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# Infectious scrapie agent is apparently not as small as recent claims suggest

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A potentially important claim that the minimum size of infectious scrapie agent is 50 000 $M_r$ or less has been investigated. Our results suggest that the agent is larger and that the original experiment was misinterpreted.

A recent report, based on the high-pressure liquid chromatography (h.p.l.c.) of a detergent-treated scrapie extract, claims that the infectious unit may have a $M_r$ of only 50 000 (1). Several studies, particularly those using proteinases (2-5), show that some protein or proteins are necessary for scrapie infectivity. Hence it has been argued that such a small size for the agent severely limits the amount of putative scrapie-specific nucleic acid that could also be present, and it has even been suggested that there is none (1). The latter suggestion conflicts with much biological data showing that scrapie agent behaves as an independent pathogen with its own coded information (6). These data make it likely that some scrapie-specific nucleic acid is present, though the amount could be very small, especially if the nucleic acid does not directly code for a specific protein (7). From these considerations it seemed essential to check whether or not the estimated value of 50 000 $M_r$ was correct.

## Materials and Methods

N-Tetradecyl-N,N-dimethyl-3-amino-1-propane sulfonate (sulfobetaine 3-14) was obtained from Calbiochem, Giessen.

H.p.l.c was performed on a TSK 4000 column (0.75 x 60 cm, LKB Instrument, Gräfelfing) which was equilibrated for 14 h with developing buffer containing 0.024% of sulfobetaine 3-14 (1). A 50-µl sample containing 5 µg of blue dextran, 20 µg of bovine serum albumin, 0.2 µg of tryptophan, and 10% sulfobetaine 3-14 was applied to the column. The eluate was monitored with a continuous-gradient collector equipped with a u.v. recording unit. Fractions of 1 ml were collected. To detect high detergent content in the eluate 0.1 ml of each fraction was mixed with 0.5 ml of reagent (8).

To study the sedimentation of scrapie agent on a sucrose gradient, brains were taken from 5 CLAC hamsters with clinical signs of scrapie

(strain 263K) and a $P_{215S}$ pellet was obtained as previously described (9). It was suspended by sonication (3 bursts for 5 s) in 0.06 M Tris-acetate (pH 7.2) containing 0.001 M EDTA and 10% sulfobetaine 3-14 and incubated for 16 h at 4°C (1). One tenth of the sample, i.e. 0.2 ml, was layered on top of a sterile, 5-20% sucrose gradient (13 ml) prepared in the same buffer but with only 0.024% sulfobetaine 3-14. Centrifugation was at 4°C, for 6 h at 200 000 **g** in a SW 41 rotor. A reference gradient containing bovine serum albumin (BSA) and ribosomal subunits from *Escherichia coli* (by courtesy of Prof. H. Wittmann, Max Planck-Institut für Genetik) was centrifuged simultaneously under identical conditions. The latter was analysed using a continuous-gradient collector equipped with a u.v. recording unit. Eight equal fractions were collected from the scrapie sample by needle puncture at measured distances down the side of the tube. The fractions were sonicated and 25 µl of each was diluted with 1 ml of phosphate-buffered saline and injected intracerebrally into 3 CLAC hamsters per fraction. All the assay animals developed scrapie and the average incubation period ± S.D. of each group was determined (9).

## Results and Discussion

In the original experiment (1), the 263K strain of scrapie was purified from hamster brain to about 100-fold with respect to protein (4). The preparation was exposed to 10% sulfobetaine 3-14 for 16 h and chromatographed on a molecular-sieve column (TSK 4000) in the presence of 0.024% sulfobetaine 3-14. Infectivity was found in a peak close to where bovine serum albumin eluted (1). However, this could be explained by adsorption of the agent to the column, in the presence of greatly reduced concentrations of detergent, and then desorption and elution with the bulk of the detergent if this migrated as a discrete micellar fraction. If this happened, then the estimated $M_r$ of 50 000 for scrapie could be clearly wrong.

Fig. 1 shows the elution profile of an h.p.l.c. experiment with TSK 4000 material run under the same conditions as those used with scrapie (1). The same sizing markers were used. Scrapie agent was not involved, as the position at which it elutes from the column has already been determined (1). Blue dextran, BSA, and the applied high amount of the detergent sulfobetaine 3-14 (10%) eluted in fractions 9, 18, and 20-21, respectively. The positions of these compounds did not change when run separately. The bulk of detergent therefore eluted exactly at the position where scrapie infectivity has been reported to come off the column (1). To prove further that the material eluting just behind BSA was the detergent, advantage was taken of the observation of Bradford (8) that detergents disturb the determination of proteins with Coomassie Blue. An analysis of the various fractions of the column eluate showed high detergent concentrations only in fractions 21-22 of the experiment. The low concentration of BSA in the applied sample did not disturb the determination of the detergent and the result was the same when sulfobetaine 3-14 was run separately. According to molecular-sieve chromatography, therefore, the size of the detergent micelles is less than $M_r$ = 65 000, a value not unexpected for polar detergents in salt solutions (10-12).

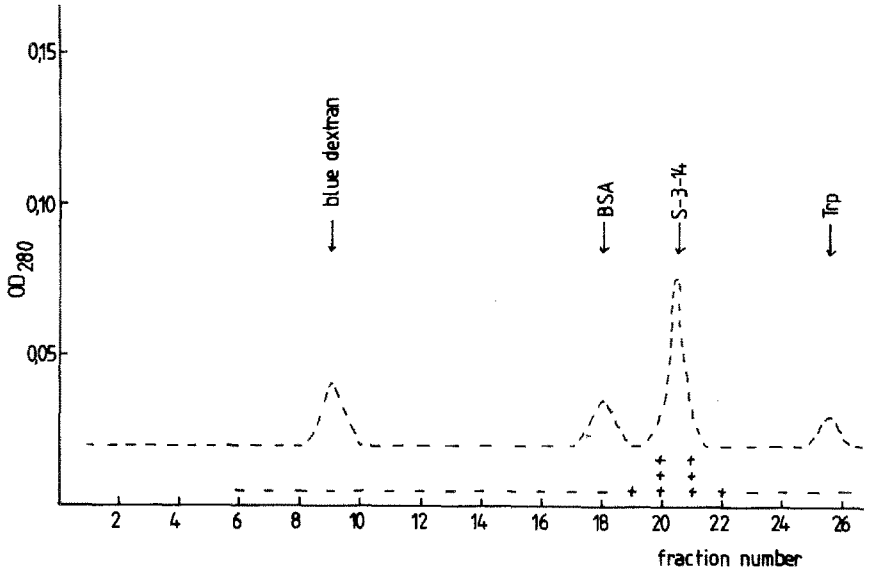


Fig. 1. H.p.l.c. profile of a sample containing blue dextran, bovine serum albumin (BSA), tryptophan (Trp), and sulfobetaine 3-14. Intensity of colour reaction with the Bradford reagent (+); negative reaction (-).

Although TSK columns are used principally for size exclusion chromatography, the column packing also displays both ion-exchange and hydrophobic interactions (13,14). Thus, on such column packing, the structure of the molecules being investigated and the experimental conditions used determine whether migration takes place solely by molecular sieving or is controlled by other properties of the column. The migration of readily soluble hydrophilic proteins such as ovalbumin and BSA, used as markers in this experiment, will not be significantly affected by non-covalent binding interactions with the column packing. However, given the poor solubility of scrapie which may be in part due to hydrophobic properties of the infectious units (4), it is probable that binding of scrapie infectivity to the column would take place. Binding would be facilitated because the column was equilibrated with only 1/400th of the detergent concentration in the sample used to solubilize scrapie infectivity.

The most likely explanation for the published result with scrapie (1) is therefore as follows: scrapie infectivity being larger than the sulfobetaine 3-14 micelles in the sample would tend to migrate in front of the detergent. In low concentrations of detergent (0.024% sulfobetaine 3-14 developing conditions), infectivity would tend to bind to the column via hydrophobic interactions. However, the high concentration of detergent (10%) added in the sample and travelling as a peak of micelles of $M_r \simeq 50\ 000$ would tend to elute infectivity. Accordingly the detergent would sweep infectivity out of the column as a peak: the size of the infectious unit would not be a factor in elution from the column.

To avoid problems of adsorption, we have carried out a similar experiment, using sucrose-density-gradient analysis instead of molecular-sieve chromatography. Infectivity was purified from scrapie brain to about 100- to 1000-fold on a protein basis, and by a modified method (9). The preparation was dissolved in 10% sulfobetaine 3-14, under exactly the same conditions, and then analysed on a sucrose density gradient made up in the same buffer as was used in the chromatography experiment (1). Eight fractions were collected after centrifugation and the relative distribution of infectivity was measured by comparing incubation periods in assay animals, as used in the original study.

Fig. 2 shows that fraction 8, at the bottom of the gradient, produced the shortest incubation time (76 ± 3 days: mean ± S.D.) indicating the highest amount of infectivity. This fraction was assigned a relative infectivity index of 100. Previous

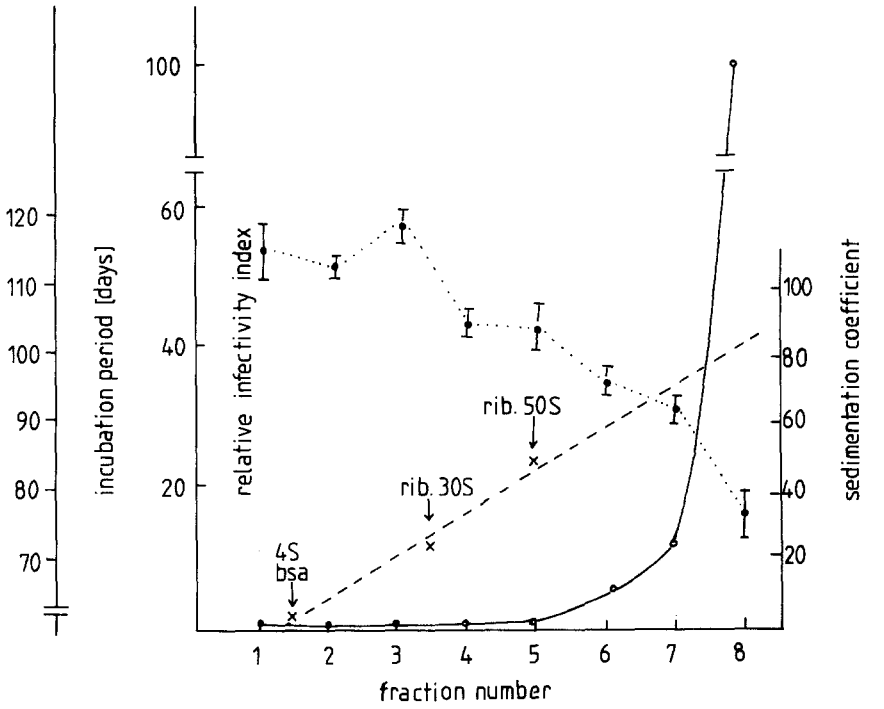


Fig. 2. Sedimentation of scrapie agent on a sucrose gradient. (••••) average incubation period ± standard deviation; (———) relative infectivity index, which was calculated from the incubation period. In the bioassay the amount of scrapie infectivity is highest in the fractions resulting in the shortest incubation period. A 12-days' increase in incubation period indicated a 90% loss of infectivity (9). (----) sedimentation coefficients of reference materials.

studies have shown that a 10-fold difference in infectivity is approximately equivalent to a 12-day difference in incubation period (9). Hence, fraction 7, which produced the next-shortest incubation time (92 ± 0 days), had a calculated infectivity index of 5, i.e. only 5% of the value of fraction 8. Fig. 2 therefore shows that at least 90% of the infectivity recovered from the gradient was at the bottom. The sedimentation of the reference materials indicated that most of the infectivity had a sedimentation value of > 70S (Fig. 2). This is in dramatic contrast to the expected result if scrapie infectivity had a size of 50 000 $M_r$: it should have remained at the top of the gradient with the BSA marker.

There are two possible interpretations of this finding. First, sulfobetaine 3-14 did not dissociate infectious scrapie agent into 50 000-$M_r$ units, in which case our suspicion that the authors misinterpreted their h.p.l.c. experiment is strengthened. Secondly, sulfobetaine 3-14 may have indeed produced very small infectious units but these reaggregated after migration into the gradient which had a much lower concentration of detergent (0.024% instead of 10%). This explanation has been used by the same authors to account for the sedimentation properties of scrapie exposed to other detergents when centrifuged through sucrose gradients containing none (15). If correct, then aggregation would also have occurred in the h.p.l.c. experiment, again invalidating their interpretation. However, reaggregation is an unlikely explanation of our results because an infectious unit of about the same size as BSA would have remained dissociated in 10% sulfobetaine 3-14 which, apart from some diffusion, stays at the top of the gradient.

We therefore find no evidence to support the claim that the infectious unit of scrapie has a molecular weight of 50 000 and we suggest that the h.p.l.c. experiment on which the claim was based was interpreted incorrectly. The minimum size of scrapie agent is probably larger than claimed although it still may be exceptionally small compared to known viruses as indicated by two further studies by the same group of authors (15,16). Unfortunately, no details of these studies have been published yet.

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