Sodium hydroxide renders the prion protein PrP\textsuperscript{Sc} sensitive to proteinase K

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Sodium hydroxide (NaOH) solutions are widely used for the purification of contaminated equipment, as they are known to inactivate a variety of pathogens. However, information about their effect on agents causing transmissible spongiform encephalopathy (TSE) is sparse and contradictory. Scrapie hamster brain homogenate, containing the disease-associated form of the prion protein (PrP\textsuperscript{Sc}), was exposed to NaOH. Kinetics studies showed that treatment of brain homogenate with millimolar concentrations of NaOH rapidly abolished the proteinase K-resistant form of the prion protein (PrP\textsuperscript{res}). NaOH treatment converted PrP\textsuperscript{Sc} into a protease-sensitive form, either in solution or when adsorbed to a metallic surface. If infectivity of TSEs is linked with PrP\textsuperscript{res}, the results imply that inactivation of TSE occurs more efficiently than currently assumed.

An important aspect in the prevention of prion disease transmission is the disinfection of potentially contaminated fluids, equipment and medical instruments. Sodium hydroxide (NaOH) solutions are commonly used as a disinfectant for surgical instruments or for the cleaning of production equipment. It is known that NaOH has the potential to lower the infectivity of different TSE agents (Brown et al., 1984; 1986; Di Martino et al., 1993; Prusiner et al., 1981). Unfortunately, these data are limited and contradictory (Tateishi et al., 1991; Taylor, 2000) and no inactivation kinetics of TSE agents by NaOH have been published so far. Therefore, the goal of this study was to investigate the effect of NaOH on PrP\textsuperscript{Sc} and to measure reduction kinetics. We have chosen an immunological approach: the measurement of PrP\textsuperscript{res} by Western blot analysis.

In a first set of experiments, hamster brain homogenate was exposed to different concentrations of NaOH. Stock brain homogenates (10% in Tris-buffered saline) from hamsters, which had been inoculated with the 263K hamster-adapted scrapie agent, were obtained from Bayer or BioReliance. The stock hamster brain homogenate was diluted with water to 0.125% and re-homogenized for 45 s with a Miccra D-8 drive and a DS-8/P dispersing tool at approximately 23 000 r.p.m. (ART-Labortechnik). NaOH concentrations were adjusted to the desired molarity by addition of 1/20 of the total sample volume using NaOH stock solution (0·2–2 M). pH values did not deviate by more than 0·3 units of pH from theoretical values, as measured by indicator sticks, and did not change significantly over the period of incubation. The suspension was neutralized after different incubation periods at room temperature by adding the same amount of HCl in 1 M HEPES (pH 7·0). Subsequently, the PK-sensitive forms of prion protein were digested as follows: the samples were incubated with PK (Roche Diagnostics) at a final concentration of 150 µg ml\textsuperscript{-1} for 1 h at 37°C. PK-resistant prion proteins (PrP\textsuperscript{res}) were detected by Western blot analysis, as described by Lee et al. (2000). The prion proteins were then sedimented by centrifugation for 1 h at 20 200 g and pellets...
were suspended in protein loading buffer, heated and serially diluted in 0.5 log₁₀ steps. Aliquots of the dilutions (15 µl) were separated on a 10% Bis/Tris NuPAGE gel (Invitrogen) and transferred onto a Nitrocellulose membrane (Invitrogen). Detection of prion proteins was performed using the 3F4 antibody (Signet) as the primary antibody and alkaline phosphatase-conjugated goat antimouse IgG₂a (Southern Biotech Associates) as the secondary antibody. Alkaline phosphatase activity was visualized using CDP-Star substrate (Applied Biosystems) and subsequent exposure to X-ray film.

The results demonstrate that treatment of the 0·125% scrapie-infected brain homogenate with 0·1 M NaOH eliminated the PrP res signal on Western blots within seconds. Incubation with 10 mM NaOH only had a minor effect on the PrP signal. Whereas with 25 mM NaOH, an intermediate reduction rate of the PrP res signal was observed; PrP res was below the detection limit after 15 min of incubation in NaOH (Fig. 1a). In control experiments, serial dilutions (0·5 log₁₀ steps) of NaOH-treated brain homogenates (0·1 M for 15 min) from infected and non-infected hamsters were analysed on Western blots without PK digestion. Prion proteins were observed in both scrapie-infected and non-infected samples, showing that NaOH treatment does not interfere with the immunological detection system (Fig. 1b). The PrP res signal was absent in samples containing non-infected brain homogenate, whether the samples were exposed to NaOH or not (data not shown).

The titre of the prion protein was determined by endpoint dilution in the Western blot assay. The relative PrP res titre of a sample was defined as the dilution factor of the sample where prion protein was still detectable on the blot. The reduction of PK-resistant prion proteins is expressed as the logarithmic reduction factor.

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LRF = \log \frac{\text{initial PrP res titre}}{\text{PrP res titre after NaOH exposure}}
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Logarithmic reduction factors were calculated for each NaOH concentration and at each time point. Treatment with 0·1 M NaOH resulted in a rapid reduction of at least 3·5–4 log₁₀ PrP res. Treatment with 25 and 50 mM NaOH also showed a reduction of 3·5–4 log₁₀ after a prolonged incubation time. With 10 mM NaOH, a minor reduction in PrP res was observed (Fig. 2).

It is known that the infectious agent of TSE may adhere to metal surfaces (Flechsig et al., 2001). Therefore, experiments were performed in which 263K-infected hamster brain homogenate was adsorbed to iron powder. Iron powder (0·6 g) (Fluka) was washed three times with water and three times with PBS. The iron powder was then re-collected either using a magnet or by low-speed centrifugation. The washed iron powder was exposed subsequently to 263K-positive hamster brain homogenate (1% in PBS) for 1 h at room temperature under constant agitation. After washing three times with PBS, the powder was split into 0·1 g aliquots and subsequently incubated with 0·1 M NaOH. After 15 min of incubation at room temperature, the samples were neutralized with HCl in 1 M HEPES. In
samples representing the starting point (T = 0 min), NaOH and HCl were mixed together prior to addition to the powder. In control samples, the homogenate-loaded powder was kept at neutral pH (no NaOH treatment).

Samples were either digested with PK (150 μg ml⁻¹ for 1 h at 37 °C in PBS) (+PK) or were kept untreated (in PBS) (–PK) followed by washing in PBS. The remaining proteins were dissociated from the iron particles by heating the iron powder samples in protein loading buffer. Proteins were then separated by SDS-PAGE and analysed by Western blotting. An aliquot representing the re-dissolved material from a surface of approximately 50 cm² was loaded per lane on the gel. Prion protein was detectable in all samples that were not treated with PK (–PK). In samples kept at neutral pH and digested with PK, a signal corresponding to PrP⁰ was detected (+PK). In samples incubated with NaOH, the PrP⁰ signal was seen only at the starting point (0 min); in samples incubated for 15 min in NaOH, no PrP⁰ signal was observed (Fig. 3).

According to the prion hypothesis, the aberrantly folded prion protein PrPSc is the infectious agent of TSEs (Safar et al., 1990). In numerous studies, it was shown that PK resistance coincides with TSE infectivity (Caughey et al., 1997; Lee et al., 2000, 2001; Race et al., 1998). However, conflicting results have also been reported. PK resistance of detergent-treated PrP⁰ did not correlate with scrapie infectivity when isolated prion rods were exposed to different solvents (Shaked et al., 1999; Wille et al., 2000) and PrPSc showed no PK sensitivity after incubation at temperatures where a significant loss in infectivity was observed (Somerville et al., 2002).

The results of the present study show that if traces of infectious prion proteins would contaminate metal surfaces, such as surgical instruments or production equipment in the blood industry, the PK resistance of these prion proteins would be eliminated efficiently by treatment with NaOH. Our results, which showed clearance of PrPSc (≥4 log₁₀ with 0.1 M NaOH at room temperature), are in good agreement with previously published single-point measurements that demonstrated loss of infectivity (≥4·8 log₁₀ of hamster scrapie and CJD in guinea pigs upon exposure to 0·1 M NaOH) (Brown et al., 1986). In addition, it seems that other groups may have obtained inactivation at high pH (>11): Somerville (2002) described inactivation at elevated temperature and stated, as unpublished results, that ‘similar effects are found with increasing pH (>pH 11)...’

Compared to earlier studies that reported contradictory results, we used a much lower concentration of prion protein, which might serve as an explanation for the differences observed. However, the final concentration of 0.125 % brain homogenate is much closer to the expected real-life situation. The results of this study, obtained in biochemical in vitro experiments, form a good basis for further investigations. Further studies, especially the direct determination of infectivity in bioassays, need to be done to confirm the in vitro results.

If the infectivity of TSE agents is obligatorily linked to PrP⁰, our results imply that a commonly used cleaning procedure for the decontamination of metal surfaces (NaOH treatment) can substantially lower the risk of TSE cross-contamination. This inactivation of TSE occurs much faster and more readily than currently assumed. Furthermore, based on this study, a method for the disinfection of metal surfaces, such as surgical instruments potentially contaminated with TSE, can be proposed: equipment should be soaked for a short period of time in NaOH and the protease-sensitive prion proteins can then be removed with PK treatment.
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REFERENCES


