Prions, infectious agents causing transmissible spongiform encephalopathy, retain infectivity even after undergoing routine sterilization processes. We found that MSK103 protease, identified in our previous study, effectively reduces infectivity and the level of misfolded isoform of the prion protein in scrapie-infected brain homogenates in the presence of SDS. The treatment therefore can be applied to the decontamination of thermolabile instruments.

Key words: prion inactivation; protease; scrapie; bioassay; protein misfolding cyclic amplification

Prions are the infectious agents of transmissible spongiform encephalopathies (TSEs), including bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep and goats, and Creutzfeldt-Jakob disease (CJD) in humans. TSE diseases are characterized by accumulation in the central nervous system of PrP\textsuperscript{Sc}, a proteinase K (PK)-resistant misfolded isoform of the cellular prion protein PrP\textsuperscript{C}. Since prions are highly resistant to conventional sterilization procedures, severe physical and chemical sterilization methods, such as high-pressure sterilization at 134°C for 18 min or strong alkali treatment, are required to reduce infectivity.

The development of a secure, simple, and effective disinfection method is required as a preventive measure against prion diseases. We recently identified a Bacillus strain that produced protease exhibiting high-degradation activity against scrapie- and BSE-derived PrP\textsuperscript{Sc}. The isolated protease (MSK103) was found to degrade PrP\textsuperscript{Sc} samples more effectively than proteinase K (PK) or a keratinase. Since this protease can be used under moderate conditions (optimum pH and temperature ranges were 9–10 and 60–70°C), it can be used to decontaminate fragile, precision, and expensive instruments that are susceptible to PrP\textsuperscript{Sc} contamination.

In the present study, we investigated the efficacy of the MSK103 protease in scrapie prion inactivation. Since protease activity is generally enhanced in the presence of SDS, we examined the additive effects obtained by the combined use of the protease and SDS. The hamster-adapted scrapie strain Sc237 was propagated in the Syrian golden hamster. The brains of hamsters at the terminal stage of the disease, titrating 5 \times 10^{8.5} LD\textsubscript{50} per gram by bioassay, were pooled and homogenized at a concentration of 10% (w/v) in 50 mmol Tris–HCl buffer (pH 9.0). The homogenates were digested with MSK103 protease at a final concentration of 0–8 units (U)/ml at 50°C for 20 h in the presence of 2% SDS. In a preliminary experiment, PrP\textsuperscript{Sc} samples containing various concentrations of SDS were digested and analyzed by western blot (WB). The addition of 2% SDS to the reaction was most effective for the degradation of PrP\textsuperscript{Sc}. Effective PrP\textsuperscript{Sc} decontamination was observed at a protease concentration of 2–3.8 U/ml against BSE-infected brain homogenate and dried Sc237 PrP\textsuperscript{Sc}, which had firmly attached to a plastic surface in our previous study. One unit of enzyme activity was defined as the amount of enzyme required to release 1 μmol of glycine per min in a 0.5% solution of human hair-derived keratin powder at 60°C for 1 h at pH 8.0.

The digested samples were centrifuged at 200,000 \times g for 30 min and a supernatant was carefully removed. No PrP\textsuperscript{Sc} signals were detected in the supernatant by WB analysis. After they were washed three times, the precipitates were resuspended in an initial volume of...
phosphate-buffered saline (PBS). The samples were separated by SDS–polyacrylamide gel electrophoresis (PAGE) and transferred to a polyvinylidene fluoride membrane (Millipore, Bedford, MA). After blocking, the membranes were incubated for 1 h with alkaline phosphatase (AP)-conjugated 3F4 antibody (Signet Laboratories, Dedham, MA; 1/10,000) or horseradish peroxidase (HRP)-conjugated T2 antibody (1/10,000). After washing, the blotted membranes were developed with Immobilon western chemiluminescent AP or HRP substrates (Millipore). Chemiluminescence signals were analyzed using a Light Capture system (Atto, Tokyo).

Figure 1 illustrates the results of WB analysis of the digested samples in the absence or presence of SDS. Residual PrPSc was detected with 3F4 or T2 monoclonal antibodies, which recognize different epitopes of the PrPSc core fragments. As expected, residual PrPSc was significantly reduced in the presence of SDS. Although the PrPSc signal was detected in the sample digested with 2 U/ml of the protease, even in the presence of SDS there were no detectable signals in the samples treated with either 4 or 8 U/ml of the protease. When concentrated to a higher level (16 U/ml), a faint signal of residual PrPSc was detected by WB analysis, suggesting that protease activity somewhat, decreased probably due to self-digestion of the protease. Hence, the samples treated with a combination of the protease (2–8 U/ml) and SDS were analyzed in further experiments.

In addition to conventional WB analysis, protein misfolding cyclic amplification (PMCA), a highly sensitive method of detecting minute amounts of PrPSc, was performed as in our previous study. Briefly, the digested sample was diluted 1:10 in 10% uninfected brain homogenate, and one round of the PMCA reaction was performed by applying 40 cycles of sonication followed by incubation at 37°C for 1 h. Next, a process diluting the PMCA product to 1:10 and its subsequent amplification was repeated twice. With regard to the PMCA products, samples (10 µl) collected before and after each round of amplification were mixed with 10 µl PK solution (100 µg/ml) and incubated at 37°C for 1 h. The digested materials were analyzed by WB using the 3F4 antibody, as described above.

Figure 2 illustrates the results of amplification of the samples treated with MSK103 protease. In the first round of amplification, weak PK-resistant PrP (PrPres) signals were detected in the sample treated with 4 U/ml of the protease in addition to the sample treated with 2 U/ml of the protease. In the second round of amplification, the signals of both these samples were enhanced, and a weak signal was observed in the sample treated with 8 U/ml of the protease. After three rounds of amplification, PrPres signals were detected in all the digested samples. These results indicate that a trace level of PrPSc, which was not detected by conventional WB, remained in the samples treated with 4 or 8 U/ml of the protease.

The digested samples were inoculated intracerebrally (20 µl per mouse) into five Tg52NSE transgenic mice that overexpressed hamster PrPSc in their nerve systems. The advantage of this bioassay is its ability to confirm the onset of the disease a short period of time after inoculation. The control mice inoculated with the source only was treated in the same manner. On the other hand, two out of the five mice that were inoculated with samples treated with either 4 or 8 U/ml of the protease survived for more than...
600 d, although the remaining mice developed the disease after an average period of 170 ± 73 d (4 U/ml) and 151 ± 62 d (8 U/ml). The survival times of the affected mice inoculated with the samples treated with the protease were statistically analyzed by one-way ANOVA. There were no significant differences among the survival times of the three experimental groups. Although several approaches to prion inactivation by enzymatic degradation have been reported, the infectivity of the degradation products was not examined in some studies.5,12,13) Where bioassay was performed, only enzymatic degradation have been reported, the infectivity of the survival times of the three experimental groups. ANOVA. There were no significant differences among the protease were statistically analyzed by one-way ANOVA. There were no significant differences among

Fig. 3. Survival Curves of Tg52NSE Mice Inoculated with Infected Brain Homogenates Treated with MSK103 Protease.

The control and digested samples were injected intracerebrally into five mice. No treatment (solid line, □), SDS alone (solid line, ●), 2 U/ml (solid line, △), 4 U/ml (broken line, ▲) and 8 U/ml (dotted line, □).


