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Prion protein 90-231 contains a streptavidin-binding motif

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Abstract

The biological function of prion protein (PrP) and the physiological relevance of its truncated subtypes and glycoforms is still enigmatic. In this paper, we adduce evidence that recombinant murine PrP fragment 90–231 (mPrP90–231) contains a biotin-mimicking sequence motif that causes binding of the bacterial protein streptavidin to mPrP90–231. As indicated by epitope mapping and proven by analysis of a deletion mutant (mPrP101–231), streptavidin binding is primarily mediated by the amino-terminus of mPrP90–231 with the core-binding sequence represented by residues 94–100. Competition with biotin significantly reduces the interaction pointing to an involvement of streptavidin's biotin-binding site (BBS). Since the BBS of streptavidin shares similarities with the active sites of proteins involved in biotin metabolism we speculate that biotin mimicry by truncated PrP-species may have an impact *in vivo*.

Keywords: Prion protein; Streptavidin; Biotin; Truncation

Spongiform encephalopathies (SEs) represent a group of transmissible neurodegenerative disorders affecting both humans and animals. They are believed to be caused solely by the so-called prions (PrP^{sc}), an abnormal conformational isomer of the cellular prion protein (PrP^{c}), which is predominantly expressed in the central nervous system, but is found at lower levels in a variety of other tissues, too [1,2].

During PrP^{c} biogenesis, the preprotein is cotranslationally directed to the endoplasmic reticulum, where the N-terminal leader sequence is cleaved off and a glycosylphosphatidylinositol (GPI)-moiety is added with simultaneous removal of the hydrophobic C-terminal signal peptide [3]. Traffic through the Golgi compartment and the secretory pathway gives rise to mature PrP^{c}, which is attached to the outer leaflet of the plasma membrane by its GPI-anchor. While the amino-terminal part of the prion protein possesses a flexible, largely undefined shape, the C-terminal half of the molecule adopts a disulfide-bridged, compact three-helix bundle structure [4] comprising two consensus sites for N-linked glycosylation that are variably occupied [5].

Although PrP^{c} is highly conserved throughout evolution, its physiological function remains poorly understood. The amino-terminal octarepeat region was shown to mediate chelation of copper ions [6]. In line with this feature there is evidence for an involvement of PrP^{c} in synaptic activity by regulation of the synaptosomal concentration of copper [7] and for a superoxide dismutase (SOD)-like activity implicating a role for PrP^{c} in cellular resistance to oxidative stress [8]. Other groups suggested a function for prion protein in signal transduction pathways [9–11] or cell adhesion and neural differentiation processes [12,13].

Besides the presence of surface-exposed full-length PrP^{c}, which, depending on the particular species, encompasses 208–220 amino acid residues, there is evidence suggesting the existence of N-terminally truncated PrP^{c} types of unknown physiological relevance. In normal human brain tissue, two major populations of truncated species exist with one containing molecules starting at His111-Met112 and the other one representing a heterogeneous mixture of PrP^{c} molecules N-terminally deleted between amino

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acids 80 and 100 [14,15]. For the chicken homolog of mammalian PrP^c, it was shown that cleavage of the full-length protein takes place in endocytic compartments and that recycling of these truncated molecules to the cell surface is part of the normal PrP^c metabolism [16]. Moreover, Perini et al. demonstrated that platelets release a soluble prion protein fragment encompassing residues 90–231 [17]. In the brains of individuals succumbed to SE, shortened forms of the infectious conformer PrP^{sc} were observed, too, but the degree of truncation was demonstrated to be more diverse and at least partly dependent on the particular disease pattern [14].

In this paper, we report a novel feature of truncated prion protein species. Using epitope mapping, blotting experiments and competition studies, we show that recombinant murine prion protein fragment 90–231 (mPrP90–231) contains an amino-terminal biotin-mimicking sequence motif that confers a streptavidin-binding activity on mPrP90–231. Streptavidin is a homotetrameric bacterial protein containing four identical biotin-binding sites (BBS) and is naturally expressed by *Streptomyces avidinii*. Besides the functional and structural analog avidin, an avian egg protein, similarities to the BBS of streptavidin were also observed in the BBS of mammalian enzymes involved in biotin metabolism (e.g., biotinidase or holocarboxylase synthase) [18–20]. In the present study, we were not able to clearly assign a biological function to the biotin-mimicking capabilities of mPrP90–231. But in light of the widespread occurrence of truncated PrP^c and PrP^{sc} *in vivo*, we speculate that there might be some physiological relevance.

Materials and methods

Purification and labeling of recombinant murine PrP90–231. Recombinant N-terminally His-tagged mouse prion protein fragment 90–231 (mPrP90–231) was expressed in *Escherichia coli* BL21(DE3) and purified using metal affinity chromatography. Briefly, mPrP90–231 containing inclusion bodies were harvested and solubilized in 50 mM Tris–HCl, 8 M urea, and 1 mM dithiothreitol (DTT) (pH 8.0). The solution containing the denatured protein was applied to a nickel–NTA–agarose column (Qiagen, Hilden, Germany), bound mPrP90–231 was eluted in 50 mM Tris–HCl, 8 M urea, and 300 mM imidazol (pH 8.0), and the solution was adjusted to 1 μM $CuSO_4$. Following overnight incubation at 4 °C on an end-to-end mixer, eluates were dialyzed against 0.2 M $NaHCO_3$–NaOH (pH 8.3) and snap-frozen in liquid nitrogen. The vector pET-15b (Merck Biosciences, Novagen brand, Bad Soden, Germany) harboring the mPrP90–231 sequence was kindly provided by M. Baier (Robert Koch-Institut, Berlin, Germany). The recombinant protein consists of the amino acids 90–231 of murine prion protein (SwissProt Accession No. P04925) preceded by an amino-terminal vector-derived sequence (MGSSHHHHHHSSGLVPRGSHM) and has a calculated molecular mass of 18.52 kDa.

Fluorescence labeling of purified mPrP90–231 with an Alexa Fluor® 680 carboxylic acid succinimidyl ester (Invitrogen, Carlsbad, CA, USA) was performed in 0.2 M $NaHCO_3$–NaOH (pH 8.3) using an equimolar dye-to-protein ratio. During all steps the preparations were protected from light. The reaction was carried out for 2 h at room temperature on an end-to-end mixer, and was terminated by the addition of glycine in a 100-fold molar excess and further incubation overnight at 4 °C. Following removal of precipitates by centrifugation and dialysis against phosphate buffer (50 mM NaH_2PO_4–NaOH (pH 8.0), 50 mM NaCl), fluorophore-labeled PrP (PrP-680) was separated from uncoupled dye by metal affinity chromatography, dialyzed against phosphate buffer, and snap-frozen in liquid nitrogen.

Labeled and unlabeled preparations of mPrP90–231 were checked for identity and purity in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE), in Western blots using the monoclonal anti-PrP antibody 6H4 (Prionics, Schlieren, Switzerland) and by mass spectrometry. The protein concentration was determined using the Micro BCA kit (Pierce, Rockford, IL, USA) and the D_C Protein assay (Bio-Rad Laboratories, Hercules, CA, USA).

Construction of the deletion mutant mPrP101–231. PCR primers containing recognition sequences of the restriction endonucleases *Nde*I (forward primer) and *Bam*HI (reverse primer) were designed flanking the coding sequence of amino acids 101–231 of mouse prion protein. A DNA fragment of 428 nucleotides was amplified using the mPrP90–231 plasmid described above as a template and hydrolyzed with *Nde*I and *Bam*HI. The purified product was ligated into the expression vector pET-15b linearized with the same enzymes and *E. coli* BL21(DE3) chemically competent cells were transformed with the plasmid. Expression and purification of the N-terminally His-tagged protein mPrP101–231 was performed as described above. The truncated protein contains the same vector-derived sequence motif as mPrP90–231 and has a calculated molecular mass of 17.32 kDa.

In vitro binding and competition studies. Purified prion protein species were immobilized on nitrocellulose membranes by electrotransfer from denaturing 12.5% (w/v) polyacrylamide SDS gels or by dot blotting using the dot blotter SRC 96D Minifold I (Schleicher & Schuell, Dassel, Germany). After blocking non-specific binding sites for 2 h at room temperature in 1% (w/v) casein (Hammarsten grade, BDH Laboratory Supplies, Poole, UK) in Dulbecco's phosphate-buffered saline (D-PBS; 2.7 mM KCl, 1.5 mM KH_2PO_4, 136 mM NaCl, and 8.1 mM Na_2HPO_4, pH 7.4), blots were incubated for another two hours with 0.2 μg/ml Alexa Fluor® 680-labeled streptavidin (SA-680, Invitrogen), with 1 μg/ml horseradish peroxidase (HRP)-labeled streptavidin (Vector, Burlingame, CA, USA) or with 0.2 μg/ml IRDye™ 800-conjugated rabbit anti-6× His epitope tag antibody (Rockland Immunochemicals, Gilbertsville, PA, USA), followed by 6 washes in D-PBS/0.1% (v/v) Tween 20 (D-PBST) and 2 washes in D-PBS. The unlabeled anti-PrP antibody 6H4 was used at a concentration of 0.1 μg/ml (2 h of incubation) and was detected by a one-hour incubation with 0.2 μg/ml of an Alexa Fluor® 680-labeled goat anti-mouse IgG (Invitrogen). All incubation steps with antibodies or with streptavidin were performed in 1% (w/v) casein in D-PBST. Blots were analyzed using the Super Signal West Pico Chemiluminescent Substrate kit (Pierce) for HRP-labeled streptavidin or the Odyssey™ Infrared Imaging System (LI-COR Biosciences, Lincoln, NE, USA) for fluorescence detection. For Western blots, 0.4 nmol of mPrP90–231 or mPrP101–231 were diluted in gel loading buffer (final concentration 10% (w/v) glycerol, 2% (w/v) SDS, 6.25% (v/v) β-mercaptoethanol, and and 0.001% (w/v) bromophenol blue), incubated at 37 °C for 30 min, and loaded on 12.5% (w/v) polyacrylamide–SDS gels along with the prestained Precision Plus Protein All Blue Standard (Bio-Rad), which is detectable in the Odyssey imager at 700 nm and, less strongly, at 800 nm. Tank blotting was performed in Towbin buffer (0.25 M Tris base, 1.92 M glycin, 0.05% (w/v) SDS, and 20% (v/v) methanol, pH 8.2) at 350 mA for 2 h at room temperature. For dot blots, either 1:2 dilution series of proteins were prepared in D-PBS and 200 μl of each was dotted with a starting amount of 0.4 nmol, or 0.15 nmol were dotted separately. In competition studies, 50 pmol of protein species were immobilized and detected with an equimolar amount of SA-680. Here, streptavidin was presaturated with different molar ratios of (+)-biotin-$(PEO)_4$-propionic acid (Molecular Biosciences, Boulder, CO, USA). Biotinylated BSA (bovine serum albumin (Sigma–Aldrich, Taufkirchen, Germany), biotinylated with a fivefold molar excess of sulfosuccinimidyl-6-(biotinamido)hexanoate (Vector, Burlingame, CA, USA)), was used as a positive control.

SPOT synthesis of peptide libraries. Solid phase peptide libraries were produced on cellulose membranes by Spot synthesis according to the method of Frank [21]. Spotting was performed with the pipetting robot ASP222 (Intavis, Köln, Germany) using Fmoc amino acids from Merck Biosciences (Novabiochem brand, Bad Soden, Germany). After depro-

tection of the amino acid side chains, membranes were stored desiccated at −20 °C.

For epitope mapping, duplicate sets of overlapping 16 mer peptides frameshifted by one residue were synthesized covering amino acids 25–183 of the streptavidin precursor (SwissProt Accession No. P22629) and amino acids 23–231 of mouse PrP (SwissProt Accession No. P04925). For a more detailed analysis of identified epitopes, truncated peptides and peptides with amino acid substitutions (Alanine-Scan) were prepared.

At least ten 16 mer random peptides cosynthesized on each filter were used for the definition of a cut-off. Random peptides were designed using the web-based program collection The Sequence Manipulation Suite [22].

Peptide library probing and quantitation of binding. After wetting in 100% ethanol and washing in D-PBS, membranes were fake-stripped (two incubations of 5 min each in dimethyl formamide and two incubations of 7 min each in strip buffer (8 M urea, 1% (w/v) SDS, and 0.5% (v/v) β-mercaptoethanol, pH 7.0) in an ultrasonic bath with an intermediate wash in water) and equilibrated in D-PBS.

Membranes were blocked for at least 4 h in 1% (w/v) casein in D-PBS and incubated overnight at 4 °C either with 0.1 μg/ml SA-680 or PrP-680 (0.001 μg per peptide spot) in 1% (w/v) casein in D-PBST. Six washes in D-PBST and two washes in D-PBS were followed by visualization with the Odyssey™ Infrared Imaging System. The fluorescence intensity of each spot was quantified using the Odyssey Application Software (version 1.2) and a cut-off was determined according to the method of Frey et al. [23] applying a confidence level of 99%.

Statistical analysis. Statistical analysis was performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA).

Results

Streptavidin interacts with mPrP90–231

The first evidence of streptavidin binding to recombinantly expressed, His-tagged murine PrP90–231 was observed in a sandwich ELISA setup. Albeit we originally aimed to analyze biotinylated PrP-ligands, we found consistently high background binding of the horseradish peroxidase (HRP)-labeled streptavidin detection system to prion protein immobilized on microwell plates (data not shown). To further analyze the interaction, we performed Western blotting experiments with purified mPrP90–231 which had been subjected to electrophoresis on 12.5% polyacrylamide SDS gels and electrotransferred to nitrocellulose membranes by tank blotting. The membranes were probed with differently labeled streptavidins, with a His6 epitope tag antibody or with the monoclonal anti-PrP antibody 6H4 as a positive control. The immobilized mPrP90–231 gave clear signals both with Alexa Fluor® 680-tagged streptavidin (SA-680) and with HRP-labeled streptavidin, indicating that streptavidin, not horseradish peroxidase, was responsible for PrP binding (data not shown).

The sensitivity of PrP-detection varied with the different detection reagents and was highest when using the 6H4 anti-PrP antibody, which we assume to be partly due to the use of an indirect detection system employing an Alexa Fluor® 680-labeled secondary goat anti-mouse antibody. The PrP detection limit determined in dot blots was as low as 0.1 pmol (∼2 ng) when using the PrP-specific 6H4 monoclonal antibody, whereas direct detection of PrP with both the IRDye™ 800-labeled 6× His tag antibody (anti-His-800) and SA-680 required at least 12.5 pmol (∼230 ng) of immobilized protein. Anti-His-800 is therefore a more suitable positive control for SA-680-mediated detection of prion protein as it can be used in an identical experimental setup. Both detection reagents were labeled directly, but with different fluorophores emitting light at different wavelengths (700 and 800 nm). This allowed their addition in equal amounts to the same blot simultaneously, two-colour detection, and direct comparison of the resulting signal intensities (Fig. 1).

Murine prion protein and streptavidin contain linear-binding sites for each other

The affinity of streptavidin to mPrP90–231 and vice versa may be mediated by linear or conformational determinants. To detect linear binding sites, we carried out solid phase mapping experiments with peptide libraries encompassing amino acids (aa) 25–183 of the streptavidin precursor and aa 23–231 of mouse prion protein. The analysis of PrP peptide-libraries treated with SA-680 revealed the existence of one major linear binding site residing at the amino-terminal part of mPrP90–231 (aa 94–100). A much weaker binding of streptavidin with approximately 15-fold lower signal intensity was detected at residues 127–140. A low but consistently detectable binding of streptavidin was also mapped to the tandem repeat region (aa 51–90) of PrP with the most prominent signal detected at amino acids 64–77. This region, however, is not included in our recombinant prion protein mPrP90–231.

Duplicates of the streptavidin peptide library were incubated with Alexa Fluor® 680-labeled mPrP90–231 (PrP-680). Signals exceeding background intensity were detected if amino acids 99–108 of the streptavidin precursor were included in the 16 mer peptides analyzed. The 10 amino acid-spanning peptide with PrP-affinity contains one of the four tryptophan residues (Trp-103 in SwissPort Accession No. P22629) that are known to be involved in biotin binding. Table 1 shows the sequence of the deduced core-

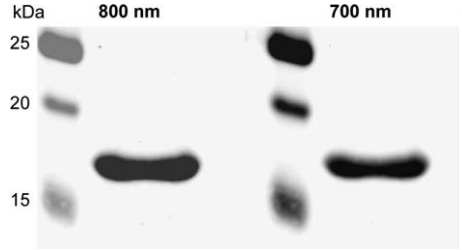

Fig. 1. Streptavidin binding to murine PrP90–231 is detectable in Western blots. 0.4 nmol purified recombinant mPrP90–231 was separated by SDS–PAGE and electrotransferred to a nitrocellulose membrane. After blocking non-specific-binding sites, the blot was incubated in 0.2 μg/ml SA-680 and 0.2 μg/ml anti-His-800 simultaneously, and analyzed at two different wavelengths in a two-color experiment. The anti-His-800-specific signal read out at 800 nm is shown on the left. Binding of streptavidin (SA-680) was analyzed at 700 nm (shown on the right) and is clearly detectable.

Table 1
Core-binding motifs of mPrP90–231 for streptavidin and vice versa

	Sequence	aa	Essential aa
mPrP90–231	THNQWNKP	94–100	W-98, K-100
	YMLGSAMSRPMIHF	127–140	Y-127, R-135, H-139
Streptavidin	WTVAWKNNYR	99–108	W-99, W-103

The numbering of the amino acid residues (aa) was done according to SwissProt Accession Nos. P04925 (murine prion protein) and P22629 (streptavidin precursor).

binding motifs of both interaction partners and essential amino acids therein, which were determined by alanine-scanning and by analyzing truncated peptides.

The two residues in the major streptavidin-binding site at the amino-terminus of mPrP90–231 that are essential for interaction (W-98 and K-100, see Table 1) are conserved in mammalian prion proteins. Amino acid substitutions at the other positions, as occurring for example in human PrP (N-99 to S-99), do not abrogate the binding of streptavidin as evidenced by screening a library of core motif homologs.

Amino acids 94–100 of mPrP90–231 are the primary mediator of streptavidin binding

To determine if amino acids 94–100 of recombinant mPrP90–231 indeed represent the primary mediator of streptavidin binding, we constructed a truncated prion protein devoid of the putative streptavidin interacting sequence motif. This protein comprised amino acids 101–231 of murine PrP (mPrP101–231) and was heterologously expressed and purified according to the procedure established for mPrP90–231. The binding of streptavidin to both proteins was analyzed by dot blotting. An identical signal intensity in the positive control assay using anti-His-800 verified that equal amounts of both protein species were immobilized. Streptavidin-binding was greatly reduced in the deletion mutant mPrP101–231 (Fig. 2). In a second experiment used to quantitate the streptavidin-binding capacities of both PrP-species, 150 pmol of the prion protein variants were immobilized on nitrocellulose membranes separately and again treated with both anti-His-800 and SA-680. The streptavidin-specific signal intensity was normalized to the mean of the signal obtained with mPrP90–231 and expressed as percent binding. Binding of SA-680 to mPrP101–231 was significantly ($P < 0.0001$; unpaired, one-tailed t test) reduced to $19.0 \pm 2.1\%$ of the binding achieved with mPrP90–231.

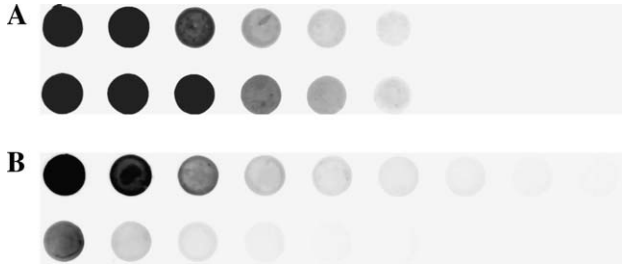

Fig. 2. Binding of streptavidin to recombinant murine PrP is significantly reduced by deletion of aa 90–100. Dilution series (1:2) of mPrP90–231 (top rows of both blots) and mPrP101–231 (bottom rows) were dot-blotted on a nitrocellulose membrane starting with an amount of 400 pmol. The blot was probed with 0.2 μg/ml anti-His-800 and 0.2 μg/ml SA-680 simultaneously. (A) shows the anti-His-800-specific signals obtained in the 800 nm channel; (B) shows the SA-680-specific signals at 700 nm. Although equal amounts were immobilized as demonstrated in (A), the signals obtained with SA-680 were much lower for the truncated protein.

Streptavidin binds to mPrP90–231 via its biotin-binding pocket

High-affinity binding of biotin is a characteristic feature of streptavidin and is the reason for its widespread use as a detection reagent in biochemical assays. Streptavidin is a homotetrameric molecule containing four identical biotin-binding sites (BBS), each of which is formed by a panel of amino acids that are spatially proximal in the tertiary fold, but sequentially distant in a monomer's primary structure. Additionally, a tryptophan residue (Trp-144 of the streptavidin precursor) from a neighboring subunit is required to establish a complete-binding site for biotin [24]. This makes the BBS of streptavidin a strictly nonlinear, conformational 'epitope' that cannot account for prion protein binding to streptavidin peptide libraries. It could, however, be partly responsible for the binding of SA-680 to PrP peptides and to recombinant mPrP90–231.

As a means to determine the contribution of the BBS of streptavidin on PrP binding, we performed competition studies by presaturating streptavidin with different molar ratios of (+)-biotin-$(PEO)_4$-propionic acid, a biotin derivative with an enhanced solubility in aqueous solutions. Fifty picomoles of SA-680, which corresponds to 200 pmol biotin-binding sites of the tetrameric protein, were incubated with the competitor for 15 min. The pre-treated SA-680 was then applied to 50 pmol of immobilized protein in a dot blot. The signal intensities were quantitated and expressed as percent binding relative to a non-competitive assay. When using biotinylated BSA, increasing amounts of (+)-biotin-$(PEO)_4$-propionic acid resulted in a continuous decline in signal intensity and a complete abolition of streptavidin binding at a BBS-to-biotin ratio of 1:20 (Fig. 3). In case of mPrP90–231, saturation of streptavidin with a 20-fold excess of biotin over BBS did not result in a complete loss of binding activity, but in a significant ($P < 0.001$, One-way ANOVA with Bonferroni's Multiple Comparison Test) signal reduction to $14.1 \pm 2.0\%$. This residual binding activity of biotin-saturated streptavidin to mPrP90–231 could not be reduced further by addition of higher amounts of the competitor. The low streptavidin-specific signals obtained with mPrP101–231 were barely influenced by competition with biotin (data not shown).

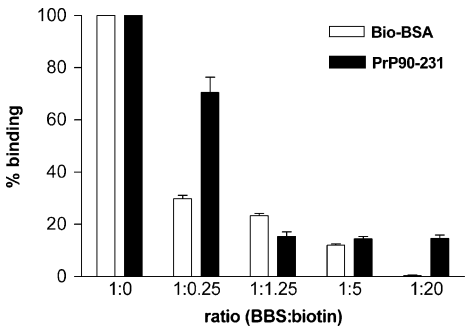

Fig. 3. Binding of streptavidin to mPrP90–231 is significantly reduced by competition with biotin. Fifty picomoles of recombinant murine PrP90–231 and biotinylated BSA (Bio-BSA) were immobilized separately on nitrocellulose membranes and treated with Alexa Fluor® 680-labeled streptavidin (SA-680) presaturated with increasing amounts of (+)-biotin-$(PEO)_4$-propionic acid (biotin). Each blot was incubated with 50 pmol of SA-680, which corresponds to 200 pmol biotin-binding sites (BBS). The amount of competitor added was calculated relative to the BBS and is given on the *x*-axis. The *y*-axis represents the binding activity of streptavidin in percent relative to a non-competitive assay. Whereas binding to Bio-BSA continuously declines and is completely eliminated at a BBS-to-biotin ratio of 1:20, mPrP90–231 shows a residual streptavidin binding of 14.1 ± 2.0%.

Discussion

Little is known by now about the physiological role of prion protein and the relevance of its truncated subtypes and glycoforms. Various PrP^c-interacting molecules (e.g., laminin [12], Bcl-2 [25], the laminin receptor [26] or the neural cell adhesion molecule N-CAM [27]) have been identified and various functions in diverse contexts like synaptic activity [7,28], metabolism of copper [29,30], signal transduction [9–11] or lymphocyte activation [31,32] have been implicated.

We show here a puzzling new feature in the mosaic of prion research and report that recombinant murine prion protein fragment 90–231 (mPrP90–231) exhibits a biotin-mimicking activity that causes binding of the biotin-binding bacterial protein streptavidin to mPrP90–231. This binding of streptavidin is primarily mediated by the amino-terminal region of the truncated prion protein with the core-binding motif represented by residues 94–100 (sequence THNQWNK) as proven by mapping of linear binding sites and by analysis of the deletion mutant mPrP101–231 which shows a decreased binding to streptavidin of 19% relative to the longer construct. Competition of streptavidin binding to mPrP90–231 with biotin leads to a significant reduction of the signal intensity to 14.1% in dot blots, whereas the weak binding of streptavidin to mPrP101–231 was barely influenced by biotin. In conclusion, this demonstrates that residues 90–100 of recombinant murine PrP90–231 interact with the biotin-binding site of streptavidin.

We assume the residual-binding activities of 19% for streptavidin-to-mPrP101–231 binding and 14.1% for streptavidin-to-mPrP90–231 binding in the biotin competition assay to be due to low-affinity interactions. These may either be mediated by the additional linear binding motif of PrP (residues 127–140) that was detected in the mapping experiments or via an unknown conformational determinant. Which part of the streptavidin molecule is responsible for these low-affinity interactions remains unclear. An involvement of the linear PrP-binding motif of streptavidin (residues 99–108 of the streptavidin precursor) is unlikely since it contributes to the biotin-binding pocket of streptavidin. The biotin-binding site (BBS) of streptavidin is a strictly conformational determinant that is only partly developed in a monomer's tertiary structure and requires a tryptophane residue from a neighboring subunit (Trp-144 of the streptavidin precursor) for completion. This way the four identical subunits in mature streptavidin complement each other forming four identical BBS. Each BBS includes four tryptophane residues that were shown to be essential for biotin binding in the so-called hydrophobic cage [33]. One of those residues (Trp-103) is contained in the above mentioned linear PrP-binding motif of streptavidin.

The involvement of the BBS in the streptavidin-mPrP90–231 interaction was confirmed by detection of immobilized mPrP90–231 with avidin, a vertebrate analog of streptavidin featuring a comparable though evolutionarily unrelated BBS. The detectability of mPrP90–231 with avidin also points out that the RYD-motif of streptavidin, which is not present in avidin, is not involved in prion protein binding. The RYD-motif mimics the universal recognition sequence RGD of fibronectin and other adhesion-related molecules, and frequently accounts for a non-specific background in histological studies [34].

The streptavidin-binding sequence motif we found resides at the amino-terminal part of our recombinant protein and localizes to the unstructured and flexible section that includes the N-terminus up to residue 124 of PrP^c [4]. Whether or not the biotin-mimicking motif is easily available on full-length PrP^c molecules remains to be elucidated, but its ready accessibility on recombinant mPrP90–231 suggests that at least truncated cellular forms of PrP are able to mimic biotin as well. Thus, it may be possible that biotin mimicry is an innate characteristic of truncated prion protein species *in vivo*.

The existence of shortened PrP-isoforms *in vivo* is well established [14–17]. One major type, designated the C1-fragment, is detectable in human brains and is N-terminally deleted at His-111, whereas a more heterogeneous population possesses N-termini starting between residues 80 and 100, and hence contains molecules that include the biotin-mimicking motif. Interestingly, in brains of individuals succumbed to SE the latter type of truncation predominates, which is hypothesized to be due to a decreased accessibility of the His-111 cleavage site in the scrapie conformer [14]. As a side effect, this would result in an accumulation of putatively biotin-mimicking prion species in infected tissues.

As a spot check to determine if truncated PrP comprising residues 94–100 might be able to interfere with cellular

biotin metabolism, we analyzed the impact of recombinant mPrP90–231 on *Bir*A-mediated biotinylation reactions. *Bir*A is an *E. coli*-derived biotin protein ligase and represents an ortholog of the enzyme holocarboxylase synthase (HCS), which catalyzes the transfer of biotin to the four mammalian apocarboxylases. The mechanism of biotin addition is highly conserved throughout evolution and accordingly, *Bir*A was shown to biotinylate target substrates derived from organisms ranging from other bacteria to plants and humans *in vitro* [35,36]. The biotin-binding domain of *Bir*A is analogous to that of mammalian HCS [19,37,38] and shares similarities with the BBS of streptavidin and avidin [19]. In our *in vitro* assay, however, which was based on measuring the *Bir*A-mediated biotinylation of a peptidic substrate in the absence or presence of mPrP90–231, we could not detect any PrP-specific inhibitory effect (data not shown).

Another occasion at which the body has to deal with truncated PrP is the ingestion of prion-contaminated food. During its passage through the gastrointestinal tract, PrP^{sc} is partially degraded by digestive enzymes, particularly by pepsin, to form a core protein resembling the proteinase K-resistant PrP27–30 fragment, which includes residues 90–231 [39]. So we speculated whether a biotin-mimicking site on PrP27–30 may facilitate intestinal invasion of PrP^{sc} and thus influence its infectivity. Enterocytes possess vitamin transport systems that actively absorb essential nutrients. For the uptake of biotin, the sodium-dependent multivitamin transporter (SMVT) and SLC19A3 (solute carrier family 19 member 3) have been described. They are both expressed in a variety of tissues including the brain, but in greatest abundance they occur in the intestinal mucosa, kidney, placenta, and liver [40,41]. Although the major substrate for these transporters is believed to be free biotin, which is liberated from biotinylated peptides in the digestive tract by the action of biotinidase, biotin derivatives may be accepted, too. Accordingly, transport of biocytin was reported [42], and the biotin transporter SMVT of enterocytes has been exploited to improve the oral absorption of peptide-based drugs by targeting it with biotinylated constructs [43]. As all mammals lack the ability to synthesize biotin and therefore entirely depend on dietary sources, the vitamin's intestinal transport system should be conserved with respect to the transporter's biotin-binding site. If recombinant murine PrP90–231 and, putatively, truncated PrP^{sc} are able to mimic biotin not only for streptavidin, but for SMVT or SLC19A3, too, they could interact with these transporters. We therefore used the human intestinal cell line Caco-2_{BBe1}, that was shown to express SMVT [44] and to be a useful model for studying biotin uptake [45], and incubated cell monolayers two weeks postconfluence with fluorescently labeled murine recombinant PrP90–231. Analysis of cell lysates in SDS–PAGE showed a weak binding and/or internalization of prion protein. However, we were not able to inhibit or decrease binding of labeled mPrP90–231 to Caco-2_{BBe1} cells by competition with biocytin in this system (data not shown).

In conclusion, the biotin-mimicking capabilities represent an interesting new feature of recombinant murine PrP90–231. Although our efforts in revealing a biological significance were inconclusive, it remains to be elucidated whether it really just represents an epiphenomenon. Whether or not, this finding will certainly be relevant for researchers who encounter high background signals when using a streptavidin-based detection system in prion research.

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