecyl sulfate (SDS) systems: more slowly than bovine serum albumin in a continuous phosphate-buffered system and more rapidly than bovine serum albumin in a discontinuous system. Estimated molecular weights in the two systems were approximately 71,000 and 64,000, respectively. There was no clear evidence for a minor virion polypeptide. Treatment of purified San Miguel sea lion virions with dimethyl suberimidate, a cross-linking reagent, preserved virion integrity during long-term storage at 4°C. Oligomeric species of the polypeptide were observed upon electrophoresis of products from cross-linked virions. Based upon a preferred polypeptide molecular weight estimate of 71,000 and distribution of oligomeric species, a calicivirion model with 120 monomeric protein units is proposed as an alternative to a 180-unit model.

San Miguel sea lion virus (SMSV), a calicivirus similar to vesicular exanthema of swine virus, has been isolated from California sea lions and Alaskan fur seals and has been implicated in vesicular disease infecting terrestrial animals (18, 23). High yields of SMSV are obtainable in cell culture, and virions are readily purified, permitting biochemical and biophysical analyses (2, 21, 24). The purified virions are rather unstable, however, upon storage; thus, a means to stabilize virion structure is of considerable interest, especially in studies involving radioimmunochemical reactions. We have investigated the stabilizing effect of dimethyl suberimidate (DMS), a reagent that forms covalent cross-links between monomers of oligomeric proteins (8, 9, 14). DMS and other bifunctional cross-linking reagents have recently been used in elucidating spatial relations of polypeptides in virions of Semliki Forest virus (12) and adenovirus (11). A study on the effect of such a reagent on viral infectivity and on structural stability of virions is reported by Bancroft and Smith (3), who used dimethyl adipimidate with two plant viruses.

The caliciviruses are provisionally classified as picornaviruses (16), though they differ in structure from other *Picornaviridae* (5, 6). Furthermore, they are unique among picornaviruses in that only one major capsid polypeptide is found by gel electrophoresis, as reported by others (2, 6) and confirmed in our laboratory (T. G. Akers, F. L. Schaffer, and M. E. Soergel, unpublished data). In pursuit of additional information on calicivirus structure, we have externally labeled SMSV with ^{125}I and used gel electrophoresis to analyze polypeptide products from DMS-treated and untreated virions.

MATERIALS AND METHODS

Virus. SMSV serotype 4 (SMSV-4), isolate 15FT, was propagated in Vero cells and harvested after 16 to 20 h of incubation at 37°C (24). Twenty milliliters of clarified tissue culture fluid from infected cells was centrifuged through 2 ml of CsCl (ρ = 1.28 g/cm^3) onto a CsCl cushion of ρ = 1.6 g/cm^3 at 22,000 rpm for 140 min in an SW25.1 Spinco rotor. The band at the density interface was further purified in a self-forming CsCl gradient of mean density 1.38 g/cm^3 centrifuged at 40,000 rpm in an SW50L rotor for 16 h. CsCl was removed by dialysis against Dulbecco phosphate-buffered saline (PBS). Alternatively, virus was purified by a polyethylene glycol precipitation-sucrose gradient procedure (2).

Radioactive labeling. Virions from density gradient bands were externally labeled with ^{125}I by a modification of the method of Greenwood et al. (13), either without cross-linking or after cross-linking. Typically, with continuous magnetic stirring, virus in 0.2 ml adjusted to pH 7.4 (with 1 M phosphate buffer or 1 M phosphoric acid) was mixed with 100 μCi of ^{125}I in 20 μl; 20 s after the addition of 10 μl of chloramine-T (3.6 mg/ml), 10 μl of sodium metabisulfite (4.3 mg/ml) was added, followed by 10 μl of

potassium iodide (90 mg/ml) and 20 μl of 1% bovine serum albumin (BSA). The labeled product was dialyzed against PBS (without Ca and Mg). It was then banded in a glycerol density gradient (see conditions below), which served to further purify the virus and separate it from residual radioiodide not remove by brief dialysis.

Conditions for propagation and ^{32}P labeling of virus were as described previously (24).

For molecular-weight marker proteins, BSA, ovalbumin, and human gamma globulin were labeled either with ^{125}I or [^{3}H]formaldehyde. With ^{125}I, the procedure was similar to that described above, omitting 1% BSA. Labeling with [^{3}H]formaldehyde was similar to the method of Rice and Means (19). Briefly, 20 μl of [^{3}H]formaldehyde (0.04 M) was added to 200 μg of protein in 200 μl of 0.2 M borate buffer, pH 9, stirred in an ice bath; after 30 s, 5 μl of sodium borohydride (4 mg/ml) was added followed by three more 5-μl additions every 40 s and finally by 25 μl after 1 additional min. The labeled products were dialyzed exhaustively against PBS (without Ca and Mg).

Cross-linking. Cross-linking of virions was based on the method of Davies and Stark (9). Briefly, 0.1 volume of freshly dissolved DMS at a concentration of 8 mg/ml in 0.2 M triethanolamine, pH 8.5 (TEA) was mixed with 50 to 500 μl purified virions (dialyzed against TEA) and held at room temperature for 3 h, followed by dialysis against TEA.

Glycerol density gradient centrifugation. As an alternative to previously described 5 to 20% sucrose gradients (24), gradients of 10 to 32% (wt/wt) glycerol in PBS (without Ca and Mg) with 0.1% BSA were used to approximate isokinetic sedimentation conditions. Virus was layered over the gradients and centrifuged for 40 min at 39,000 rpm in a Spinco SW50L rotor at 4°C. Samples, usually 4 drops each, were obtained by bottom puncture.

Acrylamide gel electrophoresis. Acrylamide gel electrophoresis of viral proteins was based on the continuous sodium dodecyl sulfate (SDS)-phosphate buffer (SDS-phos) and discontinuous SDS (SDS-disc) methods described by Maizel (15). Purified virus samples and molecular-weight standards in approximately 100 μl of dilute sample preparation buffer containing 1% SDS, 0.1% 2-mercaptoethanol, 0.005% bromophenol blue, and 5% glycerol were heated for 2 min in a boiling water bath. Gel dimensions were 0.625 by 7 cm, and concentrations were 3.5, 5, and 7.5% in the SDS-phos system and 5, 8, and 13% resolving gels with 4% spacer gel in the SDS-disc system. For radioassay, a previously described unit for slicing low-concentration polyacrylamide gels in the frozen state (22) was modified to accommodate unfrozen gels. The gels were extruded from acrylic plastic tubes (in which electrophoresis took place) by means of a hand-operated screw-driven plunger, and segments 1.6-mm thick were cut with a taut wire rigidly mounted between two surgical clamps. To optimize counting efficiency, slices were eluted for 2 to 3 days in 1 ml of 0.1% SDS in PBS (without Ca and Mg) before addition of Aquasol liquid scintillation cocktail.

Radioassay. Radioassay in standard vials (polyacrylamide gel samples) and in disposable microcentrifuge tubes (all other samples) was as described elsewhere (21). Channel spillover corrections were applied for assay of samples containing two isotopes.

Chemicals and radioisotopes. Sources were as follows: crystallized BSA and ovalbumin (for electrophoresis markers) and [^{32}P]orthophosphate (carrier free), Schwarz/Mann, Orangeburg, N.Y.; human gamma globulin fraction II, Miles Laboratories, Kankakee, Ill. (via Calbiochem); [^{3}H]formaldehyde (1% solution in water, specific activity 100 mCi/mmol), iodine-125 (protein iodination grade, carrier free as NaI in 0.1 N NaOH), and Aquasol Universal L.S.C. cocktail, New England Nuclear Corp., Boston, Mass. DMS and TEA, Aldrich Chemical Co., Milwaukee, Wis. SDS (catalogue no. L-5750), Sigma Chemical Co., St. Louis, Mo.

RESULTS

Stabilization of virions by cross-linking. To determine effects of the cross-linking and iodination procedures on SMSV virions, purified 15FT, which had been propagated in the presence of ^{32}P, was exposed to DMS treatment and labeled with ^{125}I. As a control, virus was similarly processed without DMS treatment. Upon sedimentation in glycerol density gradients, the peaks of the internal ^{32}P and external ^{125}I labels were coincident, indicating homogeneity of the viral preparations. After 3 weeks of storage, peak fractions were diluted and rerun in preparative glycerol gradients; the cross-linked preparation showed a homogeneous virion peak, whereas the non-cross-linked preparation showed a small amount of material sedimenting more slowly than the virion peak. Subsequently, small samples of the peaks from these preparative gradients were tested for stability by sedimentation in glycerol on several occasions during storage at 4°C. Figure 1a and b shows radioactivity patterns in glycerol gradients after 11 weeks of storage, when ^{32}P remained detectable by prolonged counting. In the cross-linked preparation, only trace amounts of radioactivity sedimented more slowly than the virion peak (Fig. 1a). In the non-cross-linked preparation (Fig. 1b), there was a small peak of radioiodine at approximately 118S (relative to the 183S virion peak; 20) and an appreciable amount of radiophosphorous at the top of the gradient, indicating a loss of RNA. In a similar test performed after 25 weeks of storage, when ^{32}P was no longer detectable, the cross-linked preparation showed only a minor amount of slowly sedimenting material (Fig. 1c) in contrast to the non-cross-linked preparation, which showed much greater evidence of degradation with radioio-

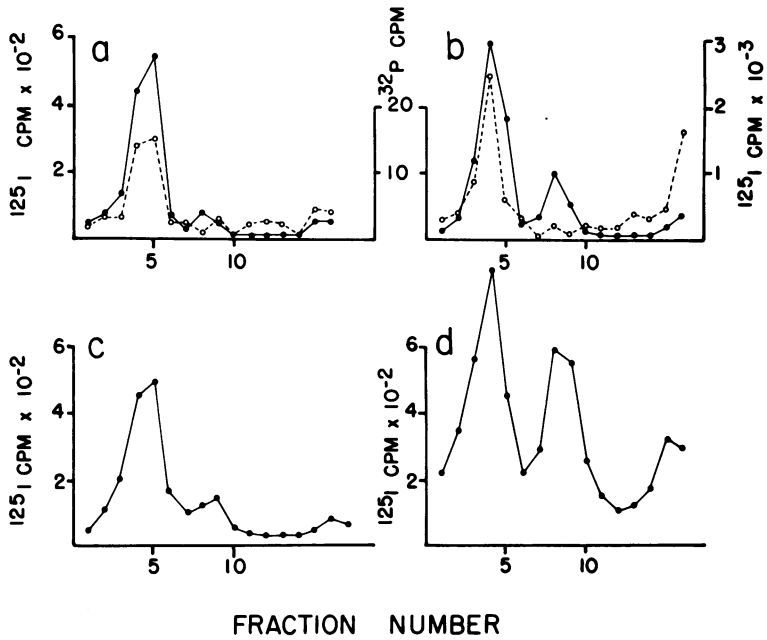

FIG. 1. *Glycerol density gradient sedimentation patterns of (a) cross-linked and (b) non-cross-linked SMSV-4 after 11 weeks of storage, and (c) cross-linked and (d) non-cross-linked SMSV-4 after 25 weeks of storage at 4°C. Symbols: ●, ^{125}I; ○, ^{32}P. ^{32}P was not detectable at 25 weeks. Top of gradients to the right.*

dine in a 118S peak and at the top (Fig. 1d). Another iodinated (not ^{32}P-labeled) cross-linked preparation showed similar or even greater stability when tested by sedimentation periodically up to about 30 weeks after storage either at 4 or −70°C.

Gel electrophoresis of non-cross-linked virion polypeptides. Preliminary experiments with both stained (Coomassie blue) and radiolabeled 15FT polypeptide revealed an electrophoretic migration partially overlapping that of BSA in 7.5% SDS-phos gels. Additionally, labeled 15FT and SMSV-1 (isolate 1MR) polypeptides had identical migration rates relative to BSA. Other experiments assured us that radiolabeling SMSV with ^{125}I and BSA with [^{3}H]formaldehyde did not alter migration rates. These preliminary results in SDS-phos gels indicated a molecular weight discrepant from the value reported for caliciviruses in SDS-disc gels (2). Therefore, we compared the migration of 15FT polypeptide relative to multiply labeled protein markers in SDS-phos and SDS-disc gels to ascertain whether the buffer system was responsible for the discrepancy. That this was the case is clearly demonstrated in Fig. 2, which shows 15FT polypeptide migrating slower than BSA in the pH 7 phosphate-buffered gel (Fig. 2a) and faster than BSA in the higher-pH Tris-glycine-buffered SDS-disc gel (Fig. 2b). Resolution, as indicated by sharpness of peaks, was somewhat better in the SDS-disc gel; however, plots of log molecular weight versus distance migrated (slice number) of marker proteins showed a better fit in the SDS-phos gel (Fig. 2c) than in the SDS-disc gel (Fig. 2d). For these plots we used the same marker molecular weights as Bachrach and Hess (2): BSA = 68,000; immunoglobulin G (IgG) heavy chain = 55,000; ovalbumin = 45,000; and IgG light chain = 23,500. Other reported values for molecular weights of the markers have a slight effect on the fit of the plots and on estimates of SMSV polypeptide molecular weight, but do not rectify the discrepancy. (IgG light chain was more reliable as a marker in the SDS-phos than in the SDS-disc gels, where it migrated with the tracking dye.) The relationships between migration rates of BSA and 15FT polypeptide were independent of gel concentration (3.5 to 7.5% SDS-phos gels, 5 to 13% SDS-disc gels) in both buffer systems.

Gel electrophoresis of cross-linked virion polypeptides. Electrophoresis patterns of labeled products from cross-linked virions in 5 and 3.5% SDS-phos gels are shown in Fig. 3, revealing various species of cross-linked products. The migrational relationships between marker BSA and the 15FT monomer from cross-linked preparations were consistent with those

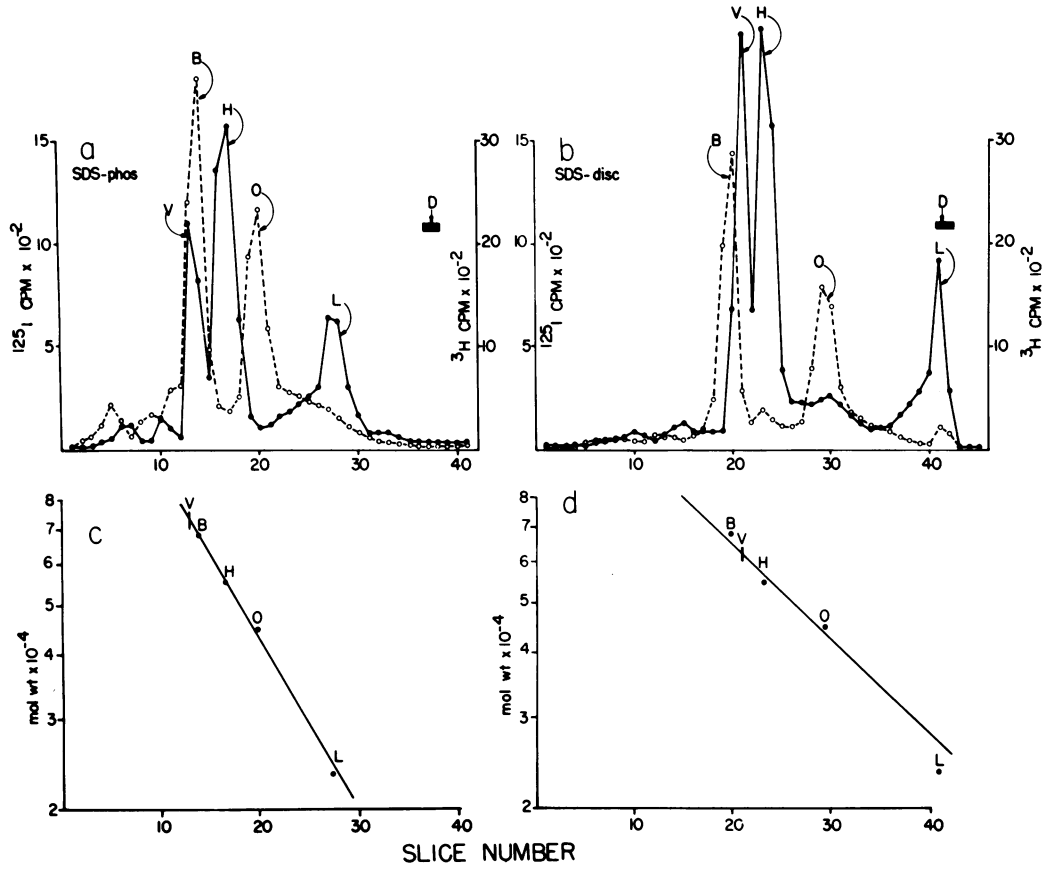

FIG. 2. *Polyacrylamide gel electrophoresis patterns of SMSV-4 polypeptide and marker proteins in (a) SDS-phos gel and (b) SDS-disc gel. V, viral polypeptide; B, BSA; H, IgG heavy chain; O, ovalbumin; L, IgG light chain; D, bromophenol blue tracking dye. (c and d) Semilog plots of molecular weight versus slice number from (a) and (b), respectively. Electrophoresis conditions: (a) 7.5% gel, 60 V, approximately 20 mA, 195 min; (b) 8% gel, 60 V, approximately 3 mA, 170 min. Symbols: ●, ^{125}I; ○, ^{3}H. Bottom of gels (anode) to the right.*

just mentioned for BSA and the polypeptide from non-cross-linked virions. Resolution of monomers, dimers, and trimers was achieved in the 5% gel, but higher-molecular-weight material remained at or near the origin. As expected (9), the 3.5% gel showed improved resolution of cross-linked species, including tetramers and pentamers. The quantity of radioactivity in the oligomeric peaks steadily decreased with increasing oligomer size. In the 3.5% gel, but not the 5% gel, the monomeric and oligomeric species fell on a straight line of a log moleculer weight versus slice number plot. In other 3.5% gels (not shown), hexamers and poorly resolved higher oligomers were observed, but attempts to achieve better resolution of higher oligomers by using 2.5% gels (sliced frozen) were unsucessful.

The double-labeled cross-linked preparation (^{32}P and ^{125}I, described in the virion stability section) was subjected to electrophoresis after SDS-mercaptoethanol treatment. The ^{125}I patterns were similar to those shown in Fig. 3; the ^{32}P failed to penetrate a 5% gel and migrated heterogeneously in the first nine fractions of a 3.5% gel. The ^{32}P heterogeneity was consistent with our earlier finding that homogeneous RNA was not obtained from SMSV by hot SDS treatment (20). These results indicate no detectable phosphoprotein in the virion and no cross-linking between RNA and protein by DMS.

DISCUSSION

In addition to usefulness in elucidating structural relationships in viruses (3, 11, 12), ribo-

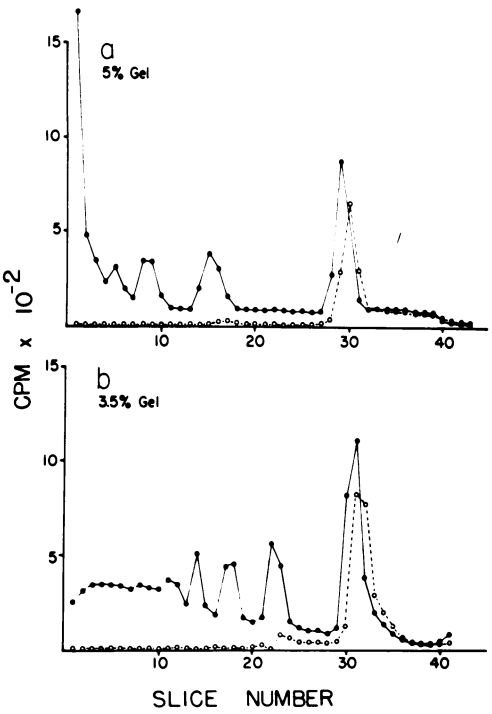

FIG. 3. *Polyacrylamide gel electrophoresis patterns of monomers and cross-linked polypeptides from DMS cross-linked SMSV-4 virions in (a) 5% and (b) 3.5% SDS-phos gels. Electrophoresis conditions: (a) 207 min, (b) 144 min, both at 50 V, approximately 17 mA per gel. Symbols: ●, ^{125}I-labeled viral protein; ○, ^{3}H-labeled BSA marker. Bottom of gels (anode) to the right.*

somes (4), and oligomeric proteins (8, 9, 14), cross-linking reagents have a potential for stabilizing labile structures. Cross-linked *Escherichia coli* 30S ribosomal subunits retained their structural integrity with only a slight loss of biological activity (4). Cross-linking protected virions of cowpea chlorotic mottle and brome mosaic viruses from disassembly upon dialysis against 1 M NaCl at pH 7.5 (3). These plant viruses lost approximately 1 $\log_{10}$ infectivity upon cross-linking, but their RNAs did not lose infectivity, leading to the conclusion that inactivation was coat related, i.e., that uncoating of covalently bonded capsids by host cells was inefficient. Based on limited observations, our results are in accord with such an interpretation; loss of infectivity of SMSV was not greater than 1 $\log_{10}$ upon cross-linking and iodination.

In an incidental preliminary experiment, cross-linking did not appear to alter the inactivation (23) of SMSV at 50°C when diluted in water or 1 M $MgCl_2$. Our primary interest in virion stabilization was in relation to long-term storage. In a study to be reported elsewhere, cross-linked SMSV retained its ability to react specifically with antiserum for at least 7 months, whereas untreated virions lost their ability to react along with loss of virion integrity. In the long-term stability study (Fig. 1), the primary products of degradation of non-cross-linked virions appeared to be RNA and a proteinaceous particle sedimenting at approximately 118S. The physical and antigenic structure of these 118S particles have not been further investigated. Similar particles in preparations of another serotype, SMSV-5, are being studied; it is likely they represent empty calicivirus capsids that others (5) have mentioned but not characterized.

Unique features of the caliciviruses among the *Picornaviridae* are the structures as seen by electron microscopy and the presence of only one major polypeptide (1, 2, 5, 6, 16). Our findings (and lack thereof) bear on three interrelated aspects of calicivirion structure: (i) possible presence of a minor polypeptide; (ii) the molecular weight of the major polypeptide; and (iii) the number and arrangement of structural subunits.

Burroughs and Brown (6) observed a minor polypeptide of about 15,000 daltons in calicivirus preparations labeled with radioactive amino acids and suggested it was a structural component of the virus. Bachrach and Hess (2) found a similar component in radiolabeled but not in stained gels and suggested that it could have been either viral or a contaminant. We did not observe a minor component in our preparations labeled with ^{125}I. However, if it did not contain tyrosine, or if its tyrosine were inaccessible under labeling conditions, we would not expect it to be labeled. (The entire virion protein contains about 4 mol% tyrosine [M. E. Soergel, T. G. Akers, F. L. Schaffer, and A. T. Noma, Virology, in press].) An unlabeled minor component might, depending upon its location in the virion, cross-link to the major polypeptide upon treatment with DMS. If such were the case, we should have observed a species at 80×10^3 to 85×10^3 daltons upon electrophoresis, but there was no clear evidence for such a species. Thus, our results do not support the suggestions of a minor virion polypeptide.

A precise estimation of the molecular weight of SMSV major polypeptide is complicated by its anomalous behavior in the two gel systems. Bachrach and Hess (2) found that the polypeptide from caliciviruses, including SMSV, migrated slightly faster than BSA in SDS-disc gels, indicating an estimated molecular weight

of 61,000. A similar value, 60×10^3 to 65×10^3, for the polypeptide of vesicular exanthema of swine virus and feline calicivirus (formerly called feline picornavirus) was reported by Burroughs and Brown (6), who did not state the buffer system used. However, in another report (5), a value of 68×10^3 was attributed to Burroughs. Our results with externally radiolabeled preparations of SMSV in SDS-disc gels are in accord with the 61×10^3- and 60×10^3- to 65×10^3-dalton estimates. On the other hand, our results in SDS-phos gels indicate a molecular weight higher than that of BSA. For comparative purposes we have calculated molecular weight values relative to BSA at 68,000 as a common marker in co-electrophoresis runs. (For these calculations we used the slopes of the lines established for multiple markers.) The results from five SDS-disc gels (8 and 5% gels) were 64,500 ± 600 daltons, and those from five SDS-phos gels (3.5, 5, and 7.5% gels) were 71,400 ± 400 daltons for the SMSV polypeptide. Anomalous behavior of other viral polypeptides in the two SDS-buffer systems has been reported (7, 17, 25). Although the SDS-disc system appears to give better resolution of polypeptides than does the SDS-phos system (15, 25), the latter has been more exhaustively investigated with regard to precision of molecular-weight estimation (10, 25, 26). Dunker and Rueckert (10) found that intrinsic charge, SDS binding, and chain folding had only minimal (or compensatory) effects on relative mobility of polypeptides in SDS-phos gels. Although Swaney et al. (25) cautioned that care must be taken in estimating the molecular weights of polypeptides from SDS gel electrophoresis, they stated that greater confidence could be placed in the SDS-phos system than in the SDS-disc system, where results vary with the source of SDS. From such considerations, and from our observation of a better fit of markers on log molecular weight versus migration plots from SDS-phos gels, we consider approximately 71,000 to be the best current estimate for the molecular weight of the SMSV polypeptide.

Electron microscopy indicates a calicivirion structure with 32 cups or "negameres" (1), and it has been suggested that it should contain 180 monomeric protein units (2, 5). One simple model with 180 units would have 20 hexamers and 12 pentamers, resulting in icosahedral symmetry. Our results are more compatible with an alternative model without hexamers and pentamers. Icosahedral symmetry could be satisfied with 32 "holes" arranged at each vertex and the center of each triangular face of the icosahedron; this would require 60 structural units (C. F. T. Mattern, personal communication). The 60 structural units could each contain dimers of identical polypeptides, resulting in 120 monomeric units or trimers with 180 units per virion. Our cross-linking studies showed no preference for trimers, pentamers, or hexamers. The predominance of dimers might be taken as evidence for a structure with 120 monomeric proteins. However, if the spatial arrangement were such that probabilities of cross-linking by DMS between and within structural subunits were equal, one would expect a steadily decreasing amount of labeled protein in increasing size of oligomers, as was observed. The mass of a calicivirion has not been precisely determined, but it has been estimated to be about 12×10^6 to 13×10^6 daltons, of which probably 2.6×10^6 daltons is RNA (20). If there are 180 polypeptides per virion, assuming absence of minor polypeptides, the mass of SMSV would be 13.6×10^6 daltons with 61,000-dalton polypeptides and 15.4×10^6 daltons with 71,000-dalton polypeptides. If, however, there are 120 polypeptides, the estimated masses would be 9.9×10^6 and 11.1×10^6 daltons, respectively, for polypeptides of 61×10^3 and 71×10^3 daltons. Thus, the model with 120 polypeptides as dimers in 60 structural units would be favored if 71,000 is the better estimate of the polypeptide molecular weight, as discussed above.

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LITERATURE CITED

1. **Almeida, J. D., A. P. Waterson, J. Prydie, and E. W. L. Fletcher.** 1968. The structure of a feline picornavirus and its relevance to cubic viruses in general. Arch. Gesamte Virusforsch. 25:105-114.
2. **Bachrach, H. L., and W. R. Hess.** 1973. Animal picornaviruses with a single major species of capsid protein. Biochem. Biophys. Res. Commun. 55:141-149.
3. **Bancroft, J. B., and D. B. Smith.** 1975. The effect of dimethyladipimidate on the stability of cowpea chlorotic mottle and brome mosaic viruses. J. Gen. Virol. 27:257-261.
4. **Bickle, T. A., J. W. B. Hershey, and R. R. Traut.** 1972. Spatial arrangement of ribosomal proteins: reaction of the *Escherichia coli* 30S subunit with *bis*-imidoesters. Proc. Natl. Acad. Sci. U.S.A. 69:1327-1331.
5. **Brown, F., and R. Hull.** 1973. Comparative virology of the small RNA viruses. J. Gen. Virol. 20:43-60.
6. **Burroughs, J. N., and F. Brown.** 1974. Physico-chemical evidence for the re-classification of the caliciviruses. J. Gen. Virol. 22:281-286.
7. **Camacho, A., J. L. Carrascosa, E. Vinuela, and M. Salas.** 1975. Discrepancy in the mobility of a protein of phage ϕ29 in two different SDS polyacrylamide-gel systems. Anal. Biochem. 69:395-400.
8. **Carpenter, F. H., and K. T. Harrington.** 1972. Intermolecular cross-linking of monomeric proteins and cross-linking of oligomeric proteins as a probe of quaternary structure. J. Biol. Chem. 247:5580-5586.

9. **Davies, G. E., and G. R. Stark.** 1970. Use of dimethyl suberimidate, a cross-linking reagent, in studying the subunit structure of oligomeric proteins. Proc. Natl. Acad. Sci. U.S.A. 66:651-656.
10. **Dunker, A. K., and R. R. Rueckert.** 1969. Observations on molecular weight determinations on polyacrylamide gel. J. Biol. Chem. 244:5074-5080.
11. **Everitt, E., L. Lutter, and L. Philipson.** 1975. Structural proteins of adenoviruses. XII. Location and neighbor relationship among proteins of adenovirion type 2 as revealed by enzymatic iodination, immunoprecipitation and chemical cross-linking. Virology 67:197-208.
12. **Garoff, H.** 1974. Cross-linking of the spike glycoproteins in Semliki Forest virus with dimethylsuberimidate. Virology 62:385-392.
13. **Greenwood, F. C., W. M. Hunter, and J. S. Glover.** 1963. The preparation of ^{131}I-labeled human growth hormone of high specific radioactivity. Biochem. J. 89:114-123.
14. **Hucho, F., and M. Janda.** 1974. Investigation of the quaternary structure of beef liver glutamate dehydrogenase with bifunctional reagents. Biochem. Biophys. Res. Commun. 57:1080-1088.
15. **Maizel, J. V.** 1971. Polyacrylamide gel electrophoresis of viral proteins, p. 179-246. *In* K. Maramorosch and H. Koprowski (ed.), Methods in virology, vol. 5. Academic Press Inc., New York.
16. **Melnick, J. L., et al.** 1974. *Picornaviridae*. Intervirology 4:303-316.
17. **Obijeski, J. F., A. T. Marchenko, D. H. L. Bishop, B. W. Cann, and F. A. Murphy.** 1974. Comparative electrophoretic analysis of the virus proteins of four rhabdoviruses. J. Gen. Virol. 22:21-33.
18. **Prato, C. M., T. G. Akers, and A. W. Smith.** 1974. Serological evidence of calicivirus transmission between marine and terrestrial mammals. Nature (London) 249:255-256.
19. **Rice, R. H., and G. E. Means.** 1971. Radioactive labeling of proteins *in vitro*. J. Biol. Chem. 246:831-832.
20. **Schaffer, F. L., and M. E. Soergel.** 1973. Biochemical and biophysical characterization of calicivirus isolates from pinnipeds. Intervirology 1:210-219.
21. **Schaffer, F. L., and M. E. Soergel.** 1974. Liquid scintillation radioassay in disposable microcentrifuge tubes: radioimmune precipitates and other applications. Appl. Microbiol. 28:280-287.
22. **Schaffer, F. L., M. E. Soergel, and D. C. Straube.** 1971. Electrophoretic analysis of ribosomal and viral ribonucleic acids with a simple technique for slicing low-concentration polyacrylamide gels. Appl. Microbiol. 22:538-545.
23. **Smith, A. W., T. G. Akers, S. H. Madin, and N. A. Vedros.** 1973. San Miguel sea lion virus isolation, preliminary characterization and relationship to vesicular exanthema of swine virus. Nature (London) 244:108-110.
24. **Soergel, M. E., A. W. Smith, and F. L. Schaffer.** 1975. Biophysical comparisons of calicivirus serotypes isolated from pinnipeds. Intervirology 5:239-244.
25. **Swaney, J. B., G. F. Vande Woude, and H. L. Bachrach.** 1974. Sodium dodecylsulfate-dependent anomalies in gel electrophoresis: alterations in the banding patterns of foot-and-mouth disease virus polypeptides. Anal. Biochem. 58:337-346.
26. **Weber, K., and M. Osborn.** 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244:4406-4412.