Copurification of Sp33–37 and scrapie agent from hamster brain prior to detectable histopathology and clinical disease

David C. Bolton,1* Raoul D. Rudelli,2 Julia R. Currie2 and Paul E. Bendheim1

Departments of 1Molecular Biology and 2Pathological Neurobiology, New York State Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, New York 10314, U.S.A.

Studies were conducted to determine whether accumulation of the scrapie agent protein Sp33–37 in brain correlated with the appearance of the scrapie agent or with pathology. The concentrations of the scrapie agent and Sp33–37 were measured in purified fraction P5 isolated from hamster brains at weekly intervals after inoculation. The scrapie agent concentration in fraction P5 was approximately $10^{-1} \text{LD}_{50} / \text{g brain}$ 1 day post-inoculation and increased to $10^{9.4} \text{LD}_{50} / \text{g}$ at day 77. Sp33–37 was first detected in P5 at day 21, when the agent titre was $10^{3.9} \text{LD}_{50} / \text{g}$. Sp33–37 concentration increased in concert with the scrapie agent concentration, although the apparent rate of increase was somewhat lower for the protein than for the agent. The histopathological evidence of disease, consisting of mild vacuolation and gliosis, was first seen at 35 days, but was not conspicuous until 49 to 56 days post-inoculation. Vacuolation and gliosis increased until termination of the experiment at day 77. Amyloid plaques were first detected at 56 days and were widespread at day 77. Clinical disease was first seen in these animals at day 66, with an average onset at day 71. Control animals inoculated with buffer alone showed some mild gliosis, but were otherwise normal. The fact that Sp33–37 purified with the scrapie agent isolated from brain 14 days prior to detectable (light microscopic) pathology supports the theory that Sp33–37 is the major structural component of the scrapie agent and not solely a product of the pathology.

Introduction

Scrapie in the laboratory hamster is a rapid and well characterized model of the natural transmissible spongiform encephalopathies (TSEs): sheep scrapie, bovine spongiform encephalopathy, and the human diseases kuru, Creutzfeldt-Jakob disease and Gerstmann-Straussler syndrome. These diseases all show similar molecular pathology and are characterized clinically by prolonged asymptomatic incubation periods followed by defined clinical phases and death. There is no measurable immune response to the aetiological agents, although the agents accumulate in the spleen and lymph nodes prior to the onset of clinical symptoms (Barry & Prusiner, 1987).

The TSE agents are clearly related and although our knowledge of their structure and chemical composition is incomplete, they appear to be biochemically unique among microbial pathogens. Many studies suggest that these agents fall outside any known virus family (Carp et al., 1989; Prusiner & McKinley, 1987; Murphy & Kingsbury, 1990). An abnormal host glycoprotein is the only macromolecule that has been shown to be specifically associated with these diseases and to purify with the agents (Bolton et al., 1982, 1987a; McKinley et al. 1983; Prusiner et al., 1982a; Prusiner & McKinley, 1987; Gabizon et al., 1987; Carp et al., 1989). This glycoprotein, Sp33–37, differs from its normal cellular form, Cp33–37, in that it aggregates to form high molecular mass structures and is remarkably resistant to protease degradation (Bolton et al., 1982, 1987a; McKinley et al., 1983; Meyer et al., 1986; Bendheim et al., 1988b; Prusiner et al., 1983; Barry et al., 1985). [This protein has been referred to variously as PrP, PrP-C, PrP-27–30, SAF protein, PrP 33–35, PrP 35–38 and Sp33–37 (McKinley et al., 1983; Bolton et al., 1985, 1987a; Kascak et al., 1986; Meyer et al., 1986; Gabizon et al., 1987; Bendheim & Bolton, 1986). PrP-C is produced from Sp33–37 by removal of 67 amino-terminal residues when proteinase K is used during isolation (Bolton et al., 1982, 1987a; Hope et al., 1986; Prusiner et al., 1982a). The term PrP has been used to denote either protease-resistant protein or prion protein.] Sp33–37 has an apparent mass of approximately 33K to 37K when isolated in the absence of exogenous proteases (Bolton et al. 1987a; Hope et al., 1986) or approximately 27K to
Sp33–37 has not been detected in normal brain at any time (Bolton et al., 1982; Prusiner et al., 1982a). There is abundant evidence that Sp33–37 plays an essential role in initiating the disease and promoting synthesis of the scrapie agent (Bendheim et al., 1983; Gabizon et al., 1987, 1988; Bendheim et al., 1988b; Carp et al., 1989; Safar et al., 1990; Brown et al., 1990). Sp33–37 aggregates into readily sedimentable forms during isolation. In vivo, at least some Sp33–37 is present in amyloid fibrils that coalesce to form amyloid plaques (Bendheim et al., 1984; DeArmond et al., 1985). The amyloid fibrils have been designated scrapie-associated fibrils (SAF) or prion rods by different groups (Merz et al., 1981, 1983, 1984; Barry et al., 1985; McKinley et al., 1986). As noted above, the properties of protease resistance and aggregation distinguish Sp33–37 from Cp33–37 (Meyer et al., 1986; Bendheim et al., 1988b). The structural basis for the difference between Sp33–37 and Cp33–37 is not known, but it is likely that Sp33–37 is derived from Cp33–37 by a covalent post-translational modification or a conformational change.

Some have argued that Sp33–37 is not a component of the scrapie agent, but a pathological by-product that adventitiously purifies with the agent (Marsh et al., 1984a, b; Dees et al., 1985; Aiken et al., 1989; Sklaviadis et al., 1986, 1989; Manuelidis et al., 1987). One group has reported that the scrapie protein appears in the brain only after replication of the agent, and that the protein is an amyloid protein induced by an unidentified virus (Czub et al., 1986, 1988; Braig & Diringer, 1985). This view is based in part on the hypothesis that the conversion of Cp33–37 to Sp33–37 is catalysed by a factor(s) present in the late-stage diseased brain. To address this question, we studied the kinetics of synthesis of Sp33–37 and the scrapie agent during the course of experimental hamster scrapie. Our results show that Sp33–37 and the scrapie agent copurify from histologically normal brains removed 21 days post-inoculation, a time point that is shown to be 2 weeks before neuropathology was first detected by light microscopy.

Methods

Experimental design. Sixty-three weanling female LVG hamsters (Charles River) were inoculated in the right cerebral hemisphere with approximately 40 μl of the 263K scrapie agent P7 fraction diluted 1000-fold in PBS containing 50 mg BSA/ml and 0.1% Zwittergent 3-14 (Calbiochem). The inoculated dose of the scrapie agent was determined by an independent incubation period assay to be 10 LD50/hamster. Another group of 14 hamsters were inoculated with 40 μl of the PBS/BSA/Zwittergent buffer alone. At day 1, day 7 and at weekly intervals thereafter, scrapie-inoculated animals were killed by asphyxiation with CO2, and the brains removed. The control animals were killed on days 7, 28, 42, 56, 70 and 77 post-inoculation. The brains were either fixed in 10% formalin or immediately frozen in a plastic bag submerged in a dry ice-ethanol bath. The frozen brains were stored at −70°C until use for purification of the scrapie agent as described below. The resulting P7 fractions were bioassayed for scrapie agent by inoculation of a 10−2 or 10−4 dilution of each sample into five hamsters, and the titres calculated using the incubation time interval method (Prusiner et al., 1982b). Sp33–37 concentration was measured by semi-quantitative immunoblotting as described in Results. Formalin-fixed brains were randomly coded and processed for histopathology as described below.

Purification of the scrapie agent and Sp33–37. The 263K isolate of the scrapie agent was propagated and purified from the brains of diseased Syrian hamsters as described previously (Bolton et al., 1987a) with subsequent modifications (Bendheim et al., 1988b) (Fig. 1). This procedure was scaled down to accommodate purification of the agent from two hamster brains at each time point as follows. All sedimentations were performed in the Beckman 60Ti rotor. The brain homogenate (H) was sedimented at 17500 r.p.m. for 10 min at 20°C. The supernatant (S1) was then sedimented at 48500 r.p.m. for 3 h at 20°C and the resuspended pellet (P1) was sedimented at 56000 r.p.m. for 2 h at 20°C. The nuclease-digested pellet (P2) was sedimented over a 1·0 M-sucrose cushion at 56000 r.p.m. for 2 h at 20°C. The nuclease-digested pellet (P3) was sedimented over a 1·0 M-sucrose cushion in the Eppendorf 5414 microcentrifuge for 30 min at room temperature. The resulting P3 fraction is highly enriched for both the scrapie agent (specific activities for hamster 263K scrapie agent are between 1014 and 1015 LD50/mg protein) and Sp33–37, which accounts for about 70 to 90% of the protein in this fraction as detected by silver staining or protein sequencing (Bolton et al., 1987a).

SDS-PAGE and immunoblotting. One-dimensional electrophoresis in SDS-PAGE gels containing 15% (w/v) acrylamide was performed as described by Laemmli (1970). The separated proteins were analysed by staining with silver (Blum et al., 1987) or transferred to nitrocellulose for immunoblotting. Immunoblots were prepared by electrophoretic transfer of proteins to nitrocellulose sheets using a Bio-Rad Trans-Blot cell apparatus (Towbin et al., 1979; Burnette, 1981). The immunoblots were developed using the 3F4 monoclonal antibody (Kascsak et al., 1987) as described previously (Leary et al., 1983; Bendheim et al., 1988b).

Histopathology. Brains were removed from hamsters killed at 7, 28, 35, 42, 49, 56 and 77 days post-inoculation with scrapie agent or inoculation buffer alone. The brains were fixed in formalin and assigned code numbers randomly. Individual sections from paraffin-embedded brains were stained with haematoxylin and eosin to determine the extent of vacuolation, and with the Holzer stain to determine the extent of gliosis. Additional sections were stained with antibodies to glial fibrillary acidic protein (GFAP) (anti-GFAP, Miles/ICN ImmunoBiological) diluted 1:500 as another means to determine the extent of gliosis. Finally, other sections were stained with...
Hamster brains

10% homogenate TEND-10S

22500 g, 10 min

S1

150000 g, 3 h

S2

P2 Resuspended in TEND-10SS

22500 g, 2 h

S3

P3 Resuspended in NDB

Digested 12-15 h
RNase A (100 μg/ml)
DNase I (20 μg/ml)
+ EDTA, NaCl, 1% Sarkosyl
225000 g, 2 h

S4

P4

Digested 2 h
RNase A
DNase I
+ EDTA, NaCl, 1% Sarkosyl
150000 g, 30 min

S5

P5 Final fraction containing scrapie agent

Fig. 1. Schematic representation of scrapie agent purification method. Routine preparations were produced from 36 g of brain as described previously (Bolton et al., 1987a; Bendheim et al., 1988b). Small-scale preparations for the time course studies were produced from two brains as described in Methods.

Table 1. Recovery of 263K scrapie agent

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Separation conditions</th>
<th>log10 Total LD50 (at each step)</th>
<th>Percentage of S1 + P1</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td></td>
<td>10.6</td>
<td>-</td>
</tr>
<tr>
<td>S1</td>
<td>22500 g</td>
<td>11.9</td>
<td>95.5</td>
</tr>
<tr>
<td>P1</td>
<td>22500 g</td>
<td>10.6</td>
<td>4.6</td>
</tr>
<tr>
<td>S2</td>
<td>150000 g</td>
<td>9.6</td>
<td>0.2</td>
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<tr>
<td>P2</td>
<td>150000 g</td>
<td>12.5</td>
<td>3.1</td>
</tr>
<tr>
<td>S3</td>
<td>225000 g</td>
<td>8.5</td>
<td>0.1</td>
</tr>
<tr>
<td>P3</td>
<td>225000 g</td>
<td>11.6</td>
<td>99.9</td>
</tr>
<tr>
<td>S4</td>
<td>225000 g</td>
<td>7.8</td>
<td>0.0</td>
</tr>
<tr>
<td>P4</td>
<td>225000 g</td>
<td>8.9</td>
<td>0.5</td>
</tr>
<tr>
<td>S5</td>
<td>150000 g</td>
<td>11.2</td>
<td>99.5</td>
</tr>
<tr>
<td>P5</td>
<td>16000 g</td>
<td>9.6</td>
<td>3.3</td>
</tr>
<tr>
<td>S6</td>
<td>16000 g</td>
<td>9.6</td>
<td>3.4</td>
</tr>
<tr>
<td>P6</td>
<td>16000 g</td>
<td>11.1</td>
<td>93.3</td>
</tr>
</tbody>
</table>

Thioflavin, Congo red, or antibodies to the scrapie agent protein (1:500 dilution) (Bendheim et al. 1984) to determine the extent of accumulation of this protein in amyloid plaques. The coded sections were examined and scored for pathology, then the code was broken and the sections were re-examined.

Results

Purification of the scrapie agent and Sp33–37

The method used in these studies to purify the scrapie agent is shown in Fig. 1. In a typical large-scale experiment, brains removed from clinically affected hamsters sacrificed near death are used as the source tissue. Bioassay results from a typical large-scale preparation show that more than 90% of the scrapie agent recovered at each step distributes in a single fraction (Table 1). Biochemical and biological analyses of the purified fraction have been reported previously (Bolton et al., 1987a; Bendheim et al., 1988b).

At this time, there are no antibodies that distinguish between Sp33–37 and Cp33–37. Since we proposed to use semi-quantitative immunoblotting to estimate the concentration of Sp33–37 in the Ps fraction, it was necessary to determine whether Cp33–37 was present in this fraction. Obviously, the presence of Cp33–37 in Ps would obscure the earliest appearance of Sp33–37 and would skew estimates of the concentration of Sp33–37 toward higher values. We have shown previously that most of the Cp33–37 remains in fraction S2 and is not detected in subsequent fractions (Bolton et al., 1987a). However, the fractions from those experiments were not examined at the high concentrations that would be required for these studies. Therefore, a preparation was made using normal hamster brains as the source tissue and fraction Ps was analysed at the highest possible concentration (Fig. 2). Cp33–37 was not detected in fraction Ps, even when 2667 mg equivalents were loaded, or 10^47 times the minimum amount (in mg brain equivalents) required to detect Sp33–37 in fraction Ps isolated from scrapie-diseased hamster brain (Fig. 2). Thus, we concluded that in fraction Ps isolated from scrapie-inoculated brain, all of the protein detected by monoclonal antibody 3F4 was Sp33–37.

Accumulation of scrapie agent and Sp33–37 in brain

The kinetics of scrapie agent and Sp33–37 accumulation in hamster brain was examined beginning 1 day after intracerebral inoculation and continuing at weekly intervals. Fraction Ps was prepared from two brains at each time point and analysed for Sp33–37 by immuno-
Fig. 2. Analysis of normal brain fraction P₅ for Sp33-37. Routine preparations were made from scrapie-diseased and age-matched normal hamster brains. The P₅ fractions were concentrated or diluted as indicated, the proteins separated by SDS-PAGE and immunoblotted using (a) monoclonal antibody 3F4 at a dilution of 1:30000 (Kascak et al., 1987) or (b) no primary antibody. Both immunoblots were developed using an alkaline phosphatase-conjugated goat anti-mouse serum diluted 1:2000. The absolute amount of fraction P₅ loaded (mg equivalents of hamster brain) is shown on the bottom line. The relative amount, where the lowest detectable amount of Sp33-37 in the scrapie-diseased fraction P₅ is defined as 1, is shown above. This band was clearly visible in the original immunoblot but did not reproduce well photographically. Sp33-37 was not detected in the normal brain P₅ even after loading 50000 times as much sample.

Fig. 3. Immunoblot analysis of fraction P₅ throughout the progression of scrapie. Fraction P₅ was prepared from brains at the times indicated and aliquots were analysed by SDS-PAGE and immunoblotting. The amounts of fraction P₅ loaded in each lane are indicated in mg brain equivalents. Sp33-37 was first detected in fraction P₅ at 21 days post inoculation. This band was clearly visible in the original immunoblot but did not reproduce well photographically.

Sp33-37 was first detected at 21 days post-inoculation and its concentration increased over time. The concentration of Sp33-37 in each P₅ fraction was estimated from the known dilutions and the staining intensity of the bands, using a dilution series of Sp33-37 as a reference. For example, the samples at 21, 28 and 35 days showed increased staining that ranged from light to intense over a tenfold increase in concentration (compare 333 mg load at day 21 with the 33 and 333 mg loads at day 28). A further increase could not be measured at a single load (see 333 mg load at days 28 and 35), but dilution of the samples showed that Sp33-37 concentration increased from day 28 to day 35. Similarly, fivefold increases in concentration were readily evident in a standard dilution series (Fig. 2), but it was difficult to distinguish between five- and tenfold differences (compare Fig. 2 and 3). Thus, we estimate that the values obtained are probably accurate within a range of two- to fourfold. It is worth noting that the value for the concentration of Sp33-37 in fraction P₅ at day 77 obtained from the immunoblots (30 µg/g) corresponds well with the values obtained by amino acid analysis of similar fractions (25 to 80 µg/g) (Bolton et al., 1987a).

Sp33-37 in fraction P₅ prepared from 28 day post-inoculation hamster brains was digested with proteinase K (5 µg/ml) at 37 °C for 2 h in the presence of 0·1% Sarkosyl to test its resistance to proteolysis. The protein was converted to PrP-27-30 under these conditions, with a yield similar to that obtained from 83 day post-inoculation P₅ fraction digested at a similar concentration (data not shown).

The concentration of scrapie agent in the P₅ fractions was measured using the incubation time interval bioassay and compared with the concentration of Sp33-37 (Fig. 4). The concentrations of scrapie agent and Sp33-37 increased concurrently, although it appears that the rate of increase of Sp33-37 was lower than that
Isolation of Sp33-37 prior to pathology

Fig. 4. Comparison of scrapie agent (△) and Sp33-37 (▲) concentrations in fraction P5. Scrapie agent concentrations were determined by incubation time interval bioassay for each P5 fraction and expressed as the geometric mean in log_{10} LD_{50} units/g brain. The error bars indicate the standard error. Sp33-37 concentrations (log_{10} pg/g brain) were estimated from the immunoblots shown in Fig. 3 by comparing the relative staining intensities with Sp33-37 standards of known concentration. Assuming a load of 333 mg equivalents, we estimated that the detection limit for Sp33-37 is about 1000 pg/g of brain.

of the scrapie agent. If correct, this observation would indicate that fewer Sp33-37 molecules are required per infectious unit late in the disease than early in the disease.

Histopathology

Brains removed at selected times after inoculation with scrapie agent or with buffer alone were examined for the pathological lesions characteristic of scrapie. All pathological lesions were represented bilaterally and symmetrically, even though inoculation was done uni-laterally into the right parietal lobe. A moderate variation in the location of lesions within brains from animals sacrificed at identical time points was noticed. This variation of localization was limited to sub-regional discrepancies, e.g. ventral-lateral thalamic nuclei in one animal and ventral-posterior thalamic nuclei in a second animal, and may have been the result of slight differences in the site or depth of inoculation.

The first evidence of scrapie-specific pathology was seen at 35 days post-inoculation and consisted of scattered vacuoles in the posterior nuclei of the thalamus and in retrolenticular fibres of the internal capsule passing through the lateral-most aspect of the thalamus (Fig. 5a). A rare vacuole was noted in the H3 field of

Fig. 5. Figurative representation of the anatomical distribution of (a) vacuolation, (b) gliosis and (c) amyloid plaques containing Sp33-37. In each panel the composite lesions detected in scrapie-affected hamster brains are shown at four time points. A single composite at the last time point is shown for control brains. Abbreviations: H, hippocampus; V, ventricle; IC, internal capsule; OT, optic tract.
hippocampus. In brains from animals 42 days post-inoculation, vacuolation was apparent also in the ventral and lateral thalamic nuclei, internal medullary laminae, and the H4 fields of hippocampus. By 56 days post-inoculation, vacuoles were numerous in the thalamic regions noted above and were more prominent in the hippocampus, including the dorsal aspect of the fimbria. Vacuoles were present also in the lateral geniculate nuclei, reticular nuclei, dorsal striatum and stria terminalis. No vacuoles were observed in any control animals at any time point.

A limited astrocytic gliosis was observed in scrapie-inoculated animals at 35 and 42 days, but was indistinguishable from that seen in control animals. The localization of this reactive astrocytosis in regions adjacent to the ventricular system suggests that an inciting component present in the inoculation buffer may have dispersed in ventricular fluid. By 56 days the astrocytosis in normal brains had peaked, but that in scrapie-inoculated brains continued to progress (Fig. 5b).

The earliest time point at which amyloid plaques could be detected was at 56 days (Fig. 5c). They were localized to periventricular and subependymal sites, in agreement with previous observations (Bendheim et al., 1984; DeArmond et al., 1987). At 77 days the number of plaques had increased considerably in those sites and additionally some were detected in the anterior thalamus.

The pathological lesions observed at 35 days post-inoculation were insufficient to make a blind neuropathological diagnosis of scrapie, but when re-examined in the context of the time course experiments these lesions could be interpreted as the earliest specific changes caused by the agent. The extent of both vacuolation and gliosis progressed steadily after 35 days, so that by 49 to 56 days, or approximately 10 to 17 days prior to the earliest clinical signs of disease, a neuropathological diagnosis of spongiform encephalopathy was made from the coded samples (Fig. 5a and b). At the time of clinical signs (after 66 days) the brain sections revealed extreme vacuolation and gliosis.

**Discussion**

The initial report identifying the scrapie agent protein suggested two possible roles for it in the disease: ‘(i) the scrapie-associated protein may be a pathological product of scrapie infection, or (ii) it may be a structural component of the scrapie agent’ (Bolton et al., 1982). One could argue that Sp33–37 is a disease-specific product of a host gene and thus a pathological product. However, the clear intent of this designation is to differentiate those molecules that are solely the end products of disease from those that have an essential role in initiating or perpetuating the disease process. Comparing the properties of Sp33–37 and GFAP (a pathologic end product of scrapie), it is clear that both proteins are produced as a result of the disease and accumulate in the brain. Unlike Sp33–37, GFAP does not purify with the agent and has not been found to be associated in any way with induction of disease.

Evidence in support of Sp33–37 as an essential (and perhaps the only) structural component of the scrapie agent is substantial and compelling (Bolton et al., 1982, 1987a; McKinley et al., 1983; Prusiner et al., 1982a, 1990; Gabizon et al., 1987, 1988; Bendheim et al., 1988b; Carp et al., 1989; Prusiner & McKinley, 1987; Safar et al., 1990; Brown et al., 1990; Scott et al., 1989; Hsiao et al., 1990). The modified-host-protein or protein-only hypothesis of scrapie (Bolton & Bendheim, 1988) has been controversial, however, and a few other studies appear to contradict this interpretation (Czub et al., 1988; Aiken et al., 1989; Sklaviadis et al. 1989). A study similar to the one reported here measured the concentration of the scrapie agent in homogenates of hamster brain and the concentration of PrP-27–30 (the proteinase K-digested form of Sp33–37) in an SAF-enriched fraction at different times after intracerebral inoculation (Czub et al., 1988). In that report, PrP-27–30 was not detected in the SAF fraction until the concentration of scrapie agent in the brain homogenate was $>10^8$ LD$_{50}$/brain (42 days post-inoculation), compared with a minimum titre of about $10^4$ LD$_{50}$/brain in our study. Although histopathology was not measured in that study, the other observations (scrapie agent concentration in brain, onset of clinical disease and time to death) were similar to ours.

The essential differences in the results presented here and those of Czub and colleagues are in the earliest time and the minimum agent titre at which the scrapie protein was detected in brain. The Czub group interpret their data (i.e. the appearance of the protein only after the agent reached high titres) to indicate that Sp33–37 (SAF protein) must be solely a pathological product induced by infection by an unidentified scrapie virus. However, the scrapie agent titres in those experiments were measured from the brain homogenate, while the scrapie protein concentration was measured from an SAF-enriched fraction. The SAF purification method used by Czub et al. (1988) relies on aggregation of the agent and resistance of the protein to degradation by proteinase K, properties characteristic of scrapie agent isolated at a late stage in the disease. The efficiency of this method for purifying the agent may be diminished at an early stage in the disease, when the concentration of the scrapie agent in the brain is low, the
protein may be less aggregated and the aggregates may be smaller. Also, aggregation may play a role in protecting the agent and protein from digestion with proteinase K. Thus, measurement of scrapie agent in the brain homogenate may not reflect the concentration of agent in the SAF fractions during the early phase of disease.

Our data suggest that the ratio of Sp33-37/LDso is higher early in the disease (i.e. more protein molecules are present per infectious dose). This finding is consistent with the hypothesis that scrapie agent purified early in the disease is more susceptible to degradation (i.e. by endogenous host proteases after inoculation for bioassay or by proteinase K during purification of the agent) than the agent obtained from the late-stage diseased brain. Another study (Kimberlin, 1979) has shown that scrapie agent isolated from preclinical mice is more sensitive to inactivation by SDS than that from clinically affected mice, in agreement with this hypothesis. Although we found that Sp33-37 in fraction P5 isolated early (28 days) was resistant to proteolysis, it is not clear whether this property resulted from aggregation induced during purification and sample handling (freezing and thawing) or was inherent in the protein structure. Also, we cannot determine whether degradation by proteinase K was a major factor contributing to reduced sensitivity in detecting Sp33-37 in the study by Czub et al. (1988).

In the present study, both the scrapie agent and Sp33-37 concentrations were measured in the purified (P5) fraction. The scrapie agent and Sp33-37 accumulated in the brain during the progression of scrapie from the time of intracerebral inoculation to development of neuropathology and clinical disease. Sp33-37 was present in brain at least 14 days prior to detectable neuropathology, and it purified with the scrapie agent at this and all subsequent times examined. In addition, the concentration of both the agent and Sp33-37 in the purified fraction increased logarithmically as the disease progressed. Within the accuracy of these assays, increases in the scrapie agent titre were accompanied by corresponding increases in the concentration of Sp33-37 in fraction P5.

Our observations on the time course of pathology in hamster scrapie are consistent with those of another blind study by Masters et al. (1984) with only minor differences. In rare cases, they observed abnormal vacuolation in the hippocampus at 32 to 35 days post-inoculation and gliosis in the thalamus and hippocampus at 35 to 39 days. We observed vacuoles in the thalamus at 35 days, with minimal gliosis. We concur with their statement that 'both gliosis and spongiform change are reliably detected at approximately the same time after inoculation (49–57 days), although rarely either change may be seen at an earlier interval (32–35 days)' (Masters et al., 1984).

Amyloid plaques were discovered to be a component of the pathology of experimental scrapie in hamsters when antibodies to Sp33-37 were used in immunohistochemical staining of scrapie-affected brain (Bendheim et al., 1984). These antibodies have consistently localized the amyloid plaques in extracellular spaces in periventricular, subependymal, subpial and perivascular sites (Bendheim et al., 1984; DeArmond et al., 1987). Our results confirm these earlier observations in end-stage scrapie and indicate that the progressive accretion of Sp33-37 into deposits large enough to be stained as amyloid is a relatively late occurrence in scrapie, with the first plaques not detectable by the techniques used here until approximately 56 days post-inoculation. Processing of Sp33-37 by astrocytes may play a role in its eventual appearance as amyloid (Diedrich et al., 1991). Whether or not the accumulation of Sp33-37 into plaques is an active or passive process is unknown. The localization of these plaques suggests that (i) Sp33-37 accumulates because export from the brain through vascular, pial or ependymal surfaces is inhibited, (ii) Sp33-37 is produced by extra-cerebral sources but becomes insoluble and deposit in these locations after transport through these surfaces, or (iii) the cell types capable of processing Sp33-37 in ways that produce amyloid plaques are found only in these locations.

The data in this report are consistent with the modified-host-protein hypothesis of the scrapie agent, which states that an abnormal host protein, Sp33-37, is responsible for inducing the TSEs and producing more of the agent by a process of pseudo-replication (Bolton & Bendheim, 1988). The idea of a protein-only agent directing its own synthesis was probably first put forward in the context of scrapie by Griffith (1967) and has subsequently been expanded by others (Prusiner, 1982, 1987; Bolton & Bendheim, 1988; Oesch et al., 1987). A fundamental premise in the modified-host-protein model is that Sp33-37 has the ability to convert the normal cellular form of the protein, Cp33-37, to the abnormal form by inducing post-translational modifications or changes in conformation (Bolton & Bendheim, 1988). Accumulation of the abnormal form is predicted to be neurotoxic. An essential prediction of this theory is that conversion of Cp33-37 to Sp33-37 and replication of the agent would coincide. This study confirms that premise and thus supports the modified-host-protein model for the scrapie agent.

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References


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