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Immunoaffinity purification and neutralization of scrapie prion infectivity

(prion protein isoforms/liposomes/detergent–lipid–protein complexes/immunoprecipitation/monoclonal antibodies)

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ABSTRACT **Prions are unusual infectious pathogens causing scrapie of sheep and goats as well as Creutzfeldt–Jakob disease of humans. Biochemical and genetic studies contend that the scrapie isoform of the prion protein (PrP^{Sc}) is a major component of the prion. Limited proteinase K digestion of PrP^{Sc} produced a protein of 27–30 kDa. After dispersion of brain microsomes isolated from scrapie-infected hamsters into detergent–lipid–protein complexes, copurification of PrP^{Sc} and scrapie infectivity was obtained with scrapie prion protein of 27–30 kDa monoclonal antibody-affinity columns. PrP^{Sc} was enriched ≈5700-fold with respect to total brain protein, whereas scrapie prion infectivity was enriched ≈4000-fold. The ratio of prion titer to PrP^{Sc} remained constant throughout purification. Heterologous monoclonal antibody columns failed to bind either PrP^{Sc} or scrapie infectivity. Polyclonal rabbit prion protein antiserum raised against $NaDodSO_4$/PAGE-purified scrapie prion protein of 27–30 kDa reduced scrapie infectivity dispersed into detergent–lipid–protein complexes by a factor of 100. These results represent direct immunologic and chromatographic demonstrations of a relationship between PrP^{Sc} and prion infectivity as well as providing additional support for the contention that PrP^{Sc} is a major component of the infectious scrapie particle. That PrP^{Sc} is a host-encoded protein is an important feature distinguishing prions from viruses.**

Scrapie is a degenerative neurological disease of sheep and goats that can be transmitted to laboratory rodents. Much evidence argues that the infectious agent is not a virus, but rather an unprecedented particle, a prion (1). The only macromolecule in preparations highly enriched for prion infectivity identified to date is a protein of 33–35 kDa, designated the scrapie isoform of the prion protein (PrP^{Sc}) (2). Removal of the N-terminal 67 amino acids of hamster PrP^{Sc} by limited proteinase K digestion yields a prion protein (PrP) of 27–30 kDa that is designated PrP 27–30 (3). Many biochemical (4–9), genetic (6, 10, 11), biophysical (12), neuropathologic (13–16), and cell biological (17) lines of evidence support the idea that PrP^{Sc} is a component of the infectious particle.

For several years, the only highly purified preparations of scrapie prions were in the form of rod-shaped amyloid polymers (4–6). Recently, we have developed a protocol to disperse these rod-shaped polymers into detergent–lipid–protein complexes (DLPC) and liposomes with full retention of infectivity (12). This protocol was subsequently adapted for DLPC formation directly from brain microsomes, and it avoids the aggregation of PrP^{Sc} (18).

We reasoned that PrP monoclonal antibody (mAb)-affinity chromatography would copurify solubilized PrP^{Sc} and prion infectivity if PrP^{Sc} were a component of the infectious scrapie particle. On the other hand, this procedure should separate PrP^{Sc} from infectivity if the two were unrelated, as suggested by some investigators (19–21). As reported here, fractions eluted from the immunoaffinity column at alkaline pH contained one protein, PrP^{Sc}, and high titers of scrapie infectivity. We also demonstrate that rabbit polyclonal PrP 27–30 antiserum (α-PrP 27–30) can neutralize scrapie infectivity.

MATERIALS AND METHODS

Materials. Sodium cholate, sodium dodecyl sarcosinate (Sarkosyl), Tris buffer, sodium deoxycholate, and Nonidet P-40 were obtained from Sigma. Protein A–Sepharose CL-4B was purchased from Pharmacia, dimethyl pimelimidate from Pierce, $NaDodSO_4$ from BDH, phosphatidylcholine (PtdCho) from Avanti Biochemicals (Birmingham, AL), and proteinase K from Beckman.

Scrapie Prion Propagation and Bioassay. Hamster scrapie prions (22) were passaged five times in golden hamsters [Lak:LVG (SYR)] purchased from Charles River Breeding Laboratories, Lakeview Colony (Wilmington, MA). Prions were propagated in golden hamsters inoculated intracerebrally with $\approx 10^7$ ID_{50} units of scrapie agent, and the animals were sacrificed 70 days later. Scrapie infectivity was bioassayed in hamsters by incubation-time measurements (23).

Analytical Procedures. Protein was measured using the bicinchoninic acid (BCA) (Pierce) dye method with crystalline bovine serum albumin as standard (24). Samples with low protein concentrations were precipitated by chloroform/methanol (1:2) before measurement with bicinchoninic acid. Radioiodination of PrP 27–30 was accomplished using ^{125}I-labeled Bolton–Hunter reagent purchased from Amersham (1 mCi, 2000 Ci/mmol; 1 Ci = 37 GBq) (25). $NaDodSO_4$/PAGE was done according to the method of Laemmli (26). Immunologic blots (27) and silver staining of gels (28) were done as described previously with 0.01% $NaDodSO_4$ in the electrotransfer buffer (17). Concentration of PrP was estimated visually by comparing the volume of a given fraction required for detection on an immunologic blot as well as the intensity of staining with that of standards.

Preparation of Immunoaffinity Resin. PrP mAb 13A5 was purified by a Protein A–Sepharose CL-4B column in the presence of 0.5 M NaCl at pH 8.5; ≈10 mg of antibody was obtained from 1 liter of hybridoma medium (29). The PrP mAbs (10 mg) were crosslinked to Protein A–Sepharose CL-4B (4 ml) using 20 mM dimethyl pimelimidate dihydrochloride (30), washed with phosphate-buffered saline, and

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Abbreviations: PrP, prion protein; PrP^{Sc}, scrapie isoform of prion protein; PrP 27–30, scrapie prion protein of 27–30 kDa derived from PrP^{Sc} by partial proteolysis; DLPC, detergent–lipid–protein complexes; mAb, monoclonal antibody; PtdCho, phosphatidylcholine; HIV, human immunodeficiency virus; α-PrP 27–30 and α-PrP mAb 13A5, antiserum against PrP 27–30 and against mAb 13A5, respectively; α-PrPs, α-PrP 27–30 and α-PrP mAb 13A5.

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Table 1. Immunoaffinity copurification of PrP^{Sc} and scrapie prion infectivity using a PrP mAb matrix

Fraction	Volume, ml	Protein*, mg/ml	Log titer*, ID_{50} per ml ± SE	Log total infectivity, ID_{50}	Protein recovery, %	Log specific infectivity, ID_{50} per mg of protein
Homogenate	150	15	8.1 ± 0.25	10.3	100	6.9
Microsome	15	5.0	8.8 ± 0.18	10.0	50	8.1
DLPC	15	5.0	8.8 ± 0.22	10.0	50	8.1
Flow-through	15	4.0	7.9 ± 0.23	9.1	6	7.3
Wash 3	15	0.13	4.8 ± 0.35	6.0	0.005	5.7
Wash 10	15	<0.00001	<0			
Eluate pH 9.5	10	0.00007	5.9 ± 0.28	6.9	0.04	10.1
Eluate pH 10.0	10	0.0005	6.9 ± 0.13	7.9	0.4	10.2
Eluate pH 11.2	10	0.0027	7.9 ± 0.23	8.9	4	10.5

*Mean of three determinations.
†Concentration of PrP estimated by volume of fraction required for detection on immunologic blot and by the intensity of staining.

stored at 4°C in a suspension containing 0.02% sodium azide. Alternatively, the IgG fraction from a polyclonal rabbit α-PrP 27–30 (RO17) (17) or a polyclonal rabbit human immunodeficiency virus (HIV)-gp120 synthetic peptide antiserum was crosslinked to Protein A–Sepharose CL-4B. This α-PrP 27–30 was raised against the prion rods that had been denatured in 0.5% $NaDodSO_4$ and boiled for 5 min. The synthetic peptide antiserum was raised against the amino acids 465–480 of the HIV-gp120 (CFRPGGGDMRDNWRSEL) and was provided by T. Krowka.

Purification of Brain Microsomes. Scrapie-infected hamster brains were homogenized with a Polytron for 10 sec in 320 mM sucrose to produce a 10% (wt/vol) homogenate. All procedures were performed at 4°C unless otherwise stated. After centrifugation at 5000 × *g* for 10 min, the pellet was discarded, and the supernatant was centrifuged for 1 hr at 100,000 × *g*. The resulting pellet was resuspended in a minimal volume of a buffer containing 10 mM Tris·HCl, pH 7.4/150 mM NaCl and then subjected to osmotic shock by a 100-fold dilution into 10 mM Tris·HCl, pH 7.4/2 mM phenylmethylsulfonyl fluoride. After 20 min the suspension was centrifuged for 1 hr at 100,000 × *g*, and the osmotic-shock step was repeated. The purified microsomal membranes were resuspended at a protein concentration of 5 mg/ml and either used immediately or stored at −70°C.

Preparation of DLPC. Ten milliliters of microsomes purified from scrapie-infected brains were added to a glass test tube containing 100 mg of dry PtdCho and mixed in a Vortex before the addition of Sarkosyl to a final concentration of 2% (wt/vol) (12, 18). The mixture was then sonicated for 10 min in a cylindrical bath sonicator and centrifuged at 100,000 × *g* for 1 hr. The supernatant fraction contained DLPC, which were used for further purification.

RESULTS

A microsomal membrane fraction was prepared from scrapie-infected brains containing 50% of the scrapie infectivity and 50% of the PrP (Table 1). The microsomes were solubilized by a combination of Sarkosyl (2% wt/vol) and PtdCho (5 mg/ml). The DLPC formed by this procedure were subjected to ultracentrifugation, and the supernatant was applied to PrP mAb-affinity matrix. mAbs raised against PrP 27–30 were crosslinked to Protein A–Sepharose to minimize the leakage of the antibodies. After overnight incubation at 4°C, the immunoaffinity matrix was washed with buffers containing various increased concentrations of salt and different detergent as well as 2% (wt/vol) PtdCho. The PtdCho was included to prevent aggregation of PrP^{Sc} while bound to the matrix and during elution.

Elution of PrP from the matrix was accomplished with higher and higher concentrations of alkali; the pH of the eluant was increased progressively from 9.5 to 11.2. At pH 9.5, the first detectable PrP was eluted. Acid was also examined, but it was not efficient in eluting the PrP. Because alkali is known to inactivate scrapie infectivity (31), samples were titrated immediately after elution to pH 7.

Selected fractions of the immunoaffinity purification procedure were analyzed by $NaDodSO_4$/PAGE as shown in Fig. 1. Most unbound PrP was eluted in the void volume; that not all of the PrP molecules are bound to the matrix may result from some PrP molecules possessing a configuration unfavorable for binding. No PrP was detected in subsequent washes before increasing the pH of the eluant buffer to 9.5 as judged by immunologic blotting (Fig. 1 *Upper*). Approximately 9% of the PrP was recovered in the alkaline eluate; this represents ≈20% of the PrP in the microsome fraction. An equal amount of PrP was found in the flow-through fraction. At least 20% of the unaccounted-for PrP remained bound to the column and was eluted by additional washes with the pH 11.2 buffer. The extent of PrP purification was ≈5700-fold, and the purity of the PrP, as judged by $NaDodSO_4$/PAGE with silver staining, was excellent (Fig. 1 *Lower*). Similar results were obtained when the IgG fraction of a polyclonal rabbit α-PrP 27–30 was coupled to Protein A–Sepharose and used in place of the PrP mAb columns.

Aliquots eluted from the immunoaffinity column at alkaline pH were examined by electron microscopy after negative staining with uranyl formate. Amorphous vesicles but no prion rods were seen (data not shown).

Some prion proteins in purified fractions are probably the cellular isoform of the prion protein because all of our PrP mAbs to date bind to both PrP isoforms. To estimate the amount of PrP^{Sc} in the purified fractions, we digested the fractions with proteinase K, which catalyzes the hydrolysis of the cellular isoform of PrP and the conversion of PrP^{Sc} to PrP 27–30. As depicted in Fig. 1 (lane 10), the majority of the purified PrP^{Sc} is converted to PrP 27–30 as evidenced by the intensity of immunostaining as well as silver staining on $NaDodSO_4$/PAGE. From these digestions, we conclude that most of the PrP in our purified fractions is PrP^{Sc}.

Aliquots of fractions from the PrP immunoaffinity column were inoculated into hamsters for bioassay of scrapie prion titer. Those fractions, which contain PrP^{Sc}, also contain scrapie infectivity, whereas those fractions with no detectable PrP^{Sc} contain either low levels of scrapie prions or none (Table 1). Moreover, the amount of PrP^{Sc} recovered from the column was a function of the eluate pH and was roughly proportional to the prion titer. Although the specific infectivity (ID_{50} units per mg of protein) increased by 4000-fold during purification, the ID_{50} units per μg of PrP remained constant. We recovered ≈4% of the total infectivity in the pH 11.2 eluate; this amount corresponds to ≈10% of infectivity found in the microsome fraction (Table 1). An additional ≈10% of the microsome infectivity was found in the flow-

Table 1. (*Continued*)

Protein purification, -fold	PrP†, μg/ml	Total PrP, μg	Prp recovery, %	Specific activity, μg of PrP/mg of protein	Prp purification, -fold	Log titer/ PrP ratio, ID_{50} per μg
1	2	300	100	0.13	1	7.8
16	10	150	50	2.0	15	7.8
16	10	150	50	2.0	15	7.8
2.5	2	30	10	0.5	4	7.6
0.06	<0.01					
	<0.01					
1600	0.05	0.5	0.2	740	5700	7.2
2000	0.5	5	1.7	1000	7700	7.2
4000	2.0	20	7	740	5700	7.6

through fraction. How much of the unaccounted infectivity remains bound to the column and how much is inactivated during alkali elution remain to be determined. The imprecision of the animal bioassay (23) for scrapie infectivity complicates attempts to determine accurately the degree of purification and the recovery for any particular step. Even though the prion titers given in Table 1 represent the means from three separate experiments, it may be more prudent to claim a 10^3- to 10^4-fold purification of scrapie infectivity than the ≈4000-fold claimed, as described above.

To establish the specificity of our immunoaffinity purification protocol, we constructed an immunoaffinity matrix using an unrelated antibody raised against a synthetic peptide of glycoprotein 120 of HIV. The results of the chromatography are depicted in Fig. 2. No PrP^{Sc} was eluted from the column regardless of the buffer pH, establishing that the binding of the PrP^{Sc} to the column was specific. Aliquots of the fractions from this experiment were bioassayed in hamsters. Prion infectivity was recovered only in the flow-through fractions; none was found in fractions eluted with alkali (Table 2). Comparing the recoveries of prion infectivity eluted by alkali for the two columns reveals an impressive difference of $\approx 10^8$ (Tables 1 and 2).

Concurrent with the development of an immunoaffinity purification protocol, we examined the immunoprecipitation of purified PrP 27–30 rods dispersed into DLPC. Polyclonal rabbit PrP 27–30 antiserum (RO18) precipitated ≈50% of the radioiodinated PrP 27–30 in DLPC in the presence of Protein A–Sepharose (Table 3). Preimmune serum precipitated 1–2% of the PrP 27–30. PrP mAb 13A5 could not precipitate a significant portion of the PrP 27–30 in the presence of Protein A–Sepharose; presumably, this was due to the relatively poor

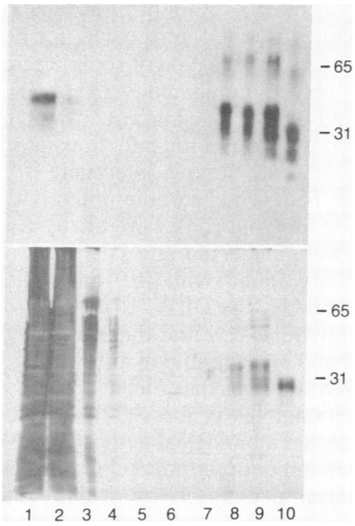

FIG. 1. Immunoaffinity purification of PrP^{Sc}. Microsomes isolated from 15 scrapie-infected hamster brains were solubilized by a combination of Sarkosyl and PtdCho. The DLPC fraction was incubated overnight at 4°C with PrP mAb Protein A–Sepharose beads on a rocking platform oscillating 30 times per min. The DLPC bound to resin was transferred to a column (2.5 × 15 cm) and washed with the following buffers: (*i*) 0.5 M NaCl/0.05 M Tris·HCl, pH 8.2/1% (wt/vol) Sarkosyl/PtdCho at 2 mg/ml (4 vol); (*ii*) the same buffer with 0.5% (vol/vol) Nonidet P-40 instead of Sarkosyl (3 vol); (*iii*) 0.15 M NaCl/0.5% (wt/vol) sodium deoxycholate/PtdCho at 1 mg/ml (3 vol). PrP was then eluted by a discontinuous gradient of alkaline pH: (*i*) 10 ml of 0.05 M triethylamine, pH 9.5/0.5% sodium deoxycholate/PtdCho at 1 mg/ml; (*ii*) the same solution titrated to pH 10.0; (*iii*) the same solution at pH 11.2. After elution, the samples were immediately titrated to neutrality with 2-[*N*-morpholino]ethanesulfonic acid (MES) buffer. Each wash was equivalent to one column volume of 15 ml. Aliquots of selected fractions were analyzed by $NaDodSO_4$/PAGE and bioassays for infectivity (Table 1). (*Upper*) Immunologic blot from $NaDodSO_4$/polyacrylamide gel stained with rabbit α-PrP 27–30 (RO18) that had been raised against $NaDodSO_4$/PAGE-purified PrP 27–30. Similar immunoblots were obtained when PrP mAb were used in place of the rabbit α-PrP. (*Lower*) Silver-stained duplicate 12% PAGE. Lanes: 1, DLPC; 2, flow-through; 3, second wash; 4, third wash; 5, seventh wash; 6, tenth wash; 7, pH 9.2 eluate; 8, pH 10 eluate; 9, pH 11.2 eluate; 10, 100-μl aliquot of the pH 11.2 eluate was incubated with proteinase K (20 mg/ml) for 30 min at 37°C before denaturation by boiling in 2% $NaDodSO_4$ buffer. M_r of protein standards are given in kDa.

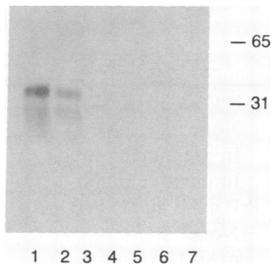

FIG. 2. Immunoaffinity chromatography of PrP^{Sc} with a heterologous antibody. An immunoaffinity resin was constructed by cross-linking the IgG fraction from a polyclonal rabbit HIV-gp120 synthetic peptide antiserum that was coupled to Protein A–Sepharose CL-4B. Microsomes isolated from 15 scrapie-infected hamster brains were solubilized by a combination of Sarkosyl and PtdCho. The resulting DLPC were incubated with constant mixing by using a rocking platform overnight at 4°C in the presence of the immunoaffinity resin. The resin was then loaded into a column and washed sequentially with the three buffers described in Fig. 1. Immunologic blot of a $NaDodSO_4$/PAGE of selected fractions stained with rabbit α-PrP 27–30 (RO18). Lanes: 1, DLPC; 2, flow-through fraction; 3, third wash; 4, fifth wash; 5, seventh wash; 6, pH 10 eluate; 7, pH 11.2 eluate. M_r of protein standards are given in kDa.

Table 2. Immunoaffinity chromatography of PrP^{Sc} and scrapie prion infectivity using a heterologous antibody matrix

Fraction	Volume, ml	Protein, mg/ml	Log titer, ID_{50} per ml ± SE	Log specific infectivity, ID_{50} per mg of protein	PrP, μg/ml	Log titer/PrP ratio, ID_{50} per μg
Homogenate	150	15	8.1 ± 0.25	6.9	2	7.8
Microsome	15	5.0	8.8 ± 0.38	8.1	10	7.8
DLPC	15	4.7	8.7 ± 0.23	8.0	10	7.8
Flow-through	15	4.1	8.6 ± 0.32	8.0	8	7.7
Wash 3	15	0.5	5.0 ± 0.13	5.3	<0.01	
Wash 10	15	0.05	<0		<0.01	
Eluate pH 9.5	10	0.002	<0		<0.01	
Eluate pH 10.0	10	0.002	<0		<0.01	
Eluate pH 11.2	10	<0.00001	<0		<0.01	

IgG fraction from a polyclonal rabbit HIV-glycoprotein 120 synthetic peptide antiserum was coupled to Protein A–Sepharose CL-4B.

binding of murine IgG molecules to Protein A. The efficacy of PrP mAb immunoprecipitation was substantially increased by using rabbit antiserum (RO21) raised against PrP mAb 13A5, as a second antibody (α-PrP mAb 13A5) to facilitate binding to Protein A–Sepharose (Table 3). With all PrP antibodies tested, high concentrations were necessary to obtain maximal immunoprecipitation of ^{125}I-labeled PrP 27–30 in DLPC.

Immunoprecipitated fractions were also assessed for scrapie infectivity (Table 3). The prion titers of samples treated with rabbit α-PrP 27–30 were reduced by a factor of 100, whereas exposure to preimmune serum did not alter the titer (Table 3). In contrast to the polyclonal rabbit α-PrP (RO18), PrP mAb 13A5 as well as polyclonal rabbit α-PrP mAb 13A5 (2021) failed to neutralize scrapie infectivity associated with the DLPC.

To extend our finding that α-PrP 27–30 can neutralize scrapie infectivity, we incubated PrP 27–30 DLPC with various concentrations of antiserum. Preimmune serum failed to alter prion titer, whereas immune serum caused a progressive decrease in titer (Fig. 3). A correlation between the ratio of α-PrP 27–30 to PrP 27–30 and diminishing infectivity was found (Fig. 3).

DISCUSSION

The immunoaffinity purification and neutralization studies reported here provide direct immunological and chromatographic demonstrations of a link between PrP^{Sc} and prion infectivity. For many years, investigators searched for a detergent that would solubilize scrapie prion infectivity (32, 33). Attempts at purification, as well as characterization of the scrapie agent, were plagued by the smearing of scrapie infectivity across centrifugation gradients, electrophoretic fractions, and chromatography profiles (34, 35). The experimental protocols described here could not have been done without functional solubilization of scrapie prions into DLPC (12, 18). Our results combined with other immunologic, biochemical, and genetic data make it difficult to contend that PrP^{Sc} is not a component of the infectious particle but rather a pathologic product of scrapie infection.

Attempts to neutralize scrapie infectivity in fractions containing prion amyloid rods were unsuccessful (36). Only after the rods were dispersed into DLPC was a reduction of scrapie infectivity found with PrP antibodies (Fig. 3 and Table 3). Presumably, the DLPC expose epitopes that allow neutralizing antibodies to bind; these same epitopes in rods may be buried and inaccessible to antibodies. Neutralization of viruses by antibodies has been widely studied and is thought to be a complex process (37). Antibodies have also been used to assign a biological activity to a specific protein using neutralization or immunoprecipitation procedures (38–40). The precise mechanism by which antibodies raised

Table 3. Immunoprecipitation of PrP 27–30 and neutralization of scrapie prion infectivity in DLPC

Antibody	[^{125}I]PrP 27–30 precipitated, %	Log prion titer, ID_{50} per ml ± SE		
		Unfractionated	Supernatant	Pellet
α-PrP 27–30 (RO18)				
None		8.5 ± 0.32		
In preimmune rabbit	1.4	8.6 ± 0.25	8.7 ± 0.13	5.6 ± 0.21
In immune rabbit	54	6.1 ± 0.22	6.6 ± 0.23	5.7 ± 0.32
PrP mAb and α-PrP mAb				
None		7.5 ± 0.39	7.6 ± 0.16	5.8 ± 0.31
mAb 13A5	1.5		7.8 ± 0.22	5.4 ± 0.27
mAb 13A5 + rabbit α-PrP mAb (RO21)	51		6.7 ± 0.21	6.8 ± 0.41
Rabbit α-PrP mAb (RO21)	4.5		7.9 ± 0.33	4.7 ± 0.20

^{125}I-labeled PrP 27–30 in purified prion rods was prepared using the Bolton–Hunter reagent (25), methanol-precipitated and solubilized into DLPC by the addition of 2% cholate and PtdCho at 5 mg/ml. Protein concentration was 5 μg/ml. DLPC were incubated with an IgG fraction of α-PrP or PrP mAb in a 1.5-ml Eppendorf tube and rocked overnight at 4°C. Protein A–Sepharose beads were added to the PrP antibody/DLPC mixture and incubated for 1 hr before centrifugation in a Microfuge for 10 min. The protein adsorbed by the beads was separated from the supernatant fluid, and the ^{125}I in each fraction was measured in a Beckman γ counter. For neutralization experiments, nonradioactive ^{127}I-labeled PrP 27–30 was solubilized into DLPC and incubated with the relevant antibody overnight. Samples were either directly inoculated into hamsters after dilution with inoculation buffer or incubated with Protein A–Sepharose before fractionation and inoculation. Prion titers in RO18 treatment represent an average of two experiments, whereas values in the mAb and α-PrP treatment are from a single experiment.

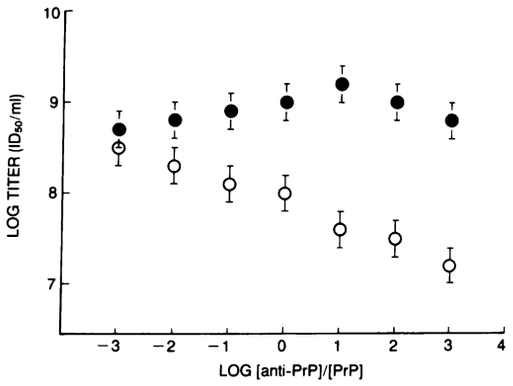

FIG. 3. Neutralization of scrapie prion infectivity with polyclonal α-PrP 27–30. DLPC were formed from purified prion rods containing PrP 27–30 at 5 μg/ml by dispersion in 2% cholate/PtdCho at 5 mg/ml. The IgG fraction (10 mg/ml) of rabbit preimmune serum (●) or antiserum (anti-PrP; RO18) (○) raised against $NaDodSO_4$/PAGE-purified PrP 27–30 was mixed with PrP 27–30 solubilized into DLPC and incubated in an Eppendorf tube on a rocking platform at 4°C overnight. α-PrP 27–30 and DLPC as well as diluent, when necessary, were added to each sample to obtain a final volume of 100 μl. Seven samples contained the following volumes (in μl) of α-PrP 27–30 and DLPC-solubilized PrP 27–30, respectively: 1, 0.05 and 50; 2, 0.5 and 50; 3, 5 and 50; 4, 50 and 50; 5, 50 and 5; 6, 50 and 0.5; 7, 50 and 0.05. The samples were then inoculated directly into hamsters for bioassay. Samples were not frozen at any time after the DLPC were formed. Scrapie prion titer is plotted as a function of the ratio of the volume of α-PrP 27–30 to the volume of DLPC containing PrP 27–30.

against $NaDodSO_4$/PAGE-purified PrP 27–30 reduce scrapie infectivity remains to be established.

In part, the need for high concentrations of PrP antiserum to demonstrate neutralization may be due to the high particle-to-infectivity ratio. We estimate that the ratio of PrP^{Sc} molecules per ID_{50} unit in our immunoaffinity-purified fractions is $\approx 10^5$. This particle-to-infectivity ratio resembles that previously reported for the prion amyloid rods, where it ranges from 10^4 to 10^6 (4, 5).

The experimental results of the complementary immunologic approaches reported here combined with results of biochemical and genetic studies mount a convincing argument for PrP^{Sc} being a major component of the infectious scrapie prion. This is an important feature, distinguishing prions from viruses, because PrP is encoded by a host gene and not by a nucleic acid within the prion particle. Our results argue neither for nor against a second molecule within the prion—such as a small nucleic acid; however, all attempts to demonstrate a scrapie-specific polynucleotide have been unsuccessful to date. The development of an immunoaffinity purification protocol and procedures for chromatography will undoubtedly facilitate structural studies of prions as well as experiments focusing on how prions multiply.

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