Sedimentation Characteristics of the Scrapie Agent from Murine Spleen and Brain†

Stanley B. Prusiner,* William J. Hadlow, Carl M. Eklund,‡ Richard E. Race, and S. Patricia Cochran

ABSTRACT: Sedimentation profiles of the scrapie agent in extracts of murine spleen and brain were determined by analytical differential centrifugation. Infectivity profiles of the agent from the two tissues were similar. Sedimentation of the agent was not substantially altered by detergent treatment with sodium deoxycholate. In the presence of detergent, centrifugation at an ω^2t value of 3.0×10^{10} rad^2/s in a fixed-angle rotor sedimented 90% of the agent. Comparative studies with radioisotopically labeled Simian virus 40 showed that centrifugation at an ω^2t value of 1.6×10^{10} rad^2/s removed 90% of the virions. The sedimentation profile of the scrapie agent was similar to that observed for cellular ribosomal RNA. Heating infectious extracts of spleen to 80 °C for 30 min resulted in the destruction of 95% of the RNA while sedimentation of the scrapie agent was unchanged. These studies establish a limited range of particle sizes for the scrapie agent.

For almost two decades the chemical structure of the scrapie agent has remained elusive (Gajdusek, 1977; Eklund & Hadlow, 1973; Kimberlin, 1976; Hunter, 1972). Interest in the agent which causes a degenerative disease of the central nervous system of sheep has increased with the recognition that it belongs to a novel class of infectious entities. Two diseases of the nervous system in humans, Creutzfeldt-Jakob disease and Kuru, appear to be caused by similar agents (Gajdusek, 1977; Eklund & Hadlow, 1973). Like many viruses the scrapie agent can be serially transmitted and propagated to substantial titers in a variety of animal hosts. But its unusual resistance to inactivation by heat, formalin, and ultraviolet irradiation, as well as its small ionizing radiation target size, have prompted several investigators to suggest that the scrapie agent may not contain a nucleic acid genome (Kimberlin, 1976; Hunter, 1972). Since attempts to purify the scrapie agent have been unsuccessful to date, information on the unusual physicochemical properties of the scrapie agent has been gathered from studies using crude extracts of infected brain.

Attempts to purify the scrapie agent have been hampered by the inconvenient titration assay in mice. Such titrations require care and observation of the mice over the 6- to 12-month period before a characteristic neurological disease develops (Chandler, 1961; Eklund et al., 1967). In order to develop a preparatory procedure for purification of the agent, we have studied its sedimentation characteristics in fixed-angle rotors using analytical differential centrifugation (Anderson, 1968; Prusiner, 1978).

In this communication our initial observations on the sedimentation properties of the scrapie agent in homogenates of murine spleen have been refined and extended (Prusiner et al., 1977). We have established that the scrapie agent sediments as infectious particles and we have also defined a limited size range for these particles.

Experimental Procedures

Materials. All materials were of the highest grade commercially available. DOC was purchased from Sigma Chemicals and lysolecithin from Koch-Light.

Source of the Scrapie Agent. Brain tissue from three naturally infected Cheviot sheep in Scotland was serially passaged ten times in sheep, mainly Cheviot stock. The 11th sheep passage was in Welsh Mountain sheep; thereafter, 8 serial passages were made in goats. A brain suspension from an 8th goat passage ("drowsy" type) was inoculated in Swiss mice by R. L. Chandler at Compton, England (Chandler, 1961). The inoculum used in studies reported here represented the 4th passage of this "Chandler" strain in mice. Brain tissue from 50 Swiss mice with clinical signs of scrapie was homogenized in 9 vol of 0.32 M sucrose and centrifuged at 121g for 10 min at 4 °C. Inocula were prepared from this supernatant fluid. Control mice were similarly inoculated with analogous suspensions prepared from the brain of healthy Swiss mice. The controls neither developed signs of neurological dysfunction nor histopathological changes of the brain as judged by electron microscopy (Prusiner & Baringer, unpublished observations).

Preparation of Homogenates. Female Swiss mice, 1 month old, purchased from Charles River Laboratories were inoculated intracerebrally with 0.03 mL of mouse-adapted scrapie agent (10^6 ID_{50} units). For studies on the agent in spleen, mice were killed 40 days after inoculation. Their spleens were removed immediately and washed in 20 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7.4, containing ice-cold 250 mM sucrose. For studies on the agent in brain, mice were sacrificed 100 days after inoculation because replication is slower than in spleen. Prior to homogenization the brains were washed in 320 mM sucrose. A Potter-Elvehjem glass homogenizer equipped with a motor-driven Teflon pestle was used to prepare a 20% homogenate of the tissue (w/v). For spleen, the homogenizing media consisted of 20 mM Tris-HCl (pH 7.4)–250 mM sucrose and for brain, 320 mM sucrose alone. All procedures were performed at 4 °C unless otherwise noted. The homogenates were centrifuged for

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TABLE I: Distribution of the Scrapie Agent in Fractions from Murine Spleen and Brain.

	spleen					brain				
fraction	vol (mL)	protein (mg)	RNA (mg)	DNA (mg)	scrapie (log ID_{50} units)	vol (mL)	protein (mg)	RNA (mg)	DNA (mg)	scrapie (log ID_{50} units)
homogenate	58	1017	64.6	380.0	9.4	72	2232	36.7	18.9	9.1
pellet (P_1)	45	432	32.3	386.0	8.8	32	704	17.4	17.3	10.0
supernatant (S_1)	100	547	46.2	16.6	9.1	134	1742	20.8	3.4	9.1

10 min at 121g in a Sorvall RC2B centrifuge equipped with an SS34 rotor and the supernatant fluids removed. The pellets were rehomogenized in additional buffer and centrifuged again. After the pellets from the second centrifugation were washed, the three portions of supernatant fluids were combined; the final suspensions were 10% (w/v). The combined supernatant fluids from the 121g sedimentations, designated "S_1", were used as the starting material for all the analytical differential centrifugation studies reported here.

Sedimentation Analysis in Fixed-Angle Rotors. All analytical differential centrifugation studies were performed in a Spinco L2-65B ultracentrifuge equipped with a 50 Ti fixed-angle rotor. Two-milliliter aliquots of the 121g combined supernatant fluid were centrifuged at specified speeds for given time periods in cellulose nitrate tubes (7.9 mm diameter × 40 mm length). The total time of centrifugation included 33% of the time required for acceleration and deceleration of the rotor. After centrifugation was completed, a long-tip Pasteur pipet was introduced along the wall of the tube that had been positioned toward the interior of the rotor during centrifugation to minimize contamination of the supernatant fraction by pelleted material. About 1.7 mL of supernatant fluid was slowly aspirated; the remaining 0.3 mL of fluid overlying the pellet was left undisturbed (Prusiner, 1978).

Sedimentation profiles were constructed by plotting the quantities of sedimenting particles remaining in the supernatant fluid as a function of $\omega^2 t$, where ω is the angular velocity of the centrifuge rotor in rad/s and t is the time of centrifugation in sec (Anderson, 1968; Prusiner, 1978). Sedimentation coefficients were estimated from these profiles using eq 1:

$$s_{obsd} = \frac{k}{\omega^2 t} \quad (1)$$

where k is a proportionality constant dependent on the geometry of the centrifuge tube in the fixed-angle rotor, and $\omega^2 t$ is the minimum value required to sediment 90% of the particles. Simian virus 40, which has a $s_{20,w}$ of 240 S as determined by rate-zonal and equilibrium centrifugation in swinging bucket rotors, was used to calibrate the system and determine k for the rotor configuration described above (Black et al., 1964; Prusiner et al., 1978). From the s_{obsd} values, $s_{20,w}$ were calculated using the equation:

$$s_{20,w} = s_{obsd} \frac{\eta_{T,M}(\rho_p - \rho_{20,w})}{\eta_{20,w}(\rho_p - \rho_{T,M})} \quad (2)$$

where $\eta_{T,M}$ and $\rho_{T,M}$ are the viscosity and density, respectively, of the centrifugation medium at the chosen temperature and ρ_p is the density of the particle (Anderson, 1968; Prusiner, 1978; Schachman, 1956; Cotman et al., 1970). The $s_{20,w}$ calculations for the scrapie agent assumed that the agent has a density of 1.2 g/cm^3 in sucrose (Siakotos et al., 1976; Brown et al., 1978; Prusiner et al., 1978). Corrections for the viscosity and density of the centrifugation medium considered only the 8.5% (w/w) sucrose in the homogenization buffer.

Assay of Biochemical Markers. RNA and DNA were measured according to the procedure of Schneider (1957) by repeated ethanol precipitations prior to colorimetric determinations using orcinol and diphenylamine reagents, respectively. Commercial preparations of yeast RNA and calf thymus DNA were extracted with phenol and used as standards, assuming an $E_{260}^{1\%} = 250$ for RNA and $E_{260}^{1\%} = 200$ for DNA. Protein was determined by the method of Lowry et al. (1951); bovine serum albumin was used as a standard.

Assay of Scrapie Agent Infectivity. Female Swiss mice of Rocky Mountain Laboratory stock were used to titrate the amount of scrapie agent in the fractions of supernatant fluid. Serial tenfold dilutions of the fractions were prepared with phosphate-buffered saline that contained 10% fetal calf serum, 0.5 unit/mL penicillin, 0.5 μg/mL streptomycin, and 2.5 μg/mL amphotericin. For each dilution, 6 mice were inoculated intracerebrally with 0.03 mL of the diluted suspension. The animals were examined weekly during the next 12 months for clinical signs of scrapie, i.e., bradykinesia, plasticity of the tail, waddling gait, and a coarse ruffled appearance of the coat. Histologic examination of the brain and spinal cord was done occasionally to confirm the clinical diagnosis. Titers were calculated by the method of Spearman and Kärber (Dougherty, 1964). The standard errors of these titrations varied between ±0.2 and ±0.4 log unit.

Preparation of Simian Virus 40 (SV40). SV40 grown in African Green Monkey kidney cells cultured in the presence of [^{35}S]methionine and purified by CsCl density gradient centrifugation was the generous gift of Drs. J. Reiser and G. Stark. The virus was further purified by rate-zonal centrifugation in a 15–30% linear sucrose gradient using an SW27.1 rotor at 27 000 rpm for 3.5 h. Only virions found in a narrow peak near the center of the gradient were used for the analytical differential studies described here. Prior to the centrifugation studies the ^{35}S-labeled SV40 was dispersed by vortexing at 4 °C in 20 mM Tris-HCl containing 250 mM sucrose and 0.5% (w/v) DOC, in the S_1 fraction from murine spleen, or in the S_1 fraction treated with 0.5% (w/v) DOC. After centrifugation 1 mL of the supernatant fluid was added to 10 mL of Aquasol solution (New England Nuclear, Boston) and the radioactivity in each sample determined using a Beckman LS233 liquid scintillation counter.

Results

Centrifugation at 121g for 10 min sedimented more than 95% and 80% of the cellular DNA in homogenates from murine spleen and brain, respectively (Table I). The majority of the RNA and protein did not sediment under these conditions and was recovered in the supernatant fractions (S_1) for both spleen and brain. Scrapie infectivity was distributed almost equally in the supernatant and pellet fractions.

The 121g supernatant fluids (S_1) were divided into 2-mL aliquots. The disappearance of RNA, protein, and scrapie agent from these fluids was determined as a function of $\omega^2 t$, where ω is the angular velocity of the centrifuge rotor in rad/s and t is the time of centrifugation in s. Sedimentation profiles

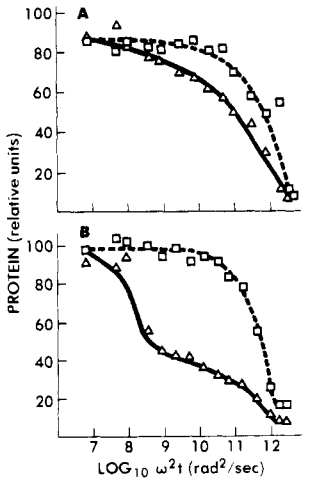

FIGURE 1: Sedimentation profiles of total cellular protein in the S_1 fraction from murine spleen and brain infected with the scrapie agent. Samples were untreated (-△-) and treated with 0.5% (w/v) sodium deoxycholate (- -□- -). For (A) spleen and (B) brain, a value of 100 relative units equals 9.3 and 14.0 mg/mL, respectively.

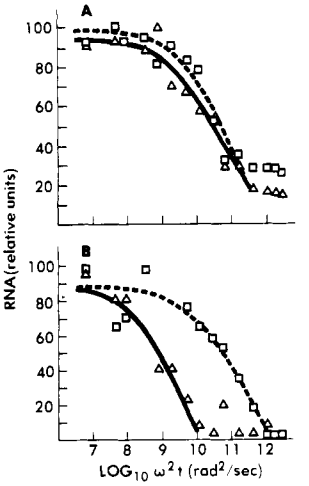

FIGURE 2: Sedimentation profiles of total cellular RNA in the S_1 fraction from murine spleen and brain infected with the scrapie agent. Samples were untreated (-△-) and treated with 0.5% (w/v) sodium deoxycholate (- -□- -). For (A) spleen and (B) brain, a value of 100 relative units equal 0.79 and 0.24 mg/mL, respectively.

for total protein in the presence or absence of DOC are shown in Figure 1. In the absence of detergent, centrifugation at an ω^2t value of 1.0×10^{11} rad^2/s was required to sediment 50% of the protein in the spleen extract (Figure 1A). In brain extract (Figure 1B) an ω^2t value of 5.0×10^8 rad^2/s was required to sediment an equivalent amount of protein. After DOC treatment, to sediment 50% of the protein required centrifugation at an ω^2t value of 1.0×10^{12} rad^2/s in the brain extract.

Sedimentation profiles for total RNA in presence or absence of DOC are graphed in Figure 2. In the absence of detergent, centrifugation at 1.0×10^{10} rad^2/s resulted in sedimentation of 50% of the total RNA from the spleen extract. An equivalent amount was sedimented from the brain extract at an ω^2t value of 1.0×10^9 rad^2/s. In the spleen extract almost no shift in the sedimentation profile for RNA was observed after treatment with 0.5% DOC (w/v), while in the brain extract sedimentation of 50% of the RNA was observed at an ω^2t value of 5.0×10^{10} rad^2/s. Like the protein profiles the differences in RNA sedimentation profiles for spleen and brain may partly reflect differences in cytoarchitecture.

The sedimentation profiles of the scrapie agent in untreated and DOC-treated extracts of murine spleen are shown in Figure 3. Centrifugation of both extracts at ω^2t values of less than 1.0×10^9 rad^2/s allowed virtually all the scrapie agent to remain in the supernatant fraction; centrifugation at 1.0×10^{11} rad^2/s or greater sedimented the agent. The data shown are from two separate experiments performed 1.5 years apart and clearly demonstrate the reproducibility of the methodology. [Data denoted by square (-□-) symbols were previously reported in a preliminary communication (Prusiner et al., 1977).] No substantial differences between the profiles for the untreated and the DOC-treated extracts could be discerned. As reported in previous studies (Prusiner et al., 1977), sonication also did not alter the sedimentation profile of the scrapie agent.

The sedimentation profiles for the scrapie agent in homogenates of brain and of spleen are similar. As shown in Figure 4, centrifugation at ω^2t values of less than 1.0×10^9 rad^2/s allowed the scrapie agent to remain in the supernatant fraction; centrifugation at 1.0×10^{11} rad^2/s or greater sedimented virtually all of it. The data clearly indicate that the tissue in which the agent replicates does not influence its sedimentation

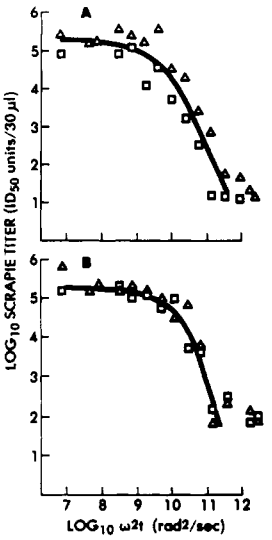

FIGURE 3: Sedimentation profiles of the scrapie agent in the S_1 fraction from murine spleen. (A) S_1 untreated, data from two separate experiments are plotted. (B) S_1 treated with 0.5% (w/v) sodium deoxycholate, data from two separate experiments denoted by (-△-) and (-□-) symbols.

behavior. Addition of DOC to fraction S_1 from brain resulted in an apparent shift in the sedimentation profile of the agent to the right.

Sedimentation profiles for ^{35}S-labeled SV40 were constructed in order to calibrate the sedimentation profiles for the scrapie agent and to establish the validity of the technique for particles the size of viruses. The purified virus was added to three different mixtures: (1) Tris-HCl buffer containing sucrose and DOC; (2) S_1 fraction from uninoculated mice; and (3) S_1 fraction from uninoculated mice treated with DOC. As shown in Figure 5, under these three conditions the sedimentation profiles were virtually identical. To permit direct comparison with the scrapie titration data in Figures 3 and 4, the date were plotted with a logarithmic scale for the ordinate. Like the scrapie agent, SV40 remained in the supernatant fluid at ω^2t values less than 1.0×10^9 rad^2/s and was pelleted at values greater than 1.0×10^{11} rad^2/s. From the visually fitted curve of the sedimentation profile, a 1 log decrement in ^{35}S-labeled SV40 corresponded to an ω^2t value of 1.6×10^{10} rad^2/s.

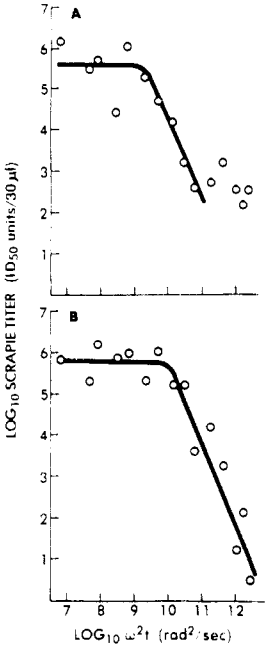

FIGURE 4: Sedimentation profiles of the scrapie agent in the S_1 fraction from murine brain. (A) S_1 untreated; (B) S_1 treated with 0.5% (w/v) sodium deoxycholate.

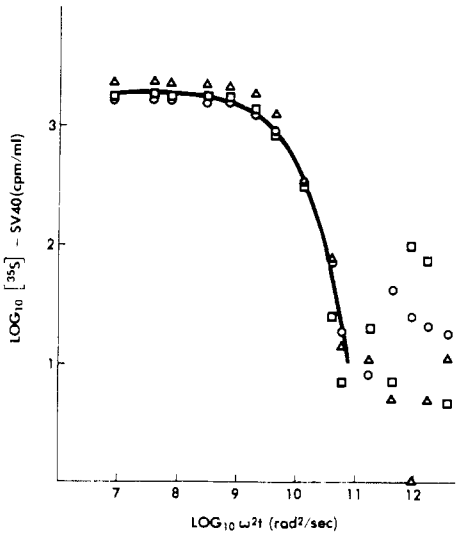

FIGURE 5: Sedimentation profiles of SV40. Radioisotopically labeled SV40 was mixed with Tris-HCl buffer containing sucrose and DOC (-Δ-), S_1 fraction from murine spleen (-□-), and S_1 fraction from murine spleen treated with 0.5% (w/v) DOC (-○-).

TABLE II: Comparison of Estimated $s_{20,w}$ Values for the Scrapie Agent from Murine Spleen and Brain.

tissue	prep	ω^2t value for 1 log infect. decrements[a] (rad^2/s) × 10^{-10}	$s_{20,w}$[b] (S)
spleen	untreated	0.30-3.4	120-1400
brain	untreated	0.39-1.1	390-1100
spleen	0.5% DOC	0.68-6.0	70-600
brain	0.5% DOC	1.10-7.1	60-380

[a] Estimated from visually fitted sedimentation profile curves. The ω^2t values were determined corresponding to 1 log infectivity decrements over a range equivalent to 1 standard deviation (±0.6 log unit). See text for details. [b] Calculations are described in text using a value for k of 0.38.

Like the sedimentation profiles for the scrapie agent, the profiles for SV40 show a small residuum of radiolabeled virus in the supernatant fluid at ω^2t values from 10^{11} to 10^{13} rad^2/s. The residuum, accounting for less than 0.5% of the radioactivity, could be due to sampling problems as well as convection of particles during centrifugation. Degradation of virions into small particles containing the radioisotopic label is an alternative explanation. However, this seems less likely since simultaneous rate-zonal sucrose gradient centrifugation studies showed a single peak of radioactivity in the center of the gradient.

Estimates of the sedimentation coefficients for the scrapie agent from the sedimentation profiles in Figures 3 and 4 must consider the imprecision of the titration assay. From the visually fitted sedimentation profile curves, ω^2t values were determined corresponding to 1 log decrements in titer over a titration range for the agent equivalent to 1 standard deviation ± 0.6 log unit. From these values, sedimentation coefficients were calculated using eq 1 and 2 as described above (see Experimental Procedures). For untreated S_1 fractions a 1 log decrement in scrapie titer after centrifugation was found to correspond to particles within a range of sedimentation coefficients from 120 to 1400 S for spleen homogenates and from 390 S and 1100 S for the brain homogenates (Table II). A 1 log decrement of scrapie titer in DOC-treated spleen extracts was observed to correspond to a range of sedimentation coefficients from 70 S to 600 S and for DOC-treated brain extracts, a range of 60 S to 380 S.

In Figure 6, the data from Figures 3B and 5 have been replotted with a linear ordinate to permit direct comparison of the profiles for the scrapie agent and SV40 with those for subcellular markers. The agent and SV40 were found in the supernatant fluid at ω^2t values of less than 1.0×10^9 rad^2/s and were virtually absent at values greater than 1.6×10^{10} rad^2/s. The sedimentation profiles for SV40 shown in Figure 6A fit quite closely the computed curve for the sedimentation behavior of an ideal particle with a $s_{20,w}$ of 240 S, assuming a particle density (ρ_p) of 1.3 g/cm^3. The sedimentation profile for the disappearance of the scrapie agent from supernatant fractions is similar to that of SV40 as illustrated in Figure 6B. This indicates that the agent sediments either as discrete particles homogeneous in size or as a series of particles differing in size over a relatively narrow range (Table II).

Because the agent in extracts of both spleen and brain exhibited a sedimentation profile similar to that for RNA, we considered the possibility that the agent was linked to ribosomal particles (deDuve, 1971; Bonanou-Tzedaki & Arnstein, 1972). To take advantage of the unusual heat resistance of the scrapie agent, extracts of spleen were heated to 80 °C for 30 min. Under these conditions greater than 95% of the RNA in the S_1 supernatant fraction was destroyed (Table III). Endogenous ribonucleases, which are known to be stable and catalytically active at high temperatures, were presumably responsible for degrading the RNA (Sela & Anfinsen, 1957). Under these conditions the infectivity of the scrapie agent was unaltered.

Prior to the analytical differential centrifugation of the heated extract, the coagulum formed during exposure to 80 °C was sonicated for three 15-s bursts in the presence of DOC or lysolecithin. Heating caused most of the cellular proteins to be sedimented at relatively low ω^2t values. Centrifugation at ~10^8 rad^2/s removed 50% of the cellular protein after heat

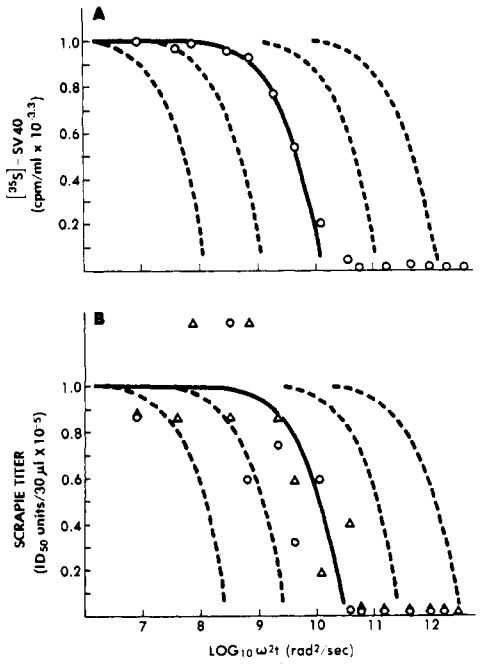

FIGURE 6: Comparison of the sedimentation profiles of SV40 and the scrapie agent in homogenates of murine spleen. The data from Figures 3B and 5 are replotted with a linear ordinate to permit comparison with the profiles computed for ideal particles. (A) Sedimentation profile for SV40 is shown by data points. Curves for ideal particles with $s_{20,w}$ values of 24 000 S, 2400 S, 240 S, 24 S, and 2.4 S from left to right as $\omega^2 t$ increases are plotted. The solid curve was computed for a 240S particle assuming a particle density of 1.3 g/cm^3 in sucrose. (B) Sedimentation profile for the scrapie agent is shown by data points. Curves for ideal particles with $s_{20,w}$ values of 8000 S, 800 S, 80 S, 8.0 S, and 0.8 S are plotted. The solid curve is for an 80S particle assuming a particle density of 1.2 g/cm^3. Symbols are defined in Figures 3 and 5.

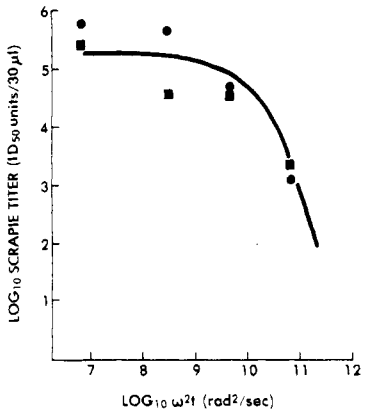

FIGURE 7: Sedimentation profiles of the scrapie agent after heat treatment of the S_1 fraction from infected murine spleen. S_1 was exposed to 80 °C for 30 min followed by sonication with 0.5% (w/v) sodium deoxycholate (-■-) or with 5 mg/mL lysolecithin (-●-). The solid curve is the sedimentation profile of the scrapie agent in S_1 after treatment with DOC at 4 °C (Figure 3B).

TABLE III: Heat Treatment of Spleen Suspensions (S_1) from Scrapie-Infected Mice.[a]

treatment	protein (mg/mL)	DNA (µg/mL)	RNA (µg/mL)	scrapie titer (log ID_{50}/mL)
control	5.0	54.2	422.5	6.4
deoxycholate	5.5	65.5	395.1	6.7
lysolecithin	5.7	52.0	440.6	
heat + sonication	4.6	51.8	30.8	
heat + sonication + deoxycholate	3.9	55.1	26.8	6.9
heat + sonication + lysolecithin	3.6	49.6	12.0	7.0

[a] Spleen suspensions were prepared by low-speed centrifugation (121g) as described in the text.

treatment. In the absence of heat treatment 5.0×10^{11} rad^2/s was needed to sediment an equivalent amount. In contrast to these alterations in the sedimentation profiles for protein after heat treatment, the sedimentation profile of the scrapie agent was unchanged (Figure 7).

Discussion

The sedimentation profiles for the scrapie agent in freshly prepared extracts of both murine spleen and brain exhibit relatively steep slopes, indicating that the agent sediments as either discrete particles homogeneous in size or as a series of particles differing in size over a small range (Table II). Using the technique of analytical differential centrifugation, the imprecision of the titration assay does not permit us to distinguish between these two possibilities. However, sucrose gradient centrifugation described in another paper does suggest that the scrapie agent may exist as a succession of particles differing in size over a limited range (Prusiner et al., 1978). The apparent range of sedimentation coefficients for the agent in DOC-treated extracts from spleen and brain is 70 S to 600 S. This is in reasonable agreement with reports by others showing that the scrapie agent was found in the void volume of an agarose gel column which excluded spherical particles of molecular weight greater than 5.0×10^7 (Kimberlin et al., 1971) and that the scrapie agent passed through a filter with pores 50 nm in diameter but was retained by filters with pores 27–30 nm in diameter (Eklund et al., 1963; Gibbs, 1967; Kimberlin et al., 1971). Comparative data on poliovirus and SV40 which are both known to be discrete infectious particles is of interest. Poliovirus has a $s_{20,w}$ of 160 S, a molecular weight of 6.8×10^6 and is spherical in shape with a 27-nm diameter (Schaffer & Schwerdt, 1959). SV40 has a $s_{20,w}$ of 240 S and is spherical in shape with a diameter of 45 nm (Black et al., 1964). The sedimentation characteristics of the scrapie agent from spleen and brain tissues are similar despite the vast differences in their cellular architecture. The spleen tissue in which the agent replicated did not have pathologic changes, whereas the brain exhibited marked spongiform alterations (Fraser, 1976). That the size of the scrapie agent is independent of the pathological condition or type of tissue in which it replicates is similar to observations made on conventional viruses (Fenner et al., 1974).

The sedimentation profile of the scrapie agent in extracts of murine spleen is similar to that observed for total cellular RNA, which is mainly ribosomal RNA, but is considerably different from that observed for mitochondria, soluble proteins, lysosomes, microsomes, and plasma membranes (Prusiner et al., 1977). To determine whether the sedimentation profile of the scrapie agent was dependent on the presence of ribosomes, we exposed the S_1 fraction of scrapie-infected spleens to 80 °C for 30 min. Under these conditions 95% of the total RNA was degraded, presumably by endogenous ribonucleases that remain active at high temperatures (Sela & Anfinsen, 1957). The heat treatment which caused degradation of ribosomal structures did not alter either the infectivity of the scrapie agent or its sedimentation profile (Table III, Figure 7). It is thus reasonable to conclude that the sedimentation behavior of the

agent is independent of polyribosomes and that the scrapie agent is not a minute particle whose sedimentation is a consequence of its attachment to polyribosomes. Destruction of ribosomal RNA by heating was also observed in suspensions of mouse spleen from control animals, indicating that this phenomenon is not dependent on prior scrapie infection. This concurs with earlier studies that scrapie infectivity was unaltered by the addition of exogenous ribonucleases and with recent studies which suggest that the scrapie agent is not a viroid-like RNA structure (Marsh et al., 1974; Ward et al., 1975).

Early studies with infected sheep brain (Gordon, 1946) demonstrated the unique resistance of the scrapie agent to inactivation by exposure to high temperatures. Experiments using mouse-adapted scrapie showed that the infectivity was unaltered by temperatures as high as 80 °C (Kimberlin, 1976; Eklund et al., 1963; Gibbs, 1967). At this temperature relatively few biological particles are stable and the infectivity of most conventional viruses is destroyed (Ginoza, 1968). Parvoviruses, which are small single-stranded DNA viruses, are the most heat-resistant conventional viruses and can survive exposure to 80 °C without significant loss of infectivity (Rose, 1974). The unusual heat stability and small size of the parvoviruses raise the possibility that the scrapie agent may resemble these viruses in several respects, i.e., the agent might have a single-stranded DNA genome protected from nuclease digestion by a coat of protein and/or lipid. In another communication we suggest that the resistance of the scrapie agent to heat inactivation may result in part from extensive hydrophobic interactions which are enhanced at elevated temperatures (Prusiner et al., 1978). The hydrophobicity of amino acid residues at selected positions in proteins seems to correlate with the heat stability of molecules (Perutz & Raidt, 1975; Yutani et al., 1977). Interestingly, ionic bonding seems to be crucial in stabilizing the proteins of thermophilic bacteria but the role of these bonds in preserving infectivity of the scrapie agent during heating remains to be elucidated (Perutz & Raidt, 1975; Biesecker et al., 1977).

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