Scrapie agent contains a hydrophobic protein

(slow viruses/protease inactivation/hydrophobic chromatography/chemical modification/diethylpyrocarbonate)

STANLEY B. PRUSINER, MICHAEL P. MCKINLEY, DARLENE F. GROTH, KAREN A. BOWMAN, NELL I. MOCK, S. PATRICIA COCHRAN, AND FRANK R. MASIAZ

Howard Hughes Medical Institute Laboratory, Departments of Neurology and Biochemistry and Biophysics, University of California, San Francisco, California 94143

Communicated by Theodor O. Diener, July 16, 1981

ABSTRACT The scrapie agent causes a degenerative nervous system disorder of sheep and goats. Considerable evidence indicates that the scrapie agent contains a protein that is necessary for infectivity [Prusiner, S. B., Groth, D. F., Cochran, S. P., Masiaz, F. R., McKinley, M. P. & Martinez, H. M. (1980) *Biochemistry* 19, 4883–4891], but direct demonstration of a protein moiety has been hampered by lack of sufficiently purified preparations. Employing preparations of the scrapie agent enriched 100- to 1000-fold with respect to protein, we found that digestion by proteinase K destroyed more than 99.9% of the infectivity. Diethylpyrocarbonate, which chemically modifies amino acid residues in proteins with high efficiency, also inactivated the scrapie agent in these purified preparations. Reductions of infectivity by proteinase K and diethylpyrocarbonate were not observed with less purified preparations. The agent bound to phenyl-Sepharose could not be eluted with 8.5 M ethylene glycol; however, a combination of ethylene glycol and detergents did release the agent. These observations provide good evidence for a protein and for hydrophobic domains within the scrapie agent. Whether the protein required for infectivity is the same protein responsible for the hydrophobic properties of the scrapie agent remains to be established.

The scrapie agent causes a degenerative nervous system disorder in sheep and goats and is considered a prototype for two similar disorders of humans: kuru and Creutzfeldt-Jakob disease (1). Many months or even years pass from the time of exposure or inoculation until onset of neurological dysfunction; hence the term "slow virus" disease. Because the unusual properties of the scrapie agent readily distinguish it from conventional viruses, we prefer to use the term "unusual slow virus-like agent". Studies on the structure of unusual slow virus-like agents are of great interest because they may provide new approaches to the investigation of many common degenerative diseases of unknown etiology.

In this communication we show that the scrapie agent possesses a protein that is required for infectivity. The titer of the scrapie agent in a purified fraction was reduced by proteolytic digestion and by chemical modification. Hydrophobic chromatography provides further evidence for the hydrophobicity of the agent (2).

Our findings that the scrapie agent contains a protein, to our knowledge, represent the most convincing identification so far of a macromolecule within the agent. The requirement for a protein to maintain infectivity clearly separates the scrapie agent from infectious "naked" nucleic acids such as plant viroids (3). To date we have been unable to identify a nucleic acid within the agent. These results raise the possibility that the genome coding for the scrapie agent protein may not reside within the infectious particle itself.

MATERIALS AND METHODS

Chemicals and Reagents. Crystalline proteinase K was purchased from Merck; micrococcal nuclease, phenylmethylsulfonyl fluoride (PhMeSO₂F), and diethylpyrocarbonate (Et₂PC) from Sigma; ribonucleases and deoxyribonucleases from Worthington; phenyl-Sepharose from Pharmacia; and alkyl agaroses from Miles. Nonidet P40 (NP40), Triton X-100, Brij 35, and sodium dodecyl sarcosinate (Sarkosyl) were obtained from Sigma. 1-Dodecylpropanediol-3-phosphocholine was purchased from Medmark (Munich, Federal Republic of Germany). Sulfobetaine 3-14 (Zwittergent), sodium deoxycholate, and sodium cholate were purchased from Calbiochem. Enzyme grade (NH₄)₂SO₄ was obtained from Schwarz/Mann.

Source and Bioassay of the Scrapie Agent. Hamster-adapted scrapie agent was propagated and prepared as described (4). The infectivity titer was determined by measuring the incubation time intervals from inoculation to onset of illness and to death; this procedure is a modification of the incubation period assay previously described (4). Weanling female hamsters (LVG/LAK) purchased from Charles River Breeding Laboratories were inoculated intracerebrally with 50 μ l of suspension at designated dilutions.

Partial Purification of Scrapie Agent. In a typical preparation, 200 weanling female hamsters (LVG/LAK) were inoculated intracerebrally with $\approx 10^7$ ID₅₀ (50% infective dose) units of hamster-adapted scrapie agent and were sacrificed 60–65 days later. All solutions were degassed under reduced pressure and purged with argon to remove oxygen. The brains were removed and immediately washed with ice-cold 320 mM sucrose. A 10% (wt/vol) homogenate in 320 mM sucrose was prepared using a Polytron device equipped with a PT35K generator. Homogenization was performed for 30 sec in a flask surrounded by ice water. The entire operation was carried out in a Baker Sterilgard biosafety hood. During homogenization the temperature of the suspension was never allowed to exceed 15°C. The titer of the homogenate varied between $10^{7.5}$ and $10^{8.5}$ ID₅₀ units/ml and the protein concentration was 10 mg/ml. All subsequent operations were performed at 4°C. The homogenate was centrifuged at 1000 rpm for 10 min in a Beckman JA-10 rotor. The supernatant fluid (S₁) was decanted and centrifuged again at 2100 rpm for 30 min. To the supernatant fluid (S₂), EDTA and dithiothreitol were added to give final concentrations of 5 mM each and the fluid was centrifuged in a Spinco Ti 15 zonal rotor at 32,000 rpm for 16 hr. The pellet (P₂) was recovered on the external wall of the rotor by using a specially designed core that permitted removal of the supernatant fluid prior to opening the rotor. The pellet was resuspended in 20

mM Tris-HOAc (pH 8.4) containing 1 mM EDTA and 1 mM dithiothreitol and adjusted to a protein concentration of 10 mg/ml. The suspension was then treated with sodium deoxycholate at a final concentration of 0.5%. The extract was centrifuged in a Spinco 50.2 Ti rotor at 50,000 rpm for 120 min (5, 6). The pellet (P_4) was resuspended in 20 mM Tris-HOAc (pH 8.3)/1 mM EDTA/1 mM dithiothreitol to give a final protein concentration of 10 mg/ml. The P_4 pellet was stored under argon at -70°C until further purification was undertaken by sequential digestions with micrococcal nuclease at 12.5 units/ml and 4 mM CaCl_2 for 16 hr at 4°C and with proteinase K at 100 $\mu\text{g}/\text{ml}$ and 0.2% Sarkosyl for 8 hr at 4°C . Both digestions were performed with constant stirring. Protease activity was terminated by addition of 0.1 mM PhMeSO_2F . A solution of sodium cholate was added slowly to give a final concentration of 2% (wt/vol) followed by addition of solid $(\text{NH}_4)_2\text{SO}_4$ to 30% saturation (5). Stirring of the sample was continued for 30 min prior to centrifugation at 20,000 rpm for 30 min at 4°C in a Beckman JA-20 rotor. The pellet (P_5) was resuspended in 20 mM Tris-HOAc (pH 8.3)/1 mM EDTA/0.2% Sarkosyl. To ensure that it was uniformly resuspended, the pellet was sonicated twice for 10 sec in a Branson 350-W bath sonicator. Residual $(\text{NH}_4)_2\text{SO}_4$ was removed by dialysis against the resuspension buffer. The titer of the P_5 fraction varied from $10^{9.0}$ to $10^{10.0}$ ID₅₀ units/ml and the protein concentration was 2–5 mg/ml.

Preparative 0.8% agarose gel electrophoresis at 4°C was used for further purification (7). The strategy for employing this procedure as well as conditions for Sarkosyl gel electrophoresis have been described (5). The sample was then electrophoresed toward the anode through a short gel having a 2-cm path length. An electronic timing device provided automatic cycling of the electrophoresis system, which was operated at 100 mA (8). After elution of a peak absorbing at 260 and 280 nm, the electrophoresis was terminated. The scrapie agent was removed by electroelution for 16 hr or by pulverizing the gel with a Brinkmann Polytron (9). These fractions (designated E_6) were eluted into 60 mM Tris-HOAc (pH 8.3)/1 mM EDTA/0.2% Sarkosyl. The titers of E_6 fractions were $10^{6.5}$ to $10^{8.5}$ ID₅₀ units/ml and the protein concentrations were 20–50 $\mu\text{g}/\text{ml}$. The degree of purification of the scrapie agent was generally 100-fold with respect to protein. In a few instances the degree of purification was as high as 1000-fold. The imprecision of the bioassay and a $10^{0.5}$ to 10^1 ID₅₀ units/ml reduction in titer upon sodium deoxycholate addition to fraction P_3 undoubtedly contribute to this variability in degree of purification.

Phenyl-Sepharose Binding and Elution. Fraction P_4 was digested with micrococcal nuclease and proteinase K and then diluted with an equal volume of buffer containing 100 mM Tris-HCl (pH 7.8)/0.8 M KCl/20% (vol/vol) glycerol/2 mM dithiothreitol/2% NP40/0.8% Sarkosyl (10). The mixture was clarified by centrifugation at 10,000 rpm for 20 min in a JA-20 rotor. The supernatant fluid was then diluted 1:10 with 50 mM Tris-HCl (pH 7.8)/1 mM dithiothreitol/10% (vol/vol) glycerol/1 M KCl. Three milliliters of the diluted preparation was then bound to 1 ml of packed phenyl-Sepharose that had been equilibrated previously with this dilution buffer. Binding was allowed to proceed for 50 min at 4°C with gentle mixing on a Vortex mixer at 5-min intervals. The phenyl-Sepharose was then separated from the diluent buffer by centrifugation at 4°C for 10 min at 1800 rpm in a Beckman J-4.2 rotor. Subsequent washes of 6 ml each were performed twice and the supernatant fluids were combined.

RESULTS

In crude preparations, the scrapie agent is resistant to inactivation by a variety of hydrolytic enzymes, including proteases

Table 1. Enzymatic digestion of scrapie agent preparations*

Enzyme	Conc., $\mu\text{g}/\text{ml}$	Time, hr	log titer, ID ₅₀ units/ml
Fraction P_4			
—		1	8.6 ± 0.21
Proteinase K	100	1	8.1 ± 0.37
RNase A	50	1	8.1 ± 0.26
DNase I	50	1	7.9 ± 0.00
Fraction E_6			
—		2	6.6 ± 0.42
Proteinase K	100	2	3.7 ± 0.09
RNase A†	100	2	6.0 ± 0.53
DNase I†	100	2	6.4 ± 0.23

* Fractions P_4 (CB 31) and E_6 (DG 170) in the buffers given in *Materials and Methods* were incubated at 37°C with the enzymes listed. The reactions of samples containing proteinase K were terminated by addition of PhMeSO_2F (0.1 mM). MgCl_2 (2 mM) was added to samples digested with DNase. Samples were stored at -20°C for 3–4 weeks prior to bioassay at 10^{-1} dilution. The log titers are given $\pm$ SEM.

† Catalytic activities of DNase I and RNase A were diminished by Sarkosyl in fraction E_6 .

and nucleases (Table 1), (5, 11, 12). In contrast, in fraction E_6 proteinase K significantly reduced the titer of the scrapie agent. Ribonuclease A and deoxyribonuclease I did not inactivate the agent, but it should be noted that the presence of Sarkosyl reduced their catalytic activities. The resistance of the scrapie agent to inactivation by these nucleases diminishes the possibility that the inactivation observed with proteinase K was caused by a nonspecific contaminant. The extent of inactivation by digestion with proteinase K at 100 $\mu\text{g}/\text{ml}$ for 2–3 hr at 37°C varied between 10^1 and $>10^3$ ID₅₀ units/ml. Our data demonstrate that the extent of inactivation was dependent on enzyme concentration and duration of digestion (Fig. 1). Digestion was optimal at 37°C , whereas at 60°C it was ineffective even though proteinase K is active at this temperature (13). Preliminary studies indicate that, in the presence of 1 M KCl, digestion at 60°C with proteinase K reduced scrapie agent infectivity more than 10^4 ID₅₀ units/ml (unpublished data). Other studies have shown that the scrapie agent is also inactivated by trypsin digestion (unpublished data).

To further probe the proteinaceous structure of the scrapie agent, the effect of chemical modification of amino acid residues on infectivity was determined. Addition of 10 mM Et_2PC to fraction E_6 led to a marked reduction in titer (Table 2). Et_2PC had no effect at 1 mM. Under the conditions of these experiments the half-life for Et_2PC was ≈ 0.4 min, so that unreacted Et_2PC was rapidly hydrolyzed (14). In crude preparations,

Table 2. Inactivation of the scrapie agent by Et_2PC

Et_2PC conc., mM	log titer, ID ₅₀ units/ml	
	Exp. I*	Exp. II†
—	7.2 ± 0.16	5.9 ± 0.28
1		5.8 ± 0.29
10		3.9 ± 0.24
14	3.7 ± 0.13	
20		3.0 ± 0.15

The log titers are given $\pm$ SEM.

* Fraction E_6 (MM 57) in 60 mM Tris-HOAc (pH 8.3)/1 mM EDTA/0.2% Sarkosyl was treated with Et_2PC for 30 min at 4°C .

† Fraction E_6 (MM 82) was dialyzed against 20 mM Tris-HCl (pH 7.4)/1 mM EDTA/0.2% Sarkosyl and then treated with Et_2PC for 30 min at 4°C . Samples were stored at -20°C for 3–4 weeks prior to bioassay at 10^{-1} dilution.

FIG. 1. Digestion of extensively purified preparations of the scrapie agent by proteinase K at 37°C (DG 213). (A) Samples were exposed to proteinase K for increasing periods of time (hr). Results with proteinase K at concentrations of 0 µg/ml (□), 100 µg/ml (Δ), and 500 µg/ml (○) are shown. (B) Results with samples exposed to increasing concentrations of proteinase K for time periods of 0 hr (□), 2 hr (Δ), 8 hr (○), and 30 hr (◇) are shown. PhMeSO₂F (0.1 mM) was added to all samples to terminate the reaction. The samples were stored at -20°C for 3-4 weeks prior to bioassay at 10⁻¹ dilution.

Et₂PC did not inactivate the agent.

Many of the unusual properties attributed to the scrapie agent can be explained by the presence of hydrophobic domains within a protein (4, 15, 16). The binding of the agent to the hydrophobic gel, phenyl-Sepharose, and elution from it are shown in Table 3. The amount of agent bound to phenyl-Sepharose varied between 60% and 99%. Washing the phenyl-Sepharose with 1 M KCl did not release the bound agent. Neither decreasing the salt concentration nor increasing the concentration of ethylene glycol to 8.5 M resulted in elution of the agent. Addition of a detergent mixture containing 4% NP40 and 2% Sarkosyl to the 8.5 M ethylene glycol released the majority of agent, but as much as 30% remained bound to the phenyl-Sepharose. The scrapie agent did not bind to Sepharose 6B or to Sepharoses with C₈ and C₁₀ alkyl chains. Longer alkyl chains were not examined, but these may prove useful, as reported for some membrane-bound enzymes (17).

The hydrophobicity of the scrapie agent was also reflected by a decrease in titer when detergents were removed from fraction E₆ by dialysis. As much as 10⁴ ID₅₀ units/ml have been lost

Table 3. Scrapie agent binding to and elution from phenyl-Sepharose*

Procedure	Total log ID ₅₀ units†	
	Exp. I‡	Exp. II§
Fraction P ₄ digested	7.6 ± 0.34	7.7 ± 0.34
Unbound supernatant	5.3 ± 0.18	5.8 ± 0.53
1 M KCl wash	5.9 ± 0.33	4.8 ± 0.49
8.5 M ethylene glycol wash	4.8 ± 0.21	6.6 ± 0.52
8.5 M ethylene glycol/4% NP40/2% Sarkosyl wash	7.7 ± 0.73	7.8 ± 0.30
Phenyl-Sepharose beads	7.2 ± 0.30	7.2 ± 0.30

* Fraction P₄ (DG 206) was digested with micrococcal nuclease followed by proteinase K. All samples were stored at -20°C for 3-4 weeks prior to bioassay at 10⁻¹ dilution.

† Total log ID₅₀ units = titer (ID₅₀ units/ml) × vol (ml). Results are given ± SEM.

‡ Phenyl-Sepharose lot no. 5720.

§ Phenyl-Sepharose lot no. 16110.

upon dialysis against buffer without detergent or against H₂O alone. The scrapie agent titer in fraction P₄ was increased routinely 10- to 50-fold by addition of nonionic detergents such as Brij 35, 1-dodecylpropanediol-3-phosphocholine, or sulfobetaine 3-14 at concentrations of 5-10%. Sulfobetaine 3-14 is a zwitterionic detergent and 1-dodecylpropanediol-3-phosphocholine is a lysolecithin analogue. Increases in titer with more commonly used nonionic detergents such as Triton X-100, NP40, or octyl glucoside were not as great.

DISCUSSION

Five separate and distinct lines of evidence now clearly show that the scrapie agent contains a protein that is required for infectivity: (i) inactivation by proteinase K digestion, (ii) inactivation by chemical modification with Et₂PC, (iii) inactivation by the denaturing detergent NaDodSO₄, (iv) inactivation by chaotropic ions such as guanidinium and SCN⁻, and (v) inactivation by the denaturing organic solvent phenol. The cumulative evidence presented here and in several earlier communications builds a compelling case for a protein within the scrapie agent (4, 15, 16).

The reduction in scrapie titer by proteinase K was shown to be a function of enzyme concentration as well as time and temperature of digestion. Proteinase K was chosen for these studies because the enzyme is available in a highly purified crystalline form (13, 18). Preliminary studies indicate that PhMeSO₂F added to proteinase K prior to digestion of fraction E₆ abolished the reduction in scrapie agent titer (unpublished data). The degree of purification of the scrapie agent was found to be important in demonstrating both its susceptibility to protease degradation and chemical modification. Inactivation of the scrapie agent by Et₂PC is concentration dependent and most likely occurs through ethoxyformylation of amino acid residues within a protein required for infectivity. We do not know whether the protein or proteins cleaved by proteinase K are the same ones that react with Et₂PC. Recent studies in our laboratory have shown that addition of exogenous protein or histidine to fraction E₆ will prevent inactivation by Et₂PC and that treatment of the inactivated agent with NH₂OH leads to complete restoration of infectivity (unpublished data). These observations argue for acylation of amino acid residues during inactivation of the agent by Et₂PC. The ethoxyformyl adducts of histidine, serine, and tyrosine residues within proteins have been shown to be sensitive to nucleophilic displacement by NH₂OH (14, 19).

Several studies have examined the inactivation of conventional viruses by Et₂PC. T4 and Qβ bacteriophages of *Escherichia coli* are inactivated by Et₂PC (20, 21). The mechanism

of inactivation of these bacteriophages by Et₂PC appears to be ethoxyformylation of coat or capsid proteins, because no reduction in infectious nucleic acid was observed. Poliovirus exhibited similar inactivation by Et₂PC, but tobacco mosaic virus and 3NT bacteriophage of *Bacillus subtilis* were resistant to inactivation by Et₂PC (20, 22–24). When Et₂PC was added to preparations of viral genomes, the double-stranded DNAs of T4 and 3NT bacteriophage as well as the double-stranded RNA of the replicative form of poliovirus were unaltered as measured by transfection (20, 22). In contrast, significant reductions in transfection were observed when the single-stranded RNA genomes of poliovirus, tobacco mosaic virus, and Q β bacteriophage were treated with Et₂PC (21–24). Et₂PC reacts with histidine residues $\approx 10^4$ times more rapidly than with adenosine moieties of nucleic acids (14, 25). Treatment of adenosine with Et₂PC has been shown to result not only in opening of the imidazole ring but also in ethoxyformylation of the amino group. The studies with conventional viruses and their genomes are not directly comparable to our studies because the half-life of Et₂PC is greatly influenced by the buffer composition and pH (14). We are unaware of any published information concerning the NH₂OH reversibility of Et₂PC-inactivated conventional viruses or nucleic acids.

Earlier studies providing evidence for a protein within the scrapie agent showed that it was inactivated by NaDodSO₄, chaotropic ions, and phenol (4). Inactivation of the agent by NaDodSO₄ occurred as the detergent-to-protein ratio approached 1.8 g of NaDodSO₄ per g of protein (4). At this ratio most proteins become completely denatured and their hydrophobic binding sites for NaDodSO₄ are saturated (26). Inactivation by NaDodSO₄ could be prevented by addition of non-ionic detergent (4). Chaotropic ions have also been found to be potent inactivators of the scrapie agent (4, 15, 27). Exposure of the agent at 4°C to 1 M guanidinium SCN reduced its titer $>10^8$ ID₅₀ units/ml in less than 6 hr. Infectivity was not restored when guanidinium SCN was removed by dialysis (15). Enzymatic activities of proteins are characteristically altered at concentrations of chaotropic ions below 1 M (28, 29). Changing the secondary structure of nucleic acids requires chaotrope concentrations of 4–5 M (30). Several investigators have shown that the scrapie agent is readily inactivated by phenol (4, 31). Interpretation of these results has varied, but denaturation of a protein necessary for infectivity is a likely interpretation (4). It is of interest that attempts to reconstitute infectious agent from both the aqueous and organic phases of phenol-extracted preparations in liposome carriers have been unsuccessful to date (unpublished observations). In contrast to the denaturing organic solvent, phenol, ethanol precipitated the scrapie agent but did not inactivate it (4, 32).

We do not know how many peptide bonds must be hydrolyzed or how many amino acid residues must be chemically modified for inactivation of the agent to occur. Nor do we have any information about the conformational state of the protein within the infectious agent that is most susceptible to protease degradation as well as chemical modification. Numerous studies in other systems have shown that the rate of proteolysis depends upon the conformation of the protein substrate (33, 34). Although Sarkosyl did not influence the titer of the scrapie agent in fraction E₆ (4), it may have shifted the agent into a conformational state that is susceptible to degradation by proteinase K. NaDodSO₄, a structural analogue of Sarkosyl, has been shown to increase the rate of proteinase K-catalyzed proteolysis (35); however, NaDodSO₄ was not used in these studies because it inactivates the agent (4). It is also possible that the scrapie agent assumes another conformational state at elevated temperatures that renders it resistant to degradation by proteinase

K at 60°C. Alternatively, increased association of the agent with cellular elements at elevated temperatures could protect it from enzymatic proteolysis (2, 16).

If we assume that the scrapie agent still represents a small fraction of the total protein present in fraction E₆, then we must address two important considerations. First, the concentration of scrapie protein substrate is low, and a 2-log reduction in titer means that 99% of the substrate has been cleaved at least once. This reduction in substrate is accompanied by a decrease in the velocity of scrapie agent peptide bond hydrolysis (36). Second, competitive inhibition of the proteolytic reaction by cellular polypeptides must be significant because they represent most of the protein.

It is of interest to contrast inactivation of the scrapie agent by proteases with inactivation of the potato spindle tuber viroid by RNases. When RNase A at 0.1 μ g/ml was added to a crude nucleic acid extract, the potato spindle tuber viroid titer decreased by a factor of $1/10^6$ in 1 hr at 25°C (37). In these extracts it is estimated that viroid RNA was $<0.01\%$ of the total RNA (38). Hydrolysis of a single phosphodiester bond within a viroid probably inactivates it (39, 40). In contrast, there are many examples or proteins that retain their biological activities after limited proteolysis (41–44).

Whereas our experimental observations provide the most convincing evidence for a protein within the scrapie agent, three earlier communications have reported reductions of infectivity with protease digestions. In these studies, trypsin at 100 μ g/ml (31), papain at 590 μ g/ml (32), and Pronase at 2000 μ g/ml (45) reduced the scrapie agent titer $10^{2.0}$, $10^{1.5}$, and $10^{2.7}$ ID₅₀ units, respectively. The digestion with Pronase was performed on a sample containing $<0.1\%$ of the initial infectivity because extraction with NaDodSO₄ reduced the titer of the scrapie agent. In addition, Pronase preparations are known to contain impurities (46). The endpoint titration data for the Pronase sample was unusual because all of the mice inoculated with a 1:100 dilution of the sample died and all the mice inoculated with higher dilutions, with one exception, never developed scrapie (45). Is it possible that these unusual titration data result from the vast amount of Pronase protein inhibiting the infectious process rather than enzymatically degrading the agent? Our studies with proteinase K control for this possibility because proteinase K added to fraction E₆, followed immediately by PhMeSO₂F addition and freezing, did not inactivate the agent (Fig. 1B). It is of interest that the infectivity of tobacco mosaic virus was immediately diminished upon addition of trypsin, chymotrypsin, or ribonuclease to the inocula (47). Similar observations have been recorded for ribonuclease added to necrotic ringspot virus (48). Because infectivity was recovered upon removal of these enzymes, inhibition was not due to hydrolysis of viral proteins or nucleic acids.

Many of the unusual properties of the scrapie agent appear to be a consequence of its hydrophobicity (4, 16). With the demonstration that the agent has a protein, it seems most likely that this hydrophobicity arises from nonpolar side chains of amino acid residues within the protein. The ability of the agent to readily associate with cellular elements when heated provided an earlier glimpse of its hydrophobicity (16). The formation of these large aggregates was observed only when detergent-extracted preparations of the agent were exposed to elevated temperatures (6, 49). Our recent findings that chaotropic ions are potent inactivators of the scrapie agent provide further evidence for its hydrophobicity (4, 15). Chaotropic ions perturb the structure of H₂O, and this leads to disruption of hydrophobic interactions within macromolecules (29). The most compelling evidence for hydrophobicity of the agent is its high affinity for phenolic groups immobilized on Sepharose. The agent binds so tightly

to phenyl-Sepharose that elution requires a mixture of 8.5 M ethylene glycol, 4% NP40, and 2% Sarkosyl. In addition, the hydrophobicity of the agent is manifested by its loss of titer in fraction E₆ when detergents are removed. Presumably the agent aggregates with itself as well as with cellular elements in such a manner as to result in a decrease in titer.

Although we now have abundant evidence that the scrapie agent contains a protein and that the protein is probably hydrophobic, we do not have evidence for a nucleic acid within the agent. The scrapie agent in purified preparations is resistant to psoralen crosslinking, Zn²⁺-catalyzed hydrolysis, and ultraviolet irradiation (unpublished data). Now that the presence of a protein has been demonstrated, an intensive search must be made for the genome of the agent (50, 51). Does a genome coding for the scrapie agent protein reside within the infectious agent itself or is the genome part of the cellular genetic material? Will the unusual properties of the scrapie agent, which distinguish it from conventional viruses, reveal unprecedented mechanisms of replication?

S.B.P. expresses his appreciation to Drs. R. Schmid, J. R. Krevans, R. C. Morris, Jr., L. H. Smith, Jr., F. A. Sooy, and G. W. Thorn for their enthusiastic support and continuing encouragement. The authors thank Ms. F. Elvin for superb administrative assistance and Ms. L. Gallagher for expert manuscript preparation. This work was supported in part by research grants from the National Institutes of Health (NS14069) and the National Science Foundation (PCM77-24076). S.B.P. is an investigator of the Howard Hughes Medical Institute.

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