JOURNAL OF VIROLOGY, Sept. 1986, p. 676–683
0022-538X/86/090676-08$02.00/0
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Vol. 59, No. 3

Immunological Comparison of Scrapie-Associated Fibrils Isolated from Animals Infected with Four Different Scrapie Strains

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Received 5 February 1986/Accepted 14 May 1986

Scrapie-associated fibrils (SAFs) are abnormal filamentous structures that are uniquely associated with unconventional slow virus diseases. The antigenic relationships of SAFs from animals infected with four biologically distinct scrapie strains were investigated by using antisera raised to purified SAF proteins. Rabbit antisera were raised to SAFs isolated from mice infected with the ME7 scrapie strain and to SAFs isolated from hamsters infected with the 263K scrapie strain. A strong antigenic relationship was shown among SAF proteins (PrPs) isolated from all scrapie-infected animals (ME7, 139A, and 87V in mice and 263K in hamsters), and this relationship was demonstrable regardless of which antiserum was used. SAF proteins were antigenically distinct from those of paired helical filaments or amyloid isolated from patients with Alzheimer disease. Distinct Western blot profiles were demonstrated for SAFs isolated from animals infected with each scrapie strain. Differences seen among SAFs were independent, at least in part, of host species or genotype, implying that certain specific structural and molecular properties of SAFs are mediated by the strain of scrapie agent.

Abnormal filamentous structures termed scrapie-associated fibrils (SAFs) were first observed in detergent-treated membrane fractions from scrapie-infected mouse brain by electron microscopy with negative stain (33). Extensive studies have established that these structures are observed exclusively in naturally occurring and experimentally induced unconventional slow virus diseases including scrapie, kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Straussler syndrome (GSS), and chronic wasting disease (31, 32, 34). SAFs copurified with scrapie infectivity in various purification protocols (16, 24, 30, 46) and may represent a form of the infectious agent. Structures with similar morphological and biophysical properties to those of SAFs have been termed prion rods by some investigators (41, 43).

A protease-resistant protein with a molecular mass of 27 to 30 kilodaltons (kDa) (PrP 27-30) has been shown to be the major component which purifies with infectivity from scrapie strain 263K-infected hamster brain (6). This protein is also the major component of SAFs and prion rods isolated from 263K-infected hamster brain (17, 24, 41, 43). Three protease-resistant proteins of molecular masses 26 to 28, 23 to 24, and 21 to 22 kDa are associated with SAFs isolated from scrapie-infected mice (7, 24) and have been designated M-PrPs (3). Using synthetic oligonucleotide probes generated from a partial sequence of PrP 27-30 (42), Oesch et al. (38) and Chesebro et al. (12) succeeded in cloning major portions of genes encoding hamster PrP 27-30 and M-PrPs. Northern and Southern blot analyses revealed that the coding information for these proteins were present in both normal and scrapie-infected animals. Non-protease-resistant proteins of molecular masses 54 (3) and 33 to 35 kDa (27, 36, 44) have been suggested as the primary gene products related to PrP 27-30 and M-PrPs.

Several scrapie strains have been identified which can be distinguished by the specific parameters of incubation period, histopathological change, and species susceptibility (9, 14, 18, 25). Differences in the biochemical properties of SAFs associated with a number of scrapie strains have been described and may reflect the different biological and pathological characteristics of these strains (24). SAFs isolated from different scrapie strains differ morphologically and with respect to their susceptibility to degradation by proteinase K (24). SAFs are composed of one to three PrPs, depending upon the scrapie strain (5, 7, 24). PrPs have been shown to be glycoproteins by periodic acid-Schiff staining (8), digestion with deglycosylating agents (8, 37), and binding of specific lectins (27). Rabbit sera had previously been raised to PrP derived from hamsters infected with strain 263K (2, 5). These sera react specifically with PrP, as shown by Western blot (WB) analysis (2, 5), and with SAFs (prion rods), as shown by immunoelectron microscopy (1, 36). Immunocytochemical staining with antisera to PrP 27-30 indicates that amyloid plaques present in the brains of certain scrapie agent-host strain models are composed of PrPs or related proteins (13). Antisera have now been raised to the SAF proteins from mice infected with scrapie strain ME7. This study describes the use of two antisera, anti-263K PrP 27-30 and anti-ME7 M-PrPs, to examine the antigenic interrelationships of SAF proteins from different scrapie models and to further establish the role of individual strains of scrapie in determining the properties of SAFs.

MATERIALS AND METHODS

Animals and scrapie agents. Mouse scrapie strains ME7 and 87V were kindly provided by Alan G. Dickinson, ARC and MRC Neuropathogenesis Unit, Edinburgh, Scotland. Hamster scrapie strain 263K and mouse scrapie strain 139A were kindly provided by Richard H. Kimberlin, ARC and MRC Neuropathogenesis Unit. Strains ME7 and 139A, used to infect C57BL/6J mice, were obtained from Jackson Laboratory, Bar Harbor, Maine, strains ME7 and 87V, used to infect IM/Dk mice, and strain 263K, used to infect LVG/LAK hamsters, were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Maine. Preparation of the inoculum, injection, scoring, and sacrificing of animals were performed as previously described (10). All

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TABLE 1. Biological and biochemical properties of the scrapie strains used in this study

Scrapie agent	Host	Incubation period (days) (mean ± SD)[a]	Histopathological changes[a]	SAF polypeptides (silver stain)[b]	Sensitivity of SAFs to proteolysis[b]
ME7	Mice s7s7 p7p7	153 ± 1 300 ± 3	Grey-matter vacuolation, with slight white matter changes, low plaque formation in p7p7 mice	3	Moderately sensitive
139A	Mice s7s7 p7p7	125 ± 1 163 ± 1	Grey- and white-matter vacuolation, no plaque formation	3	Highly sensitive
87V	Mice s7s7 p7p7	467 ± 21 311 ± 5	Grey-matter vacuolation, high plaque formation in p7p7 and s7s7 mice	1	Not done
263K	Hamsters	60 ± 5	Low vacuolation, low plaque formation	1	Highly resistant

[a] Taken from Carp et al. (11) and Kimberlin and Walker (25). Histopathological changes apply to mouse agent in either p7p7 or s7s7 mice, except when otherwise specified (18).
[b] Taken from Kascsak et al. (24).

animals used in this study were at the clinical stage of disease.

Purificatin of SAFs. SAFs were isolated from brains of infected animals by a modification (R. Rubenstein, R. Kascsak, P. Merz, and H. M. Wisniewski, manuscript in preparation) of the procedure of Hilmert and Diringer (23). A 10% homogenate of infected brain (usually 12 g) was prepared in 10% sarcosyl in a Tekmar model TR-10 Tissumizer (two 30-s homogenizations). After incubation for 30 min at room temperature, homogenates were clarified by centrifugation at 22,000 × *g* for 30 min at 20°C. The pellets were reextracted and clarified as described above. Combined supernatants were centrifuged at 200,000 × *g* for 2.5 h at 20°C. The pellet was suspended by sonication (Branson model 185 sonifier with microtip at 25 W) in TBS (10 mM Tris hydrochloride, 133 mM NaCl [pH 7.4]) containing 10% NaCl and 1% sarcosyl (TBSNS) and incubated overnight at room temperature. The suspension was pelleted at 200,000 × *g* for 3.5 h at 20°C. The pellet was suspended as described before in TBSNS and shaken at 37°C for 2 h. The suspension was pelleted in a Microfuge (Beckman Instruments, Inc., Fullerton, Calif.) for 15 min at room temperature. The pellet was resuspended in TBSNS containing 50 μg of proteinase K (Merck Co., Inc., Rahway, N.J.) per ml and shaken at 37°C for 2 h. The suspension was pelleted in the Microfuge for 15 min and resuspended in H_2O containing 0.1% sarcosyl and 1 mM phenylmethylsulfonyl fluoride. The suspension was diluted to 8 ml, layered over a cushion of 20% sucrose, and centrifuged at 210,000 × *g* for 5 h at 20°C. The final pellet was suspended in 100 μl of TBS containing 0.1% sulfobetaine 3-14 (Calbiochem-Behring, La Jolla, Calif.). This final pellet contained 100 to 500 μg of SAF protein and 1 to 10% of the infectivity found in the original homogenate (R. Rubenstein, manuscript in preparation).

Antisera to PrPs. Antisera to ME7 PrPs was prepared by immunizing a New Zealand White rabbit with gel-eluted protein. ME7 SAFs were purified as described above, and the solubilized proteins were separated on 12% Laemmli gels. Areas containing SAF protein (21 to 28 kDa) were cut from the gel, and the proteins were eluted by H_2O extraction. Primary immunization consisted of 40 μg of eluted protein in complete Freund adjuvant (1:1) (Difco Laboratories, Detroit, Mich.) injected into the footpads and intramuscularly near hindleg lymph nodes. Each subsequent immunization was given in the same manner but in the presence of incomplete Freund adjuvant. The rabbit received four additional immunizations at approximately 2-week intervals. The final immunization consisted of purified but non-gel-eluted SAFs. Antiserum to 263K PrP was obtained as previously described (5).

Non-SAF antigens. Microtubules and neurofilaments were prepared from bovine brain as previously described (22). Paired helical filaments (PHFs) were prepared by the purification protocol of Rubenstein et al. (44a). Core amyloid from Alzheimer- and GSS-infected brains were isolated by using pepsin and collagenase treatment as previously described (35).

Slot-Blot (SB) analysis. Antigens (50 ng) were applied to nitrocellulose (NC) by using a minifold II slot-blotter (Schleicher & Schuell, Inc., Keene, N.H.) (2). The NC paper was blocked with 2% normal goat serum–2% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 30 min at room temperature. Primary antiserum (anti-ME7 PrPs at 1:5,000 or anti-263K PrP at 1:10,000) was added in PBST (PBS with 0.2% Tween 20) containing 1% normal goat serum for 1.5 h at 37°C. After being washed in PBST, secondary antiserum (goat anti-rabbit immunoglobulin G conjugated with alkaline phosphatase [Tago, Burlingame, Calif.]) (1:1,000) was added in PBST containing 1% normal goat serum for 1.5 h at 37°C. The reaction was visualized by addition of substrate (Nitro Blue Tetrazolium [0.33 mg/ml; Sigma Chemical Co., St. Louis, Mo.] and 4-bromo-4-chloro-3-indolyl phosphate [0.17 mg/ml; Sigma] in 0.1 M Tris–0.1 M NaCl–0.05 M $MgCl_2$ [pH 9.5]) (26) and was stopped by washing in H_2O.

WB analysis. Purified SAFs (approximately 200 ng) were solubilized in 2% sodium dodecyl sulfate–0.5% β-mercaptoethanol and electrophoresed on 12% Laemmli gels as previously described (24) by using a mini-slab-gel apparatus (Hoeffer, San Francisco, Calif.). Proteins were electrophoretically transferred to NC by using a trans-blot apparatus (Bio-Rad Laboratories, Richmond, Calif.) (47). Blots were processed for reactivity with antisera to ME7 PrPs or 263K PrP, as described for the SB procedures.

Affinity purification of antibodies. Rabbit antisera (anti-ME7 MPrPs and anti-263K PrP) were purified by elution from SAF proteins bound to NC strips. ME7 M-PrPs antiserum (1:5,000) was added to a blocked WB of 200 ng of purified ME7 SAF protein. 263K PrP antiserum (1:10,000) was added to a blocked WB of 200 ng of purified 263K SAF protein. As negative controls, these antisera were added to blocked WBs of 200 ng of neurofilament protein. Antibodies were allowed to react with these strips for 2 h at 37°C. Blots were washed four times for 10 min each in 25 ml of PBST.

Antibodies were eluted from the strips by two washes in 5 ml of 100 mM glycine–20 mM sodium acetate–50 mM KCl (pH 2.2) (39) for 20 min at room temperature. Eluates were brought to pH 7.4 by the addition of 1 N NaOH, made 2 mg/ml with respect to BSA, and dialyzed overnight against PBST. These affinity purified antibodies were analyzed for reactivity by WB analyses, as previously described.

Lectin binding. SAF proteins were solubilized, electrophoresed, and transferred to NC as described above. The blot was blocked in PBS containing 3% BSA, which was pretreated with perchloric acid (21) for 30 min at room temperature. The lectin wheat germ agglutinin, conjugated with biotin (Vector, Burlingame, Calif.) (1:1,000), was added in PBS containing 1% pretreated BSA and 0.1% Tween 20 for 2 h at 37°C. Secondary reagent consisted of avidin conjugated with alkaline phosphatase (1:1,000) (Tago) and was added at 37°C for 1.5 h. The binding of lectin was visualized by addition of substrate as described in the SB procedure.

Electron microscopy. A 1:10 to 1:50 dilution of purified SAF preparations was applied for 1 min to freshly glow-discharged 400-mesh carbon-coated grids. Excess fluid was drained with filter paper, and the sample was washed with distilled H_2O, stained for 45 s with 2% uranyl acetate, and washed again with distilled H_2O. The grid was air dried and examined in a Philips EM 300 or EM 420 electron microscope at 80 kV and a magnification of ×10,000 to ×50,000.

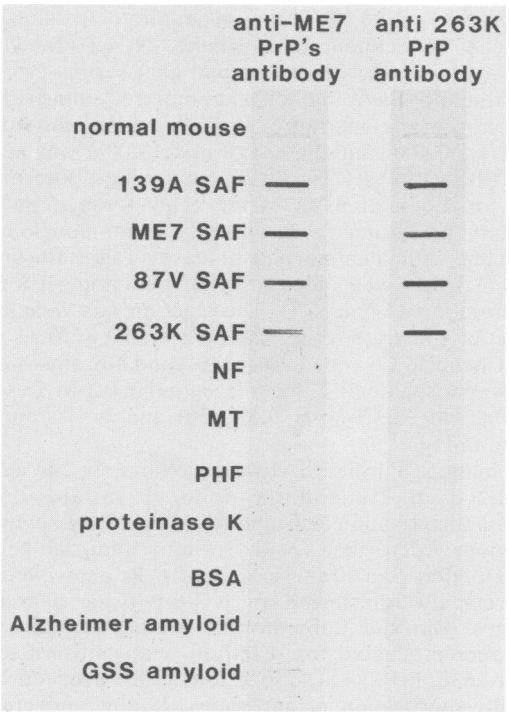

FIG. 1. SB analysis of SAF and other proteins. Proteins (50 ng) were applied to NC with a minifold II slot blotter. Following blocking of the NC, primary antiserum (anti-ME7 PrPs at 1:5,000 or anti-263K PrP at 1:10,000) was added for 2 h at 37°C. Goat anti-rabbit antiserum conjugated with alkaline phosphatase was added for 1.5 h at 37°C. The reaction was visualized by addition of Nitro Blue Tetrazolium-based substrate. Antigens were purified as described in Materials and Methods. Symbols: SAF, scrapie-associated fibrils; NF, neurofilaments; MT, microtubules; PHF, paired helical filaments; BSA, bovine serum albumin.

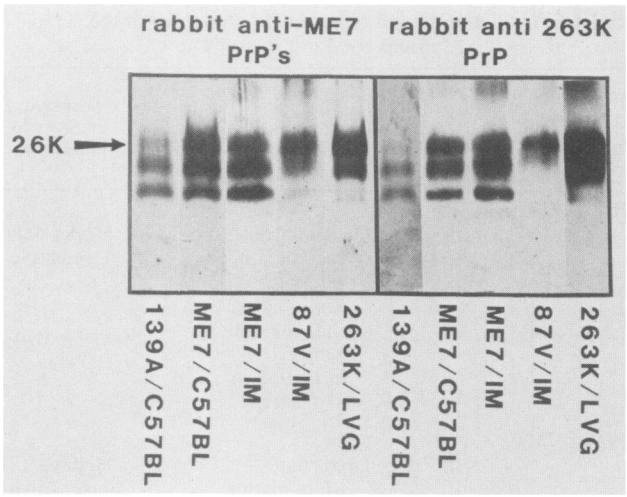

FIG. 2. WB analysis of SAF proteins isolated from animals infected with four different scrapie strains. SAFs from mouse or hamster brains infected with strain ME7, 139A, 87V, or 263K were isolated as described in Materials and Methods. SAF proteins (approximately 200 ng) were solubilized and electrophoresed on 12% Laemmli gels as previously described (24). Proteins were electrophoretically transferred to nitrocellulose with a Bio-Rad trans-blot apparatus. Blots were reacted with antisera raised to ME7 and 263K SAF proteins as described in the legend to Fig. 1. Lane designations: scrapie strain/host strain.

RESULTS

SAFs were purified from brains of mice or hamsters infected with four different scrapie strains: ME7, 139A, 87V, and 263K. These strains differ in a variety of biological and pathological properties (Table 1). Mouse scrapie strains differ in their incubation periods in mice of defined Sinc genotypes (11, 15). Mouse strains with a short incubation period for scrapie strain ME7 have a genotype termed s7s7, whereas the genotype of those with a long incubation period is termed p7p7. In this study C57BL/6J mice represent the s7s7 genotype and IM/Dk mice represent the p7p7 genotype. The degree of pathological change, including vacuolation and plaque formation, is often characteristic of a given scrapie strain in mice of a particular Sinc genotype (18). The SAFs isolated from animals infected with different scrapie strains have also been shown to differ in their polypeptide profile, as revealed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and in their susceptibility to degradation by proteolytic enzymes (24).

Antibodies to SAF proteins were prepared in rabbits. PrP 27-30 from 263K-infected hamsters and M-PrPs (26 to 28, 23 to 24, and 21 to 22 kDa) from ME7-infected mice were used. The reactivity of these antibodies to SAFs isolated from animals infected with various scrapie strains was first examined by the SB procedure (2). Endpoint titers of each of the antisera, obtained with 50 ng of purified homologous antigen in the SB procedure, were 1:20,000 for anti-ME7 M-PrPs and 1:40,000 for anti-263K PrP. These antisera were used at 1:5,000 and 1:10,000 dilutions, respectively, for SB and WB analyses of SAF proteins. Both anti-ME7 M-PrPs and anti-263K PrP antisera reacted strongly with SAFs from all scrapie models examined by the SB procedure (Fig. 1), regardless of the biological or pathological properties of the individual agents. Reactivity was, however, stronger in the homologous system, i.e., antisera raised to ME7 SAF pro-

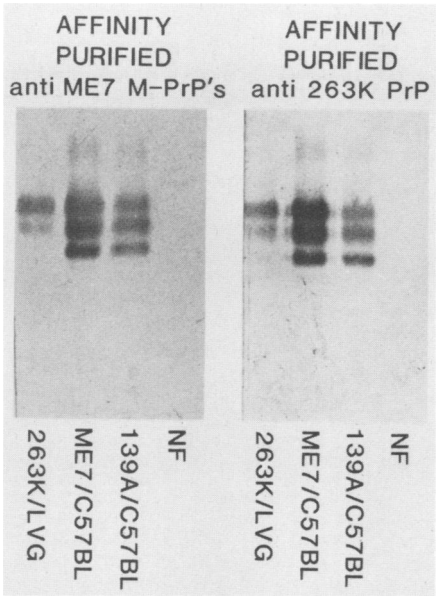

FIG. 3. WB analysis of affinity-purified antisera to PrP and M-PrPs. Antisera to Me7 M-PrPs and 263K PrP were affinity purified by using NC-bound antigen, as described in Materials and Methods. These purified antisera were examined by WB analysis for reactivity to 139A, ME7, and 263K SAF (PrP) proteins and, as a negative control, neurofilament (NF) proteins. Antigen preparations, electrophoresis, and WB staining were as described in the legend to Fig. 2.

tein reacted more strongly with ME7 than with 263K SAFs and antisera raised to 263K SAF protein reacted more intensely with 263K than with ME7 SAFs. A slight reaction was seen with normal brain material processed in the same manner as infected brain. However, this reaction appeared to be nonspecific in that it was similar to the reaction seen with preimmune sera (data not shown). Neither antiserum reacted with neurofilaments, microtubules, BSA, or proteinase K (50 ng each) by this procedure.

Certain ultrastructural similarities exist between SAFs and the individual fibrils which compose the abnormal filamentous structures found in Alzheimer disease, PHF and amyloid. Antisera raised to SAF proteins were used to investigate the antigenic relationship between SAFs and the structures associated with Alzheimer disease. Antisera to ME7 or 263K SAF proteins did not react with purified Alzheimer PHF or amyloid (Fig. 1). Solubilized proteins of PHF and amyloid also did not react with these antisera by the WB procedure (results not shown). These results are in agreement with immunocytochemical and immunoelectron microscopic studies, suggesting that these structures are not antigenically related (31a). These antisera also did not react with purified amyloid cores isolated from a patient with GSS.

The WB procedure was used to study the antigenic relationships among the individual SAF proteins derived from animals infected with the different strains of scrapie. Subtle but unique differences were seen in the WB profile for SAF proteins isolated from animals infected with different strains of scrapie (Fig. 2). These results were independent of the antiserum used. As seen in the SB results, each antiserum tended to be more reactive with the antigen to which it was raised. The WB profile of SAFs derived from ME7-infected C57BL/mice (ME7/C57BL) revealed three bands with molecular masses of 26 to 28, 23 to 24, and 21 to 22 kDa. All bands stained with equal intensity. SAFs derived from 139A-infected C57BL/6J mice (139A/C57BL) displayed a profile similar to that of ME7 SAFs, except that the 26- to 28-kDa band was much less reactive with the antisera. This is in contrast to silver-stained gels, in which SAFs isolated from animals infected with ME7 and 139A reveal three equally staining protein bands (24). The WB profile of SAF protein derived from 263K-infectd hamsters (263K/LVG) was quite different from that of ME7 or 139A SAF. The profile exhibited three bands, 26 to 28, 23 to 24, and 19 to 20 kDa, with the 26- to 28-kDa band being the most intense and the 19- to 20-kDa band being lightly stained and less diffuse. This profile is not merely a reflection of species differences between hamster- and mouse-derived SAFs, since the WB profile of SAFs isolated from IM/Dk mice infected with strain 87V (87V/IM) was very similar to that of SAF proteins isolated from hamsters infected with strain 263K. The 87V SAF proteins were less reactive with the antisera, in that the 87V SAF proteins stained with less intensity than the 263K SAF proteins. These differences in WB profile were highly reproducible and consistent among many different SAF preparations which had been isolated by the same purification protocol. In all, five preparations of SAFs isolated from C57BL/6J mice infected with ME7, six preparations of SAFs isolated from C57BL/6J mice infected with 139A, six preparations of SAFs isolated from IM/Dk mice infected with 87V, three preparations of SAFs isolated from IM/Dk mice infected with ME7, and five preparations of SAFs isolated from LVG hamsters infected with 263K were examined. Each preparation was Western blotted at least twice, and often several times, with similar results.

These studies allowed the examination of host influences on the WB profiles of SAF proteins. SAFs isolated from ME7- and 139A-infected animals could be distinguished by their WB profiles even when they were derived from the same strain of mice (C57BL/6J) under the same isolation conditions (Fig. 2). SAF proteins derived from C57BL/6J mice infected with ME7 were indistinguishable from SAF proteins isolated from IM/Dk mice infected with ME7 (Fig. 2) but were quite different from SAF proteins isolated from IM/Dk mice infected with 87V. The WB profile of 87V SAFs isolated from IM/Dk mice more closely resembled that of 263K SAFs isolated from hamsters. These results suggested that there are agent-directed properties of SAFs which are independent of the host species or genotype.

Both the SB and WB results suggested a strong antigenic relationship among SAFs isolated from different species of animals infected with different strains of agent. However, since these studies involved the use of polyclonal sera, the possibility existed that different antibodies were binding to the SAF proteins derived from different scrapie strain-host strain combinations. Rabbit anti-ME7 M-PrP and anti-263K PrP antisera were affinity purified by using NC-bound antigen. Each serum was reacted with its homologous antigen bound to NC, and the eluted antibodies were tested for reactivity against homologous and heterologous antigens (Fig. 3). Affinity-purified anti-ME7 M-PrP antisera reacted with PrPs of 139A, ME7, and 263K SAFs and not with neurofilament proteins. Affinity-purified anti-263K PrP antisera reacted with PrPs of 263K, 139A, and ME7 SAFs and not with neurofilament proteins. These results establish that similar antibodies bind to SAF (PrP) proteins in the various SAF preparations and that differences seen in WB profiles are not the result of the binding of different antibodies.

Evidence suggests that PrP 27-30 and M-PrPs are coded for by the host (12, 38). Posttranslational modifications to

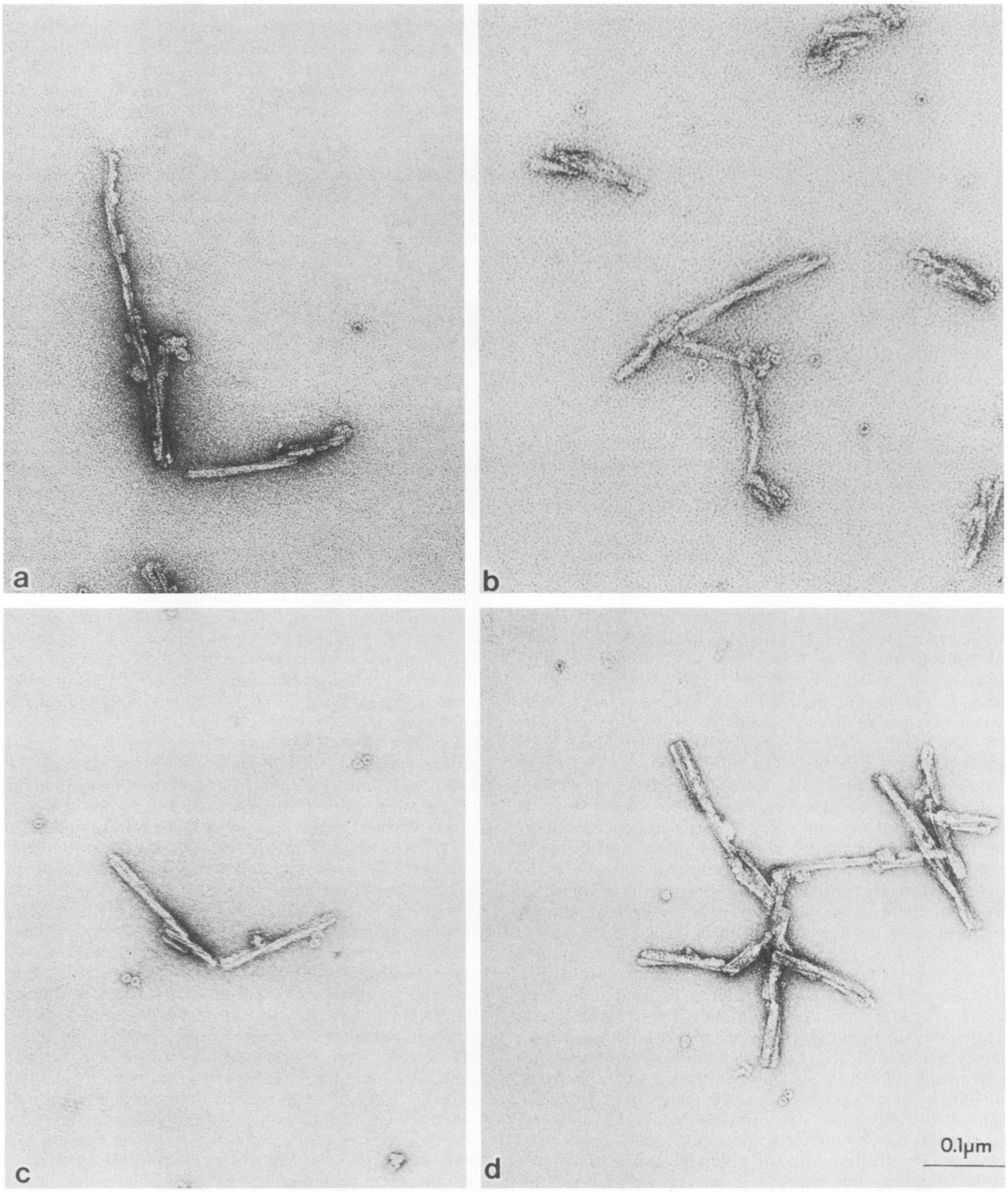

FIG. 4. Electron micrographs of purified SAFs isolated from animals infected with four different scrapie strains. SAFs were purified and processed for negative-stain electron microscopy as described in Materials and Methods. (a) 139A SAFs isolated from C57BL/6J mice; (b) 263K SAFs isolated from LVG/LAK hamsters; (c) 87V SAFs isolated from IM/Dk mice; (d) ME7 SAFs isolated from C57BL/6J mice.

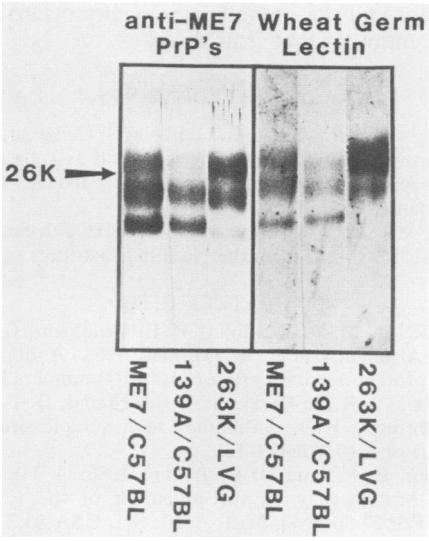

FIG. 5. Comparison of the reactivity of SAF PrPs with antisera to ME7 SAF PrPs and with wheat germ agglutinin. SAFs were purified and electrophoresed as described in the legend to Fig. 2. Samples were electrophoresed in duplicate, and one set was stained with antisera to ME7 PrPs as described in the legend to Fig. 2. The second set was used for lectin binding. Following blocking, wheat germ agglutinin conjugated with biotin was added for 2 h at 37°C. the secondary reagent consisted of avidin conjugated with alkaline phosphatase and was added at 37°C for 1.5 h. Reactivity was detected as described in the legend to Fig. 1. Lane designations: scrapie strain/host strain.

host proteins may occur as a consequence of the scrapie infection process. Such modifications could affect the assembly or packaging of proteins into the SAF structure. Structural differences were found by negative-stain electron microscopy among SAFs isolated by the same procedure (Fig. 4). 263K and 87V are tightly packed structures in which individual fibrils are not easily seen (Fig. 4b and c, respectively). In contrast, ME7 SAFs are more loosely packed, with each fibril being easily distinguishable (Fig. 4d). 139A SAFs are also composed of loosely packed fibrils, but appear more fragile and brittle than ME7 SAFs (Fig. 4a). Such structural differences may arise as a result of agent-directed processing of host protein. One posttranslational modification known to occur is glycosylation (8). The lectins wheat germ agglutinin and ricin have been shown to bind to SAF protein isolated from hamsters infected with CJD (27). Wheat germ agglutinin was used to investigate the glycosylation of SAF proteins from animals infected with ME7, 139A, and 263K (Fig. 5). In contrast to the results with antibody staining in which ME7 and 139A SAF proteins can be distinguished, all three protein bands from both ME7 and 139A SAF reacted strongly with this lectin, indicating that these proteins are glycosylated. The 19- to 20-kDa 263 SAF band did not bind lectin under these conditions, while the 26- to 28-kDa and 23- to 24-kDa bands strongly bound lectin. Such a pattern indicates processing differences between 263K and both ME7 and 139A SAF proteins. Blocking studies between lectin and antibody revealed that bound lectin was not able to inhibit antibody binding. However, previously added antibody was able to partially block lectin binding. These results suggest that lectin- and antibody-binding sites are not identical but may be located close to each other.

DISCUSSION

Immunological analyses with antisera raised to PrP 27-30 and M-PrPs demonstrated a strong antigenic relationship among SAF proteins isolated from animals infected with four different scrapie strains, 139A, ME7, and 87V in mice and 263K in hamsters. Studies with affinity-purified antisera demonstrated that similar antibodies were binding to SAF proteins isolated from the various scrapie strain-host strain combinations. These antisera have also been shown to react to SAF proteins isolated from humans with CJD (4, 5, 20, 27) and guinea pigs and hamsters infected with CJD (27). Anti-ME7 M-PrP antiserum has also been shown to react with SAFs isolated from naturally occurring or experimentally induced scrapie in sheep (Rubenstein, in preparation). Since it is now known that these proteins are coded for by the host (12, 38), it would seem that these proteins are highly conserved over a wide range of species.

Antisera raised to SAF PrPs did not react with PHF or amyloid isolated from patients with Alzheimer disease. This lack of antigenic similarity is in agreement with previous morphological data, suggesting that these structures are similar but not identical (31, 45). It is also in agreement with immunoelectron microscopic and immunocytochemical studies (Merz et al., in press). It had been suggested that, because of morphological similarities, amyloid may represent an aggregated form of infectious agent in Alzheimer disease (40). Despite ultrastructural similarity, the abnormal fibrils seen in Alzheimer disease and those seen in the unconventional slow virus diseases do not appear to be composed of the same proteins. A comparison of the sequence data between scrapie PrP (42) and amyloid plaque core protein in Alzheimer disease (29) also indicates that these structures are composed of different proteins. The close association of SAF proteins and the infectious agent in unconventional slow virus diseases (30) is in contrast to the lack of antigenic similarity between SAFs and abnormal fibrils in Alzheimer disease and suggests that Alzheimer disease is not caused by a similar agent. However, the possibility remains that a putative infectious agent associates with different host proteins in different disease states, and our results do not necessarily rule out an infectious etiology for Alzheimer disease.

Antisera to SAF proteins also did not react with purified amyloid cores isolated from a patient with GSS. This finding was unexpected in that immunocytochemical staining of amyloid plaques in experimentally transmitted cases of scrapie is seen with these antisera (13; P. A. Merz, manuscript in preparation) and GSS appears to be caused by an agent similar to scrapie agents (28). The failure of these antisera to react may be related to the harsh conditions used to isolate amyloid cores. Amyloid plaques isolated from human cases of GSS cannot be stained with antisera to SAF protein unless the amyloid plaques are first treated with proteolytic enzymes (31a). Amyloid plaques in tissue from patients with Alzheimer disease do not stain under these conditions (31a). The composition of CNS amyloid deposits in a variety of degenerative diseases and their relationship to PrPs are not fully understood. Factors such as host strain- or strain-directed protein modifications, along with alterations in other biochemical events occurring as a consequence of the disease process, may influence the antigenic properties of these plaques.

Although SAF proteins isolated from animals infected with different scrapie strains were closely related antigenically, there were distinct differences in the WB profiles of

these SAFs. SAFs from animals infected with each of the scrapie strains used in this study, 139A, ME7, 87V, and 263K, could be distinguished in this manner. Such WB profiles may represent structural as well as posttranslational modification differences among SAFs and their constituent polypeptides. All PrP proteins are host encoded (12, 38) and may be related to a host protein of 33 to 35 kDa (27, 36, 44) or possibly a higher-molecular-mass form of 54 kDa (3). Proteolytic enzymes used in the SAF purification procedure would appear to convert these higher-molecular-mass forms into the lower-molecular-mass forms that are seen in association with purified SAFs by silver staining and WB analysis (36, 44). Posttranslational modifications of PrPs may influence both their assembly into SAFs and their specific WB profiles. Lectin-binding studies with wheat germ agglutinin indicate glycosylation differences in the polypeptide profile of purified SAFs from mice infected with strain ME7 or strain 139A and hamsters infected with strain 263K. The 19- to 20-kDa protein associated with SAFs isolated from animals infected with strain 263K does not appear to be glycosylated and may represent the nonglycosylated form of PrP. Chemical deglycosylation studies on PrP appear to yield a similar protein (D. Bolton, personal communication). Other studies suggest that the deglycosylated form may be of a lower molecular mass, approximately 7 kDa (37). Studies are under way to investigate the carbohydrate moieties of SAF proteins isolated from animals infected with various scrapie strains and to determine whether such modifications are controlled by scrapie strain, host strain, or both. Glycosylation may play an important role in the stability of SAF proteins and may influence their structure, assembly, and perhaps also their biological activity.

In addition to glycosylation, other posttranslational modifications of SAF protein may also occur prior to the formation of SAFs in the scrapie-infected animals. It cannot yet be ruled out that other components, possibly additional protein or nucleic acid, also contribute to the structural conformation of different SAFs. A 4.3S RNA has been found associated with fractions enriched for scrapie infectivity (19). Differences in the properties of SAFs may also reflect biochemical events occurring as a consequence of the biological and pathological differences among strains of scrapie. Although PrPs are host encoded, the properties of SAFs appear to be controlled by both the host strain (5, 36) and the scrapie strain, as demonstrated in this study. The genotype of the host, including Sinc (15), does not appear to influence certain properties of SAFs. Thus, alterations in the host-encoded protein must be generated, at least in part, by the scrapie infection process, and the resulting structure and composition of SAFs must be specifically directed in some way by each scrapie strain.

The stable structure of SAFs and their close association with infectivity may help to confer upon the infectious agent its unusual stability to chemical and physical inactivation (11, 41). Infectivity is known to be sensitive to proteolytic enzymes (11, 41), and PrPs are the only proteins consistently associated with infectivity. On the other hand, it has been suggested that SAFs are a pathological structure which merely copurify with infectivity owing to the high carbohydrate content of PrPs (36; R. H. Kimberlin, Neuropathol. Appl. Neurobiol., in press). In either case, at present purified SAFs represent the only marker other than infectivity itself for the presence of the scrapie infectious agent. Continued study of the molecular characteristics of SAFs isolated from animals infected with these various scrapie strains will contribute to our understanding of both the nature of the scrapie agent and the pathological events occurring within the unconventional slow virus diseases.

ACKNOWLEDGMENTS

We thank C. Scalici, M. Papini, C. Fama, S. Callahan, R. Fersko, and M. Tonna-DeMasi for their fine technical assistance. We also thank R. Weed for photographic assistance and A. Monaco for typing the manuscript.

The work was supported in part by Public Health Service grants NS21349 and AGO 4220 from the National Institutes of Health.

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