Electrophoretic properties of the scrapie agent in agarose gels
(hydrophobic viral-like agents/Sarkosyl gel electrophoresis/stability in detergents)

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ABSTRACT The molecular properties of the scrapie agent were investigated by subjecting partially purified preparations to electrophoresis on agarose gels. When electrophoresis was performed at room temperature in the presence of sodium dodecyl sulfate (NaDodSO4), most of the recoverable agent was found at the top of the gel, consistent with previous studies indicating aggregation of the agent upon exposure to elevated temperatures. In addition, less than 5% of the agent applied to the gel was found after electrophoresis, even though the study was performed with a low concentration of NaDodSO4 (0.1%). Further studies on the inactivation of the agent by NaDodSO4 suggest that this may be, in part, a function of the NaDodSO4:protein ratio in the sample. In contrast, sodium N-lauroyl sarcosinate (Sarkosyl) did not inactivate the agent in concentrations as high as 5% (wt/vol). Virtually all of the infectivity could be recovered after electrophoresis of the agent into 0.6% agarose gels at 4°C in the presence of 0.2% Sarkosyl. Digestion of the preparations with micrococcal nuclease and proteinase K prior to Sarkosyl electrophoresis caused a substantial portion of the agent to migrate ahead of DNA fragments of 1 × 10^6 daltons. The behavior of the scrapie agent in electrophoretic gels is consistent with earlier studies showing that the monomeric form of the agent has a sedimentation coefficient of ≤40 S. Thus, the smallest or monomeric form of the agent is smaller than any known animal virus.

Four transmissible slow infections comprise the spongiform encephalopathies: scrapie of sheep and goats, encephalopathy of mink, and kuru and Creutzfeldt-Jakob disease of humans (1). These diseases are characterized by prolonged incubation periods of 2 months–20 years, by spongiform degeneration of the central nervous system, and by unusual viral-like agents. These unusual agents have eluded isolation mainly because of the availability of only a slow, cumbersome, and imprecise bioassay for the agent in animals and the lack of a homogeneous cell culture source for replication of the agent for high titers. In addition, the hydrophobic nature of the agent, which has become apparent only recently (2, 3), also complicates purification.

To minimize these obstacles, we have studied the scrapie agent in hamsters, which develop neurological dysfunction as early as 55–65 days after inoculation (4). At this time, their brains contain 10^9–10^10 mean infective dose (ID50) units of the agent per g of wet weight. By carefully determining the time from inoculation to the onset of clinical illness, we have been able to relate the titer to the length of the incubation period and thus obviate the need for conventional and more cumbersome endpoint titration assays.

In this communication, we report on the electrophoretic behavior of the scrapie agent in agarose gels in the presence of detergents. In a partially purified preparation from hamster brain, the agent migrates into agarose gels when electrophoresed at 4°C in the presence of 0.2% sodium N-lauroyl sarcosinate (Sarkosyl). These studies show that the smallest or monomeric form of the agent migrates ahead of DNA fragments of <1 × 10^6 daltons in an electrophoretic gel. Thus, both by earlier observations on the sedimentation characteristics of the agent (2, 3) and now by electrophoresis, the monomer of the scrapie agent is considerably smaller than the smallest known animal virus (2, 3).

MATERIALS AND METHODS

Materials. Electrophoresis-grade NaDodSO4 was purchased from BDH Chemicals (London), sodium deoxycholate (DOC) from Schwarz/Mann, and Sarkosyl and Triton X-100 (TX-100) from Sigma. Proteinase K was obtained from Merck, DNases and RNases from Worthington, and micrococcal nuclease from Sigma. Acrylamide, bisacrylamide, N,N,N',N'-tetramethyl-ethylenediamine (Temed), and ammonium persulfate were purchased from Bio-Rad; agarose was obtained from Marine Colloids (Rockland, ME). The remaining chemicals were of the highest grades commercially available. Nucleic acid standards were obtained from New England BioLabs.

Source of Scrapie Agent. Hamster-adapted scrapie agent was obtained from Richard Marsh in its 6th passage in hamsters. Extracts of hamster brain containing the agent were inoculated into weanling, random-bred Syrian female hamsters (LVG/LAK) obtained from Charles River Laboratories. After two serial passages, animals with clinical illness were killed, their brains were removed and homogenized in 320 mM sucrose, and the 10% (wt/vol) homogenate was stored at −70°C after clarification by centrifugation at 1000 × g for 10 min at 4°C.

Partial Purification of Scrapie Agent. Weanling female hamsters (LVG/LAK) were inoculated intracerebrally with 50 μl of brain extract containing 10^7 ID50 units. The animals were killed 55–65 days later by cervical dislocation and their brains were removed and washed in 320 mM sucrose. A 10% (wt/vol) homogenate was made by using a Polytron for three bursts of 15 sec each. The homogenization was performed in a Baker Sterilgard biosafety hood and the flask was surrounded by ice water. The temperature was never allowed to exceed 15°C. The cell nuclei were removed by centrifugation at 2700 rpm for 10 min in a Spinco JA-10 rotor. The pellet was washed with 320 mM sucrose, and the combined supernatants were centrifuged at 4000 rpm for 30 min to remove mitochondria and large membranous fragments. DOC was added to the supernatant fluid to give a final concentration of 0.5%, and the suspension was stirred for 30 min at 4°C. The suspension was then centrifuged at 50,000 rpm for 120 min in a Spinc<sub>o</sub> Ti50.2 rotor or at 32,000 rpm for 220 min in a Spinc<sub>o</sub> Ti15 zonal rotor. The pellets designated P<sub>3</sub> were collected and resuspended in

*Abbreviations:* Sarkosyl, sodium N-lauroyl sarcosinate; ID<sub>50</sub>, mean infective dose; TX-100, Triton X-100; DOC, sodium deoxycholate.
20 mM TrisOAc (pH 8.3) and stored at -70°C. The P₃ fraction contained 12% of the RNA and DNA found in the homogenate and less than 2% of the protein. The yield of scrapie infectivity was 10–90%. By electron microscopy, the preparation contained numerous membrane fragments, ribosomes, and amorphous structures.

Assays. Protein, RNA, and DNA were assayed as described (3, 5). Determination of the scrapie titer used measurements of the incubation period, which is the time interval from inoculation to the onset of clinical disease. Samples, undiluted or diluted 10-fold unless otherwise noted, were inoculated intracerebrally into four weanling female hamsters. The animals were scored for the development of clinical scrapie as judged by an ataxic gait, difficulty in righting themselves from a supine position, or repetitive bobbing of the head (or all three).

Several investigators have described a lengthening of the incubation period as a function of increasing sample dilution (4, 6, 7). We have extended these observations by constructing a plot of the product of titer and the dilution as a function of the incubation period (8). The points suggest two straight lines which were chosen to ensure a good approximation at the shortest and longest incubation periods. The following equation describes these lines and was used to calculate the titer of the scrapie agent:

\[
\log_{10} \text{titer} = \begin{cases} 
17.27 - \log_{10} D - 0.1424x & \text{for } x \leq 100, \\
8.27 - \log_{10} D - 0.0524x & \text{for } x > 100
\end{cases}
\]

where \(D\) is the dilution of the sample, \(x\) the incubation period in days, and the titer is expressed as ID₅₀ units/ml. This assay gave titer within ±0.5 \(\log_{10}\) ID₅₀ units/ml of those obtained by endpoint titration.

**Gel Electrophoresis.** Vertical slab gels 28 cm wide, 10 cm high, and 1.5 or 3.0 mm thick with five sample wells were prepared with an SE 500 apparatus obtained from Hoefer Scientific Instruments (San Francisco). The gels were composed of either 0.6% agarose or a combination of 2.5% polyacrylamide and 0.5% agarose. A 15% polyacrylamide gel barrier (2 cm deep) was used to support the gels using Sarkosyl buffer. Frosted glass plates were used to prevent slippage of the gels during electrophoresis. The buffer in the upper and lower chambers as well as in the gel itself contained 60 mM TrisOAc (pH 8.3), 1 mM EDTA, and either 0.1% NaDodSO₄ or 0.2% Sarkosyl. Electrophoresis with NaDodSO₄ was performed at room temperature for 2 hr with composite gels and for 1.5 hr with agarose gels at a constant voltage of 120 V. Gels were run with nucleic acid molecular weight standards: total *Escherichia coli* RNA or bacteriophage λ DNA digested with the restriction enzyme *HindIII*. Electrophoresis with Sarkosyl was performed at 4°C for 4.5 hr at a constant voltage of 90 V after slowly increasing the voltage during the preceding hour. To facilitate entry of materials into the gels, electrophoresis at 4°C was begun at 10 V for 5 min, followed by 20 V for 5 min, and then maintained at 30 V for the next 45–50 min while the bromophenol blue dye migrated 1.5 cm from the origin.

Upon termination of the electrophoresis, lanes to be assayed for infectivity were cut into sections of 2 cm or less and eluted into 2–4 ml of buffer containing 20 mM Tris-HCl (pH 7.4). The gels were homogenized at 4°C with a Polytron for 15 sec at full speed. The pulverized gel, suspended in buffer, was placed on a gyrotary shaker at 4°C for 15 hr. The suspension was clarified by centrifugation at 3300 X g for 30 min at 4°C. Fifty microliters of supernatant fluid was then injected intracerebrally into hamsters either undiluted or diluted 10-fold for measurement of the scrapie incubation period.

Lanes to be stained for nucleic acids and proteins were immersed in ethidium bromide solution (1 µg/ml) for 30 min, rinsed with water, and photographed with Polaroid type 55 film. The ethidium bromide-stained gels were illuminated from below with an ultraviolet light source (excitation maximum, 254 nm) and the emission was filtered before photography with a Kodak Wratten filter (no. 9). Protein was visualized in the gels by staining with Coomassie brilliant blue R.

**RESULTS**

From previous sedimentation studies of the scrapie agent, we learned that the smallest or monomeric form of the scrapie agent had a sedimentation coefficient of ≤ 40 S and that the agent could readily aggregate with cellular elements (2, 3). We reasoned that purification of the agent should be facilitated by the high degree of resolution achievable by gel electrophoresis if conditions for disaggregation of the agent with retention of infectivity could be found. In our initial studies, a mixture of TX-100 plus DOC or NaDodSO₄ alone was used with the intent of disaggregating the agent prior to electrophoresis. TX-100 and DOC were chosen because the agent in fraction P₃ is stable in both these detergents (unpublished data) and mixed micelles of these two detergents have been shown to be useful in the electrophoresis of hydrophobic proteins (9). Although the scrapie agent has been shown to be labile in NaDodSO₄ (10, 11), electrophoresis with NaDodSO₄ was performed because of a report describing the entry of the scrapie agent into electrophoretic gels in the presence of low concentrations of the detergent (12). The partially purified P₃ fraction was dialyzed to equilibrate it with the electrophoresis buffer containing detergent and then subjected to electrophoresis at room temperature. When a composite gel containing 2.5% polyacrylamide and 0.5% agarose was used (Fig. 1A), greater than 90% of the agent recoverable from the gel was found in the uppermost region. However, the total recovery of the agent from the gel was 5% of that applied to the sample well. Similar results were obtained when a gel of greater porosity composed of 0.6% agarose was used in order to facilitate the entry of a greater proportion of the recoverable agent (Fig. 1B). Again, greater than 90% of the recoverable agent was found in the top portion.

**Fig. 1.** NaDodSO₄ gel electrophoresis of the scrapie agent. (A) Composite 2.5% polyacrylamide/0.5% agarose gel: 40 µl of fraction P₃ was loaded onto a slab gel 1.5 mm thick with each sample well 2.1 cm in length. (B) 0.6% agarose gel: 40 µl of fraction P₃ was loaded. Details of the electrophoresis are given in Materials and Methods and Table 1.
of the gel and less than 1% of the total infectivity applied to the gel was recovered. Similar results were obtained when the mixture of TX-100 and DOC was used in place of NaDodSO4 and the electrophoresis was performed at room temperature.

From the results of these studies, we concluded that NaDodSO4 gel electrophoresis at room temperature not only resulted in substantial losses of infectivity (Table 1), but also did not provide conditions for monomerization of the agent because previous studies indicated that the monomeric form was 40 S or less. Also as noted in Table 1, predigestion of the samples with RNases A and T1 followed by proteinase K did not alter the recovery of the agent or its distribution in the electrophoretic gels.

To investigate the lability of the scrapie agent during NaDodSO4 gel electrophoresis, we determined the infectivity of the P3 fraction as a function of the NaDodSO4:protein ratio (Table 2). We found that up to 0.8 g of NaDodSO4 per g of protein, the scrapie titer in P3 was unaltered. However, progressively increasing the detergent:protein ratio led to inactivation of the agent. More than 90% of the agent was inactivated at a detergent-to-protein ratio of 1.8 whether the agent was incubated with NaDodSO4 for 3 hr or 24 hr at room temperature. Virtually all proteins examined showed saturation of their hydrophobic binding sites between 1.4 and 2.0 g of NaDodSO4 per g of protein with concomitant denaturation (13–15).

Examination of a wide variety of nonionic detergents (including TX-100) and non-denaturing, anionic detergents (including DOC and Sarkosyl) has shown that the scrapie agent is stable at concentrations up to 5% (wt/vol) at 4°C. The stability of the scrapie agent in Sarkosyl at concentrations up to 5% (wt/vol) for 24 hr is shown in Table 2.

Based on the stability of the scrapie agent in Sarkosyl at 4°C and on our previous findings that elevated temperatures promote aggregation of the agent with cellular elements (2, 3), we turned to agarose gel electrophoresis at 4°C in the presence of 0.2% Sarkosyl. As shown in Fig. 2, considerably higher titters of the agent were found in the eluates of 0.6% agarose gels under these conditions, but more than 90% of the agent was again recovered from the top of the gel.

Because virtually all of the agent could be recovered under these conditions of electrophoresis, we proceeded to examine the stability of scrapie infectivity in the P3 fraction to enzyme digestion. Such digestions might then be performed prior to electrophoresis in order to remove nonessential molecules and permit improved penetration of the gels. As shown in Table 3, digestion of the P3 fraction with micrococcal nuclease, which catalyzes the digestion of both DNA and RNA, did not alter the infectivity of the agent. Preservation of scrapie infectivity was observed when the nuclease digestions were performed at 4°C for 16 hr followed by proteinase K digestion in the presence of 0.2% Sarkosyl for 8 hr at 4°C.

To take advantage of the stability of the agent to nucleases and proteases, the P3 fraction was first digested with micrococcal nuclease and then with proteinase K in the presence of Sarkosyl. Under these conditions, virtually all of the nucleic acids detectable by ethidium bromide staining were degraded to polynucleotides of Mr < 1 × 10^6 as illustrated by comparing the gel patterns in Figs. 2 and 3. In addition, large protein ag-

### Table 1. Recoveries and distribution of scrapie agent after NaDodSO4 gel electrophoresis

<table>
<thead>
<tr>
<th>Detergent/protein* (g/g ratio)</th>
<th>Exposure time† (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaDodSO4 at 25°C</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>9.6, 8.7</td>
</tr>
<tr>
<td>0.05</td>
<td>0.09, 8.7</td>
</tr>
<tr>
<td>0.1</td>
<td>0.18, 9.9</td>
</tr>
<tr>
<td>0.2</td>
<td>0.35, 9.3</td>
</tr>
<tr>
<td>0.5</td>
<td>0.88, 8.9</td>
</tr>
<tr>
<td>1.0</td>
<td>1.75, 7.8</td>
</tr>
<tr>
<td>3.0</td>
<td>5.26, 5.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sarkosyl at 4°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>0.2</td>
</tr>
<tr>
<td>0.6</td>
</tr>
<tr>
<td>1.0</td>
</tr>
<tr>
<td>1.5</td>
</tr>
<tr>
<td>2.0</td>
</tr>
<tr>
<td>3.0</td>
</tr>
<tr>
<td>5.0</td>
</tr>
</tbody>
</table>

* Fraction P3 contained 5.7 mg of protein per ml for NaDodSO4 treatment and 3.2 mg/ml for Sarkosyl treatment.
† Values are log10 ID50 units/ml. Titer was estimated from incubation periods at 10^-1 dilution of samples.

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The values in Table 1 were determined by incubating fraction P3 with NaDodSO4 or Sarkosyl and then performing electrophoresis under conditions that permitted improved penetration of the gels. The recoveries of the agent in the eluates of fraction P3 were determined by inoculating into scrapie-free animals. The recoveries were expressed as a percentage of undiluted undigested P3. The gels were stained with ethidium bromide and Coomassie blue. The electrophoresis was performed at 4°C in the presence of 0.2% Sarkosyl; 60 μl of fraction P3 was loaded onto a 0.6% agarose slab gel 3.0 mm thick with each sample well 2.1 cm in length. Details are given in Materials and Methods.
gregates were also degraded, as shown by Coomassie blue staining. Most striking, the entry of the scrapie agent into the gel was clearly enhanced. Under these conditions, the titer of the agent in the lower portions of the gel was as great as that at the top of the gel. Quite similar results were obtained whether the digested preparation contained 0.2% or 1.0% Sarkosyl. In both cases, the electrophoresis was performed with 0.2% Sarkosyl in the agrose gel and the electrophoresis buffer.

The recoveries of scrapie infectivity from four agarose gel electrophoreses at 4°C in the presence of Sarkosyl from undigested and digested P3 samples are given in Table 4. In addition, the distribution of the recoverable agent is also given. In all cases, more than 30% of the agent applied to the gel was recovered. With undigested samples, less than 1.5% of the agent could be recovered from the lower two regions of the gels, where restriction fragments of λ bacteriophage DNA of 2 × 10⁶ daltons or less migrate. In contrast, more than 30% of the recoverable agent from digested samples migrated into this region of the electrophoretic gel. Because the shape of the scrapie agent is unknown, no correction for changes in electrophoretic mobility due to differences in shape between the agent and the DNA restriction fragments used for calibration was attempted. However, our observations on the electrophoretic mobility of the scrapie agent from hamster brain are consistent with previous sedimentation studies on the agent from murine spleen indicating the monomeric form of the agent has a \( s_{20,w} \leq 40 \) S (2, 3).

**DISCUSSION**

Although the scrapie agent continues to elude isolation and determination of its chemical structure, the studies reported here provide considerable information about the molecular properties of the agent which may prove of importance in its eventual isolation. Our experimental data have defined certain conditions for electrophoresis of the agent into agarose gels with good recovery of infectivity. Digestion of the fraction P3 by micrococcal nuclease and proteinase K prior to electrophoresis has demonstrated that the smallest or monomeric form of the agent must be approximately 1 × 10⁶ daltons or less. Although conditions for complete disaggregation or monomerization of the scrapie agent have not been found, it may be possible to use gel electrophoresis in the presence of Sarkosyl at 4°C to obtain a considerable purification of the agent. As shown in Fig. 3, almost 70% of the recoverable agent was found in the upper four portions of the gel, where virtually no nucleic acid or protein was detected by ethidium bromide and Coomassie blue staining, respectively.

Our results contrast with those reported recently by Malone et al. (12), who found a peak of scrapie infectivity migrating with 4S and SS RNA in a NaDodSO₄/polyacrylamide/agarose gel similar to that described in Fig. 1A. Using either their preparative procedure prior to electrophoresis or the one described in this communication, we have been unable to obtain titers of the scrapie agent in the lower portions of NaDodSO₄ composite gels in excess of 10⁴ ID₅₀ units/ml. It is of interest that Malone et al. (12) discarded the top portion of their NaDodSO₄ composite gels, the region where we have found 90% of the recoverable scrapie titer. The explanation for these discrepancies in the electrophoretic migration of the scrapie agent is unclear, but one possibility lies in their application of large samples (200 µl) on the gel (0.6 cm in diameter) and subsequent shrinkage of the agarose matrix during electrophoresis. Our samples are considerably smaller when expressed as the ratio of sample volume to unit area of gel surface at the bottom of the sample well.

Poor recovery of the scrapie agent after NaDodSO₄ gel electrophoresis may in part be explained by the lability of the agent in NaDodSO₄ as reported previously by others (10, 11) and shown in Table 2. During gel electrophoresis in the presence of 0.1% NaDodSO₄, the ratio of NaDodSO₄ to protein may have undergone marked change. In other words, as cellular proteins were separated from the putative scrapie protein during electrophoresis, the ratio of NaDodSO₄ to scrapie protein presumably increased, thus leading to inactivation of the agent.

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**Table 3.** Enzyme digestions of P₃ fraction containing scrapie agent.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Digestion*</th>
<th>Enzyme</th>
<th>log₁₀ ID₅₀ units/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>Micrococcal nuclease</td>
<td>9.8</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>None</td>
<td>9.5</td>
</tr>
<tr>
<td>A</td>
<td>2</td>
<td>Proteinase K</td>
<td>9.3</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>None</td>
<td>9.9</td>
</tr>
</tbody>
</table>

* The first digestion was with micrococcal nuclease (50 µg/ml) for 16 hr at 4°C in the presence of 2 mM CaCl₂. The second digestion was with proteinase K (100 µg/ml) for 8 hr at 4°C in the presence of 0.2% Sarkosyl.

† Scrapie titers were calculated from incubation period measurements at 10⁻¹ dilution.

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**Table 4.** Recoveries and distribution of scrapie agent after Sarkosyl gel electrophoresis.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrophoretic gels†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Titer of sample applied</td>
<td>8.4</td>
<td>9.1 (100)</td>
<td>8.3 (75)</td>
<td>8.2 (58)</td>
</tr>
<tr>
<td>Distribution of recoverable agent in gels</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gel region</td>
<td>1 (top)</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>9.1 (96)</td>
<td>7.6 (29)</td>
<td>6.6 (22)</td>
<td>6.4 (0.2)</td>
</tr>
<tr>
<td></td>
<td>8.2 (88)</td>
<td>7.2 (11)</td>
<td>7.4 (18)</td>
<td>6.1 (0.7)</td>
</tr>
<tr>
<td></td>
<td>7.6 (49)</td>
<td>6.6 (22)</td>
<td>7.1 (9.0)</td>
<td>7.4 (18)</td>
</tr>
<tr>
<td></td>
<td>7.2 (11)</td>
<td>7.4 (18)</td>
<td>6.4 (3.0)</td>
<td>7.3 (24)</td>
</tr>
<tr>
<td></td>
<td>6.6 (49)</td>
<td>6.6 (22)</td>
<td>6.4 (3.0)</td>
<td></td>
</tr>
</tbody>
</table>

All titers were estimated from incubation periods. Samples inoculated were undiluted.

* Values are given as log₁₀ ID₅₀ units (% in parentheses).
† All gels were composed of 0.6% agarose. Gels A and C were loaded with samples containing 0.2% Sarkosyl. Gels B and D were loaded with samples containing 1% Sarkosyl. Samples placed on gels C and D were digested with micrococcal nuclease (50 µg/ml) for 16 hr at 4°C, followed by proteinase K (100 µg/ml) digestion for an additional 8 hr at 4°C in the presence of 0.2% Sarkosyl.

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**FIG. 3.** Sarkosyl gel electrophoresis of scrapie agent in fraction P₃ predigested with micrococcal nuclease and proteinase K. Details are described in Fig. 2 legend.
Electrophoresis at room temperature appears to be another factor contributing to the poor recovery of the scrapie agent because electrophoretic studies with mixed micelles of TX-100 and DOC at room temperature also resulted in poor yields of the agent (unpublished data).

The size heterogeneity of scrapie agent from hamster brain shown by gel electrophoresis is consistent with our observations on the agent from murine spleen by rate-zonal sedimentation (2, 3). In those studies, the infectivity of a partially purified fraction P2 subjected to sedimentation through a sucrose gradient showed a continuum of agent sizes with sedimentation coefficients ranging from 40 S to 500 S. The studies reported here also demonstrate a continuum of agent sizes ranging from 1 × 10^6 to 2 × 10^7 daltons.

The stability of the scrapie agent to nuclease and protease digestion provides an effective purification procedure that can be performed at 4°C, thus minimizing conditions favoring aggregation of the agent. As shown in Table 3, the agent was stable during digestions by micrococcal nuclease and proteinase K, which effectively degrade nucleic acids and proteins at 4°C. In addition, the agent in both P2 preparations from hamster brain and the P3 fraction from murine spleen was unaffected by digestions with DNases I and II as well as RNases A and T1 (unpublished data). These findings are consistent with those reported by others (16–18) except for two published observations. Marsh et al. (19) found that the eluate from the lower portions of NaDodSO₄ gels contained a form of the scrapie agent whose infectivity was destroyed by DNases I and II, but remained unharmed by RNases A and T1, proteinase, and proteinase K. These results are puzzling because DNase I is readily inactivated by concentrations of NaDodSO₄ as low as 0.01% (20) and no procedure was described to remove residual NaDodSO₄ from the gel eluate prior to digestion. In contrast, the catalytic activity of RNases and of proteinase K is unaltered or enhanced by low concentrations of NaDodSO₄. Hunter et al. (21) found a significant reduction in scrapie titer after digestion of detergent-treated extracts with papain, whereas ficin and trypsin did not reduce the infectivity. Further studies are needed to clarify these findings.

Although the animal bioassay requiring prolonged incubation periods for determination of scrapie infectivity continues to impede progress in the isolation of the agent, the ability to measure scrapie titer reliably in partially purified fractions as a function of incubation period represents an important advance (8). Calculation of titer from the length of the incubation period substantially reduces the number of animals required for bioassay of the agent and permits examination of a much greater number of variables than was previously possible with endpoint titrations. Titors obtained by incubation period measurements appear to have a precision not significantly different from that found with quantal endpoint titrations with four to six animals per dilution (8). Further studies are clearly required to establish more firmly the precision of the bioassay as measured by incubation period length under a wide variety of conditions.

All of the data presented here appear to be consistent with the hypothesis that the scrapie agent is a small, hydrophobic macromolecular complex (2, 3). This small complex is presumably composed of a hydrophobic protein tightly bound or covalently linked to the putative scrapie nucleic acid genome. Firmly anchored to the hydrophobic protein may be a shell of protective lipid, presumably phospholipids. This model is consistent with observations we and others have made about the scrapie agent, including its small monomeric size, its unusual heat stability, its ability to aggregate upon heating, and its propensity to bind lipids as well as cellular proteins and possibly nucleic acids (22). The agent is stable in nonionic and nondenaturing, anionic detergents as well as in weak chaotropic ions. However, it is labile in the presence of denaturing detergents such as NaDodSO₄, strong chaotropic ions such as SCN⁻, and denaturing organic solvents such as phenol and 2-chloroethanol (refs. 10 and 18; unpublished data). Although all of these properties are most consistent with the presence of a hydrophobic protein that is critical for infectivity, we cannot exclude the possibility of an unusual nucleic acid structure exhibiting all these features.

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