Solubilization of the Semliki Forest Virus Membrane with Sodium Dodecyl Sulfate†

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ABSTRACT: The dissociation of Semliki Forest virus induced by increasing concentrations of the anionic detergent sodium dodecyl sulfate was studied using density gradient centrifugation. Detectable binding to the virus started well below the critical micellar concentration of the detergent and increased thereafter with increased detergent concentration. At 4° there were about 11,000 binding sites per virus particle with an average association constant of about 10^5 M^{-1}. The extent of virus dissociation could be controlled both by the detergent concentration and by the temperature. At 4° only disruption ("lysis") of the virus membrane could be observed. At 20° most of the membrane was solubilized into lipoprotein complexes, and the nucleocapsid dissociated into RNA and protein. Complete delipidation of the viral membrane proteins was achieved at 30° at a detergent concentration still below the critical micellar concentration.

Recent progress in the study of membrane protein composition has been aided considerably by the use of the anionic detergent sodium dodecyl sulfate (SDS).[1] High concentrations of this detergent dissociate membranes into their lipid and protein components (Engelman et al., 1967; Jones and Kennedy, 1969; Simons and Kääriäinen, 1970; Shapiro et al., 1967; Weber and Osborn, 1969). In this process most proteins are denatured and lose their biological activities (see Tanford, 1968; Steinhardt and Reynolds, 1969). Drastic conformational changes are induced at free SDS concentrations above 1.5×10^{-4} M resulting in massive SDS binding by the proteins (Pitt-Rivers and Impiombato, 1968; Reynolds and Tanford, 1970).

We are investigating the mechanisms by which detergents solubilize biological membranes and lipoproteins. We have previously studied the mode of actions of the nonionic detergent, Triton X-100, and of the bile salt, sodium deoxycholate (Helenius and Simons, 1971, 1972; Helenius and Söderlund, 1973; Simons et al., 1973a). In contrast to SDS, these detergents can usually be used to extract proteins from membranes without disruption of native structure or loss of biological activity (see Helenius and Simons, 1975).

As our experimental membrane model we have used the membrane of Semliki Forest (SF) virus because it is easy to handle, to purify, and to label radioactively, and it is homogeneous and it is simple in structure (Simons et al., 1973b). SF virus is composed of a spherical nucleocapsid, which is surrounded by a lipid membrane (Acheson and Tamm, 1967). The nucleocapsid consists of the 42S viral RNA (molecular weight about 4×10^6) and one lysine-rich protein species (molecular weight 3.3×10^4) (Levin and Friedman, 1971; Simons and Kääriäinen, 1970). The viral membrane has a lipid composition similar to that of the host cell plasma membrane (Renkonen et al., 1971), and its lipids are arranged in a bilayer structure (Harrison et al., 1971). Three glycosylated proteins have been found in about equimolar ratios in the SF virus membrane: E1, E2 (both with a molecular weight of about 5×10^4), and E3 (molecular weight about 10^4) (Garoff et al., 1974). The glycoproteins form projections, spikes, on the external surface of the virus (Compans, 1971; Gahmberg et al., 1972). These are attached to the bilayer by hydrophobic peptides present in both E1 and E2 (Utermann and Simons, 1974). One or both of these segments extend across the membrane into close contact with the nucleocapsid (Garoff and Simons, 1974).

In this paper we have studied how the SF virus membrane is solubilized by increasing concentrations of SDS. Although SDS is widely used to solubilize membranes, few systematic studies of its mode of action have been published.

Experimental Section

Materials. RNase free sucrose was purchased from Mann (New York, N.Y.); [^{35}S]methionine and [^{3}H]uridine were from Radiochemical Centre (England); [^{3}H]leucine, [^{3}H]isoleucine, and [^{3}H]valine were from New England Nuclear (Boston, Mass.); ^{32}P-labeled carrier-free orthophosphate was from Institutt för atomenergi (Norway). D_2O was from Norsk Hydro, Norway.

Virus. A prototype strain of SF virus was grown in monolayer cultures of baby hamster kidney cells (BHK 21) and purified as described previously (Kääriäinen et al., 1969). The purity of each isolated virus batch was determined by SDS–polyacrylamide gel electrophoresis and electron microscopy. The chemical composition used in the calculations was RNA, 6%; nucleocapsid protein, 12%; membrane protein, 45%; lipid, 31%; protein-bound carbohydrate, 6%. The molecular weight of the virus is approximately 6.4×10^7 (Laine et al., 1973). Labeling of virus with ^{32}P-labeled orthophosphate (20 μCi/ml), [^{3}H]leucine, [^{3}H]isoleucine, and [^{3}H]valine (33 μCi of each/ml), with [^{35}S]methionine (107 μCi/ml), and with [^{3}H]uridine (125 μCi/ml) was carried out as described previously (Kääriäinen et al., 1969; Helenius and Söderlund, 1973). In the ^{32}P-labeled virus about half of the radioactivity was found in the RNA and half in the phospholipids.

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[1] Abbreviations used are: SDS, sodium dodecyl sulfate; SF virus, Semliki Forest virus.

Virus Membrane Protein. The viral membrane proteins were isolated lipid-free from SF virus labeled with [^{3}H]leucine, [^{3}H]isoleucine, and [^{3}H]valine in a water-soluble form by a simple procedure which will be published separately (A. Helenius, C-H. Bonsdorff, and K. Simons, manuscript in preparation). Essentially, the method consists of adding enough Triton X-100 to SF virus to delipidate the membrane proteins (Simons et al., 1973a). This mixture is layered on a 12-ml sucrose gradient (20–50% in 0.05 *M* Tris (pH 7.2)–0.1 *M* NaCl, with a 0.3-ml zone of 1% Triton X-100 in 15% sucrose on top) and centrifuged in an MSE 6 × 14 ml rotor at 40,000 rpm for 22 hr at 20° in an MSE superspeed 75 ultracentrifuge. The membrane proteins are recovered from the gradient as a soluble complex. The molecular weight of the complex is about 900,000 and it contains less than 1% phospholipid and less than 1% of Triton X-100. Each complex probably contains eight polypeptides of E1, E2, and E3.

Liposomes. The phospholipids were isolated from BHK 21 cells as described by Renkonen et al. (1971). Cholesterol was added to give approximately the same molar ratio of phospholipid to cholesterol (1.2:1) as in the virus assuming an average of 750 for the molecular weight of the phospholipids. A small aliquot of lipid extract from ^{32}P-labeled BHK cells was included. The lipids were dried under nitrogen and suspended in buffer to give a phospholipid concentration of 0.5 mg/ml. The suspension was allowed to equilibrate for 2 hr at room temperature and was then sonicated for 15 min (Disintegrator 80, Ultrasonic Industries) at 40° under nitrogen, concentrated at 4° by ultrafiltration using an Amicon XM 100 A filter, and subjected to Sepharose 4B gel filtration (Huang, 1969). The included peak of ^{32}P radioactivity containing the single-shelled liposomes was pooled, concentrated as above, and used immediately for SDS binding experiments.

Sodium Dodecyl Sulfate. SDS was purchased from Fluka (Switzerland) and crystallized three times from ethanol, washed with cold diethyl ether, and dried above phosphorus pentoxide. It was shown to be pure by thin-layer chromatography on silica gel G plates using chloroform–methanol–7 *N* NH_4OH (65:25:4, v/v) as the solvent. ^{35}S-Labeled SDS was purchased from New England Nuclear. It was purified by preparative thin-layer chromatography using the solvent system described above. The purified ^{35}S-labeled SDS was stored in chloroform–methanol (1:1, v/v) at 4°. All aqueous solutions of SDS were freshly prepared before use to diminish the risk of ester bond hydrolysis (Motsavage and Kostenbander, 1963). The following buffer was used throughout this work (unless otherwise stated): 0.05 *M* sodium phosphate–0.1 *M* NaCl (pH 7.4) (pH 7.4 buffer).

The critical micellar concentration of SDS was determined by measuring the surface tension by the drop weight method at different temperatures (Mukerjee and Mysels, 1971). The solubility and the critical micellar temperature (see Carey and Small, 1972) of SDS were determined by equilibrating SDS in pH 7.4 buffer in concentrations ranging from 0.1 to 80 m*M* overnight in a cryostat (Lauda, Germany) at 4°. The temperature was then raised, and the solution was allowed to equilibrate at each temperature for at least 2 hr. Solubility was recorded by visual inspection. The effect of sucrose on the solubility was studied either by adding sucrose to the SDS solutions in the method described above or by performing density gradient centrifugations in linear 5–45% (w/w) sucrose gradients containing 1 m*M* ^{35}S-labeled SDS in pH 7.4 buffer at 4 or 20°. After 16 hr at 40,000 rpm in an MSE Superspeed 75 ultracentrifuge using a swingout (6 × 14 ml) rotor, fractions were collected from below. The concentration of soluble SDS was obtained from the radioactive base line throughout the gradient; crystalline SDS banded at about 36–38% sucrose at 20°, whereas at 4° two or more bands of SDS were seen in the lower region of the centrifuge tube at sucrose concentrations higher than 35%.

Determination of SDS Binding. The binding of SDS to SF virus under equilibrium conditions was determined by sucrose gradient centrifugation essentially as described earlier for Triton X-100 binding to SF virus (Helenius and Söderlund, 1973). SF virus samples (0.05 ml) labeled with [^{3}H]leucine, [^{3}H]isoleucine, and [^{3}H]valine of known specific activity containing either 0.05 or 0.1 mg of virus protein were centrifuged to their isopycnic positions in linear sucrose density gradients, 10–47%, containing varying concentrations (5×10^{-6} to 2×10^{-4} *M*) of ^{35}S-labeled SDS in pH 7.4 buffer using a Spinco SW50 rotor in a Spinco Model L 3 ultracentrifuge. The centrifugation times were 90 min at 45,000 rpm or 16 hr at 27,000 rpm. The virus binds SDS when it enters the gradient. The amount of virus was determined from the ^{3}H radioactivity and the amount of SDS bound from the ^{35}S radioactivity, subtracting the base line (see Figure 3). All operations were done at 4°.

SDS binding to the lipid-free virus membrane protein complexes labeled with [^{3}H]leucine, [^{3}H]isoleucine, and [^{3}H]valine and to liposomes of ^{32}P-labeled phospholipids and of cholesterol (extracted from the viral host cells) was determined by equilibrium dialysis experiments. These were performed in Union Carbide dialysis tubing which had been boiled in 10% $NaHCO_3$–5 m*M* EDTA, rinsed, soaked overnight in 5 m*M* EDTA–10 m*M* 2-mercaptoethanol, and rinsed again with water. Samples (0.5 ml) containing 0.4 mg/ml of protein or 0.25 mg/ml of liposomes were put into the dialysis bags and dialyzed with effective mixing for 48 hr at 4° against 20 ml of ^{35}S-labeled SDS in pH 7.4 buffer of varying concentrations. Free and bound SDS, protein, and lipid were determined from the ^{35}S, ^{3}H, and ^{32}P radioactivities inside and outside the bags, and their known specific activities.

Virus Breakdown. The breakdown of the virus particle induced by increasing concentrations of SDS was followed by centrifugation in three different density gradients. (1) Twelve-milliliter linear sucrose gradients (5–20%) containing varying concentrations of SDS in pH 7.4 buffer were centrifuged in the MSE swing-out rotor (6 × 14 ml) at 27,000 rpm at 4° for 1 hr and 50 min, and at 20° for 1 hr and 30 min. Under these conditions the free 42S viral RNA sedimented to the position indicated by an arrow in Figure 6J. (2) Twelve-milliliter linear sucrose gradients (5 –45%) containing varying amounts of SDS in pH 7.4 buffer were centrifuged in the same rotor at 40,000 rpm at 4 or at 20° for 16 hr. Samples of labeled SF virus (no carrier virus was added) in a volume of 0.05 ml were applied to the gradients. (3) To demonstrate separation of the lipids from the membrane proteins, a three-layered "sandwich" gradient was used (Engelman et al., 1967), in which H_2O was exchanged for D_2O to make the pH 7.4 buffer. The lower layer was a 6-ml linear sucrose gradient (24–35%), the middle layer consisted of 0.3 ml of an SF virus labeled with both [^{32}P]orthophosphate and [^{3}H]leucine, [^{3}H]isoleucine, and [^{3}H]valine in 20% sucrose, and the upper layer was a 6-ml linear sucrose gradient (3–15%). Lipids float to the top and the

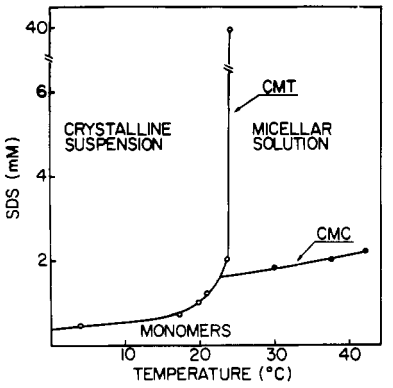

FIGURE 1: Temperature–concentration phase diagram of SDS determined in 0.1 *M* NaCl–0.05 *M* sodium phosphate buffer (pH 7.4): CMC, critical micellar concentration; CMT, critical micellar temperature; (○) maximum solubility of SDS; (●) CMC measured by the drop weight method.

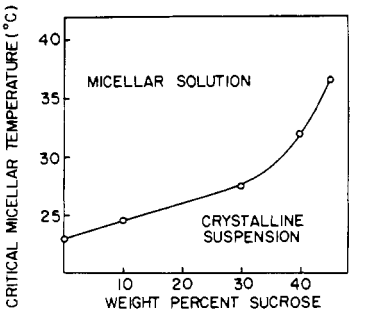

FIGURE 2: Effect of sucrose concentration on the critical micellar temperature of SDS. Temperature–concentration phase diagrams similar to that in Figure 1 were determined at different sucrose concentrations. The critical micellar temperature given is for a SDS concentration of 2 m*M*.

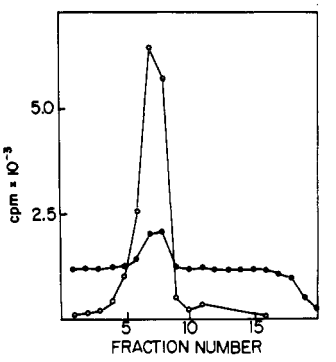

FIGURE 3: Determination of SDS bound to SF virus by sucrose density gradient centrifugation. Ice-cold SF virus (100 μg of protein) labeled with [^{3}H]leucine, [^{3}H]isoleucine, and [^{3}H]valine was applied to a precooled sucrose gradient (10–47%) containing 3.3×10^{-5} *M* ^{35}S-labeled SDS and centrifuged at 4° for 90 min at 165,000*g*: (●) ^{35}S radioactivity; (○) ^{3}H radioactivity. The amount of bound SDS was calculated from the ^{35}S peak after subtracting the base-line ^{35}S radioactivity. Sedimentation to the left.

42S RNA sediments to the bottom of the gradient. The entire gradient including the sample contained 0.45, 0.75, 1, or 3 m*M* SDS. The gradients were centrifuged in the MSE rotor for 48 hr at 40,000 rpm at 4, 20, or 30°. Fractions were collected from below and assayed for radioactivity.

Other Methods. Radioactivity was determined in glass vials containing Triton X-114–xylene cocktail (Anderson and McClure, 1973) using a Wallac scintillation counter 81,000. Quenching was determined from the channels ratio, and corrections were made for overflow in double labeled samples.

Protein was determined by the method of Lowry et al. (1951) with 0.1% SDS in the reaction mixture, and by quantitative amino acid analysis with a Beckman 120 C amino acid analyzer using norleucine as the internal standard. Phospholipids were determined as inorganic phosphorus by the method of Bartlett (1959). The amount of phosphorus was multiplied by 25 to obtain the amount of phospholipid.

SDS–polyacrylamide gel electrophoresis was carried out in 10% acrylamide gels as described by Weber and Osborn (1969). The samples were treated with 1% SDS and 5% 2-mercaptoethanol at 100° for 3 min before electrophoresis. For liquid scintillation counting, the gels were cut into 2-mm slices, solubilized with NCS (Radiochemical Centre, England), and counted (Caliguiri and Mosser, 1971).

Sucrose concentrations were determined by refractometry.

Results

Characterization of SDS. The temperature–concentration phase diagram of our SDS preparation is shown in Figure 1. At 4° only 4.5×10^{-4} *M* of the detergent was soluble. The solubility increased to 7.5×10^{-4} *M* at 20°, and at about 23° there was a large increase in solubility due to micelle formation. A critical micellar temperature of 23° for SDS has earlier been reported by Corkill and Goodman (1962). However, most earlier values are significantly lower (Pallansch and Briggs, 1954; Nakayama et al., 1966). Impurities in the SDS preparation are known to increase the solubility and lower the critical micellar temperature (Nakayama et al., 1966). The critical micellar concentration determined in the buffer we used throughout this work (0.05 *M* sodium phosphate–0.1 *M* NaCl, pH 7.4) was 2×10^{-3} *M* at 37°.

As most of the subsequent studies on the effects of SDS on SF virus were done in media containing sucrose, its effects on the properties of SDS were also investigated. The critical micellar concentration measured at 37° was not affected by 30% sucrose. Nor was the solubility of SDS at temperatures below the critical micellar temperature appreciably changed by sucrose concentrations up to 30% as studied by sucrose density gradient centrifugation. The region above 30% sucrose was more difficult to study due to banding of crystalline SDS (see Experimental Section). The critical micellar temperature increased, however, with increasing concentrations of sucrose (Figure 2), especially at concentrations higher than 30%. Most of the subsequent work was done at temperatures below the critical micelle temperature.

Binding of SDS to the Virus, and to Its Membrane Components. Binding of SDS to the SF virus was studied by density gradient centrifugation at 4° using radioactively labeled detergent and virus. A typical gradient is shown in Figure 3. Figure 4 gives the number of SDS molecules bound per virus particle plotted against the logarithm of the free equilibrium detergent concentrations. After binding of about 6000 molecules of SDS, there was an increase in binding which began at a free SDS concentration of about 5×10^{-5} *M*, and coincided with the beginning of virus disruption (observed as a change in virus sedimentation pattern; see below). Binding of SDS to isolated viral membrane protein complexes and to single-walled liposomes made from host cell lipids was studied by equilibrium dialysis at

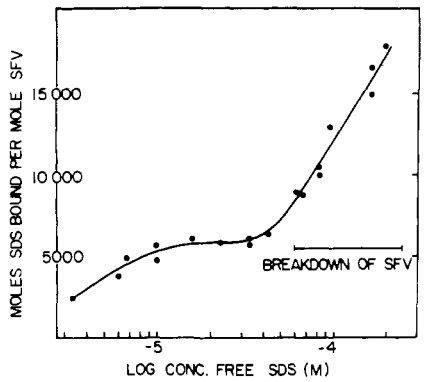

FIGURE 4: SDS binding to SF virus at 4° determined by sucrose gradient centrifugation in 0.1 *M* NaCl-0.05 *M* sodium phosphate (pH 7.4).

4°. The binding data were analyzed as described by Klotz (1953) using double reciprocal plots (see Figure 5). The binding of ligands to a set of identical sites is at equilibrium determined by the equation:

$$\frac{1}{\bar{\nu}} = \frac{1}{nKc} + \frac{1}{n}$$

where $\bar{\nu}$ = the average number of bound ligands per particle; K = the intrinsic association constant; c = free ligand concentration; and n = the number of binding sites in the set. The low SDS concentration region in the reciprocal plots (with SF virus less than 2×10^{-5} *M*) was analyzed by least-squares analysis to give K and n for the high affinity binding (Table I). The association constants of SDS binding to the virus particle, to the virus membrane protein, and to the lipid were all in the same range. The number of high affinity sites in the virus and in the equivalent amount of liposomal lipid were similar within the accuracy of the data. The high affinity binding sites on the isolated membrane proteins, however, accounted for only about 17% of the sites observed in the virus.

Virus Disruption. The gradual disruption of the virus with increasing SDS was studied by sucrose gradient centrifugation. Rather than determining separately the bound and free SDS in the various gradients (which would have been difficult because of the heterogeneity of the viral breakdown products) we used trace amounts (less than 1 μg) of highly radioactive virus labeled with [^{32}P]orthophosphate and with ^{3}H-labeled amino acids. As the amount of SDS bound to the small amount of virus was only a negligible fraction of the total SDS, it did not appreciably change the free SDS concentration in any part of the gradient.

Disruption at 4°. In sucrose gradients containing 5×10^{-5} *M* SDS or less the virus sedimented as a homogeneous particle (Figure 6A,B). The only change with increasing SDS concentrations in this low concentration range was a small decrease in the sedimentation rate of the virus (Figure 6A,B) and a decrease in viral buoyant density from 1.18 to 1.15 g/cm^3 (determined under isopycnic conditions; not shown). A similar density decrease occurs when Triton X-100 binds to the virus (Helenius and Söderlund, 1973). At 6.7×10^{-5} *M* SDS the viral ^{32}P and ^{3}H radioactivities were heterogeneously distributed in the upper part of the gradient (Figure 6C) indicating viral breakdown. Little further change in the sedimentation pattern occurred when the SDS concentration in the gradients was increased to the solubility limit (4.5×10^{-4} *M*) or above it (7.5×10^{-4} *M*) (Figure 6D,E). The distribution of ^{32}P and ^{3}H radioactivities in the gradients (Figure 6C–E) showed no clearcut separation between protein, lipid, and RNA, nor was separation of these components observed in isopycnic "sandwich" gradients at 4° where most of the ^{3}H and ^{32}P activities banded as a sharp peak (Figure 7A). SDS-polyacrylamide gel electrophoresis of the separate fractions throughout the gradients in Figures 6B, D, and E showed, furthermore, that no separation of the viral proteins had occurred. These data indicate that viral breakdown at 4° induced by SDS proceeds to a point where the virus disrupts but where no major dissociation of the components takes place.

Disruption at 20°. At 20°. the solubility of SDS was 7.5×10^{-4} *M* and the breakdown of the virus could proceed much further than at 4°. This is shown in the velocity gradient in Figure 6J, the "sandwich" gradient in Figure 7B, and the 10–45% sucrose gradient in Figure 8B, all of which contained 7.5×10^{-4} *M* free SDS. It was observed that: (1)

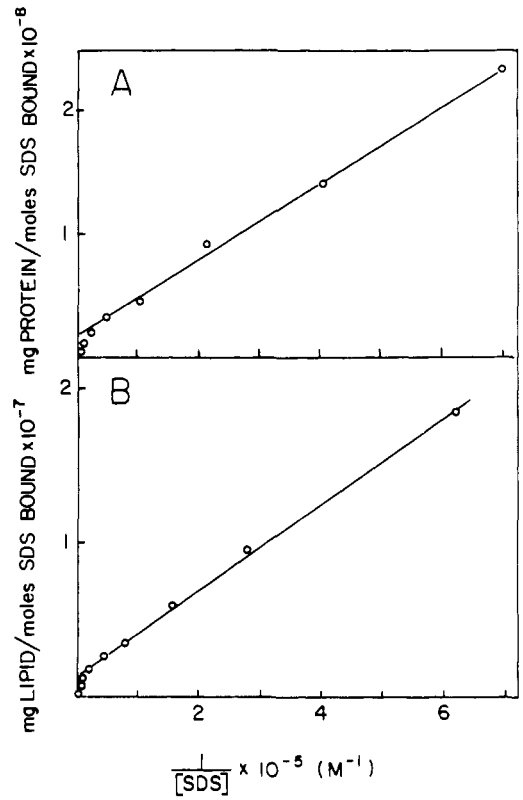

FIGURE 5: Reciprocal plots ($1/\bar{\nu}$ vs. $1/c$) of the SDS binding determined at 4° of: (A) the isolated virus membrane protein and of (B) liposomes prepared from BHK 21 cell lipids.

Table I: High Affinity Binding of SDS to SF Virus, Isolated Membrane Protein, and Liposomes at 4°.

	Assoc. Constant (K) (M^{-1})	No. of Binding Sites[a] (n)
SF virus	8×10^4	11,400 ± 3900
Membrane protein[b]	6×10^4	1,900 ± 500
Liposomes[c]	6×10^4	12,200 ± 2000

[a] Number of sites expressed per virus particle, or per membrane protein or membrane lipid equivalent. The molecular weight of the virus is 6.4×10^7, the membrane protein equivalent 2.9×10^7, and the lipid equivalent 2.0×10^7. [b] Water-soluble membrane protein complexes virtually free from lipid (see Experimental Section). [c] Single-walled liposomes prepared from BHK-21 cell phospholipids and cholesterol in a molar ratio of 1.2: 1.

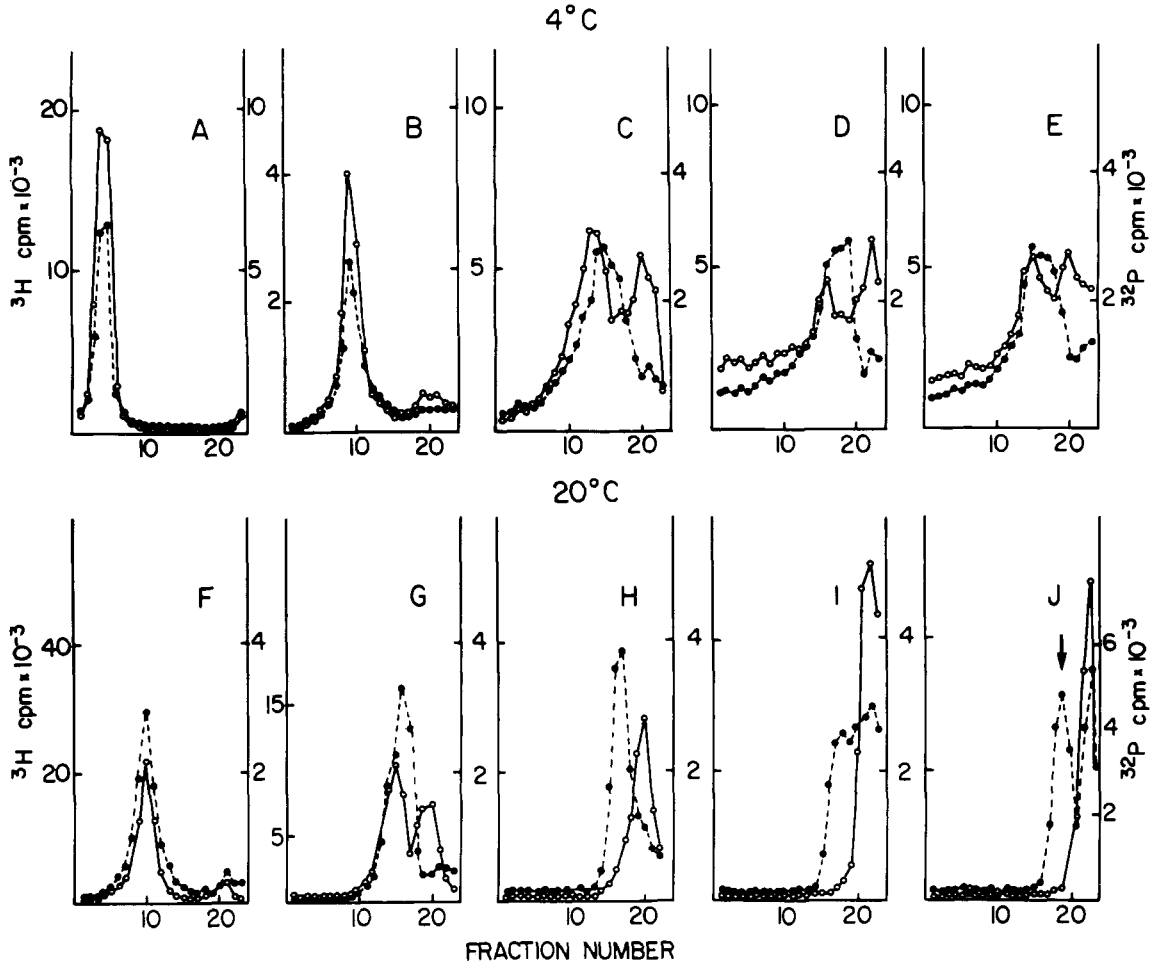

FIGURE 6: Velocity gradient centrifugation of SF virus in the presence of increasing SDS concentrations at 4 and 20°: (A), no SDS; (B) 2.5×10^{-5} *M* SDS; (C) 6.7×10^{-5} *M* SDS; (D) 4.5×10^{-4} *M* SDS; (E) 7.5×10^{-4} *M* SDS; (F) 2.5×10^{-5} *M* SDS; (G) 5×10^{-5} *M* SDS; (H) 1.7×10^{-4} *M* SDS; (I) 4.5×10^{-4} *M* SDS; (J) 7.5×10^{-4} *M* SDS. Trace amounts of SF virus labeled with [^{3}H]leucine, [^{3}H]isoleucine, [^{3}H]valine, and with [^{32}P]orthohophosphate were applied to 5–20% sucrose gradients, and centrifuged at 27,000 rpm for 110 min at 4° and for 90 min at 20°. The arrow in J indicates where the free 42S RNA sediments under these conditions; sedimentation to the left: (O–O) ^{3}H radioactivity; (● - - - ●) ^{32}P radioactivity.

the viral RNA was released in essentially protein-free form, RNA release was also demonstrated using SF virus specifically labeled in its RNA by [^{3}H]uridine (not shown); (2) the nucleocapsid protein was present in a slowly sedimenting form (the protein was found in fractions 21–26 in the gradient in Figure 8B by SDS–polyacrylamide gel electrophoresis); (3) the membrane proteins E1, E2, and E3 (detected by SDS–polyacrylamide electrophoresis) occurred as relatively small complexes containing 40–45% of the viral phospholipid (Figure 7B fractions 18–24 and Figure 8B fractions 16–21); (4) the rest of the phospholipid was released from the membrane proteins and found on top of the gradients probably in the form of SDS–lipid mixed micelles (Figures 6J, 7B, and 8A); (5) if the SDS concentration was increased above the solubility limit (to 3×10^{-3} *M*) in the gradients no further dissociation of the lipid from the protein occurred.

Using lower SDS concentrations the viral disintegration could be arrested at earlier stages (Figures 6F–I and 8A). We demonstrated that already at about 1.1×10^{-4} *M* SDS the virus had dissociated further than the maximum disruption seen at 4° (Figure 8A). With virus specifically labeled in its RNA with uridine it was shown that the bulk of the RNA was in the pellet (not shown). Most of the viral membrane protein and phospholipid sedimented together at 1.1 $\times 10^{-4}$ *M* SDS indicating that they formed lipoprotein complexes or membrane fragments (Figure 8A). With increasing SDS concentration the lipid content of these lipoprotein complexes and the sedimentation rate decreased (Figures 6H–J and 8B).

Disruption at 30°. In order to obtain complete separation of membrane protein and phospholipids it was necessary to increase the temperature. Figure 7C shows that at 30° the separation was virtually complete.

Discussion

The present study shows that the solubilization of SF virus by SDS proceeds through different stages with increasing SDS concentration. The first is the binding of detergent to the virus particle without any signs of disruption. Detectable binding begins at SDS concentrations far below the critical micellar concentration. Thus, the detergent is bound in its monomeric form (and not as micelles) in conformity with the general conclusions presented by Tanford (1972) concerning detergent binding and experimentally verified (Reynolds and Tanford, 1970; Makino et al., 1973; Helenius and Söderlund, 1973; Nozaki et al., 1974).

Each SF virus particle has about 10,000 SDS binding sites with an average association constant of about 10^5 M^{-1} at 4° (Table I). The number of high affinity binding sites is

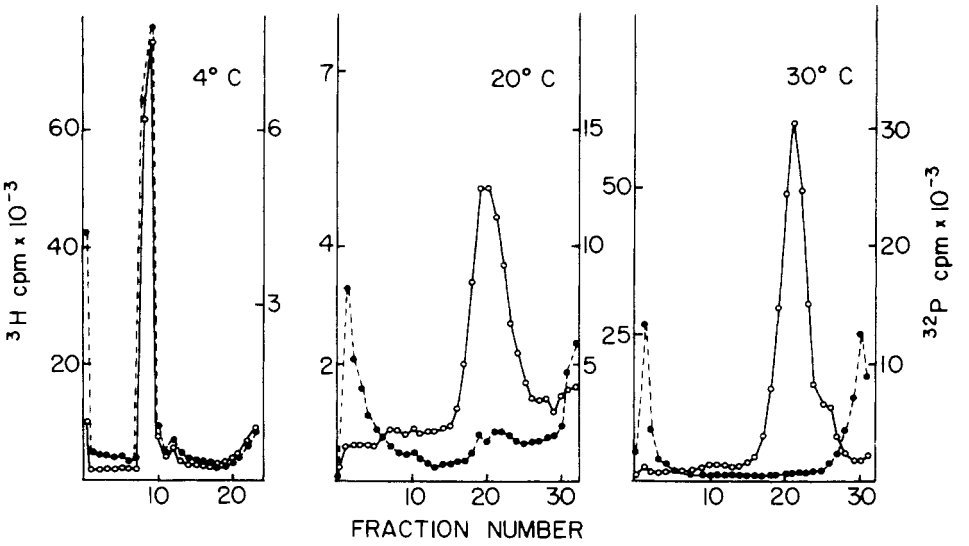

FIGURE 7: Isopycnic density gradient centrifugation of SF virus in the presence of 7.5×10^{-4} *M* SDS at 4, at 20, and at 30°. Trace amounts of SF virus labeled with [^{3}H]leucine, [^{3}H]isoleucine, [^{3}H]valine, and with [^{32}P]orthophosphate were applied to D_2O–sucrose sandwich gradients and centrifuged for 48 hr at 40,000 rpm: (O–O) ^{3}H radioactivity; (● - - - ●) ^{32}P radioactivity.

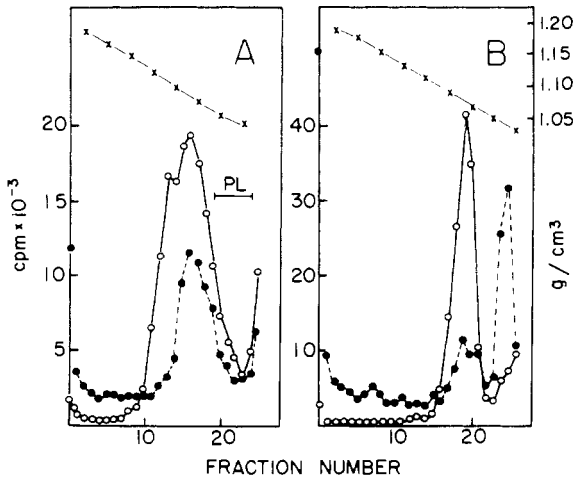

FIGURE 8: Isopycnic density gradient centrifugation of SF virus in the presence of: (A) 1.1×10^{-4} *M* SDS and (B) 7.5×10^{-4} *M* SDS at 20°. Trace amounts of SF virus labeled with [^{3}H]leucine, [^{3}H]isoleucine, [^{3}H]valine, and with [^{32}P]orthophosphate were applied to 5–45% sucrose gradients and centrifuged for 16 hr at 40,000 rpm. PL indicates the position where phospholipids alone sediment in the gradient under similar conditions. Free 42S RNA sediments to the bottom of the gradient: (X—X) sucrose density; (O–O) ^{3}H radioactivity; (● - - - ●) ^{32}P radioactivity.

very large considering that there are only about 16,000 phospholipids and 16,000 cholesterols in the virus membrane and that all other viral components occur in much lower numbers (Laine at al., 1973). If the binding were to protein only, there would be about 30 high affinity binding sites per 100,000 daltons of protein. The binding of SDS to isolated water-soluble membrane protein complexes revealed only two high affinity sites per 100,000 daltons. On the contrary, the binding of SDS to single-walled liposomes [prepared from host cell lipids which were similar but not identical in composition with the viral lipids (Renkonen et al., 1971)] was equivalent both in affinity and number of sites to the high affinity binding of the virus. Taken together these data suggest that SDS is initially bound mainly to the lipids in the virus membrane, but some binding to the proteins is also possible.

At free SDS concentrations of about 5×10^{-5} *M*, when about half of the high affinity sites for SDS were filled, virus disruption ("lysis") started. At this stage the viral components still sedimented together forming a heterogeneous population with no clearcut separation of protein, lipid, and RNA. The viral RNA was now available to RNase digestion in contrast to the intact virus particle where the RNA is protected against enzymatic attack by the viral membrane (unpublished experiments).

The third stage of virus breakdown was reached when the viral nucleocapsid dissociated into RNA and protein. The stability of capsids in different viruses against SDS disruption appears to depend on the type of interactions that stabilize their structure. Capsids stabilized by RNA–protein interactions (such as the SF virus nucleocapsid; Söderlund and Kääriäinen, 1974) appear to be very sensitive to SDS, whereas capsids stabilized mainly by noncovalent protein–protein bonds are resistant (Boatman, 1973).

Viral capsid disruption is followed by the solubilization of the membrane into lipoprotein complexes, which decrease in size and in lipid content with increasing SDS concentration. The final stage in the solubilization process is reached when the membrane proteins are completely delipidated. The hemagglutinating activity of the viral membrane proteins is lost during SDS solubilization apparently due to protein denaturation (Simons and Kääriäinen, 1970). The exact SDS concentration needed for inactivation could not be determined because of assay difficulties due to hemolysis. Overall, the solubilization scheme found for the virus membrane is similar to that previously reported by Engelman et al. (1967) for Acholeplasma cell membranes. They found that solubilization caused by increasing concentrations of SDS proceeds through a continuum of states from disruption into lipid–protein complexes of decreasing size to the formation of separate lipid- and protein–detergent complexes.

There are two noteworthy features concerning the action of SDS on the virus. One is that complete solubilization of the SF virus membrane into separate lipid and protein complexes can be obtained at free SDS concentrations below the critical micellar concentration (for a general discussion of this aspect of membrane solubilization by detergents, see

Helenius and Simons, 1975). The other feature is that the degree of disruption can be controlled not only by the free monomeric SDS concentration but also by the temperature. Temperature affects both the detergent and the membrane. The monomer solubility at different temperatures (Figure 1) sets a limit for the chemical potential of the detergent molecules and thus also for the solubilization efficiency (Tanford, 1972). Important to note is that different SDS preparations differ in their solubility properties depending on the purity of the detergent (Nakayama et al., 1966). Temperature must also influence the properties of the membrane. The extent of virus dissociation at the same free SDS concentration is different at different temperatures (e.g., Figure 7). At 4° only lysis of the virus can be achieved with our SDS preparation; at 20° solubilization stops at the lipoprotein stage, whereas at 30° delipidation is complete.

The major difference in the effects of Triton X-100 on SF virus compared to that of SDS is the milder action of the nonionic detergent. With Triton X-100 the nucleocapsid can be isolated intact, and the hemagglutination activity is preserved during solubilization (Helenius and Söderlund, 1973). This is in line with what is generally known of the effects of these two detergents: SDS is a denaturing agent, whereas Triton X-100 is usually not (see Makino et al., 1973).

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