ELSEVIER

Journal of Chromatography B, 697 (1997) 223–229

JOURNAL OF CHROMATOGRAPHY B

Short communication

Use of capillary sodium dodecyl sulfate gel electrophoresis to detect the prion protein extracted from scrapie-infected sheep

Mary Jo Schmerr[a,*], Allen Jenny[b], Randall C. Cutlip[b]

[a]*National Animal Disease Center, 2300 Dayton Road, Ames, IA 50010, USA*
[b]*National Veterinary Services Laboratory, Animal and Plant Health Inspections Service, Veterinary Services, 1800 Dayton Road, Ames, IA 50010, USA*

Abstract

Scrapie in sheep and in goats is the prototype of a group of transmissible spongiform encephalopathies (TSE). A feature of these diseases is the accumulation in the brain of rod shaped fibrils that form from an aggregated protein that is a protease-resistant form of a modified normal host cell protein. In this study, we compared SDS gel capillary electrophoresis to conventional SDS-PAGE and Western blot to detect the monomer of this aggregated protein. This prion protein was extracted from the sheep brain by homogenizing the brain stem (10%, w/v) in 0.32 *M* sucrose and by using a series of ultracentrifugation steps and treatment with sodium lauroyl sarcosine and proteinase K. After the final centrifugation step, the pellet was resuspended in 0.01 *M* Tris pH 7.4 in a volume equivalent to 0.1 ml/g of brain used. This resuspended pellet was treated with 1% SDS and 5% 2-mercaptoethanol and boiled for 10 min. The analysis was done in a Beckman P/ACE 5500 using a SDS gel capillary (eCap SDS14-200 Beckman capillary). In infected sheep brain samples, but not normal sheep, a major peak at a molecular mass of 16.1 kDa and a minor peak with a leading shoulder were observed. Since the molecular mass determined for this protein was lower than that estimated on Western blot (22.4 kDa), a Ferguson plot was made to determine if there were abberations in the molecular mass determination. After correction, the major peak was estimated to be 19.2 kDa. This has a better correlation with that determined by SDS-PAGE and Western blot. The equivalent amount of brain sample in the capillary was ~50 μg. For Western blot, the amount of brain sample was ~20 mg. For this assay, this is ~100 times less than that needed for Western blot for sheep samples.

Keywords: Proteins; Prion protein

1. Introduction

Transmissible spongiform encephalopathies (TSE) found in humans and in animals cause progressive degenerative disorders of the central nervous system resulting in death [1]. Scrapie in sheep is the prototype of these TSE. Since there is no known treatment for these diseases, detection of the presence of the disease causing agent early in infection before clinical signs appear could lead to better control of the disease. Scrapie has been described and known in sheep for 200 years [2] and there is no documented transmission to humans of this disease. TSE are relatively rare in other species including humans, but concern has increased recently because of the diagnosis in the British Isles of TSE [3] in cattle and the possibility of transmission to humans. This infection in the cattle has been attributed to the feeding of bone meal contaminated with the disease agent [4].

*Corresponding author.

PII S0378-4347(97)00133-3

The mechanism of pathogenesis of TSE is not clearly understood [5,6] but an accumulation in the brain of infected individuals of rod shaped fibrils that are aggregates of a modified normal cellular protein is characteristic of this disease [7,8]. This protein is modified by removal of 30–50 amino acids of the host cellular protein at the N-terminus [6,9]. The normal protein is ~40% α helix and contains 3% β pleated sheet, and the abnormal form is ~45% β pleated sheet with less α helix [10]. After this change in conformation, the abnormal protein becomes resistant to proteases and aggregates. These properties are used to partially purify the fibrils from infected brains [11,12]. When the aggregates are subjected to SDS-PAGE in the presence of β-mercaptoethanol and Western blot, a distinct pattern of banding occurs [13]. A major band is observed with a molecular mass of 21–29 kDa depending upon the source of the sample. Two bands of lesser intensity and smaller molecular masses are observed as well. There are many available antiserums that react with the dissociated aggregates on Western blot analysis [14]. Although Western blot is an adequate analytical method to detect these proteins, many species of animals (including sheep) have very low levels of abnormal protein present. Because of this problem, new sensitive methods of detecting this protein are needed. Reports on SDS-gel capillary electrophoresis for the analysis of immunoglobulins [15] and other proteins [16,17] led us to investigate the use of SDS-gel capillary electrophoresis for this protein. By using similar preparation methods that are used for conventional SDS-PAGE and Western blot, we were able to detect at least 100 times less prion protein than these methods.

2. Experimental

2.1. Preparation of sheep brain material

Scrapie infected brains were obtained from sheep that were confirmed positive by histological examination and by immunohistochemistry [18]. Normal brains were obtained from sheep from a scrapie free flock and confirmed negative by the above tests. The brain material was prepared by a modification of Bolton et al. [12]. Briefly, the brain stem was dissected from the whole brain, weighed, placed in 0.32 *M* sucrose (10% w/v), and homogenized for 60 s with a Brinkman Polytron (Kinematica AG, Lucerne, Switzerland). A 0.7 cm disposable generator was used at the highest speed setting. The homogenate was centrifuged at 10 000 *g* to remove particulates, and the resultant supernatant fluid was centrifuged at 230 000 *g* for 1 h. The pellet was resuspended in 0.02 *M* Tris pH 7.4, 0.15 *M* NaCl and centrifuged at 230 000 *g* for 1 h. This pellet was resuspended in 0.01 *M* Tris pH 7.4 containing 10% sodium N-lauroyl sarcosine and centrifuged at 230 000 *g* for 1 *h*. The pellet was resuspended in 0.01 *M* Tris pH 7.4 and incubated with proteinase K (10 μg/ml) for 1 h at 37°C and held overnight at 4°C and then centrifuged at 230 000 *g* for 1 h. The final pellet was resuspended in 0.01 *M* Tris pH 7.4 (100 μl/g of the initial brain sample).

2.2. Capillary electrophoresis conditions

Capillary SDS gel electrophoresis was performed on a Beckman P/ACE 5500 (Beckman Instruments, Fullerton, CA, USA) controlled by System Gold software (Beckman Instruments). An eCap SDS14-200 kit was purchased from Beckman as well. The effective length of the capillary to the detector was 47 cm. To prepare the capillary for sample injection the capillary was rinsed for 4 min with 1 *M* HCl and then with capillary gel buffer for 5 min. The test mixture provided with the kit was used as markers for the capillary. The markers included proteins of molecular masses of 14.2, 29, 45, 66, 97.4, 116, and 205 kDa. Samples were injected for 60 s by hydrodynamic flow. The voltage was 300 V/cm and the current was from 30–40 μA. Samples were prepared by boiling for 10 min in 0.06 *M* Tris–HCl 0.05% SDS pH 6.6 and 5% 2-mercaptoethanol. Orange G was added as a reference marker. The concentration of the SDS-gel buffer was used at 100% for all of the samples except when other concentrations are given. Because the prion protein is a glycoprotein, Ferguson plots were made according to the directions on the eCap SDS14–200 kit. Four different concentrations of the SDS-gel buffer (Beckman) were used, undiluted, 75, 67 and 50%. The test mixture (Beckman) and a sample from an infected sheep were run at each of the gel concentrations. The relative migration

time was calculated and used to generate the Ferguson plots. These calculations were then used to generate the curve for the corrected molecular masses. The capillary used to generate the electropherograms for the individual sheep was 57 cm to the detector. The voltage was adjusted so that it was 300 V/cm.

2.3. SDS-PAGE and immunoblot

Biotinylated low molecular mass markers (molecular masses 97.4, 66.2, 45.0, 31.0, 21.5 and 14.4 kDa) were purchased from Biorad Laboratories (Hercules, CA, USA). Prepared 10–15% gradient acrylamide gels were purchased from Pharmacia Biotech AB (Uppsala, Sweden) and run on the PhastGel system (Pharmacia Biotech AB) according to the instructions of the manufacturer. Immediately after electrophoresis, the gel was blotted onto nitrocellulose paper (Pharmacia Biotech AB) by diffusion. After placing the nitrocellulose paper on the gel, the temperature was raised to 70°C on the cooling plate of the Phastgel system for 20 min. The nitrocellulose paper was removed and placed in 5% non-fat dry milk in 0.02 *M* Tris–HCl pH 7.5 containing 0.5 *M* NaCl and 0.05% Tween-20 (TTBS) for 1 h. After washing the blot twice with TTBS, the blot was incubated with antibodies made to peptides of the prion protein for 2 h. After washing with TTBS, the blot was developed using the Amplified Alkaline Phosphatase Immun-Blot® Assay system (Biorad Laboratories).

3. Results and discussion

The molecular mass of the major band observed on Western blot was estimated to be 22.4 kDa (Fig. 1). Two other bands were observed at 18.2 kDa and at 16.4 kDa. These values for the molecular masses were the same whether a homogeneous gel at a concentration of 12% acrylamide or a gradient gel of 10–15% acrylamide was used. In Fig. 2, the plot of the molecular mass vs. 1/relative migration time for the SDS-gel capillary is shown. An inset shows the actual electropherogram of the markers used in the capillary and the arrows mark the positions at which the two peaks are observed for the scrapie prion protein. The molecular mass that was calculated for the prion protein using this method was 16.1 kDa. The Ferguson plot of the markers in the SDS-gel capillary at different concentrations of the buffer gel is shown in Fig. 3. A computer graphics program was used to calculate a linear curve fit for each molecular mass marker. The slopes were calculated from each of the linear curve fits and were used to calculate the retardation coefficient [$K_r = -1(\text{slope})$]. The r values had a correlation of 0.96 or greater. The same calculations were made for the prion protein. The plot of the square root of the retardation coefficient for the markers vs. the log of the molecular mass is shown in Fig. 4. The molecular mass of the prion protein is estimated to be 19.2 kDa by this method.

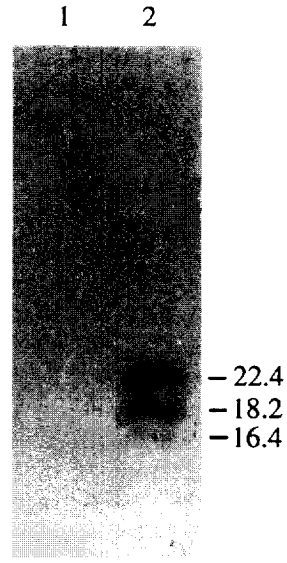

Fig. 1. Western blot of prepared samples of normal and scrapie infected sheep. Lane 1: brain sample from a normal sheep. Lane 2: brain sample of a scrapie infected sheep. The markers 97.4, 66.2, 45, 31, 21.5 and 14.4 kDa were used to make a standard curve. The molecular masses of 22.4, 18.4 and 16.4 kDa for the bands in lane 2 are indicated by the arrows.

Electropherograms showing samples from 4 normal sheep are shown in Fig. 5. As can be seen, the peak at 26.5 min is very low. A peak at 24.1 min is not present in these samples. The results showing the electropherograms of samples from 3 individual scrapie infected sheep and from a pool (SBT123) of 3 scrapie infected sheep is shown in Fig. 6. A major

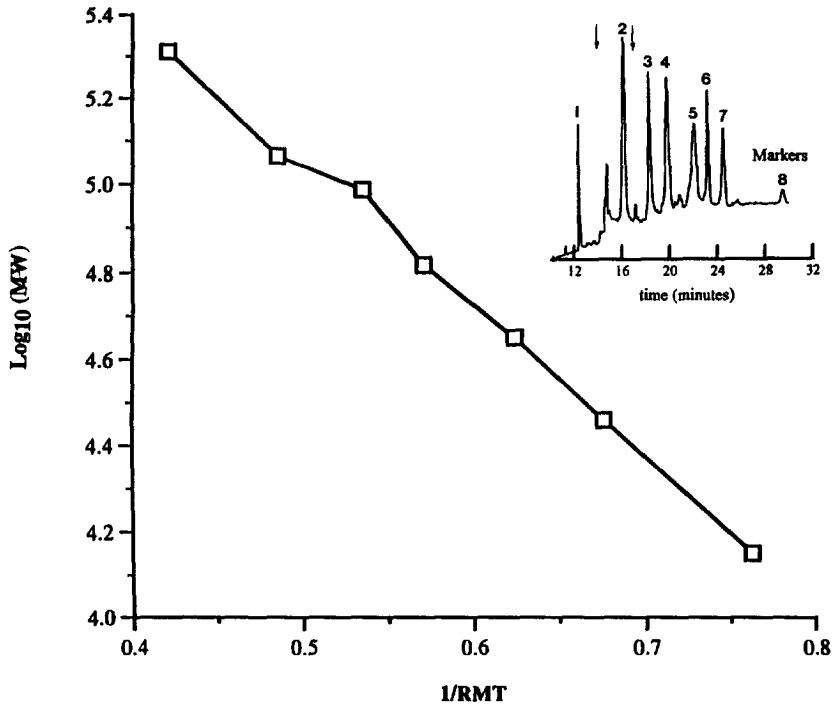

Fig. 2. Plot of the $\log_{10}$ molecular masses vs. 1/relative migration time. The molecular mass of the scrapie prion protein as calculated by this method was 16.1 kDa. Inset: markers from the test mixture provided in the eCap SDS14-200 kit. (1) Orange G, the reference marker, (2) 14.2 kDa, (3) 29 kDa, (4) 45 kDa, (5) 66 kDa, (6) 97.4 kDa, (7) 116 kDa and (8) 205 kDa. The two arrows indicate where the two peaks are observed for the scrapie prion protein.

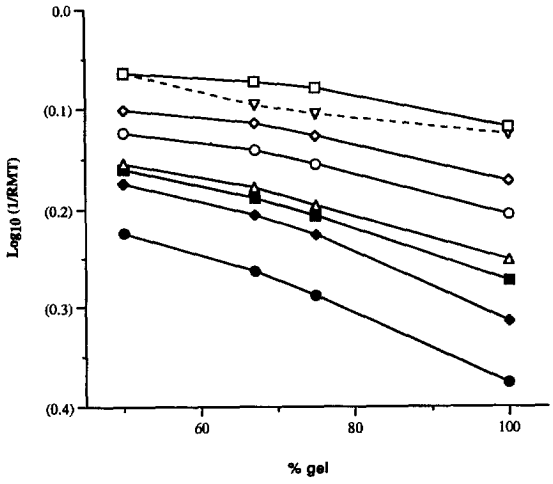

Fig. 3. Ferguson plot. Plot of the $\log_{10}$ [1/RMT] for the individual markers vs. the percentage of gel in the capillary. ▽ represents the plot of the scrapie prion protein at the different concentrations of gel in the capillary.

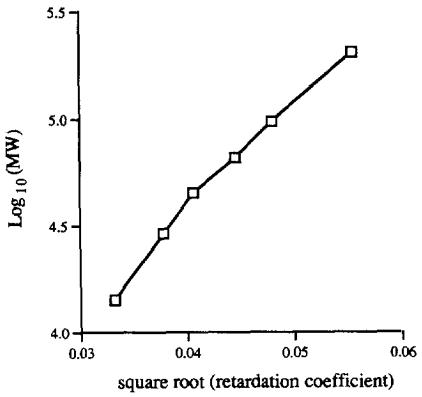

Fig. 4. Plot of the $\log_{10}$ molecular masses vs. the square root of the retardation coefficeint. The molecular mass of the prion protein as calculated by this method was 19.2 kDa.

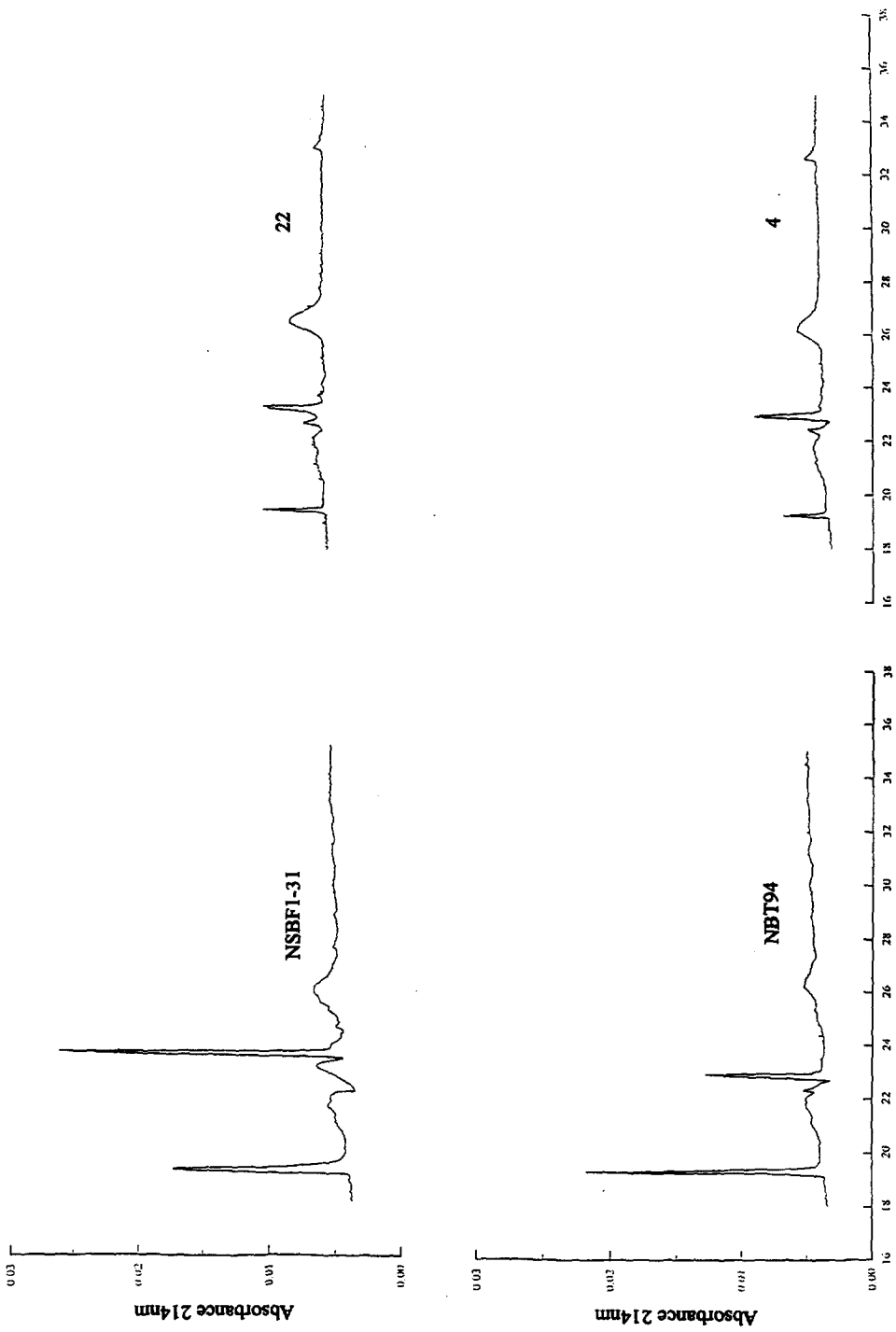

Fig. 5. Electropherograms of brain samples from normal sheep. The peak observed at 26.5 min for the scrapie infected sheep are either not present or are present in a very small amount as noted. Each panel represents an individual sheep.

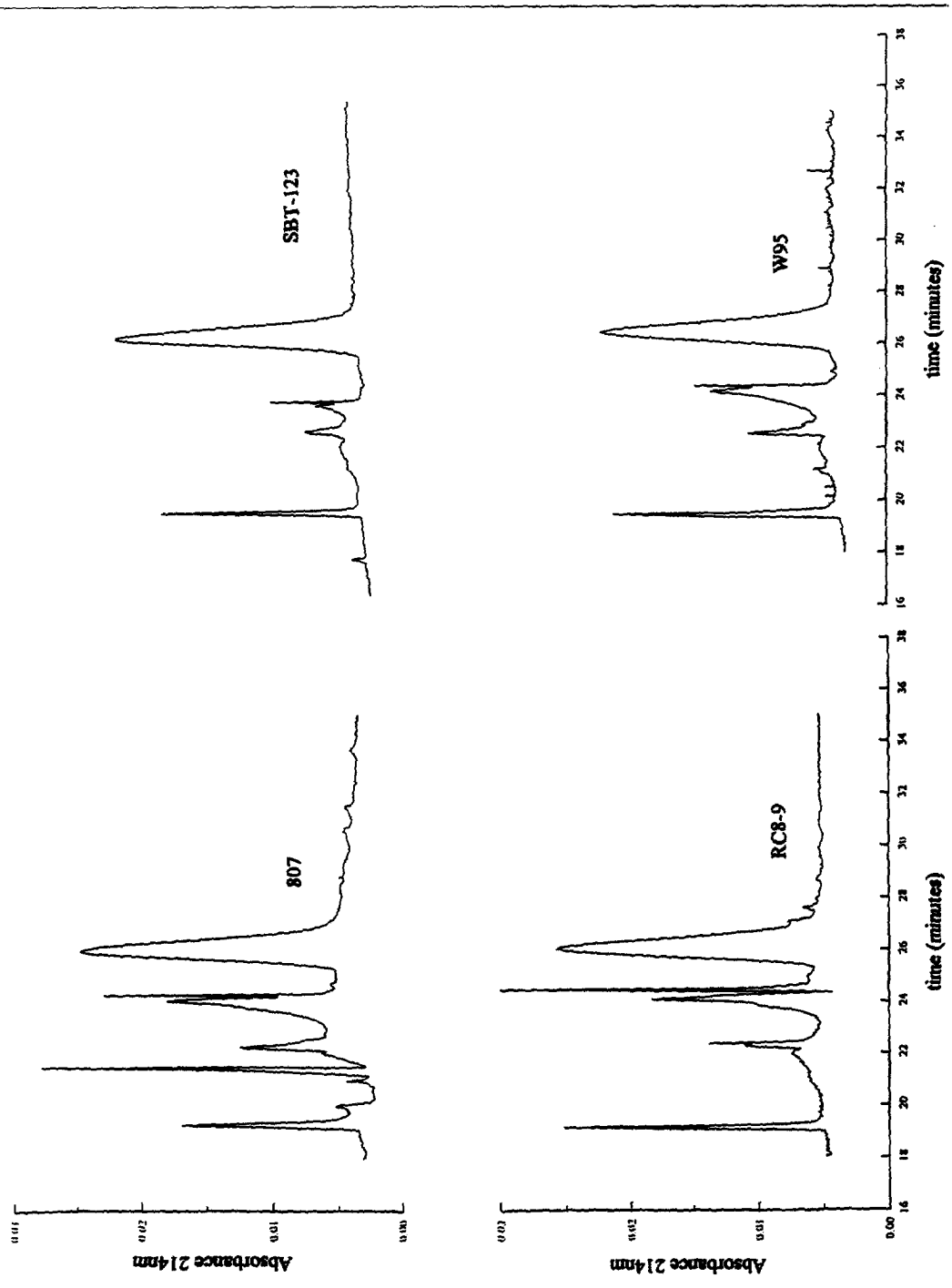

Fig. 6. Electropherograms of brain samples from scrapie infected sheep. One major peak at 26.5 min and a smaller peak with a shoulder are consistently observed. The second smaller peak at 24.1 min represents minor species of the prion protein and is outside the sizing capacity of this column. Each panel represents individual sheep, except for the panel SBT123 which represents a pool of three scrapie infected sheep.

peak is observed at 26.5 min. A smaller peak with a shoulder is observed at ~24.1 min. When these electropherograms are compared to those samples obtained from the normal sheep, the difference is obvious from the peak at 26.5 min. An accurate molecular mass could not be determined for the peak at 24.1 min, since this peak is outside the measuring range of the capillary. Occasionally, an unexplained peak appears between 22–23 min in all of the electropherograms. This peak may be a result of a carry-over of the detergents or reagents used to prepare the brain samples.

In these experiments, we found that SDS-gel capillary electrophoresis was a simple method that could be used to measure the amounts of prion protein. When brain samples from scrapie infected sheep were run using this method, it was easy to distinguish samples from these sheep from those of normal sheep. Although in this study we did not quantitate the minimum amounts of scrapie prion protein that can be detected by Western blot, others have reported this information for sheep [14,19]. In these reports, it was estimated that 100–800 mg of sheep brain may be required to detect the scrapie prion protein. In a few sheep, scrapie prion protein could be detected for amounts less than 100 mg of brain tissue. Larger tissue samples are required to perform immunohistochemistry [18]. This method did underestimate the molecular mass of the protein by about 3 kDa. After correction using a Ferguson plot, the molecular mass was reasonably close to that estimated by SDS-PAGE.

Although this method is not as sensitive as the capillary electrophoresis immunoassay based on competition for fluorescent labelled peptides from the prion protein [20,21], the preparation is rather simple in that it only requires denaturation in SDS. This method will be useful to monitor recoveries of prion protein as new methods are developed to extract the prion protein from tissues from infected animals, as well as determining the scrapie status of an individual sheep.

Disclaimer: No endorsements are herein implied. Brand names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standards of the products, and the use of the names by the USDA implies no approval of the products to the exclusion of others that may also be suitable.

References

[1] S.B. Prusiner, Biochemistry 31 (1992) 12277.
[2] I.H. Pattison, Vet. Rec. 123 (1988) 661.
[3] G.A. Wells, A.C. Scott, C.T. Johnson, R.F. Gunning, R.D. Hancock, M. Jeffrey, M. Dawson, R. Bradley, Vet. Rec. 121 (1987) 419.
[4] J.W. Wilesmith, J.B.M. Ryan, W.D. Hueston, L.J. Hoinville, Vet. Rec. 130 (1992) 90.
[5] D.A. Kocisko, J.H. Come, S.A. Priola, B. Chesebro, G. J Raymond, P.T. Lansbury, B. Caughey, Nature 370 (1994) 471.
[6] C. Weissmann, FEBS Lett. 389 (1996) 3.
[7] M.P. McKinley, R.K. Meyer, L. Kenaga, F. Fahbar, R. Cotter, A. Serban, S.B. Prusiner, J. Virol. 65 (1991) 1340.
[8] D.W. Fairbairn, R.N. Thwaits, G.R. Holyoak, K.L. O'Neill, FEMS Microbiol. Lett. 123 (1994) 233.
[9] B. Caughey, G.J. Raymond, D. Ernst, R.E. Race, J. Virol. 65 (1991) 6597.
[10] S.L. Kazmirski, D.O.V. Alonso, E.E. Cohen, S.B. Prusiner, V. Daggett, in D.R. Marshak (Editor), Techniques in Protein Chemistry VII, Academic Press, San Diego, 1996, p. 469.
[11] S.B. Pruisner, M.P. McKinley, K.A. Bowman, D.C. Bolton, P.E. Bendheim, D.F. Groth, G.G. Glenner, Cell 35 (1983) 349.
[12] D.C. Bolton, M.P. McKinley, S.B. Prusiner, Biochemistry 23 (1984) 5898.
[13] J. Collinge, K.C.L. Sidle, J. Meads, J. Ironside, A.F. Hill, Nature 383 (1996) 29.
[14] U. Oberdieck, Y.-G. Xi, M. Pocchiari, H. Diringer, Arch. Virol. 136 (1994) 99.
[15] J. Liu, S. Abid, M.S. Lee, Anal. Biochem. 229 (1995) 229.
[16] K. Tsuji, J. Chromatogr. B 662 (1994) 291.
[17] L.E. Bennett, W.N. Charman, D.B. Williams, S.A. Charman, J. Pharm. Biomed. Anal. 12 (1994) 1103.
[18] J.M. Miller, A.L. Jenny, W.D. Taylor, R.F. Marsh, R. Rubenstein, R.E. Race, J. Vet. Diagn. Invest. 5 (1993) 309.
[19] R. Race, D. Ernst, A. Jenny, W. Taylor, D. Sutton, B. Caughey, Am. J. Vet. Res. 53 (1992) 883.
[20] M.J. Schmerr, K.R. Goodwin, R.C. Cutlip, A.L. Jenny, J. Microcol. Chromatogr. 7 (1995) 521.
[21] M.J. Schmerr, K.R. Goodwin, R.C. Cutlip, A.L. Jenny, J. Chromatogr. B 681 (1996) 29.