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Nucleic acid binding proteins in highly purified Creutzfeldt–Jakob disease preparations

(scrapie/Northwestern blots/spongiform encephalopathy)

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ABSTRACT **The nature of the infectious agent causing human Creutzfeldt–Jakob disease (CJD), a slowly progressive dementia, is controversial. As in scrapie, no agent-specific proteins or nucleic acids have been identified. However, biological features of exponential replication and agent strain variation, as well as physical size and density data, are most consistent with a viral structure—i.e., a nucleic acid–protein complex. It is often assumed that nuclease treatment, which does not reduce infectious titer, leaves no nucleic acids of >50 bp. However, nucleic acids of 500–6000 bp can be extracted from highly purified infectious complexes with a mass of ≈1.5 × 10^7 daltons. It was therefore germane to search for nucleic acid binding proteins that might protect an agent genome. We here use Northwestern blotting to show that there are low levels of nonhistone nucleic acid binding proteins in highly purified infectious 120S gradient fractions. Several nucleic acid binding proteins were clearly host encoded, whereas others were apparent only in CJD, but not in parallel preparations from uninfected brain. Small amounts of residual host Gp34 (prion protein) did not bind any ^{32}P-labeled nucleic acid probes. Most of the minor "CJD-specific" proteins had an acidic pI, a characteristic of many viral core proteins. Such proteins deserve further study, as they probably contribute to unique properties of resistance described for these agents. It remains to be seen if any of these proteins are agent encoded.**

Virually all viruses package their genetic material together with specific proteins. Within the viral core, nucleocapsid proteins are most numerous, although other minor nucleic acid binding proteins that function in viral replication and transcription can be present. Binding proteins from different viruses exhibit different modes of interaction with, and affinities for, nucleic acids. Some viral proteins show extreme sequence specificity (see, e.g., ref. 1), whereas many others do not even differentiate RNA from DNA (see, e.g., ref. 2). The capsid proteins bind to the viral genome to produce tightly folded viral cores (nucleic acid–protein complexes) that are resistant to degradation both outside and inside the cell. Different viral cores exhibit different levels of resistance to adverse conditions, such as acid hydrolysis, heat, and nuclease attack, probably as a consequence of precise features of binding and folding within the core complex. In Creutzfeldt–Jakob disease (CJD) and scrapie the infectious agent is often considered to exhibit unusual resistance to physical and chemical treatments. These indirect data, as well as the assumption that nuclease or Zn^{2+} treatment effectively cleaves all nucleic acids to fragments <50 bp (3), is often cited in the litany of "evidence" for an infectious host protein or "prion" (4, 5). However, such preparations have contained as much as 1–10 μg of nucleic acid per ≈10^8 infectious units (IU) (4), and more recent infectious CJD and scrapie preparations, including those treated with Zn^{2+} and nucleases, invariably show nucleic acids of 500–6000 bp by conventional ^{32}P labeling (6–8). We considered that nucleic acid binding proteins might be constituents of these infectious agents that could protect a viral-like genome from nuclease attack and additionally endow these agents with unique attributes of resistance. We therefore searched for nucleic acid binding proteins in highly purified infectious fractions of CJD. This approach has not, to our knowledge, been previously utilized in scrapie or CJD, and it has the potential to uncover specific host proteins, as well as agent-encoded binding proteins, that may protect an agent genome.

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MATERIALS AND METHODS

Clarified supernatants from brain homogenized in 10% Sarkosyl were centrifuged at 215,000 × *g* for 2 hr, and the resuspended pellet, treated with excess micrococcal nuclease and Ca^{2+} (7, 8), was disaggregated at pH 8.9 (9). The supernatant was centrifuged on a linear 10–30% sucrose gradient to resolve the infectious 120S peak, yielding an ≈100,000-fold purification of infectivity with respect to starting nucleic acids (8) and >11,000-fold with respect to protein (7). The 120S peak sucrose fractions were diluted ≈3-fold and were concentrated by pelleting at 215,000 × *g* for 2 hr with no loss of titer (10). Initial homogenization releases only ≈20% of the brain infectivity, almost all of which is recovered in the final concentrates—i.e., 1.5–3 × 10^7 IU/g (7, 8, 10). For reference and standardization, quantities of material are expressed in gram equivalents of starting hamster CJD brain, which contains ≈10^8 IU/g.

An RNA ladder (Bethesda Research Laboratories) was 3′-^{32}P-end-labeled with T4 RNA ligase to a specific activity of ≈10^6 cpm/μg (8). The Syrian hamster intracisternal A particle (SHIAP) retroviral insert [gift of E. Kuff (11)], purified by agarose gel electrophoresis, was end-labeled with [γ-^{32}P]ATP by polynucleotide kinase or was random primed with Klenow fragment (8) to yield higher specific activity probes (≈2 × 10^8 cpm/μg). A hamster CJD cDNA probe was generated as follows. Fraction 2 of the 120S CJD gradient peak was digested with proteinase K/SDS, total nucleic acids were extracted with phenol/chloroform, and DNase I digestion was then used to produce undegraded RNA (8). This RNA was reverse transcribed by using random primers, the resultant cDNAs were converted to double-stranded DNA molecules, and then designed PCR adaptor/primers were blunt-end ligated to the cDNA for sequence-independent amplification (12). The total PCR product was end-labeled with ^{32}P to produce the CJD cDNA probe. This cDNA pool, rather than cloned cDNA derivatives, was used to maximize sequence complexity. Optimal conditions for nucleic acid

Abbreviations: CJD, Creutzfeldt–Jakob disease; VSV, vesicular stomatitis virus; IU, infectious units; SHIAP, Syrian hamster intracisternal A particle; 1D and 2D, one- and two-dimensional.

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binding were determined empirically, and probes were used at 2.5×10^5 cpm/ml. Binding, done as described (13), was appreciably reduced by incubation in ≥150 mM NaCl or at a pH of ≥8.0. DNA probes were tested both in native double-stranded form and as single-stranded molecules produced by boiling. Autoradiography was done at 22°C, or at −70°C with an intensifying screen, for up to 24 hr.

RESULTS AND DISCUSSION

CJD infectivity can be substantially purified by using mild disaggregation procedures which have no effect on infectious titer (9). After disaggregation, Gp34 cosedimenting with infectivity loses its resistance to limited proteolysis, a presumed characteristic of the "infectious prion" form of this host protein (5), and infectivity migrates with reproducible virus-like size and density characteristics in sucrose gradients (7–9). Exhaustive nuclease digestion, which does not reduce infectivity or alter these physical characteristics, is used to substantially increase agent purification (7, 8). In sucrose gradients, almost all applied infectivity is recovered within a single gaussian peak centered at 120S (8). In contrast, the majority of host Gp34 (prion protein) remains with other small proteins at the top (≈5S portion) of the gradient. This 5S region contains <5% of the loaded infectivity (8) as determined for CJD established in hamsters (14) with well-defined titration characteristics (15). HPLC and sedimentation field flow fractionation of the infectious 120S peak indicate an agent mass of $\approx 1.5 \times 10^7$ daltons with a mean radius of ≈30 nm (10). This peak contains protected nucleic acids of significant size (8) but is still insufficiently purified (≈7 ng of nucleic acid per $1.5–3 \times 10^7$ IU/g) to directly detect an agent-specific nucleic acid. Here we studied nucleic acid binding proteins in this infectious 120S peak and in parallel fractions from uninfected brain. Previous studies have shown a similar yield of proteins in 120S fractions from CJD and uninfected brain, but in CJD preparations there is a small amount of residual Gp34, estimated to be ≈1% of that in starting brain (unpublished data).

We isolated the 120S peak from three sources by using an established protocol (8). Human CJD brain, which cannot be titered directly but which had been verified by rodent transmission and/or neuropathology, was used as a species control. Analytical studies of colloidal gold stained proteins, Gp34 detected with antibodies, and ^{32}P-labeled nucleic acids showed human CJD gradient profiles were comparable to those previously described for hamster CJD (8, 10). Hamster CJD brains from clinically ill animals were compared with uninfected brains to find if binding proteins were specific for CJD or were host encoded. Several different nucleic acid ^{32}P-labeled probes were used to evaluate protein (Northwestern) blots of SDS-denatured preparations. These probes included a nonspecific RNA ladder, a cloned endogenous retroviral sequence (11), and a cDNA probe made from nucleic acids extracted from the 120S infectious hamster CJD peak (see *Materials and Methods*). The last probe was included because a subset of nucleic acid sequences within a purified infectious CJD preparation might have an enhanced or selective affinity for proteins of viral origin.

Fig. 1 shows a representative Northwestern profile for an entire sedimentation gradient profile made from human CJD brain. This human CJD gradient, probed with hamster CJD cDNA, is typical for all probes and all preparations, including uninfected hamster brain preparations. For orientation, fractions 1–4 cover the range of 70–250 S, with the 120S peak largely confined to fraction 2. Fractions 9–14 cover the range of 4–10 S and contain most soluble proteins, including >70% of the loaded Gp34 in CJD preparations, as previously shown (8, 10). It can be seen that this upper region of the gradient contains the vast majority of nucleic acid binding proteins. Residual micrococcal nuclease, a protein known to bind nucleic acids, is also clearly seen in this same region, but it does not contaminate the 120S peak. It can be appreciated that a large number of host-encoded binding proteins will contaminate infectious preparations that are not purified by gradient centrifugation. In contrast to the upper portion of the gradient, only a limited subset of binding proteins are detectable in the 120S region, even when fractions 1–4 are pooled and concentrated (Fig. 1 *Left*). Notably, human proteins within the 120S gradient peak can bind hamster CJD-associated cDNA sequences.

In hamster CJD, titered infectious fractions 1–4 also contained small amounts of nucleic acid binding proteins with a similar apparent size and pattern by electrophoresis and were studied in more detail. The relatively low level of nucleic acid binding proteins in the infectious peak is not surprising in view of the low nanogram levels of protected nucleic acids remaining in gradient-purified CJD preparations as well as the small amounts of proteins other than Gp34 in colloidal gold-stained blots. The small amount of cosedimenting host Gp34, detected only in fraction 2, is shown in Fig. 2, lane 12. The identity of this colloidal gold-stained protein was verified with specific antibodies (as in Fig. 3, lane 4). Because fraction 2 was centered within the infectious 120S peak and also contained all the detectable Gp34 cosedimenting with infectivity, we used this single fraction for initial evaluation of different nucleic acid probes. A parallel fraction from uninfected brain was compared. Several control proteins, including reverse transcriptase, micrococcal nuclease, and purified

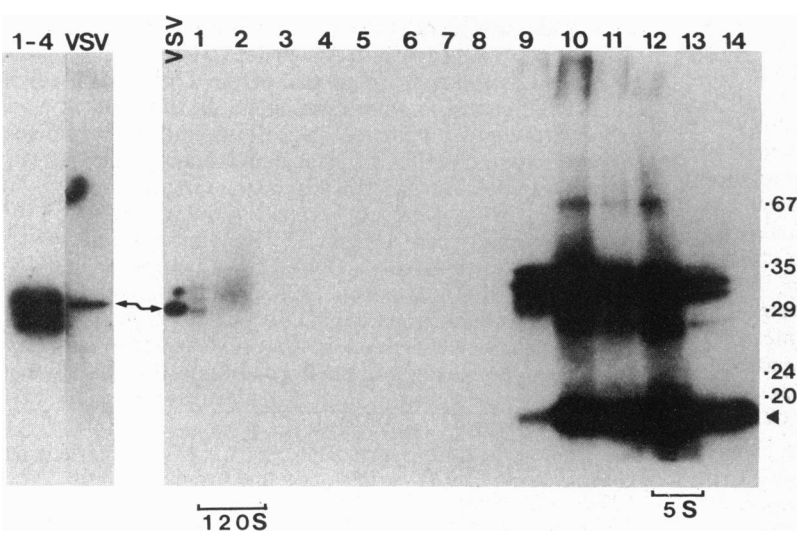

FIG. 1. Northwestern profile of nucleic acid binding proteins in fractions from a representative sucrose gradient. All individual gradient fractions (lanes 1–14, 0.35 g equivalents of brain each) are shown in this human CJD example, probed with ^{32}P-labeled CJD hamster cDNA. The majority of binding proteins segregate at 4–10 S, together with residual micrococcal nuclease (arrowhead, 16.8 kDa). In hamster CJD gradients the majority of Gp34 is in this same region, which contains negligible infectivity (8, 10). At left, the pooled human 120S peak (fractions 1–4) is shown in parallel with the vesicular stomatitis virus (VSV) lysate, which contains a 29-kDa binding protein. Marker proteins are indicated in kDa, and gel blots were made as described (16).

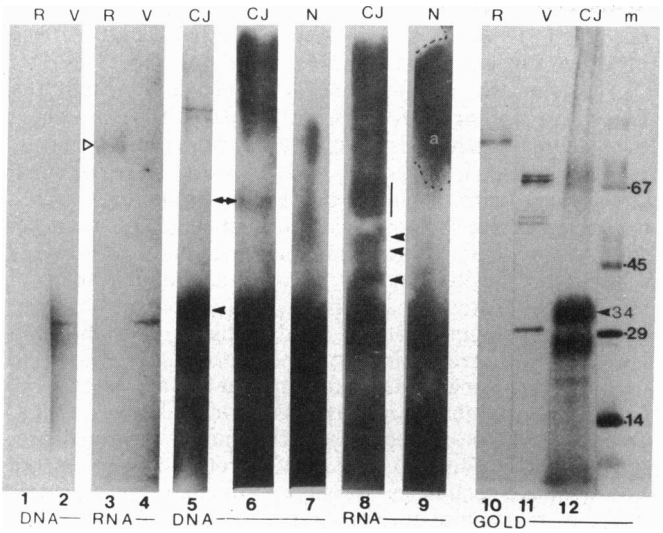

FIG. 2. Parallel gel lanes with Moloney murine leukemia virus reverse transcriptase (R), ≈50 ng of VSV lysate (V), and hamster gradient fraction 2 (0.5 g each) from CJD (CJ) or uninfected (N) brain. Probes are [^{32}P]cDNA (DNA) either native (lanes 1, 2, and 5) or denatured (lanes 6 and 7) and ladder RNA (RNA, lanes 7 and 8); corresponding protein profiles are shown with colloidal gold (GOLD). The control ≈71-kDa reverse transcriptase binds RNA, but not DNA (compare lanes 1 and 3, open triangle). The VSV 29-kDa M protein binds both the RNA and cDNA probes (lanes 2 and 4), whereas other VSV proteins in the gold profile (lane 11) show no binding; these are the ≈48-kDa nucleocapsid protein, the ≈50-kDa NS dimer, and the heavily glycosylated G protein (two bands at ≈67 kDa). In CJD lane 5 the most intense binding proteins comigrate with Gp34 at the arrowhead (see lane 12). Minor nucleic acid binding proteins are at ≈80 kDa in this short-exposure autoradiograph, and bands at ≈60 kDa (double arrow) and ≈48 kDa were seen on 4-fold longer exposures. The 60-kDa band is also seen in CJD lane 6, where the ≈80-kDa bands are obliterated by a smear of binding proteins. Neither is seen in the same or longer exposures of the uninfected control (lane 7). Long exposures with the RNA probe in CJD (lane 8) show an ≈37-kDa band and an ≈48-kDa doublet (arrowheads), as well as a smear at ≈60 kDa (line), that are not detectable in the uninfected control (lane 9, with an artefactual smudge at a, outlined with broken lines). Despite the 0.5-g gel loads, few proteins other than Gp34 and its dimer (at ≈67 kDa) are detectable with gold (lane 12). The identity of Gp34 was verified with specific antibodies as in Fig. 3, lane 4. Molecular mass markers (kDa) are shown in lane m.

VSV, were simultaneously included to assess sensitivity and sequence specificity of different binding proteins in Northwestern blots.

The control protein reverse transcriptase, as expected, did not bind DNA, but did bind to RNA (Fig. 2, lanes 1 and 3). Micrococcal nuclease, which digests both RNA and DNA, predictably bound to both RNA and DNA probes (as in Fig. 1). VSV lysates showed nucleic acid binding to only one of several major viral proteins resolved by electrophoresis. The binding of this single 29-kDa viral M protein, which contains a highly basic amino-terminal domain (13), was nonspecific with respect to nucleic acid sequence, because it bound both the hamster CJD cDNA probe and the unrelated RNA ladder probe (Fig. 2, lanes 2 and 4). Interestingly, the ≈50-kDa nucleocapsid VSV protein, although present in similar amounts as the M protein (Fig. 2, lane 11), did not bind either of these probes. This lack of binding may indicate that a highly specific nucleic acid sequence is required for binding the VSV nucleocapsid protein, unlike many viral capsids (2). In hamster CJD fraction 2, all probes showed strong binding to a group of proteins between 14 and 34 kDa and did not discriminate between the RNA or DNA probes (Fig. 2). Longer exposures of CJD but not normal brain showed several additional bands (Fig. 2, lanes 6–9). These included an ≈80-kDa doublet, a band or smear at ≈60 kDa, and bands at ≈48 and ≈37 kDa.

We attempted to determine the identity and/or special characteristics of the detected CJD nucleic acid binding proteins. These studies were directed at exploring (*i*) the binding capacity of sedimenting Gp34, by definition the "prion form" of the protein; (*ii*) the possibility that a subset of binding proteins was host encoded; and (*iii*) the nature of the binding interactions. Three independent preparations each of CJD and uninfected hamster brain were studied in detail. A prominent subset of nucleic acid binding proteins comigrated electrophoretically with Gp34 (Fig. 2, lanes 5 and 12). Although Gp34, a membrane protein, would not be expected to bind nucleic acids, it possesses basic amino acid domains that might interact nonspecifically with nucleic acids, as demonstrated for the basic M protein of VSV. It was therefore pertinent to empirically evaluate the ability of Gp34 to bind nucleic acids. Comparison of parallel sucrose fractions prepared from uninfected hamster brain were most informative in this context. Host Gp34 in uninfected hamster brains does not sediment when centrifuged at 215,000 × *g* for 2 hr, in contrast to Gp34 derived from brains of clinically ill CJD hamsters (16). Therefore 120S gradient fractions made from uninfected brain contain no detectable Gp34, unlike parallel fractions 1–4 from CJD brains (Fig. 3, lanes 3 and 4). When equal gram equivalents of both CJD and normal 120S peak fractions were blotted and probed with ^{32}P-labeled nucleic acids, identical bands were detectable in the same region as Gp34 (Fig. 2, lane 7) even when no Gp34 was present (Fig. 3). Thus these comigrating nucleic acid binding proteins are not Gp34. Furthermore, because they are present at similar levels in uninfected brain fractions they are

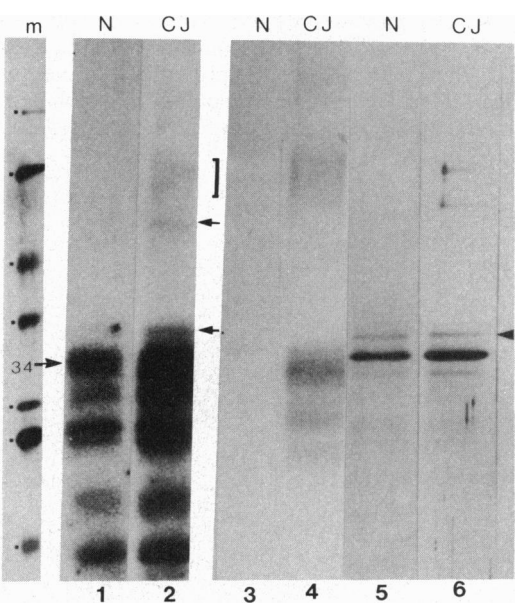

FIG. 3. Comparison of parallel uninfected (N) and bioassayed hamster CJD fractions 1–4 (CJ) probed with the [^{32}P]RNA ladder (lanes 1 and 2) or with antibodies to Gp34 (lanes 3 and 4) as described (16). Lanes 1–4 are each loaded with 0.125 g of brain, and controls for protein loads (lanes 5 and 6, 0.05 g of brain) are shown with gold staining in the same blot. Note the equivalent load of a 35-kDa protein (arrowhead) in lanes 5 and 6. Several minor nucleic acid binding bands at ≈37 and ≈48 kDa (arrows) as well as a 60- to 65-kDa smear (bracket) are apparent in CJD but not in uninfected samples in this parallel exposure, or with longer exposures (not shown). Only the CJD samples contain proteins that react with antibodies to Gp34, but both N and CJ lanes contain comparable nucleic acid binding protein bands in the 22- to 34-kDa region. Markers (lane m) are at 20, 24, 29, 36, 45, 67, and 94 kDa.

clearly host encoded, and their sedimentation is unrelated to CJD pathology.

Again, comparison of CJD and control preparations revealed several binding proteins that could potentially be either agent-specific or agent-associated. These minor nucleic acid binding species were visible only in CJD, but not in parallel control fractions even with longer autoradiographic exposures. One can additionally see that parallel lanes from the normal and CJD preparations contain comparable loads of protein, with a band at ≈35 kDa (Fig. 3, lanes 5 and 6) that is unrelated to the "CJD-specific" 37-kDa band. Even in this less heavily loaded gel an ≈48-kDa band and an ≈60-kDa smear were visible only in the CJD sample (Fig. 3, lanes 1 and 2). Because these CJD-associated nucleic acid binding proteins were not simply related to colloidal gold-stained bands, they could not be quantified with precision. However, based on nucleic acid binding intensity, these proteins probably are present at ≤1–10 ng in these blots.

To relate these potentially CJD-specific binding proteins to infectious units, we titered the CJD agent, using dilutions in the linear range of the bioassay as described (15). Pooled concentrated hamster brain fractions 1–4 from the same preparative fractions used for Fig. 3 contained 1.5×10^7 IU/g equivalents of starting brain. This level of infectivity was comparable to that determined for other similar micrococcal nuclease-treated fractions [range $1.5–3 \times 10^7$ IU/g in six independent CJD preparations (7–9)]. Thus this blot loaded with 0.125 g equivalents of brain contains roughly 25 ng of these minor CJD proteins per 10^7 IU. Since there are often multiple copies of nucleocapsid proteins for each viral genomic copy, the levels of the minor binding proteins detected here are high, but not inconsistent with a virally encoded product. However, until the sequence of these peptides is determined, alternative interpretations are equally valid. These proteins may be only differentially detectable in CJD infectious fractions due to (*i*) an increased expression of host-encoded (but potentially protective) proteins, (*ii*) a modification of specific host proteins that enhances their nucleic acid binding capacity, or (*iii*) abnormal sedimentation of selected host proteins caused by complex pathological changes in the brain at later stages of disease, as has been proposed for host Gp34 (15–17).

Although the origin (host or virus) for these CJD-associated binding proteins is not yet clear, it was pertinent to evaluate the mechanisms underlying these protein–nucleic acid interactions, especially because one or more host-encoded proteins could participate in protecting a CJD-specific genome. Two-dimensional electrophoresis was useful for elucidating charge interactions, and this strategy additionally resolved a few separate binding proteins of similar size. Moreover, a trivial explanation for the observed nucleic acid binding proteins could be discounted. The binding proteins detected in one-dimensional (1D) gels could derive from strongly basic proteins that are abundant in all cells. At least some chromatin-associated histones, for example, would be expected to contaminate any nuclease-treated preparation and consequently protect only a subset of host genomic sequences. In contrast to such strongly basic proteins, many viral core proteins that interact with nucleic acids can be resolved at a pI of 4–8. We therefore analyzed large amounts of the gradient-purified CJD material (2.3×10^7 IU derived from 1.5 g of brain) in each two-dimensional (2D) minigel (18) designed to resolve more neutral or slightly acidic proteins. These studies (Fig. 4) showed that most of the "CJD-specific" binding proteins detected in 1D gels were acidic, and thus not derived from strongly basic proteins, including histones. In contrast, the common host proteins of <25 kDa are not present in this pH range and could be histonelike. The pI of the "CJD-specific" binding proteins indicates that interactions with nucleic acid probes are not a consequence of net charge.

One of the blots shown was probed with a SHIAP insert of 3.6 kb (Fig. 4*A*). This ^{32}P-labeled probe contained the long terminal repeat (LTR) and coding regions for the nucleocapsid gag protein, the Cys–His nucleic acid binding motif, and the 5′ portion of reverse transcriptase (11). We used this probe for the following reasons: (*i*) we had hypothesized that LTR and *gag*-like retroviral elements might drive and protect

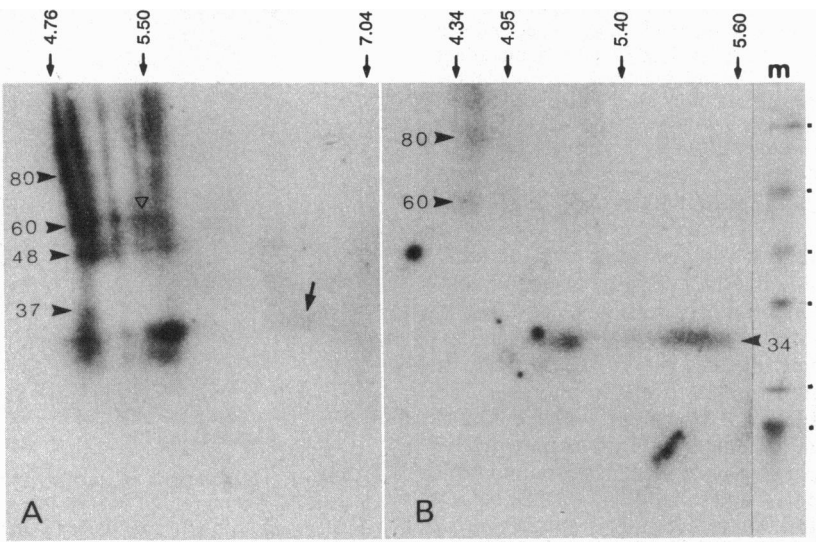

FIG. 4. Two-dimensional electrophoresis of CJD concentrated gradient fractions 1–4 probed with the SHIAP insert (*A*) and the RNA ladder (*B*). Each blot represents a load of 1.5 g equivalents of brain. A major host binding protein (at ≈34 kDa in 1D gels) is detected as two separate spots (pI ≈5 and 5.5) in both blots. The SHIAP probe highlights several additional minor "CJD-specific" acidic species at a pI of ≈4.8. The ≈37-kDa spot is well resolved, and the spots at ≈48, ≈60, and ≈80 kDa (arrowheads) are within a smear of reactive material. Only the very minor spot at 60 kDa which separates at a pI of ≈5.3 (open triangle) has been found in both control and CJD hamster preparations by using immunological methods (unpublished data). Blot A also shows the location of a more neutral ≈35-kDa host protein [at arrow corresponding to the intense gold band in Fig. 3 (lanes 5 and 6). This host protein, previously characterized in 2D gels (17 and 18), shows minimal nucleic acid binding]. Molecular mass markers for blot *B* (lane m) are 24, 29, 36, 45, 66, and 94 kDa and pI markers are noted at the top of each gel. First-dimension equilibrium electrophoresis was done in 200-μl capillary tubes (18) with LKB 3-10 ampholytes for 20 hr at 400 V followed by 1 hr at 800 V.

the CJD agent genome (19); (*ii*) several protected retroviral LTR sequences have been identified in infectious fractions (20); and (*iii*) although the sequence is not specific for CJD, more than 900 bases of the protected SHIAP RNA genome have been cloned and/or PCR amplified from infectious CJD fractions (21). This probe therefore seemed most relevant for study in the absence of any CJD-specific sequences. An enhanced sensitivity and/or additional binding proteins were not detected with the SHIAP probe, which showed binding and sensitivity characteristics similar to those of the hamster cDNA probe. It is shown for comparison with a lower specific activity RNA ladder probe (Fig. 4*B*). In both 2D blots two strong ≈34-kDa binding spots were resolved at a pI of ≈5.0 and 5.5. Both of these spots correspond to the major host-derived proteins shown in both CJD and control 1D gels (Fig. 3). A very faint trace of the nonspecific ≈35-kDa protein is also resolved (Fig. 4*A*). The SHIAP probe also clearly delineated the more minor "CJD-specific" ≈37-kDa species at a pI of ≈4.9, and the other "CJD-specific" bands in Figs. 2 and 3, at ≈48, ≈60, and ≈80 kDa, were also visible within a smear of binding proteins at a pI of ≈4.9. These spots, as well as the smear, were less apparent with the RNA ladder probe, probably as a consequence of the relatively less efficient incorporation of ^{32}P into RNA by 3′ end labeling. Interestingly, one very minor acidic peptide spot resolved at ≈60 kDa, with a pI of 5.3 (Fig. 4*A*, open triangle), is within the predicted region for an intracisternal A particle gag protein (11). Immunological studies indicate this spot represents a retroviral gag protein (unpublished results). At least some of the minor "CJD-specific" proteins in these 2D blots are likely to be present in amounts approximately one order of magnitude less than those visualized in 1D blots, because >10-fold amounts of infectious material were analyzed, and different minor protein species with the same molecular mass were resolved from each other.

The molecular nature of the infectious agent in CJD and scrapie remains a matter of speculation because only host proteins and nucleic acids have been detectable in any infectious preparation. The intensely investigated prion protein (Gp34) has failed to show any primary or post-translational modifications in infected animals or cell cultures and is now hypothesized to have some conformational change that can make it infectious (22). Neither a conformational change nor infectivity of this protein in any purified, recombinant, or transgenic form has yet been demonstrated, and our disaggregation experiments indicate that partial proteolytic resistance may not be an intrinsic property of the sedimenting protein (9). In this context, the abnormal behavior of Gp34 would seem to reflect late stages of agent-induced pathology, with altered pathways for processing damaged membranes (15, 17). Although the resistance of Gp34 is a useful marker of late-stage infection, many biochemical (7–9, 15) as well as *in vivo* experiments (23–25) have shown a lack of correlation of prion protein levels with infectious titers. It is also difficult to reconcile the host prion hypothesis with compelling biological data showing viruslike properties for these agents. Such properties include exponential agent replication (23–25), unique agent strains in both CJD and scrapie (19, 26–28), conventional viremic modes of infection (29–31), and viruslike cross-species transmission characteristics (32, 33). Such attributes are more consonant with an agent-specific genome.

The present direct data demonstrating nucleic acid binding proteins, as well as previous characterizations showing significant amounts of bound nucleic acids in all purified CJD and scrapie preparations (4, 6–8, 34, 35), are consistent with a viral hypothesis for the nature of the infectious agent(s). Infectivity has never been separated from nucleic acids, even when only size-selected molecules are analyzed with low-sensitivity detection methods (34). Clearly reproducible filtration and physical studies indicate a viruslike core structure of significant size (7, 8, 10). The present demonstration of nucleic acid binding proteins is further evidence for protected nucleic acid–protein complexes in highly purified infectious fractions. Enhanced detection and isolation strategies will be advantageous for further defining the specific binding proteins that can associate with or identify the infectious agent.

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