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ORIGINAL ARTICLE

A rapid method for detection of PrP by surface plasmon resonance (SPR)

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Abstract Surface plasmon resonance was used to develop a rapid, label-free and sensitive immunoassay for detection of Prion protein (PrP). Anti-PrP monoclonal antibodies immobilized on the biosensor surface were allowed to bind various concentrations of cellular prion protein (PrP^C), followed by a pulse with additional soluble anti-PrP polyclonal antibodies to intensify the signal. The interaction of antibody with antigen was monitored in real time. With this method, it was possible to detect PrP^C with a limit of 31.7 ng/ml in serum and 13.1 ng/ml in HEPES-saline, requiring 1 h for a single sample measurement. The assay also detected misfolded prion protein (PrP^{Sc}) in spiked serum and PrP^{Sc} in scrapie-infected mouse brains. This is a rapid and sensitive assay for the detection of PrP in serum that could be developed into a platform for a serum-based TSE test.

Abbreviations

PrP	Prion protein
PrP^C	Cellular prion protein
PrP^{Sc}	Misfolded prion protein
SPR	Surface plasmon resonance

Introduction

Prion diseases, also called transmissible spongiform encephalopathies (TSEs), are a group of infectious and invariably fatal neurodegenerative diseases, including Creutzfeld–Jakob disease (CJD), bovine spongiform encephalopathy (BSE), chronic wasting disease (CWD) and scrapie. Prions are comprised mainly or exclusively of a misfolded prion protein named misfolded prion protein (PrP^{Sc}), which is a post-translationally modified version of the normal prion protein (PrP^C) [1, 2]. PrP^C is present in abundance in most tissues of the central nervous system (CNS), although its precise physiological function remains unknown. PrP^{Sc} differs from PrP^C in its insolubility, its higher content of β-sheet structure, its partial resistance to protease digestion, and its tendency to form large aggregates [3]. Scrapie has been known in sheep for over 250 years. BSE was first identified in 1986, with over 2,00,000 confirmed cases in cattle to date. Both have had major agricultural and economic impact in Western Europe and the USA [4, 5]. In 1996, a variant of CJD (vCJD) was described in young human adults that had atypical clinical features and neuropathology [6] and was transmitted by the consumption of beef contaminated by the BSE infectious agent [7]. The establishment of a link between vCJD and BSE has raised considerable concern about a potential epidemic in the human population [7, 8]. Additionally, a risk of human-to-human transmission has now been clearly identified following the detection in Great Britain of four cases linked to blood transfusions [9]. Within the last decade, BSE has become a substantial health problem affecting many countries [10], and there is a pressing need for rapid and sensitive diagnostic methods to identify infected animals in order to define the extent of the epizootic and to prevent transmission to humans. In addition, scrapie has become a diagnostic focus due to the postulated danger of BSE transmission to sheep [11].

Current prion detection methods employ post-mortem analysis after suspicious animals manifest one or more

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symptoms of the disease. There are five commercially available BSE testing kits that are approved by the Commission of the European Communities for the testing of bovine brain material [12]. These utilize either a western blot or a variation of a standard ELISA (i.e., enhanced chemiluminescent or sandwich immunoassay) for the post-mortem detection of PrP^{Sc} in CNS tissue. Although such tests are currently widely used, they are expensive and time-consuming and not easily automated or standardised for routine large-scale diagnostic screening. Consequently, prion infectivity bioassays remain the most sensitive and specific methods for detecting prion diseases. However, bioassays are limited in scope because of the long time required for disease to develop in test animals (usually rodents) and possible limitations due to cross-species barrier effects on transmission [13].

Based on estimates of PrP^{Sc} levels during the incubation period, it seems unlikely that the current analytical methods can identify cattle that are in the early phases of disease [14, 16]. It is therefore possible that infected animals without clinical signs and negative by the available biochemical tests could enter into the food chain, thereby imposing a serious risk to human health. Pre-symptomatic detection of CJD in living patients is also not possible [15, 16]. To improve food safety and public health, it would be beneficial to screen all animals for prion disease before death, whether or not symptoms are present. For such testing to be performed on pre-symptomatic animals, it would be necessary to be able to detect extremely small amounts of Prion protein (PrP) circulating in blood samples and to differentiate between PrP^{C} and PrP^{Sc} [17]. Some recent approaches for ante-mortem diagnosis include application of newly developed ligands against PrP^{Sc} [18], conformation-dependent immunoassays [19], spectroscopic techniques [20], and PrP^{Sc} protein misfolding cyclic amplification (PMCA) [21].

Of these methods, PMCA has the greatest potential for development as a blood test. However, PMCA has not been clinically useful for prion detection, since several issues remain. For example, PMCA is extremely time-consuming and laborious, requiring days or weeks to attain maximal sensitivity, and it requires whole-brain homogenates. PrP^{Sc} molecules derived from some prion strains might also prove difficult to amplify in vitro.

For these reasons, a rapid, simple and sensitive screening and testing method for PrP^{Sc} is still required. Recently, surface plasmon resonance (SPR)-based sensing has been used for studies on biomolecular interactions [22]. SPR sensing is based on the detection of a small refractive index change on a thin metal film caused by interactions between biomolecules such as antigen–antibody complexes [23]. SPR sensors have been applied to the detection of pathogens, toxins, proteins and nucleotides [24–27]. SPR-based biosensor detection provides the advantage of being able to monitor biomolecular interactions rapidly in real time without the need for labels, while, being an automated system that provides high accuracy, sensitivity and reliability [28]. In addition, optical biosensors based on SPR show promise for development of high-throughput systems for parallel measurements [29].

In the present study, PrP has been detected using a SPR biosensor, and the lowest detectable concentrations of PrP have been established. This was achieved by covalently immobilizing primary antibody against PrP on a thin gold film through amide bonding in a CM5 biosensor chip. Then, buffer or serum containing PrP and a secondary antibody solution were sequentially applied in a sandwich-type immunoassay. By these means, we succeeded in detecting PrP at the nanogram level, as would be required for the food industry and for clinical diagnosis. This investigation also highlights some of the challenges facing development of a serum-based TSE assay.

Materials and methods

Chemicals

HBS-EP2 buffer [10 mM HEPES (pH 7.4), 150 mM NaCl, and 3 mM EDTA], the CM5 sensor chip, the Biacore amine-coupling kit, which includes *N*-hydroxyl-succinimide (NHC), *N*-ethyl-*N*-(3-diethylaminopropyl) carbodiimide (EDC), and ethanolamine hydrochloride, and glycine/HCl buffer were obtained from Biacore AB (Uppsala, Sweden). Recombinant mouse prion protein (Chemicon, Temecula, CA, USA), primary antibodies (monoclonal antibodies 6H4) (Pronics, Schlieren, Switzerland) and secondary antibodies (polyclonal antibodies) against amino acids 23–231 of mouse PrP^{C}($mPrP^{C}$) (Chemicon, Temecula, CA, USA) were all obtained from commercial sources. Ethanolamine, sodium acetate (NaOAc) and sodium dodecyl sulfate (SDS) were obtained from Sigma-Aldrich (St. Louis, MO 63103, USA). Other chemicals were purchased from standard commercial sources at analytical grade.

Immobilization of antibodies

SPR-BIA was performed with a Biacore 3000 instrument (Biacore AB, Sweden) using HBS-EP2 as a running buffer. The flow rate of the HBS-EP2 buffer and the temperature of the SPR system were maintained at 5 μl/min and 25°C, respectively. Primary antibodies were covalently immobilized on the carboxymethyl dextran of a CM5 sensor chip in flow cells using the amine-coupling method. Initially, the CM5 dextran matrix was activated by passing an equal mixture of freshly prepared 0.4 M EDC and 0.1 M NHS

for 7 min at 5 µl/min to produce receptors (NHS esters) capable of binding to the amino group of primary antibody 6H4 to form a covalent amide bond.

Prior to 6H4 immobilization, studies of pre-concentration of 6H4 on the CM5 sensor chip surface were performed to determine the optimum pH of the antibody immobilization buffer. These studies were carried out under a continuous flow of HBS-EP2 buffer (5 µl/min). Twenty micrograms per ml of 6H4 diluted in acetate buffers of various pHs were injected over the sensor chip for 2 min. Ten millimolar acetate buffers ranging from pH 4.0 to 5.5 were used in these pre-concentration experiments.

Next, after the immobilisation of 6H4, the surface was repeatedly washed with HBS-EP2 buffer to remove unbound antibodies. A 7-min injection (at 5 µl/min) of 1 M ethanolamine (pH 8.5) was used to quench excess active succinimide ester groups. The effectiveness of protein coupling was monitored according to the sensorgrams obtained after 6H4 injection. The difference in the resonance units (RU) at the beginning and the end of the coupling reaction represents the quantity of 6H4 bound to the chip.

Detection of PrP^C in buffer and chip regeneration

The interaction of PrP^C with immobilized primary antibodies was monitored at 25°C using HBS-EP2 as a running buffer at a flow rate of 5 µl/min. Dilutions of PrP^C were prepared in HBS-EP2 to 20, 50, 100, 200, 500, 1,000 ng/ml and injected for 2 min. As a control, buffer alone was injected in the same way and its reading subsequently subtracted from the PrP^C response signal. In an effort to enhance the signal obtained by direct assay, a sandwich assay was performed with different concentrations of PrP^C. The concentration of secondary antibodies was 500 µg/ml. The direct assay was followed by a 3-min pulse of HBS-EP2 to wash off unbound PrP^C from the surface. Secondary antibodies in HBS-EP2 were then added to complete the sandwich assay. The sensorgrams obtained for the different concentrations of PrP^C and blank at 50 µg/ml primary antibody and 500 µg/ml secondary antibodies concentrations were compared. The lowest concentration of PrP^C that produced a signal distinguishable from the next lower concentration defined the limit of detection sensitivity. Antigen-secondary antibody complexes were then dissociated, and the surface was regenerated by passing HBS-EP2 for 3 min, followed by 20 mM NaOH for 7 min. Sensorgrams were corrected by subtracting the signal from the reference flow cell, and changes in the SPR signal were measured to evaluate the binding of PrP^C to the primary antibody, the complex to PrP^C, and the secondary antibody to the primary antibody.

Detection of PrP^C in mouse serum

The surface on which the primary antibody had been immobilized was used as the sensing surface for the detection of PrP^C in mouse serum. To reduce non-specific protein adsorption, sera were diluted with HBS-EP2 buffer (serum: HBS-EP2 buffer = 1:5, v/v). PrP^C was resuspended in serum at various concentrations and passed over the sensing surface by injecting a 25-µl volume. Subsequently, 25 µl of polyclonal antibody (0.5 mg/ml) was injected to enhance the response signal of SPR.

Detection of PrP^{Sc} in mouse serum

Mice at advanced stages of disease were killed using exposure to carbon dioxide. Scrapie brain homogenates were prepared as described by Castilla et al. [30]. Samples were diluted 1:200 (v/v) with mouse serum. To differentiate PrP^{Sc} from PrP^C, samples were incubated with 50 µg/ml proteinase K (PK, Roche, Indianapolis, IN, USA) for 45 min at 45°C. The digestion was stopped by adding HBS-EP2 buffer (serum: HBS-EP2 buffer = 1:5, v/v), and samples were quantified individually by SPR and western blot, the latter being determined as described by Castilla et al. [30]. Each experiment included a negative control consisting of normal brain homogenate. This was essential in order to check that PK digestion of PrP^C was complete, so as to avoid confusion between undigested PrP^C and the signal from the PK-resistant core of PrP^{Sc}.

Application of SPR to analysis of PrP^{Sc} in infected mouse brain

The SPR assay was then further tested using brain homogenates from healthy mice and mice infected with the scrapie strain. Ten percent brain homogenates were prepared using the general protocol described previously [30] in 50 mM Tris–HCl buffer, pH 7.6, containing 100 mM NaCl. The homogenate was mixed with an equal volume of detergent buffer (50 mM Tris–HCl buffer, pH 7.6, containing 4% Zwittergent 3–14 [Calbiochem], 1% sarkocyl and 100 mM NaCl) and then incubated with 50 µg/ml PK at 45°C for 45 min. The digestion was stopped by adding 200 volumes of HBS-EP2 buffer, and samples were quantified by SPR and western blot, with the latter being carried out as described by Castilla et al. [30].

Statistical analysis

Triplicate experiments were run for every SPR experiment. The software SPSS (Version 11.0) was used for statistical analysis.

Results

Immobilization of antibodies

When the immobilization buffer containing monoclonal antibody 6H4 flows over the CM5 sensor chip surface, the relative response level indicates the amount of absorbed antibody during pre-concentration. The amount of absorbed capture antibody 6H4 increased with increasing pH of the immobilization buffers and reached a maximum (26,508.5 RU) at pH 4.75, above which a decrease in the amount of absorption was observed. The amount of protein adsorption also depends on the protein concentration, an increase in which should lead to increased electrostatic adsorption. The ideal protein concentration for immobilization is the lowest concentration that gives maximum electrostatic adsorption. In the following experiments, primary antibody (0.1 mg/ml) in HBS-EP2 containing 10 mM sodium ions was chosen for immobilization. The response of 0 mg/ml 6H4 antibody in HBS-EP2 was 5.1–8.5 RU and served as a control, and all of the RU values of 6H4 antibodies were significantly different ($P < 0.05$).

Detection of PrP^C in HBS-EP2 buffer

After immobilization of primary antibody 6H4 on the CM5 sensor surface, the analyte (PrP^C) in HBS-EP2 buffer was allowed to flow over the sensing surface. Response of the antigen (PrP^C)-monoclonal antibody (6H4) reaction was measured in real time using the BIAcore 3000 system. Subsequently, polyclonal antibody was injected over the sensor surface to achieve better sensitivity and specificity. This situation is comparable to sandwich-type ELISAs. Figure 1 shows a typical sensorgram for the detection of PrP^C and enhancement assay (100 ng/ml PrP^C in HBS-EP2 buffer). In phase a of Fig. 1, the increased signal shows the binding of 100 ng/ml PrP^C to the surface-immobilized primary antibody. Then, the signal was enhanced by injecting 0.5 mg/ml polyclonal antibody over the PrP^C binding surface, as shown in phase b of Fig. 1. The primary response (ΔRU_1 in Fig. 1) is 66.5 RU; and the enhanced response (ΔRU_2 in Fig. 1) is about 179.3 RU.

The results for the detection and enhancement assay of PrP^C in HBS-EP2 buffer are illustrated in Figs. 2, and 3a shows the linear range of the calibration data. For the primary response, the linear regression equation was (ΔRU) $y = 0.4172x + 21.669$ ($R^2 = 0.9991$, $n = 5$), where y and x are the relative RU response and analyte concentration, respectively. The relative standard deviation (RSD) of the blank sample was 0.96. For the amplified response, the linear range was 20–500 ng/ml PrP^C, and the linear regression equation was (ΔRU) $y = 0.9036x + 89.853$ ($R^2 = 0.9998$, $n = 5$). The RSD of the blank sample was 3.87. The lower detection limit of PrP^C, defined as the concentration corresponding to the mean blank reading plus twice the SD, was calculated as 13.1 ng/ml for the signal-amplified immunoassay and 18.9 ng/ml for the direct immunoassay. The error bars illustrate the RSD for three replicates in the concentration range 20–500 ng/ml and 10 replicates of the blank sample. The noise can be seen from the intercepts of the regression equations (Fig. 3a), where the primary and enhanced response of the blank sample were 21.669 and 89.853 RU, respectively. For SPR biosensor assays, the sandwich method can be used to amplify the response by introducing the analyte-specific antibody for better sensitivity and specificity of the analyte concentration assay. From the above results, we know that the sensitivity was improved from 0.4172 to 0.9036 RU (ng/ml) after sandwich amplification.

Detection of PrP^C added to mouse serum

Results for the detection and enhancement assay of PrP^C in mouse serum are given in Fig. 2, and 3b reveals a good linear relationship at low concentrations of PrP^C added to mouse serum. In Fig. 3b, the linear regression equation for the primary response in serum is $y = 0.2862x + 72.655$ ($R^2 = 0.9979$, $n = 4$). The RSD of the blank sample is 4.63, and the limit of detection (LOD) is 45.3 ng/ml. Using polyclonal antibody for the enhanced response, the linear regression equation is $y = 0.5339x + 94.314$ ($R^2 = 0.9997$, $n = 4$), with RSD 4.06 and LOD 31.7 ng/ml. The slopes of the equations show that sensitivity improved from 0.2862 to 0.5339 RU per concentration unit (ng/ml). The sensitivity was improved by a factor of 1.87 (from 0.2862 to 0.5339 RU).

Detection of PrP^{Sc} added to mouse serum

To study the feasibility of using the proposed SPR to measure PrP^{Sc} levels in mouse serum, we added various concentrations of PrP^{Sc} (high, medium and low) to mouse serum samples for a recovery test. Aliquots of 25 μL PK-treated mouse serum were then applied to the sensing surface and assayed as described above. As shown in Table 1, satisfactory recovery test values were realized within the linear concentration range. A 97.3% recovery was achieved for the high-concentration spiked samples and a 95.2% recovery for the 36 ng/mL samples. The mean recovery of PrP^{Sc} was 96.3% ($n = 9$). Recovery was not achieved for PK-treated samples containing 50.0 ng/mL PrP^C, and a 47.1% recovery was achieved for PK-treated samples containing 50.0 ng/mL PrP^C and 50.0 ng/mL PrP^{Sc}. The experimental results confirm that the proposed sensing system is applicable for PrP^{Sc} detection.

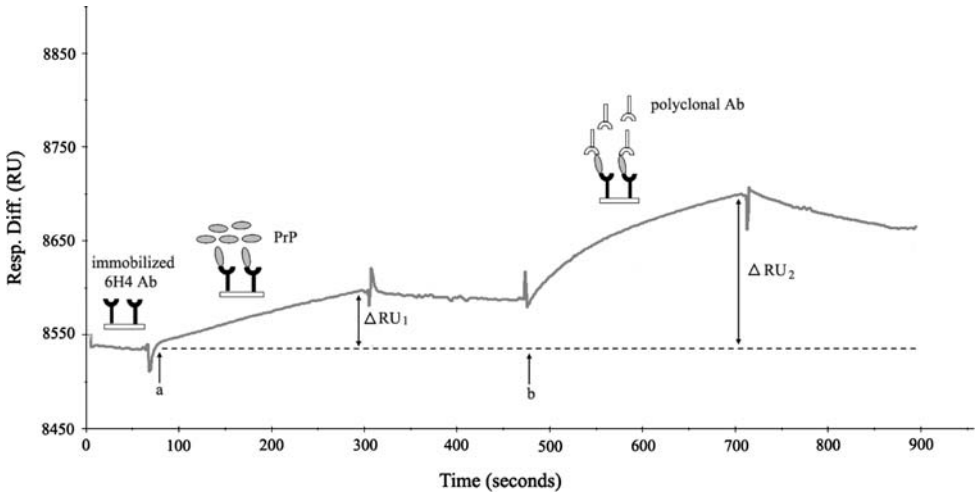

Fig. 1 *Sensorgram* showing the primary immune reaction and enhanced assay by polyclonal antibody: (*a*) injection of 25 µl 100 ng/ml PrP^C, (*b*) injection of 25 µl 0.5 mg/ml polyclonal antibody. ΔRU_1 and ΔRU_2 represent the relative primary and enhanced response, respectively

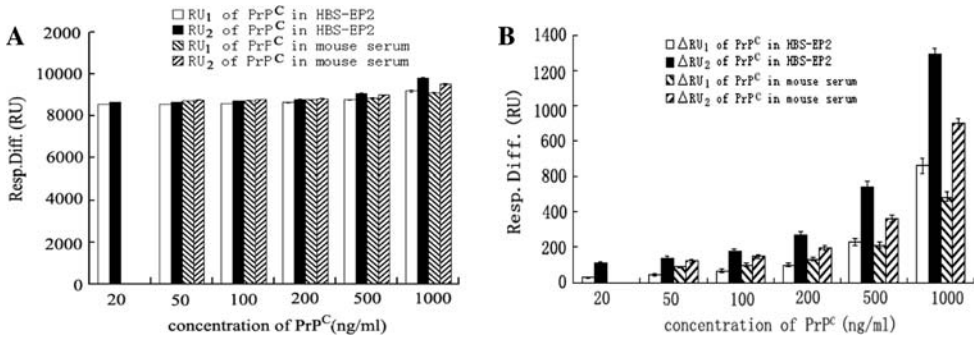

Fig. 2 Detection of PrP^C in HBS-EP2 and mouse serum. **a** RU_1 and RU_2 represent the average absolute primary and enhanced response, respectively; absolute primary response (RU_1) corresponds to data after the end of sample injection and after the background subtraction; absolute enhanced response (RU_2) corresponds to data after the end of polyclonal antibody injection and after the background subtraction. **b** ΔRU_1 and ΔRU_2 represent the average relative primary and enhanced response, respectively; this is a quantitative calculation in RU that is made by subtraction of the baseline response from the absolute response of the binding. Differences between RU_1 and RU_2, and between ΔRU_1 and ΔRU_2, have been found to be statistically significant ($P < 0.05$, $n = 3$)

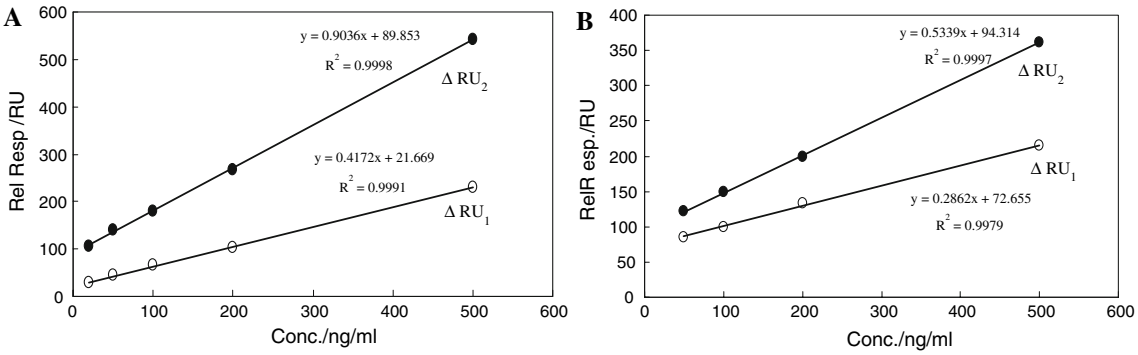

Fig. 3 **a** Linear range of the immunoassay in HBS buffer. **b** Linear range of the immunoassay in mouse serum. ΔRU_1 and ΔRU_2 represent the relative primary and enhanced response, respectively; each value represents a mean of three separate samples

Application of the SPR to analysis of PrP^{Sc} in infected mouse brain

In addition to the detailed assay validation for PrP-spiked mouse serum, the SPR assay was also successfully validated for PrP^{Sc} concentration in infected and non-infected mice brains. For this experiment, it was necessary to make substantial dilutions of PrP containing untreated and PK-treated 10% mice brain homogenates in HBS-EP2 buffer. Twenty-five microliters samples without and with PK treatment were used for the assay. As shown in Fig. 4, significant signals were obtained from untreated normal

Table 1 Recovery test for the determination of PrP^{Sc} in spiked mouse serum with PK treatment ($n = 3$)

PrP^{Sc} spiked (ng/mL)	ΔRU_2	PrP^{Sc} detected (ng/mL)	Recovery (%)	Coefficient of variability (%)
36.0 (PrP^{Sc})	112.6	34.3	95.2	0.76
72.0 (PrP^{Sc})	131.4	69.4	96.4	1.01
216.0 (PrP^{Sc})	206.5	210.2	97.3	1.23
50.0 (PrP^{C})	0	0	0	0
50.0 (PrP^{C}) + 50.0 (PrP^{Sc})	119.5	47.1	47.1	1.09

and scrapie-infected brain homogenates, whereas no significant signal was present in normal brain homogenate treated with PK.

Discussion

The sandwich SPR immunoassay described here required a matched pair of different anti-PrP antibodies. The two anti-PrP antibodies used, monoclonal antibody 6H4 and polyclonal antibody, have sufficiently different epitopes to provide adequate binding sites for both. In an Ag–Ab interaction measured by SPR, it is generally accepted that the specificity of the assay is enhanced by a sandwich technique, because the amplified antibody can thereby interact most effectively with its antigen. The sensitivities in serum and HBS-EP2 buffer were improved by a factor of 1.87 (from 0.2862 to 0.5339 RU) and 2.17 (from 0.4172 to 0.9036 RU), respectively. After addition of mouse serum spiked with PrP^{C} onto the SPR chip coated with primary antibody 6H4, a significant change in RU was observed. In the primary response, the RU values were much larger than those of SPR chips coated with primary antibody incubated with HBS-EP2 buffer alone. This was because serum

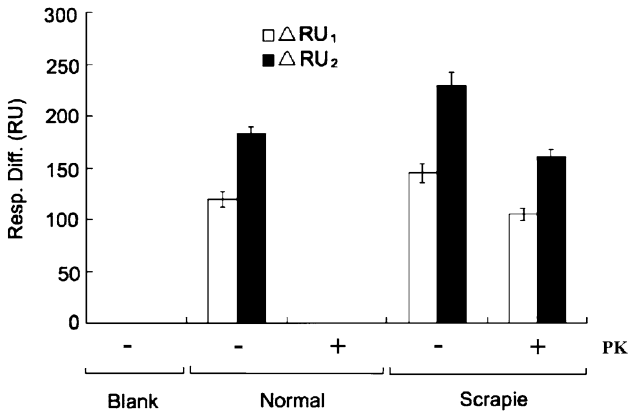

Fig. 4 Validation test of the SPR for the detection of PrP^{Sc} in the brain homogenates. The dilutions of normal ($n = 3$) and 22L-infected mouse ($n = 3$) brain homogenates without or with PK treatment (50 μg/ml for 45 min at 45°C) were applied to the SPR. As a negative control, a sample lacking brain homogenate was included. *Data* are expressed as mean ± SD

contains high concentrations of numerous proteins (72 mg/ml total protein), leading to a high non-specific adsorption of these extraneous proteins. For example, at 100 ng/ml, the primary response was 66.5 RU in HBS-EP2 buffer but 99.8 RU in serum. Although the serum used for preparing the sample had been diluted with HBS-EP2 buffer, which reduced the non-specific adsorption of protein due to its high ionic strength and surfactant activity, the non-specific adsorption was still high (33.3 RU). To date, a few studies have been reported in which biomolecules were analyzed in diluted serum using SPR [31]. Typically, dilution of the serum by a factor of 1/100 [32], 1/50 [33], or 1/20 [34] is performed. At these dilutions, the nonspecific signal from the serum proteins becomes negligible. At dilutions lower than 1/20, the nonspecific signal from serum proteins is large enough that its effect on the SPR output must be considered, and a dilution factor of 1/5 was used in the present study.Therefore, no difference was recorded at low concentrations of PrP^{C} (20 ng/ml), and the analyte range was only linear from 50 to 500 ng/ml in the primary response. Despite the high concentration of protein in the crude sample (PrP^{C} in serum), the lower detection limit of the assay using mouse serum was found to be as low as 31.7 ng/ml PrP^{C} after sandwich enhancement, and the lower detection limit of the assay using HBS-EP2 buffer was as low as 13.1 ng/ml PrP^{C}. The detection limit for PrP^{C} with the SPR assay was almost as low as that of ELISA [20, 35] and was lower than that reported by other authors [36], while the concentration detection limit for PrP^{C} was higher than those of other assays [37, 38]. While the lower detection limit of current SPR biosensors is about 1–100 ng/ml of proteins [39], sandwich-type immunoassays using additional interacting materials, such as nanoparticles, liposomes, and avidin, have been developed to improve the sensitivity of SPR biosensors [40, 41]. Therefore, further reduction of nonspecific binding and improved SPR sensitivity using gold nanoparticles could potentially further lower the detection limit of PrP in serum. Biochemically, PrP^{Sc} can be distinguished from PrP^{C} by its partial resistance to proteolysis and its marked insolubility in detergents. When incubated with PK, PrP^{C} is completely digested, whereas with PrP^{Sc}, only up to 97 amino acids are removed from the amino terminal region.

PK-treatment of prion-infected tissues results in molecular degradation of the di-, mono- and non-glycosylated PrP^{Sc} isoforms by 6–8 kDa. The fraction resulting from the treatment of PrP with PK is termed PrP^{Sc}, and most diagnostic methods are based on its detection. The primary antibody 6H4 was able to capture PrP^{Sc} efficiently and, therefore, it was chosen as the capture antibody. Polyclonal anti-PrP antibody was selected based on its ability to form efficiently a complex with PrP^{Sc} bound to antibody 6H4. Infectivity studies have shown that PrP^{Sc} is abundant only at late stages of the disease in the primary target organ—the brain, and is also present in minute amounts in peripheral tissues, such as lymphoid organs and blood [42]. The sensitivity of a PrP^{Sc}-based assay is strongly affected by the high background from the tissue matrix (e.g., brain, plasma) [43]. One means of increasing assay sensitivity and reducing the background is through enrichment and concentration of PrP^{Sc}. It has been reported that sodium phosphotungstic acid selectively precipitates PrP^{Sc} from different sources [44]. This could provide an alternative to increasing the sensitivity of PrP^{Sc} detection by increasing the amount of PrP^{Sc} present in a sample.

The time required for the analysis of each sample, including the primary antibody immobilisation step, using the SPR device is only 1 h, whereas a commercially available ELISA, Prionics-Check LIA test, requires about 4–5 h incubation time, and a commercially available western blot, Prionics-Check Western test, requires 6–8 h. There is also the possibility of further reducing this analysis time if the SPR chips are pre-coated with primary antibody and made available as ready-to-use chips. Therefore, our data indicate that sandwich amplification can be used for fast detection of PrP. Additionally, the benefit of having real-time visualisation of binding events as a sensogram makes the SPR-based assay more robust. It is possible to quickly identify the cause of any deviation in the assay by analysing the sensograms. This is not possible with existing commercial immunological methods in which identification of the cause of failure may be time-consuming and dependent on the experience of the operator. In addition, the configuration of the applied SPR biosensor allows the simultaneous detection of different proteins in a single run in a single sensor channel or in separate sensor channels on the same sensor chip. The present SPR assay, with the advantages of being label-free and requiring no sample preparation, can therefore be used for high-throughput screening of a large number of samples. The simplicity of this assay for detection of PrP clearly demonstrates the potential of the SPR technique for use as a rapid detection tool in the food industry and clinical diagnosis.

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