

Polymeric Ligands with Specificity for Aggregated Prion Proteins, Amin Lane,* Christopher J. Stanley, Stephen Dealler,† and Stuart M. Wilson (Microsens Biotechnologies, 2 Royal College St., London NW1 0TU, UK; * author for correspondence: fax 44-20-7691-2036, e-mail amin.lane@microsens.co.uk; † current address: Department of Medical Microbiology, Royal Lancaster Infirmary, Ashton Road, Lancaster LA1 4RP, UK)

The misfolding of normal proteins and their subsequent aggregation in the brain is characteristic of a group of related neurologic diseases, the "protein conformational disorders", including Parkinson disease, Alzheimer disease, and the transmissible spongiform encephalopathies (TSEs) (1). The development of highly sensitive and specific diagnostic assays for these diseases is a high priority as effective therapies become available or as food safety regulations mandate the removal of infected animals from the food chain.

Current test development effort in the TSE field has been focused mainly on designing screening tests for the aggregated prion proteins in bovine, ovine, and cervid brain tissue. Several immunoassays for bovine spongiform encephalopathy (BSE) have been evaluated by the European Union (EU) (2), and three are now approved for routine use in testing laboratories. Those approved include two ELISAs in the microplate format and reagents for a Western blot analysis. The abnormal, or rogue, prion protein detected by these tests is considered to be the best indicator of TSE infection (2), but the anti-prion protein antibodies used in these assays cannot distinguish the normal, globular, proteinase K-sensitive (α -helix-rich) form of the prion protein (PrP^{sen}) from the abnormal, aggregated, and relatively proteinase K-resistant form (PrP^{res}), in which the secondary structure is dominated by β -sheet. Thus, a complex sample preparation procedure is required to eliminate the normal prion protein in the brain homogenate before immunoassay, usually involving a proteinase K digestion step carried out under closely controlled conditions. Under- or overdigestion problems with the protease step can compromise the specificity and sensitivity of the subsequent immunoassay.

A further complication in the current TSE test procedures is a requirement for a PrP^{res} denaturation step, after protease digestion of the brain homogenate, to expose the appropriate epitopes in the protein for binding by the currently available monoclonal antibodies used in the assays. Clearly the availability of an antibody with specificity for the rogue prion protein in its native state would be a major advantage in assay development, but although there have been reports of such antibodies (3), as yet there are no reagents or assays commercially available.

As an alternative to seeking PrP^{res} -specific antibodies, we have been screening synthetic polymeric ligands to identify compounds with specificity for the native conformation of the rogue prion protein. The recombinant prion protein (produced in *Escherichia coli*) has binding sites for heparan and related polyanions (4). Other polyanions (such as pentosan polysulfate) and polycationic dendrim-

ers reduce concentrations of PrP^{res} produced by TSE-infected cell lines (5, 6), although the nature of the interaction between the abnormal aggregated form of the protein and the polyions is currently unknown.

We have identified several polymeric compounds (designated as the "Seprion" ligands) that, under the appropriate reaction conditions, have the ability to bind the PrP^{res} present in BSE, scrapie, chronic wasting disease, and Creutzfeldt-Jakob disease-infected brain tissue with high selectivity in the presence of a large excess of the normal PrP^{sen} form (7). The mechanism of binding of PrP^{res} to the Seprion ligands is unclear at this stage because information is currently not available on the detailed structure of the protein aggregates, although the optimal ligands were found to be of high molecular mass (>50 000 Da), presumably facilitating multiple ionic interactions with the aggregated protein structure (which would be expected to have multiple repeating regions of positive or negative charge). The Seprion ligands are mixed-mode in character and predominantly polyionic, but they are also able to bind strongly to solid-phase materials in a simple procedure suited to large-scale manufacturing processes. One polyionic ligand (designated v2) was chosen for inclusion in a BSE test development program.

The BSE assay was performed as described by Lane et al. (7) and is illustrated schematically in Fig. 1A. Briefly, 200 mg of bovine brain tissue (taken from the obex region) was homogenized in a ribolyser (four 30-s disruptions, with cooling in ice for 60 s between steps), and 100 μL of the homogenate was then added directly to the wells of a Seprion v2-coated microplate and incubated for 2 h. After capture of PrP^{res} , the wells were washed, and the prion protein was denatured on the surface for 10 min by a standard chemical method, followed by an additional wash to remove denaturant. One hundred microliters of a commercially available prion protein-specific monoclonal antibody/peroxidase conjugate was then added and incubated for 60 min. After removal of excess conjugate, 100 μL of 3,3',5,5'-tetramethylbenzidine substrate was added and incubated for 30 min. After the reaction was stopped, the absorbance at 450 nm was measured.

Shown in Fig. 1B are the results obtained with the Seprion immunometric assay from a panel of UK-derived bovine brain samples obtained from 26 animals (supplied by the TSE Archive, Veterinary Laboratories Agency, Weybridge, UK). The presence of BSE infection was confirmed in 11 animals by observation of the characteristic pathology in the brain and also by an immunohistochemistry procedure. Fig. 1B also includes data obtained from the same sample set with an EU-approved ELISA assay that uses a proteinase K digestion, a denaturation step (by boiling), and a centrifugation procedure to concentrate the abnormal prion protein before detection in a standard, microplate-based immunometric procedure.

Both test procedures correctly identified the positive BSE-infected samples, and the negative noninfected brain tissue gave only a background signal value in the Seprion immunometric assay. In additional experiments, the

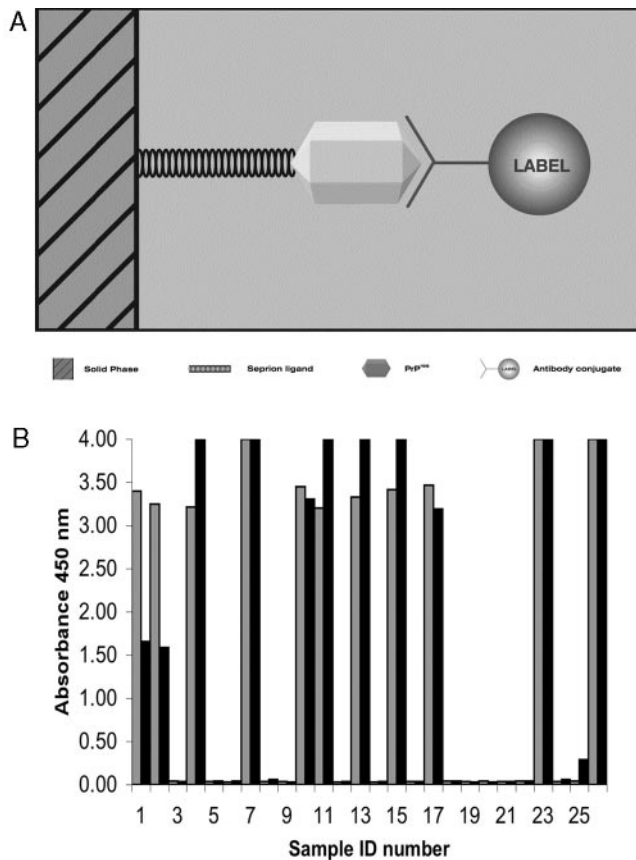


Fig. 1. Seprion immunometric assay for PrP^{Sc} (A), and comparison of the Seprion immunometric assay (▨) with an EU-approved ELISA (■) for a panel of BSE-infected and noninfected bovine brain samples (B).

(B), the immunometric procedure was carried out as described in the text, and the absorbance at 450 nm recorded in the microplate well is shown for each brain homogenate sample.

Seprion immunometric assay achieved 100% specificity with >100 negative brain tissue homogenates tested (data not shown). Dilution curves prepared with positive brain homogenate dispersed in negative brain material have indicated that the Seprion assay has a detection limit comparable to that of the EU-approved ELISA (data not shown).

In summary we have identified polyionic compounds that, under the appropriate reaction conditions, exhibit high specificity for the rogue form of the prion protein present in the brain of BSE-infected animals. The Seprion immunometric assay procedure, using enzyme-conjugated antibodies of appropriate specificity, can eliminate the complex and potentially variable proteinase K sample preparation step currently used in TSE screening tests. On the limited number of samples tested to date, the Seprion immunometric assay has a sensitivity and specificity comparable to those of an EU-approved ELISA, with the additional advantage that the assay procedure is ideally suited to automation on standard ELISA processor instrumentation. Further investigation on a larger number of infected animals will be needed to determine whether the Seprion immunometric assay could be useful in the rou-

tine detection of TSEs. We have recently prepared magnetic microparticles coated with the Seprion ligands, and studies are underway on the capture of the abnormal prion protein from larger quantities of brain homogenate, urine, and blood from animals infected with scrapie.

References

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