determine whether or not R-photopsin has another anion binding site that does not affect the absorption spectrum.

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Identification of Cellular Proteins Binding to the Scrapie Prion Protein†

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ABSTRACT: The scrapie prion protein (PrP^{Sc}) is an abnormal isoform of the cellular protein PrP^{C}. PrP^{Sc} is found only in animals with scrapie or other prion diseases. The invariable association of PrP^{Sc} with infectivity suggests that PrP^{Sc} is a component of the infectious particle. In this study, we report the identification of two proteins from hamster brain of 45 and 110 kDa (denoted *Pr*P *li*gands Pli 45 and Pli 110) which were able to bind to PrP 27-30, the protease-resistant core of PrP^{Sc} on ligand blots. Pli 45 and Pli 110 also bound PrP^{C}. Both Pli's had isoelectric points of ~5. The dissociation rate constant of the Pli 45/PrP 27-30 complex was 3×10^{-6} s^{-1}. Amino acid and protein sequence analyses were performed on purified Pli 45. Both the composition and the sequence were almost identical with those predicted for mouse glial fibrillary acidic protein (GFAP). Furthermore, antibodies to Pli 45 reacted with recombinant GFAP. The identification of proteins which interact with the PrP isoforms in normal and diseased brain may provide new insights into the function of PrP^{C} and into the molecular mechanisms underlying prion diseases.

Infectious particles causing scrapie and other prion diseases are composed largely of the scrapie prion protein (PrP^{Sc}),[1] which is an abnormal isoform of a cellular protein (PrP^{C}) (Bolton et al., 1982; Prusiner et al., 1983; Oesch et al., 1985). Extended proteolysis destroys PrP^{Sc} (or PrP 27-30) and diminishes infectivity with similar kinetics (McKinley et al., 1983). Genetic analysis in mice has linked different alleles of the PrP gene to a gene controlling the incubation time (Carlson et al., 1986; Westaway et al., 1987). In humans, a point mutation that leads to an amino acid substitution in PrP has been linked to familial Gerstmann-Sträussler syndrome (Hsiao et al., 1989).

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[1] Abbreviations: PrP^{Sc}, scrapie isoform of the prion protein; PrP 27-30, protease-resistant core of PrP^{Sc}; PrP^{C}, cellular isoform of the prion protein; GFAP, glial fibrillary acidic protein; DLPC, detergent-lipid-protein complexes; Pli, PrP ligand.

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While considerable progress has been made in recent years deciphering the structure of the infectious particle and the genetic factors controlling scrapie incubation time, little is known about the molecular mechanisms of entry, replication, and intercellular transmission of prions. It is thought that prions spread along axonal pathways as judged by the pattern of lesions (Kimberlin et al., 1983; Fraser & Dickinson, 1985). To explore the etiology of scrapie as well as the molecular mechanism leading to the scrapie form of PrP, we have attempted to identify proteins that bind PrP.

There are a number of structural features within PrP that might allow it to interact with other proteins. Near the middle of the molecule, there is an amphipathic helix; in other proteins, such helices have been implied in protein–protein interaction (Kaiser & Kezdy, 1984; Bazan et al., 1987). This may result in formation of homologous (dimers) or heterologous complexes. By cross-linking, no homologous complexes were found with PrP 27–30 solubilized in liposomes (R. Gabizon, personal communication). Another target for binding might be the glycolipid anchor; a similar structure has been implicated in binding and internalization of heparan sulfate proteoglycan (Ishihara et al., 1987). The glycan may also deliver specific signals as described for the insulin mediator (Saltiel & Cuatrecasas, 1986).

To probe the interaction of purified PrP with other proteins, we bound radioiodinated PrP 27–30 to hamster brain proteins separated by SDS–PAGE and transferred to nitrocellulose (ligand blot). Previously, radioiodinated ligands like low-density lipoprotein, interferon α/β or γ, lactotransferrin, interleukin I, and lutropin as well as α-bungarotoxin have been shown to bind to their receptors on ligand blots (Wilson et al., 1984; Critchley et al., 1985; Mazurier et al., 1985; Soutar et al., 1986; Bird et al., 1988; Keinanen, 1988; Schwabe et al., 1988). This method has clear advantages: the molecular weight of a ligand can be determined directly, the specificity of the interaction may be assessed by the number of proteins to which the probe binds, and the binding characteristics may be determined easily. In addition, it provides an assay for purification. Applying this technique, we were able to identify two PrP ligands of 45 and 110 kDa (Pli 45 and Pli 110). These experiments were made possible by the reconstitution of PrP 27–30 into detergent–lipid–protein complexes (DLPC; Gabizon et al., 1987) which provided a soluble probe for ligand blotting.

Materials and Methods

Materials. Transfer membranes were purchased from Bio-Rad, Richmond, CA (nitrocellulose, 0.45 μm). $Na^{125}I$ (carrier-free; Amersham, Arlington Heights, IL) and iodobeads (Pierce, Rockford, IL) were used for radioiodination. Ampholines (pH 3.5–10) were obtained from Pharmacia, Milwaukee, WI. Chemicals for electrophoresis were purchased from Bio-Rad. Egg L-α-lecithin was obtained from Avanti-Polar, Pelham, AL. HPLC columns (Brownlee) were used with a Rainin HPLC system. Monoclonal antibodies were purchased from Amersham (anti-GFAP, anti-68 kDa, and anti-160 kDa neurofilaments) or ICN (anti-actin). Anti-rabbit or mouse Ig antibodies coupled to alkaline phosphatase were obtained from Promega, Madison, WI. Isopropyl β-D-thiogalactopyranoside (IPTG) was purchased from Sigma, St. Louis, MO. The glial fibrillary acidic protein (GFAP) cDNA clone was a generous gift of Dr. N. Cowan. Molecular weight standards for SDS–PAGE were obtained from Bio-Rad; the molecular weights ($\times 10^{-3}$) were as follows: trypsin inhibitor, 21; carbonic anhydrase, 31; ovalbumin, 43; bovine serum albumin, 66; phosphorylase *b*, 97; β-galactosidase, 116; and myosin, 200. The positions for neurofilament proteins used as molecular weight markers (68K and 160K) were determined by Western blotting.

Ligand Blots. Whole brain homogenates [20 μL of 10% homogenate/2× SDS sample buffer (1:1) per lane] were separated on SDS–PAGE (10% acrylamide) and transferred to nitrocellulose as described (Towbin et al., 1981). Nonspecific binding to membranes was blocked by incubation in TBST (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 0.05% Tween 20)/5% blotto (nonfat dry milk). For two-dimensional gel electrophoresis, total brain proteins were separated by isoelectric focusing (IEF, ampholines pH 3.5–10) followed by electrophoresis on 10% SDS–polyacrylamide gels (O'Farrell et al., 1977; O'Farrell & O'Farrell, 1978). Individual IEF tube gels were placed above a two-gel sandwich so that proteins were separated into two parallel SDS–polyacrylamide gels; each gel would therefore contain approximately half of total proteins. The paired gels were then either silver stained (Merril et al., 1981) or transferred to nitrocellulose. As probes purified PrP 27–30 (Prusiner et al., 1983; Stahl et al., 1987) or PrP^{C} (immunoaffinity-purified on monoclonal anti-PrP antibody column as described for PrP^{Sc}; Gabizon et al., 1988) were radioiodinated to a specific activity of $(2–5) \times 10^7$ cpm/μg. Unincorporated iodine was separated by precipitation of PrP with 9 volumes of absolute ethanol. For solubilization, labeled PrP was reconstituted into DLPC (1× DLPC: 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, and 1 mg/mL L-α-lecithin) at 0.1–0.5 μg of PrP/mL by bath sonication for 20 min. Binding of probes to ligand blots was in TBST/0.5% blotto at $(1–5) \times 10^4$ cpm/mL for 12–16 h at room temperature. Blots were washed in TBST/0.01× DLPC for 2–4 h, dried, and exposed to Kodak XAR film with an intensifying screen at −70 °C.

Determination of Dissociation Rate Constant. Scrapie brain homogenate was separated by SDS–PAGE and ligand-blotted as described above. However, individual strips were washed for different lengths of time. After autoradiography, the areas corresponding to Pli 45 and to the 20-kDa region were cut out, and bound radioactivity was determined. After subtraction of background (20-kDa region), the value at 0.5 h was arbitrarily set at 1 in order to standardize individual experiments. The dissociation rate constant (k_{off}) was determined from the half-life of the Pli 45/PrP 27–30 complex according to the equation $N = N_0 e^{-k_{off} t}$. For $N = N_0/2$, $t = t_{1/2}$; $k_{off} - \ln (0.5) t_{1/2}^{-1}$.

Purification and Analysis of Pli 45. Scrapie hamster brain was homogenized in 0.32 M sucrose/1 mM PMSF and centrifuged at 5000*g* for 20 min. The pellet was resuspended in 20 mM Tris-HCl, pH 7.5, 150 mM NaCl (TBS), and 1% Triton X-100 followed by centrifugation at 5000*g* for 20 min; the pellet was reextracted with TBS/1% Triton X-100. The pellet was then solubilized in TBS/2% sarcosyl; insoluble material was removed by centrifugation at 100000*g* for 20 min. Solubilized proteins were recovered by addition of 4 volumes of absolute ethanol and centrifugation at 5000*g* for 20 min. Precipitated proteins were dissolved in SDS sample buffer and loaded onto preparative gels. After SDS–PAGE, proteins were visualized by staining in Coomassie blue, the region containing Pli 45 was cut out, and proteins were electroeluted as described (Jacobs & Clad, 1986). For HPLC, SDS–PAGE-purified Pli 45 was dissolved in formic acid (99%); chromatography was on a reverse-phase C_{18} column employing a gradient of 0–70% acetonitrile/0.04% trifluoroacetic acid (TFA). Amino acid analyses were performed by manual gas-phase hydrolysis and automated phenylthiocarbamyl (PTC)-amino acid analysis using an Applied Biosystems Model 420A/130A derivatizer.

Briefly, 6 pmol (nominally) of Pli 45 in 150 μL of 0.1% TFA/39% acetonitrile was dried in vacuo in a 6 × 50 mm glass tube. The tube was then sealed, in vacuo, inside an 18 × 150 mm Pyrex tube containing 300 μL of 6 M HCl. Hydrolysis was performed for 90 min at 165 °C. The tube was then dried in vacuo, and the free amino acids were dissolved in 15 μL of 0.025% Na_2H_2EDTA and applied to the glass reaction slide of the Model 420A derivatizer. Phenylthiocarbamylation and reverse-phase HPLC analysis were performed essentially according to the manufacturer's protocols. The composition divergence, D (Harris & Teller, 1973), was calculated by using the formula

$$D = 10^{-2}\sum_{i=1}^{16}[(\text{mol \% GFAP}_i - \text{mol \% Pli 45}_i)^2]^{0.5} \quad (1)$$

The $S\Delta Q$ index (Marchalonis & Weltman, 1971) was calculated according to the formula $S\Delta Q = 10^4D^2$. Aspartic and glutamic acid values correspond to the sums of aspartate and asparagine and glutamate and glutamine, respectively. Cysteine and tryptophan were not determined.

For tryptic peptides, SDS–PAGE-purified Pli 45 (~50 μg) was digested with 2 μg of sequencing-grade trypsin (Boehringer Mannheim, Indianapolis, IN) in 200 μL for 24 h at 37 °C. Digestion was verified by the disappearance of protein on silver-stained SDS gels (12% acrylamide). Digested Pli 45 was injected onto a reverse-phase HPLC column (C_{18}) and eluted with a gradient of 0–80% acetonitrile/0.04% TFA. Individual peaks were collected and sequenced on an applied Biosystems Model 477A/120 instrument, essentially as suggested by the manufacturer. Sequence interpretation was done manually.

Production of Antibodies to Pli 45/Affinity Purification. A rabbit was immunized with 10 μg of SDS–PAGE-purified Pli 45 first in complete and then twice in incomplete Freund's adjuvant. Blood was collected, and sera were tested on Western blots. For affinity purification of anti-Pli 45 antibodies, sarcosyl-soluble proteins (see above) were run on 10% SDS–PAGE and transferred to nitrocellulose. Nonspecific binding was blocked by incubation in 0.1% bovine serum albumin (BSA) in TBS. Binding of antiserum was at 1:100 dilution in TBST for 6 h followed by extensive washing in TBST. During washing, small strips from either side of the blots were incubated in anti-rabbit antibody conjugated to alkaline phosphatase. After visualization of bound antibodies on the side strips, the area corresponding to Pli 45 was cut out, and antibodies were eluted twice in glycine hydrochloride (pH 2.8)/0.1% BSA for 10 min. The pH of eluates was immediately adjusted to 7.5 by the addition of Tris-HCl, pH 8.2. The titer of eluted antibodies was determined on Western blots.

Expression of Recombinant GFAP in Escherichia coli. The GFAP cDNA cloned in pUC9 (Lewis et al., 1984) was transfected into *E. coli* strain DH1 (Hanahan, 1983). For expression of GFAP, two parallel cultures were grown in L broth at 34 °C for 15 h in the presence or absence of IPTG (5 mM). Cells were harvested by centrifugation (2000*g*, 10 min) followed by resuspension in 0.01 culture volume of TBS. For SDS–PAGE, resuspended cells were diluted 1:4 with SDS sample buffer and heated for 5 min in a boiling water bath. Insoluble residues were removed by centrifugation at 100000*g* for 15 min. Typically, 10 μL of cleared lysate was loaded per lane.

RESULTS

Detection of PrP Ligands. PrP is expressed in a variety of tissues; the highest levels of mRNA or protein are found in brain (Oesch et al., 1985; Doi et al., 1988). We therefore looked for ligands of PrP in hamster brain by the ligand blot technique. Briefly, proteins from whole brain homogenates were separated by SDS–PAGE, transferred to nitrocellulose, and incubated with a radioiodinated probe. Bound radioactivity was detected by autoradiography after washing. As a probe, we used purified PrP 27–30, the protease-resistant core of PrP^{Sc}, which was ^{125}I-labeled and solubilized in DLPC (Gabizon et al., 1987). Two major Pli's with apparent molecular weights (M_r) of 45000 and 110000 were observed in scrapie-infected as well as normal brain (Figure 1A, lanes 1 and 2). Other Pli's, with molecular weights ranging from 32000 to 200000 were also observed.

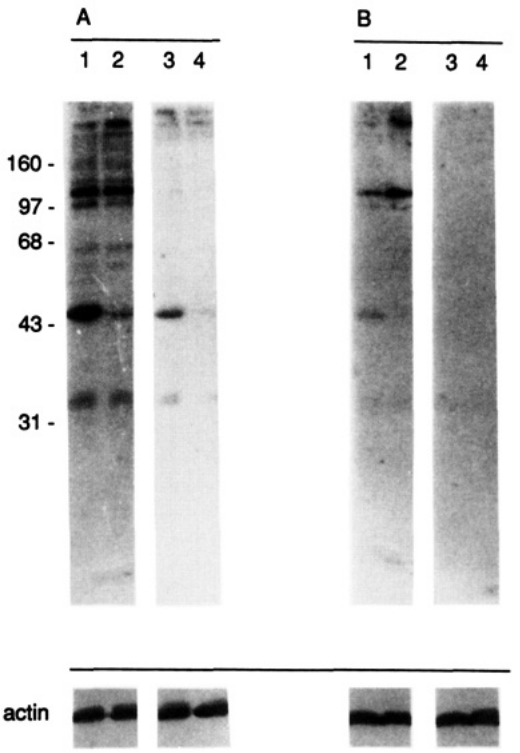

FIGURE 1: Binding of scrapie and cellular PrP to proteins on ligand blots. PrP 27–30 (A) or PrP^C (B) were radiolabeled and used to probe ligand blots of scrapie (lanes 1 and 3) or normal brain homogenates (lanes 2 and 4). Binding of both probes was competed with unlabeled PrP 27–30 (0.5 μg/mL; lanes 3 and 4). Bottom: the same nitrocellulose filters were stained for actin to show loading of lanes. Positions of marker proteins are shown in kilodaltons.

Interestingly, the 45-kDa protein (Pli 45) was more abundant in scrapie-infected than in normal brain. The binding of radiolabeled PrP 27–30 was competed by the addition of unlabeled PrP 27–30 (0.5 μg/mL) in DLPC (Figure 1A, lanes 3 and 4) while the presence of a large excess of milk proteins (0.2 mg/mL) did not affect the binding. No binding was observed with aggregated ^{125}I-labeled PrP 27–30 in rods (results not shown). To determine whether PrP^C would bind to the same proteins as PrP 27–30, immunoaffinity-purified PrP^C was radiolabeled under the same conditions as PrP 27–30, reconstituted into DLPC, and used as a probe in ligand blotting (Figure 1B). PrP^C bound to Pli 45 and Pli 110 (Figure 1B, lanes 1 and 2) under the same conditions as PrP 27–30. Binding of PrP^C was efficiently competed by unlabeled PrP 27–30 (Figure 1B, lanes 3 and 4). The same filters were also probed with anti-actin antibodies to show that each lane contained similar amounts of brain extract (Figure 1, bottom).

Brain homogenates from normal hamsters were then subjected to two-dimensional gel electrophoresis. Proteins were either visualized by silver staining or transferred to nitro-

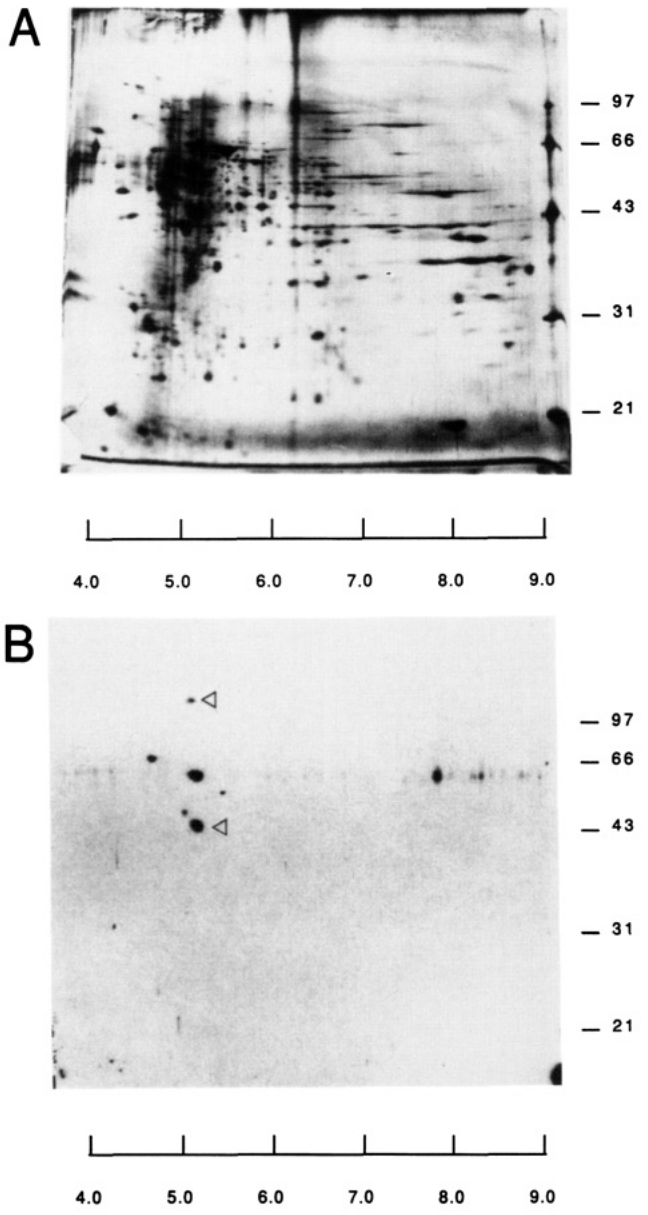

FIGURE 2: Analysis of normal hamster brain proteins by two-dimensional gel electrophoresis. Proteins in parallel gels were either stained with silver (A) or transferred to nitrocellulose and ligand-blotted (B). The p*I* gradient of a parallel IEF tube gel is indicated on the bottom. Positions of marker proteins are indicated (in kilodaltons).

cellulose and ligand-blotted. Whereas numerous proteins were visible by silver staining (Figure 2A), only a few proteins bound PrP 27–30 (Figure 2B). In addition to the 45- and 110-kDa proteins (Figure 2B, open arrowheads), other PrP binding proteins of 50, 56, 64, and 72 kDa were observed. The intensity of Pli 110 was markedly reduced in comparison to Pli 45 while Pli 64 was very prominent. It is possible that sample preparation for isoelectric focusing favors limited proteolysis of higher molecular weight Pli's. Pli 64 appeared to be less abundant in samples that were immediately boiled in SDS after homogenization (Figure 1A, lane 2). Brain homogenates kept at room temperature for 30 min selectively lost binding to Pli 110 but not Pli 45. The signals visible at ~60 kDa/p*I* 7–9 were not observed reproducibly. All the PrP ligands had a p*I* around 5.

Stability of the Pli 45/PrP 27–30 Complex. To investigate the stability of Pli 45/PrP 27–30 complexes formed on nitrocellulose, strips containing equivalent amounts of scrapie-infected brain homogenate were incubated with radioiodinated PrP 27–30 and then washed for increasing periods of time. After autoradiography, the regions corresponding to Pli 45 were cut out, and bound radioactivity was quantitated (Figure 3). Equivalent areas were cut out from the 20-kDa region as controls for background binding. Half of the bound radioactivity washed off after 60 h, corresponding to a dissociation rate constant of 3×10^{-6} s^{-1} which is in the range of rate constants observed for the dissociation of antibody–antigen complexes (10^{-8}–10^{-4} s^{-1}; Wong, 1985; Djavadi et al., 1986; Noe et al., 1988). Previously, the same procedure has been used to determine the binding constant of interleukin I and interferon, yielding constants comparable to the binding of ligands to native receptors on cells (Bird et al., 1988; Schwabe et al., 1988).

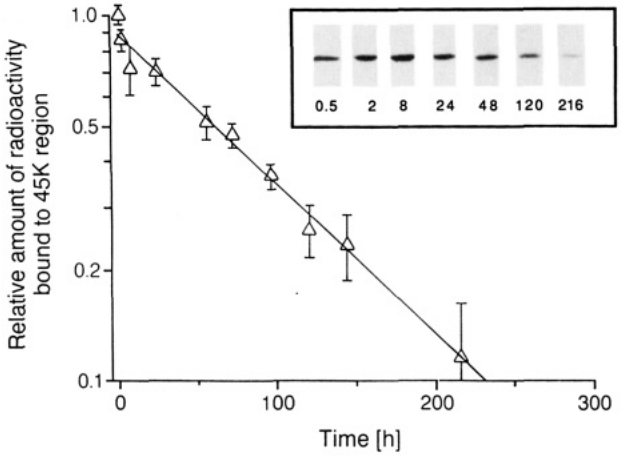

FIGURE 3: Dissociation of Pli 45/PrP 27–30 complexes formed on nitrocellulose. PrP 27–30/Pli 45 complexes formed on nitrocellulose were washed for increasing lengths of time. The amount of radiolabeled PrP 27–30 bound to Pli 45 was determined as described under Materials and Methods. The value at 0.5 h was set at 1 in order to standardize three independent experiments. The insert shows the autoradiographic signal observed at various time points (hours) for one particular experiment. Exposure was for 48 h.

Table I: Amino Acid Composition of Pli 45 and Murine GFAP[a]

amino acid	GFAP (mol %)	Pli 45 (mol %)	amino acid	GFAP (mol %)	Pli 45 (mol %)
A	10.2	10.3	L	12.4	12.7
C	0.2	ND	M	2.6	1.7
D/N	9.0	9.5	P	2.3	2.2
E/Q	20.5	21.3	R	10.2	10.4
F	2.1	1.9	S	5.5	5.6
G	2.6	3.6	T	5.0	6.0
H	1.6	1.7	V	4.7	4.4
I	2.6	3.2	W	0.4	ND
K	4.7	3.7	Y	2.3	1.8

[a] Mole percents of each amino acid are presented. Amino acids are listed by using their standard single-letter abbreviations. ND, not determined. The composition of GFAP was deduced from the sequences of Lewis et al. (1984) and Balcarek et al. (1985). The composition of Pli 45 was determined as described under Materials and Methods.

Identification of Pli 45 as Glial Fibrillary Acidic Protein (GFAP). Purification of Pli 45 was accomplished as described under Materials and Methods. The insolubility of Pli 45 in nonionic detergents allowed rapid enrichment for Pli 45. After preparative SDS–PAGE, purified Pli 45 showed a single band at 45 kDa by silver staining (Figure 4A, lane 1) which in a ligand blot contained the PrP 27–30 binding activity (Figure 4A, lane 2). SDS–PAGE-purified Pli 45 eluted from a reverse-phase HPLC column as a single major peak (Figure 4B) which also contained the PrP binding activity (results not shown). The smaller peaks were also observed in blanks.

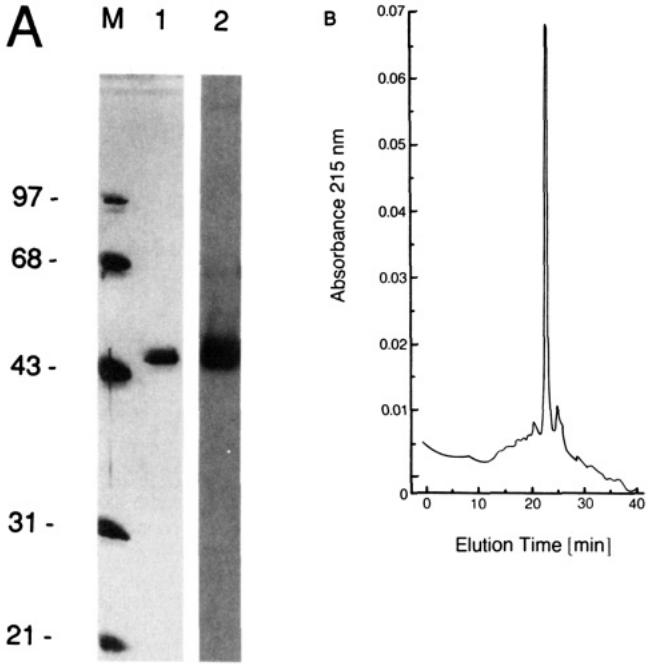

FIGURE 4: (A) Analysis of SDS–PAGE-purified Pli 45 (0.1 μg) by silver staining (lane 1) and ligand blotting (lane 2). (B) Reverse-phase HPLC of SDS–PAGE-purified Pli 45. The absorbance at 215 nm is plotted against the elution time. A gradient from 0 to 70% acetonitrile was applied over 40 min, starting at 0 min.

HPLC-purified Pli 45 had an amino acid composition which was extremely similar to that predicted for murine GFAP (Table I). The composition divergence, D (Harris & Teller, 1973), and the $S\Delta Q$ index (Marchalonis & Weltman, 1971) for these proteins were 2.4×10^{-2} and 5.6, respectively. These values suggested that the two proteins were almost certainly related ($p < 0.01$; Cornish-Bowden, 1983).

Protein sequence analysis was performed on Pli 45 and on tryptic peptides derived therefrom. No sequence information was obtained from the native molecule; however, sequences from a total of 130 amino acids, contained within 14 tryptic peptides, were determined. Computerized homology searches using the alogorithm of Lipman and Pearson (1985) and the Protein Identification Resource database (National Biomedical Research Foundation, Washington, D.C.) revealed an excellent match (94.6%) of the Pli 45 sequences with those in murine GFAP. The Pli 45 peptide sequences, aligned with the GFAP sequence, are presented in Figure 5. All the peptides except peptide 50.6 showed excellent matches with the GFAP amino acid sequence. The mismatches in 50.6 may be explained by species differences or technical limitations.

Immunochemistry of Pli 45 and GFAP. Polyclonal antibodies against SDS–PAGE-purified Pli 45 were produced in a rabbit and tested on immunoblots. The immune serum showed strong reactivity at 45 kDa (Figure 6A, lanes 2) relative to the preimmune serum (Figure 6A, lanes 1). In order to improve specificity, anti-Pli 45 antibodies were affinity purified on antigen immobilized on nitrocellulose. Eluted antibodies recognized only a 45-kDa protein (Figure 6A, lanes 3). The amount of Pli 45 appears to be increased in scrapie-infected brain relative to normal brain. The loading of individual lanes was similar with respect to actin (Figure 6A, lanes 5). The staining patterns of polyclonal anti-Pli 45 and monoclonal anti-GFAP antibodies were similar with scrapie brain; however, anti-GFAP stained normal brain less than did anti-Pli 45 antibodies (Figure 6A, lanes 3 and 4). It is possible that the monoclonal anti-GFAP antibody recognizes only a subset of GFAP molecules or due to a lower affinity is less

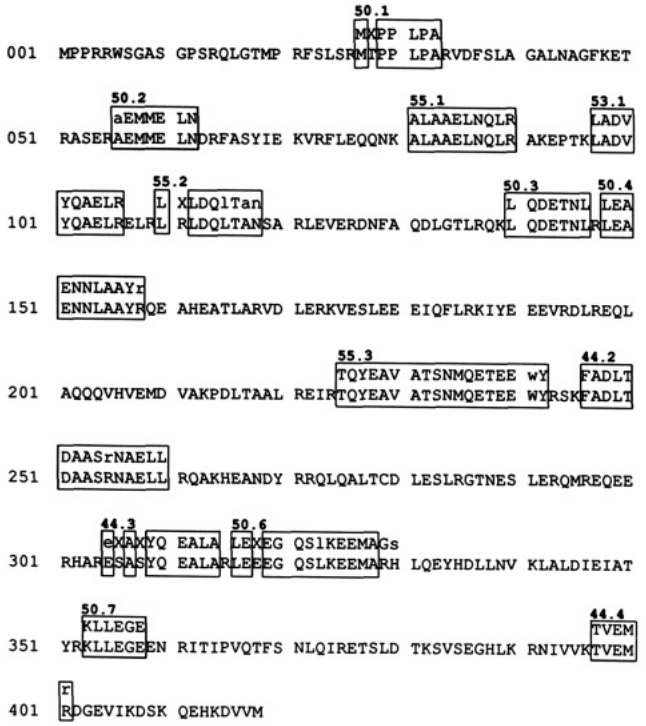

FIGURE 5: Sequence alignments of Pli 45 tryptic peptides and murine GFAP. The sequence of GFAP according to Lewis et al. (1984) and Balcarek et al. (1985) is presented. Peptides are denoted by their fraction number followed by an arbitrary number to distinguish individual sequences within a single fraction. Identical residues are boxed. X denotes residues where no assignment could be made confidently. Lower case letters signify residue assignments which were tenuous due to low signal levels, the presence of mixed sequence, or technical problems.

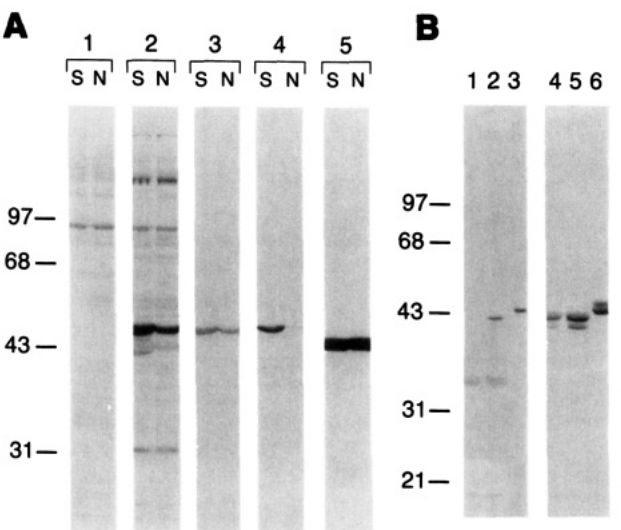

FIGURE 6: Reactivity of anti-Pli 45 and anti-GFAP antibodies. (A) Western blots of whole brain homogenates (S, scrapie-infected; N, normal) were probed with preimmune (1) or immune rabbit antiserum R111 (2), affinity-purified anti-Pli 45 antibodies (3), monoclonal anti-GFAP antibodies (4), or monoclonal anti-actin antibodies (5). (B) Western blots of recombinant GFAP produced in *E. coli* (lanes 1, 2, 4, and 5) or of scrapie-infected brain homogenate (lanes 3 and 6). Blots were probed with anti-Pli 45 antibodies (lanes 1–3) or anti-GFAP antibodies (lanes 4–6). Production of recombinant GFAP was induced in *E. coli* cultures by IPTG. Cultures were harvested by centrifugation, and extracts were run on SDS–PAGE followed by Western blots. The level of GFAP was compared for uninduced (lanes 1 and 4) and induced cultures (lanes 2 and 5).

efficient in recognizing GFAP. To assess the relatedness of Pli 45 and GFAP, we tested the reaction of anti-Pli 45 and anti-GFAP antibodies with recombinant GFAP expressed in

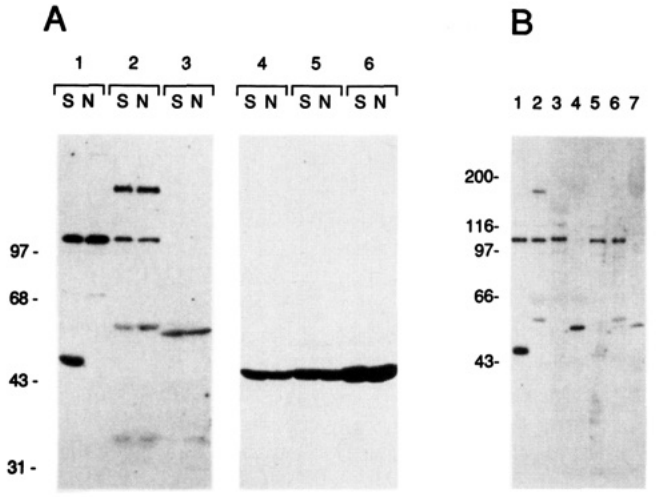

FIGURE 7: Ligand blots of extracts from various hamster organs. (A) Equivalent amounts of brain (lanes 1 and 4), lung (2 and 5), or heart (3 and 6) extracts from scrapie-infected (S) or normal (N) hamsters were ligand-blotted (lanes 1–3). The same nitrocellulose filter was probed with anti-actin antibodies to reveal loading of individual lanes (lanes 4–6). (B) Ligand blot of scrapie-infected hamster organs: brain (1), lung (2), liver (3), heart (4), pancreas (5), spleen (6), and skeletal muscle (7).

E. coli under the control of the β-galactosidase promoter (Figure 6B). After induction of the β-galactosidase promoter with IPTG, a higher amount of recombinant GFAP should be produced. Both antibodies stain a 43-kDa protein which is more abundant after induction (Figure 6B, lanes 1, 2, 4, and 5). Recombinant GFAP migrates with a lower molecular weight in comparison to GFAP from scrapie-infected hamster brain (Figure 6B, lanes 4 and 6). This difference in migration may be due to the lack of the 15 N-terminal amino acids in recombinant GFAP and/or limited proteolysis of the translation product since we did not use a protease-deficient strain of *E. coli*. In relation to scrapie hamster brain (Figure 6B, lanes 3 and 6), both antibodies recognized recombinant GFAP approximately equally well. No binding of radioiodinated PrP 27–30 to recombinant GFAP was observed (results not shown). This may be due to the modified N-terminus of GFAP (fusion to β-galactosidase, lack of N-terminus of GFAP; Lewis et al., 1984; Balcarek & Cowan, 1985). Recombinant GFAP may also lack posttranslational modifications necessary for binding of PrP; it is also possible that limited proteolysis at the N- or C-terminus destroys the binding site. Expression of full-length GFAP in eukaryotic cells will give the answer.

Tissue Distribution of Pli's. Various tissues from scrapie or normal hamsters were homogenized and ligand-blotted with radioiodinated PrP 27–30 (Figure 7A, lanes 1–3). Pli 45 was found exclusively in brain, and, consistent with the data above, it was more abundant in scrapie than in normal brain. This was confirmed by Western blotting with affinity-purified anti-Pli 45 antibodies (results not shown). Equal amounts of Pli 110 were found in brain and lung but not in heart of scrapie-infected or normal animals. As a control, the same blot was stained with anti-actin antibodies (Figure 7A, lanes 4–6). The same amount of actin was present in individual organs from scrapie and normal animals; as expected, the highest amounts of actin were found in muscle (Figure 7A, lanes 6). Pli 110 was also found in liver, spleen, and pancreas but not in muscle of normal animals (Figure 7B). Pli's of different molecular weights were observed in lung (56K and 170K), heart (52K), spleen (56K), and skeletal muscle (52K). Other Pli's of 116 and 200 kDa are only faintly visible (Figure 7B).

Discussion

PrP^{Sc} is the only macromolecule linked genetically as well as biochemically to prion diseases, to date (Bolton et al., 1982; McKinley et al., 1983; Carlson et al., 1986; Hunter et al., 1987; Westaway et al., 1987; Gabizon et al., 1988). The lack of knowledge concerning the cellular function of the PrP isoforms has impeded new approaches to investigate the molecular mechanisms causing this unique class of neurologic diseases. In this paper, we identify two proteins, Pli 45 and Pli 110, capable of binding to PrP. Pli 45 appears to be identical with GFAP by peptide sequencing as well as immunological methods.

Binding Specificity of PrP Ligands. It has previously been shown that a variety of ligands like LDL, interferon, or interleukin I bind to their receptors with the same specificity on ligand blots as in vivo (Cardin et al., 1984; Soutar et al., 1986; Rendon et al., 1987; Bird et al., 1988; Schwabe et al., 1988). Two proteins (Pli 45 and Pli 110) were identified on ligand blots using PrP 27–30 as a probe; this probably does not represent nonspecific protein–protein interactions since the incubation was done in the presence of a large excess of other proteins (0.2 mg/mL milk proteins). In addition, binding could be competed effectively by the addition of unlabeled PrP 27–30 (0.5 μg/mL; Figure 1). The reconstitution of PrP into DLPC was required, suggesting that specific areas of the protein have to be exposed which are not available when PrP 27–30 is aggregated into rod-shaped structures. The dissociation rate constant of the Pli 45/PrP 27–30 complex is in the range observed for antibody/antigen interactions (Figure 3; Noe et al., 1988; Wong, 1985). All these observations suggest that the binding of PrP to its ligands is a specific interaction. However, it is possible that specific structures like the glycolipid anchor or the amphipathic helix interact with a few proteins on the blot but that this interaction does not occur in vivo and may therefore not be relevant to the biology of PrP. Independent techniques such as cross-linking or affinity chromatography will be used in the future to confirm our results.

Pli 45 and GFAP Are Identical. The relatedness of two proteins is most readily revealed by their amino acid sequence. Peptides of purified hamster Pli 45 were found to be virtually identical with mouse GFAP (Figure 5). Even though the homologies are distributed along the whole GFAP sequence, Pli 45 may be encoded by a different, but GFAP-related gene. This seems unlikely since no GFAP-related genes were detected by Southern blot (Lewis et al., 1984; Balcarek & Cowan, 1985). In addition, Pli 45 and GFAP share a large number of properties. Both proteins are exclusively expressed in brain (Figure 7; Eng & Smith, 1985). The purification of Pli 45 indicates that it is associated with the cytoskeleton, as is GFAP. Both have an isoelectric point of approximately 5 (Figure 2; Vorgias & Traub, 1983). The amino acid composition of purified Pli 45 is very similar to that of murine GFAP (Table I). Statistical comparisons of the compositions showed that they were highly related. Recombinant GFAP is recognized by affinity-purified anti-Pli 45 antibodies (Figure 5B). Commercially available bovine GFAP binds PrP 27–30 (B. Oesch, unpublished results). If Pli 45 and GFAP were distinct proteins, they would share an unusual number of physical characteristics. If this were the case, a minor fraction of the purified protein may be Pli 45 which would bind PrP but would not be detected by amino acid analysis or sequencing.

Role of Pli's in Scrapie. GFAP expression is increased in scrapie-infected brain (Mackenzie, 1983; Weitgrefe et al., 1985; Kretzschmar et al., 1986). It has been hypothesized that high local concentrations of the infectious agent may lead to increased GFAP mRNA expression (Manuelidis et al., 1987). GFAP-positive processes of astrocytes are noticeably increased in the regions close to scrapie amyloid plaques, which are depositions of PrP^{Sc} (DeArmond et al., 1987). It has also been found that infectivity is associated with cytoskeletal proteins, namely, the fraction containing GFAP (VanAlstyne et al., 1987); however, no direct interaction between the infectious particle and the GFAP was demonstrated. Our results show that PrP^{Sc}, which appears to be a component of the infectious particle, bound GFAP. If this interaction were to occur in scrapie-infected brain, we would have predicted that infectious particles and GFAP should copurify.

GFAP is produced in reactive astrocytes while it is not known where PrP^{Sc} or scrapie prions are produced. In tissue culture, neuronal as well as fibroblast cell lines are able to produce infectious particles (Clarke & Millson, 1976; Butler et al., 1988). In order to interact with GFAP, PrP^{Sc} either has to be produced by astrocytes or has to be released from the producing cell and taken up by astrocytes. In either case, astrocytes might be the target cell for scrapie leading to astrocyte dysfunction which in turn may lead to nerve cell degeneration. This would be supported by the finding that gliosis is independent of spongiform changes (nerve cell vacuolation) in prion-infected animals (Manuelidis et al., 1987). Alternatively, astrocytic gliosis and concomitant changes in GFAP expression may be simply a response to lesions of nerve cells. In diseases unrelated to scrapie, gliosis occurs in response to brain injury (Pietrini et al., 1983; Gray et al., 1984; Roessmann & Gambetti, 1986; Rosenberg et al., 1989).

The role of Pli 110 and other Pli's is unclear since we lack information on their molecular properties. The almost ubiquitous tissue distribution of Pli 110 suggests a function which may be needed outside of the nervous system. It is clear that PrP mRNA is expressed in a wide variety of tissues, though at a lower level than in brain (Oesch et al., 1985). Molecular characterization of Pli 110 will show whether it is a member of the intermediate filament family or whether it is located at the cell surface, making it a potential receptor for PrP. Pli's may also be involved in the conversion of PrP^{C}, or a PrP precursor, into PrP^{Sc}. Different alleles of such PrP-converting enzymes might alter the incubation time; genetic analysis has shown that more than one gene controls scrapie incubation time (Dickinson et al., 1968; Kingsbury et al., 1983; Bruce & Dickinson, 1985; Carlson et al., 1986, 1988).

In conclusion, we have identified cellular proteins (denoted Pli's) capable of interacting with PrP. Pli 45, which appears to be GFAP, and Pli 110 specifically bound to the scrapie prion protein and its cellular isoform. The specificity of this interaction has been confirmed by the finding that PrP, but not other proteins, competes for the binding sites. These data provide a foundation for further biochemical investigation into the roles of Pli's in the genesis of prion diseases and/or in normal brain function. We are currently purifying and sequencing Pli 110. The production of antibodies to Pli 110 and cloning of its gene as well as further genetic analysis, for example, of linkage of incubation time and Pli-encoding genes will extend our knowledge of scrapie and potentially other neurodegenerative disorders.

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