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Quantitative Analysis of Prion-Protein Degradation by Constitutive and Immuno-20S Proteasomes Indicates Differences Correlated with Disease Susceptibility

Stefan Tenzer,* Lars Stoltze,† Birgitt Schönfisch,‡ Jörn Dengjel,* Margret Müller,* Stefan Stevanović,§ Hans-Georg Rammensee,* and Hansjörg Schild2* §

The main part of cytosolic protein degradation depends on the ubiquitin-proteasome system. Proteasomes degrade their substrates into small peptide fragments, some of which are translocated into the endoplasmic reticulum and loaded onto MHC class I molecules, which are then transported to the cell surface for inspection by CTL. A reliable prediction of proteasomal cleavages in a given protein for the identification of CTL epitopes would benefit immensely from additional cleavage data for the training of prediction algorithms. To increase the knowledge about proteasomal specificity and to gain more insight into the relation of proteasomal activity and susceptibility to prion disease, we digested sheep prion protein with human constitutive and immuno-20S proteasomes. All fragments generated in the digest were quantified. Our results underline the different cleavage specificities of constitutive and immunoproteasomes and provide data for the training of prediction programs for proteasomal cleavages. Furthermore, the kinetic analysis of proteasomal digestion of two different alleles of prion protein shows that even small changes in a protein sequence can affect the overall efficiency of proteasomal processing and thus provides more insight into the possible molecular background of allelic variations and the pathogenicity of prion proteins. The Journal of Immunology, 2004, 172: 1083–1091.

The degradation of aged, misfolded, or no longer needed cytosolic proteins depends largely on the ubiquitin-proteasome system. 20S proteasomes represent ~1% of total cellular protein (1). They degrade their substrates to peptides of 3–20 aa (2), most of which are further broken down by aminopeptidases. The resulting single amino acids are then recycled for neosynthesis of proteins. A small part of the proteasomically produced peptides are translocated into the endoplasmatic reticulum (ER)3 by the transporter associated with Ag processing where they associate with MHC class I molecules, which are then presented at the cell surface (3). The peptides presented on the MHC class I molecules are predominantly generated in the cytosol by proteolytic digestion of proteins by 20S and 26S proteasomes (4, 5). Some of the MHC ligands are generated directly by the proteasome to fit into the MHC class I binding groove. However, others are generated as precursor peptide and require additional processing by further proteases (6–8). CTLs are able to recognize MHC class I peptide complexes and—if activated—will lyse the target cell when recognizing a nonself peptide.

The proteasome is a 700-kDa complex composed of 14 different subunits, which are arranged in four stacked rings with the stoichiometry of α2β1β2α2. The proteolytically active subunits are found in the β-rings. Their active centers face the inner hollow center of the 20S proteasome (9, 10), which may associate together with the 19S cap to form the 26S proteasome.

Upon stimulation with IFN-γ, the three active β-subunits, Y, Z, MB1, are exchanged to their immunocounterparts LMP-2, LMP-7, and MECL-1 (11). This results in a change in proteasomal specificity (12–15), which influences the generation of CTL epitopes (16–20).

First cleavage motifs for constitutive and immuno-20S proteasomes have been described based on data from in vitro degradation of yeast enolase-1 (21). Based on these data, prediction algorithms have been developed (22), but the reliability of proteasomal cut predictions (~60% when comparing the predicted cleavage sites to cleavage sites identified by in vitro digests of 24–27 mer peptides) needs to be improved by generating additional cleavage data for the training of the prediction algorithms available (22–24).

Recently, it has been described that prion protein (PrP) is an in vivo substrate of the proteasome (25). PrP is a 27-kDa GPI-anchored cellular glycoprotein, which plays a key role in transmissible spongiform encephalopathies (26, 27). The cellular function of PrP is not yet established, but it may have a function in copper transport or signal transduction (28–30). Two different forms of PrP are described: the normal cellular form, called PrPc, which is rich in α-helices, and the disease-associated form, PrPSc, which consists of mainly β-sheet conformation (31) and reveals a higher protease resistance than PrPc (32, 33). The C terminus contains the main structural domains of PrPc, whereas the N terminus seems not to possess a highly ordered conformation as determined by nuclear magnetic resonance spectrometry (34). In sheep, several...
alleles of PrP exist, which are strongly correlated to scrapie susceptibility (scrapie represents the transmissible spongiform encephalopathy form of sheep) ranging from very high susceptibility for the V136-R154-Q171 variant (VRQ) to resistance for the A136-R154-R171 variant (ARR) (35–37). It has been described that the unfolding pathways of the different PrP variants can partly explain the differences in disease susceptibility (38).

PrPc is processed in the secretory pathway and primarily found on the cell surface. Normal recycling of PrPc involves N-terminal trimming and degradation in acidic compartments (39). Approximately 10% of newly synthesized PrPc molecules are diverted to the cytosol by ER-associated degradation, which involves translocation by a modified sec61p translocon, deglycosylation, and ubiquitination, followed by degradation by the proteasome (25). If proteasomal activity is blocked, PrPc accumulates in the cytosol (40), which seems to provide an environment favoring the conversion of PrPc to a protease-resistant form of PrP (41, 42).

This study characterizes the in vitro digestion of PrP by constitutive and immuno-20S proteasomes. Our data demonstrate that the N terminus of PrP is more efficiently digested by both species of 20S proteasomes than the more structured C terminus. The analysis of the digestion products showed an extended size range from 3 to 150 aa of the identified peptides. The quantitative analysis of the digestion products provides additional data for the training of more potent prediction algorithms for the two different species of 20S proteasomes. Furthermore, the comparison of the degradation kinetics of two different sheep PrP variants may explain differences in the susceptibility to pathogenic PrP accumulation.

Materials and Methods

Purification of 20S proteasomes

20S proteasomes were isolated following a modification of the purification scheme described for 26S proteasomes described previously (43). Briefly, frozen pellets of LCL-721 cells or LCL-721.174 cells were lysed in a buffer containing 0.1% Triton X-100 on ice and homogenized in a Dounce homogenizer. The 40,000 × g supernatant of the lysate was bound to DEAE-52-Servacel (Serva, Heidelberg, Germany). After batch elution with 300 mM NaCl, the proteins were precipitated with 30–70% (NH4)2SO4 and the precipitate dissolved in low salt buffer and subjected to fast protein liquid chromatography anion exchange chromatography with 100 ml of TSK-DEAE-650S Toyopearl resin ( Tosohas, Stuttgart, Germany) in a HR 16/60 column (Amersham Pharmacia Biotech, Piscataway, NJ). Proteins were eluted with a gradient (80–250 mM NaCl) in 250 min at a flow rate of 0.2 ml/min. The fractions of 4 ml were collected and tested for proteasomal activity using the fluorogenic substrates succinyl-leucyl-leucyl-valyl-tyrosyl-7-amino-4-methylcoumarin (Bachem, Heidelberg, Germany). Fractions containing at least half maximal activity were pooled, concentrated, and loaded onto a 10–35% glycerol gradient. After centrifugation at 100,000 × g for 18 h (Beckman ultracentrifuge Optima L-80, SW40Ti; Fullerton, CA), gradient fractions were tested for proteasome activity. Fractions containing at least half-maximal activity were pooled and concentrated to a concentration of 1 mg/ml. The purity of the proteasome preparations, checked by SDS-PAGE, was above 95%.

Protein quantification

Quantification of native protein was determined by a variation of the Bradford Method (Roti-Nanoquant; Roth, Karlsruhe, Germany) using BSA (Roth) as a standard. Absorption was measured at 590 nm and 450 nm with a spectrophotometer (Ultropec 3000; Amersham Pharmacia Biotech).

Immunoblotting

Five micrograms of purified proteases were separated by 12% SDS-PAGE by standard techniques (44) and transferred to polyvinylidifluoride (DuPont, Wilmington, DE) with a semidy transfer system. Human LMP-7 was detected using a rabbit polyclonal antiserum (PWS200; Affiniti Research Products, Exeter, U.K.); LMP-2 was detected using a rabbit polyclonal antiserum (PWS205; Affiniti Research Products); β1(Y) was detected by a mouse mAb (PWS8140; Affiniti Research Products) in conjunction with goat-anti-mouse-HRP (Dianova, Hamburg, Germany) or goat-anti-rabbit-IRP (Dianova) and chemiluminescence (Western Lightning; PerkinElmer, Wellesley, MA).

Recombinant PrPs

Pure recombinant PrP (24–234), alleles VRQ and ARR, were a gift of P. Deby (Institut National de la Recherche Agronomique; Unité 806/EA2703, Muséum National d'Histoire Naturelle, Paris, France). In the recombinant proteins, the N-terminal cysteine residue (position 24) is replaced by a serine (45). Before the digestion experiments, the proteins were extensively dialyzed against digestion buffer (30 mM Tris-HCl, pH 7.6, 10 mM NaCl, 10 mM KCl, 2 mM MgCl2) and concentrated to 1 mg/ml using an Ultracare-15-cell (10-kDa cutoff; Millipore, Bedford, MA).

In vitro degradation of PrP

Two-hundred fifty micrograms of recombinant PrP (VRQ) were incubated in digestion buffer (20 mM Tris-HCl, pH 7.6, 10 mM NaCl, 2 mM MgCl2) with 25 μg of 20S proteasomes (a molar ratio of 250:1). Digestions were stopped after 12 h by freezing the samples at −80°C when 50–70% of the substrate was digested.

Separation and analysis of cleavage products

For the separation of degradation products, unfractionated PrP digests were subjected to μRPC 2/10 columns (Amersham Pharmacia Biotech) on a Microbore HPLC system (SMART; Amersham Pharmacia Biotech). Buffer A contained 0.1% trifluoroacetic acid (TFA); buffer B contained 0.081% TFA and 80% acetonitrile. Gradients were 0% in 15 min, in 75 min to 50% in buffer B, in 10 min to 75% in buffer B and, up to 100% in another 10 min at a flow rate of 150 μl/min. Fractions were collected by peak fractionation with a maximal volume of 500 μl/peak. Peak fractions were dried and dissolved in 50 μl of 40% methanol, 1% formic acid, and subsequently analyzed by matrix-associated laser desorption ionization (MALDI) time of flight mass spectrometry (MS) (G2025A; Hewlett-Packard, Palo Alto, CA) and NH2-terminal sequencing (Edman degradation) (pulsed liquid protein sequencer procise 494A; Applied Biosystems, Foster City, CA). Alternatively, peptides were analyzed on a hybrid quadruple orthogonal acceleration tandem mass spectrometer (Waters, Milford, MA). All these techniques were applied as described previously (21). Picomole amounts for each peptide detected in the HPLC fraction were determined by Edman sequencing and used for the quantitative analysis of the data. For fragments derived from the octarepeat region which could not be unambiguously identified, picomole amounts were equally assigned to the possible positions in the substrate sequence for statistical analysis.

Statistical analysis–frequencies of amino acids

To detect statistically significant features in the amino acid distribution flanking the cleavage sites, we compared percent values using a classic χ2 test for four tables (variance assumed due to counting) as described before (21). Only χ2 values above 3.841 are considered to be significant.

Results

Purification of 20S proteasomes

Proteasomes were purified from frozen cell pellets of EBV-transformed B cells. As source for constitutive proteasomes (c20S) the cell line LCL-721.174 was chosen, which lacks LMP-2 and LMP-7 due to a chromosomal deletion in the MHC region (46). The incorporation and processing of MECL-1 is prevented by the lack of LMP-2 and LMP-7 (47). Therefore, proteasomes isolated from this cell line carry only constitutive β-subunits expressing activity. Immunoproteasomes were isolated from the parental cell line LCL-721, which expresses high amounts of the immunosubunits. As expected, the immunosubunits LMP-2 and LMP-7 could only be detected in the immunoproteasome preparation (Fig. 1, A and B). Western blotting against the constitutive subunit β1(Y) (Fig. 1C) revealed only very low amounts of this constitutive subunit in the immunoproteasome preparation, a fact which was further confirmed by the very low ability to release the fluorogenic group from the substrate Z-LLE-β-naphthylamide compared with constitutive proteasomes (data not shown).
Digestion of PrP

Most cleavage data available from constitutive and immunoproteasomes have been generated by using fluorogenic substrates or small peptides of up to 27 aa. In contrast, limited information exists on the selection of cleavage sites in intact proteins by 20S proteasomes. To enlarge the existing data pool, we used recombinant sheep PrP (allele VRQ, aa 24–234) as a substrate for the proteasome. In contrast to the digestion of yeast enolase-1 by 20S proteasomes in vitro (21), no SDS is required for the digestion of PrP.

Incubation of 20S proteasomes with a 200-fold molar excess of PrP leads to complete disappearance of the substrate after 16 h and digestion can be fully inhibited by the addition of the proteasomal inhibitor lactacystin (Fig. 2).

For the quantitative analysis of the digestion products, 250 µg of PrP were incubated with 25 µg of 20S proteasome (a 250-fold molar excess) for a time of 10 h, which had been determined in previous experiments to yield 50–70% degradation of the substrate (data not shown). Subsequently, the digestion products were separated by reversed-phase HPLC (Fig. 3).

Fractions were collected by automatic peak fractionation. All fractions were analyzed by MALDI-MS and Edman degradation, allowing a quantitative identification of all peptide fragments. Identified fragments with an amount of 30 pmol or more, as well as all identified cleavage sites are shown in Fig. 4.

In the digest of PrP with constitutive proteasomes (c20S), 104 different fragments were detected with a total amount of 8604 pmol and a mean fragment length of 20.2 aa. The most frequently used cleavage site of the 84 identified was at position 76 with a cleavage intensity of 1055 pmol.

For the digest of PrP with immunoproteasomes (i20S), similar data were obtained. One-hundred sixty-two peptides were identified with a total amount of 13,040 pmol and a mean fragment length of 17.5 aa. The most prominent cleavage site was identified at position 115 with an intensity of 1163 pmol. Overall, 113 individual cleavage sites were identified in the digest of PrP with i20S proteasomes.

In both digests, cleavage intensity varied substantially between individual cleavage sites, indicating that the quantification of the fragments will provide important information for cleavage site prediction. As the above numbers indicate, substrate turnover was higher with immunoproteasomes under otherwise identical conditions.

Analysis of cleavage preference of c20S and i20S

Of the 162 identified peptides generated by immunoproteasomes, only 55 (34%) were also found in the digest with constitutive proteasomes, showing that constitutive and immunoproteasomes are able to create substantially different pools of peptides as reported previously for the digestion of yeast enolase-1 (21). When comparing individual cleavage sites, we find that only 55 of 113 (49%) cleavages made by immunoproteasomes are also found in the digest using c20S proteasomes.

FIGURE 1. Characterization of proteasome species. 20S proteasomes were purified from LCL 721 and LCL 721.174 cells as described in Materials and Methods. Proteasomal subunits were separated by 12% SDS-PAGE and blotted to a PVDF membrane and probed with Abs against the immunosubunits LMP-2 (A) and LMP-7 (B) as well as the constitutive subunit Y (C). LMP-2 and LMP-7 can only be detected in i20S proteasomes, whereas the constitutive subunit Y is detectable only in c20S proteasomes.

FIGURE 2. Recombinant PrP (VRQ) is digested in vitro by c20S and i20S. Four micrograms of PrP-VRQ were incubated with 0.5 µg of 20S proteasome (a 250-fold molar excess) for a time of 10 h, which had been determined in previous experiments to yield 50–70% degradation of the substrate (data not shown). Subsequently, the digestion products were separated by reversed-phase HPLC (Fig. 3). Fractions were collected by automatic peak fractionation. A, Digestion using constitutive proteasomes. B, Digestion using immunoproteasomes. Solid line, OD at 214 nm; dashed line, concentration of buffer B.

FIGURE 3. OD214 of eluted digestion products from reversed phase HPLC. Two hundred fifty micrograms of PrP (VRQ) were incubated with 25 µg of 20S proteasomes for 10 h at 37°C. The reaction was stopped by adding TFA to a final concentration of 0.1%. The digestion mixture was separated on a µRPC-C2-C18 column. Peptides were eluted with an acetonitrile gradient (0–50% in 75 min, absorption was monitored at 214 nm). Fractions were collected by peak fractionation. A, Digestion using constitutive proteasomes B, Digestion using immunoproteasomes. Solid line, OD at 214 nm; dashed line, concentration of buffer B.
To allow a more accurate study of the influences of individual amino acids around a potential cleavage site, we compiled the quantified data of all observed cleavages (Tables I and II; Fig. 5). Closer examination by \( \chi^2 \) analyses (data not shown) revealed several deviations from background level (as defined by the frequency of the respective amino acid in PrP). Only preferences with an enrichment factor of \( \geq 3.0 \) were regarded as significant.

In the digest with immunoproteasomes, the highest preferences include W in P1 and H in P4. In general, large, hydrophobic amino acids are enriched at P1; A, P and small, polar amino acids like S and T are preferred at P1'. Reduced frequencies in certain positions are found for G at P1 and P at P1, P2, P3.

We analogously examined the cleavage data obtained for constitutive proteasomes and found preferences including again W (\( \chi^2 = 34.1 \)) and other hydrophobic amino acids like L, F and Y at P1. As observed for i20S proteasomes, A and G are preferred at P1', but not the polar amino acids S, T, and K. In addition, V at P1' and G at P1 were disfavored.

**Distribution of fragments**

Starting from fragment data of the two individual digests, we computed the position frequencies of each amino acid in the substrate sequence. (The position frequency of a single amino acid position is defined by the sum of picomoles of all fragments harboring this particular amino acid). As shown in Fig. 6, position frequencies decrease from the N to the C terminus of PrP VRQ. This is more evident for the digest with constitutive proteasomes (correlation coefficient of \( R^2 = 0.66 \)) than for immunoproteasomes (\( R^2 = 0.54 \)).

**FIGURE 4.** Proteasomal cleavage maps. Digestion map of fragments generated by degradation of PrP with c20S (A) or i20S (B) proteasomes. Horizontal bars indicate fragments found with an amount of at least 30 pmol. Arrows indicate the identified cleavage sites. (Small: 1–29 pmol; medium: 30–99 pmol; large: \( >100 \) pmol).
Both species of proteasomes generated a larger amount of fragments from the less structured N terminus of PrP than from the C terminus. The highest position frequencies are found in and immediately after the octarepeat region (position 60°C terminus. The highest position frequencies are found in and immediately after the octarepeat region (position 60–94) for immunoproteasomes and also in the vicinity of the N terminus for constitutive proteasomes.

The low frequency of fragments found from the C terminus shows that the C terminus of PrP is more resistant to proteasomal degradation. This was confirmed by direct identification of a 15-kDa C-terminal fragment by gel extraction from SDS-PAGE and MALDI-MS (data not shown). A corresponding fragment of the same size was detected by SDS-PAGE in digests of PrP ARR with c20S and i20S proteasomes (data not shown).

Kinetic analysis of the digest of two different allelic forms of PrP by 20S proteasomes

To gain more insight into the effects of small mutations in PrP sequences, we compared the kinetics of proteasomal digestion of two naturally occurring genetical variants of the sheep PrP, namely ARR and VRQ. These two variants differ only in two amino acid positions, but have very different phenotypes ranging from very high susceptibility (homozygous for VRQ) to resistance to scrapie (homozygous for ARR). Both recombinant proteins were dialyzed against the digestion buffer, adjusted to equal protein concentrations, and digested with c20S and i20S proteasomes.

After different periods of time, aliquots were taken, and the reaction was directly stopped by freezing the samples at −80°C. All aliquots were loaded onto a 14% SDS-PAGE and stained by Coomassie Blue. The bands were quantified by image densitometry, and the values obtained were plotted against the digestion time (Fig. 7B). Regression analysis revealed a linear decrease of substrate over a time period of 6 h, indicating constant proteasomal activity. When comparing the rate of degradation, we found that PrP-ARR (slope = −0.014) is degraded ~63% faster than the VRQ-variant (slope = −0.019) (Fig. 7B).

Similar data were obtained for c20S proteasomes; the slopes determined by linear regression analysis were −0.014 (ARR) and −0.009 (VRQ) corresponding to a 55% faster degradation rate of ARR. This is consistent with the higher unfolding energy of VRQ.

Discussion

Our data demonstrate that recombinant PrP can be digested by constitutive and immuno-20S proteasomes in vitro without the addition of SDS. The observed cleavage motifs for c20S and i20S are in good accordance with the cleavage motifs derived from the
Table II. Absolute amounts of amino acids and enrichment factors, i20S

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* Absolute amounts of amino acids (white background) and enrichment factors (bold) found in positions P6 to P1 and P1’ to P6’ of the cleavage sites identified in the digestion of PrP-VRQ with i20S.

However, different cleavage sites observed in the cleavage motifs of c20S vs i20S were not as prominent as in the analysis of the digestion of yeast enolase-1. For example, the preference of c20S for acidic amino acids in P1 was not as strong as observed before. This may be due to the amino acid sequence of PrP, which harbors acidic amino acids only in the C-terminal part, which appears to be more resistant to proteasomal digestion (Fig. 6). This finding demonstrates the relative influence of the substrate on the observed cleavage preferences of proteasomes.

Furthermore, we observe a higher substrate turnover for immunoproteasomes compared with constitutive proteasomes under otherwise identical conditions. This effect might be specific for PrP, but could also point toward an enhanced catalytic activity of immunoproteasomes.

Our data also show a decrease of the amount of fragments derived from the C terminus vs the N terminus of PrP. This finding can be explained by a directional processing of the substrate starting at the N terminus, but is also compatible with the possibility of endoproteolytic cleavages (48), as described recently. As no SDS is present in the digestion reaction, the differences in degradation efficiency could also be attributed to the higher degree of secondary structure of the C terminus of PrP, which may interfere with the unfolding of certain substrate molecules or for opening the substrate access channel of the 20S proteasome. When we compare the cleavage motifs of c20S vs i20S, we observe again that amino acids like I, L, F are preferred by i20S proteasomes at P1, and small and polar amino acids like S and T are enriched at P1’, whereas both proteasomes prefer G and A at P1’.
access of parts of the protein to the active sites of the 20S proteasome. However, this should affect only processing efficiency, but not cleavage specificity.

Current prediction algorithms for proteasomal cleavages are limited in their performance by insufficient amounts of training data. Until now, the reliability of proteasomal cleavage predictions cannot match those of MHC-binding predictions. A significant improvement of the accuracy of prediction algorithms for proteasomal cleavages can be achieved by incorporating the cleavage data presented in this paper. Furthermore, our data provide the basis for the development of a differential prediction of constitutive vs immunoproteasomal cleavages (S. Tenzer, B. Peters, H. G. Rammensee, H. G. Holzhuetter, and H. Schild, manuscript in preparation).

Additionally, after the recent identification of CD4+ T cell epitopes in PrP (49), our data will directly support the identification of CTL epitopes in PrP. Recently, PrP specific CD8+ T cells have been detected in PrP-infected mice using MHC tetramers (50). However, these T cells failed to lyse target cells or to synthesize intracellular TNF-α or IFN-γ in response to the PrP-derived peptides. Whether or not this is due to the induction of tolerance or anergy remains to be investigated. The use of additional CTL epitopes identified by a combination of cleavage data and MHC-binding predictions will allow a more detailed assessment of CTL immune responses against PrP and may help to answer questions regarding the induction of tolerance against peptides derived from PrP and allow the development of strategies to break this tolerance.

The recombinant PrP used for the digestion experiments was purified from Escherichia coli and is therefore not glycosylated. However, this situation is quite comparable to the one in a living cell. During protein synthesis, PrP is cotranslationally translocated into the ER. Approximately 10% of the newly synthesized PrP never reach the cell surface, but are subjected to retrograde transport into the cytosol by ER-associated degradation (25). This process involves deglycosylation, translocation into the cytosol by a modified sec61p translocon, followed by ubiquitination and degradation by the proteasome. Therefore, PrP targeted for proteasomal digestion is also not glycosylated inside a living cell. In contrast, glycosylated PrP from the cell surface is degraded in acidic compartments of the cell (39).

The reduction of proteasomal activity results in the accumulation of a protease-resistant form of PrP in the cytosol (41, 42). Our data show that two PrP variants, VRQ and ARR, are digested with different efficiencies by both constitutive and immunoproteasomes. Remarkably, the variant associated with higher susceptibility to prion infection (VRQ) is degraded significantly slower than the variant associated with resistance to prion infection (ARR), which is also in good correlation to the higher unfolding energy of VRQ. This finding supports the idea that the kinetics of proteasomal degradation may directly contribute to the differences in pathogenicity.
observed in vivo for the two alleles. It might well be possible that the reduced degradation of PrP-VRQ by c20S as well as i20S proteasomes observed in vitro (Fig. 7) may also lead in vivo to higher steady state levels of PrP-VRQ in the cytoplasm, as compared with PrP-ARR. As the cytoplasm provides an environment which promotes conversion of PrPc to PrPsc, these higher levels of protein present may in turn favor aggregation and conversion to a more protease-resistant form of PrP and thus contribute to the pathogenic effect of PrP.

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References


