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Copper-induced structural changes in the ovine prion protein are influenced by a polymorphism at codon 112

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Abstract

Prion diseases are associated with conformational change in the copper-binding protein PrP. The copper-binding sites in PrP are located in the N-terminal region of the molecule and comprise a series of tandem repeats of the sequence PHGGGWGQ together with two histidines at residues 96 and 111 (human PrP numbering). The co-ordination of copper ions within the non-octapeptide repeat metal ion-binding site involves Met109 (human numbering, which corresponds with Met112 in ovine PrP) and the binding of copper to this site leads to an increase in β-sheet formation in PrP. Here we have investigated the influence of the M112T polymorphism on copper-induced structural changes in ovine recombinant PrP. M112ARQ and T112ARQ ovine PrP show similar secondary structure although M112ARQ appears more thermostable than T112ARQ. Following treatment with copper, M112ARQ showed a greater increase in β-sheet content than did T112ARQ when measured by CD spectroscopy and by ELISA using anti-PrP monoclonal antibodies. These biochemical and biophysical differences between M112ARQ and T112ARQ correlate with similar differences seen between allelic variants of ovine PrP associated with susceptibility and resistance to classical scrapie. These observations suggest that T112ARQ may provide a measure of resistance to classical scrapie pathogenesis compared to M112ARQ.

Keywords: PrPC; Polymorphism; Transmissible spongiform encephalopathies; β-sheet; Conformational stability

1. Introduction

Prion diseases, or transmissible spongiform encephalopathies (TSEs), are a group of fatal neurodegenerative diseases that affect both animals and humans. This group of diseases includes scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle and Creutzfeldt-Jakob Disease (CJD) of humans. Collectively, these diseases may be inherited, arise sporadically or occur through infection with prion contaminated material [1]. Prion diseases are associated with misfolding of the host protein PrPC into an abnormal isomer termed PrPSc. The protein-only hypothesis predicts that the transmissible prion agent comprises solely, or principally, of proteinaceous material and in this context PrPSc is considered to be the infectious prion agent [2]. Mammalian PrPC is a cell surface glycoprotein attached to the membrane by a glycosylphosphatidyl inositol (GPI) anchor [3]. Although the relationship of PrP with prion diseases is well established, considerably less is known about the physiological function of this protein. Mice devoid of PrPC expression show no major anatomical or developmental deficits [4]. The proposed functions of PrPC include a role in the protection against apoptosis and oxidative stress [5], transmembrane signalling [6], formation and maintenance of synapses [7] and adhesion to the extracellular matrix [8]. A significant amount of information suggests that PrPC is a copper-binding protein and that this metal ion may play a role in the function of the molecule [9].

Structural analyses show that PrPC consists of a flexible N-terminal region followed by a globular C-terminal domain, both approximately 100 amino acids in length [10–14]. The C-terminal domain of PrPC comprises three α-helices, interspersed by a short anti-parallel β-sheet region. The copper-binding regions of PrP are located in the N-terminal region of the molecule. These are situated in the octapeptide repeat region [15], which, depending upon the species, comprises 4 - 5 tandem repeats of the sequence PHGGGWGQ, and also in the vicinity of amino acid residues His96 and His111 (human numbering, which correspond to amino acid residues His99 and His114 in ovine PrP) [16,17]. At

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maximum copper occupancy PrP can bind up to six copper (Cu2+) ions, each co-ordinated by a single histidine imidazole group and two deprotonated backbone amide nitrogen atoms [18,19]. In this co-ordination mode, each of the octapeptide repeats binds a single Cu2+ ion, as do sites that involve His96 and His111 (human PrP numbering). However, at low copper occupancy only one or two Cu2+ ions appear to be bound by PrP in a manner that involves multiple histidine co-ordination. In this mode, Cu2+ co-ordination is shared between the six N-terminal histidine residues within an ensemble of PrP-Cu2+ interactions [16,20–22]. Furthermore, during multiple histidine co-ordination of copper, some of the PrP-Cu2+ complex may exist in an oligomeric form as a consequence of a single Cu2+ ion co-ordinated by histidine residues from more than one PrP molecule. These observations suggest that conformational variation in PrP structure, at least within the region that binds Cu2+, will be influenced by metal ion occupancy. Prion diseases are characterized by a metal imbalance in the central nervous system, which is associated with alterations in the capacity of PrP to bind copper as it converts to PrPSc [23,24]. It is important therefore to identify those Cu2+ binding sites in PrP that contribute towards this process in order to understand mechanisms by which activity of these sites may influence PrP stability and conformation.

The interaction of Cu2+ with the unstructured N-terminal domain of PrP causes conformational change in the N- and C-terminal regions of the molecule [17,25–27]. These metal ion-induced conformational changes in PrP are likely to involve interactions between different regions of the protein. For example, residues close to the C-terminal of helix-1 and the nearby loop between β-strand-1 and helix-1 do interact with the N-terminal Cu2+ co-ordination groups following the binding of copper [21]. This occurs whether full-length or PrP90-231 are used to co-ordinate copper, which indicates Cu2+ co-ordination involving His96 and His111 (human PrP numbering) in these processes. The Cu2+-binding sites that involve amino acid residues His96 and His111 (human PrP numbering) are in a region of the PrP molecule that is considered to be essential for amyloid formation and prion infectivity during prion disease pathogenesis [28,29]. Cu2+ has been implicated in the neurotoxicity of the fragment of PrP that comprises amino acid residues 106–126 [28,30]. Furthermore, Cu2+ co-ordination by His96 and His111 (human PrP numbering) induces β-sheet formation in the unstructured amyloidogenic region of the prion protein [17]. This implicates Cu2+ co-ordination involving amino acid residues His96 and His111 (human PrP numbering) in the formation of disease-related features of PrP, as are other amino acids that participate in this particular PrP-copper complex. One such candidate is Met109 (human numbering, which corresponds to Met112 in ovine PrP), which is predicted to be part of the high affinity Cu2+ multiple histidine that occurs at low copper occupancy of PrP [31]. Met109 (human PrP numbering) may serve as an electron source to drive the redox chemistry of copper bound to His96 and His111 (human PrP numbering) [32]. Importantly, Met109 (human PrP numbering) is considered to be a critical amino acid in the formation of the increased β-sheet structure in PrP as it converts from PrPC to PrPSc [33]. This provides a link between metal-ion occupancy and the acquisition of abnormal conformations in the prion protein.

In the ovine PrP sequence three amino acid residues at positions 136, 154 and 171 are most commonly associated with susceptibility and resistance to scrapie disease [34,35]. Animals that express the allelic variant V136R154Q171 (in short, VRQ, where V, R, and Q stand for valine, arginine, and glutamine) or A136L141R154Q171 (AL141RQ, where A and L stand for alanine and leucine, respectively) show susceptibility to classical scrapie while those with A136R154R171 (ARR) show resistance. We have previously demonstrated that different genotypes of ovine PrP protein show distinct structural responses following exposure to Cu2+ [27]. Ovine VRQ PrP demonstrated a significant increase in β-sheet structure whereas ARR PrP remained relatively unchanged following exposure to Cu2+. This showed that structural changes in response to Cu2+ binding correlated with classical scrapie disease susceptibility. The M112T dimorphism in ovine PrP (which corresponds with amino acid residue 109 in human PrP) is associated with a potential increase in resistance to classical scrapie disease [36,37]. Since this dimorphism occurs in a structurally important Cu2+ co-ordination site, any alteration in susceptibility to classical scrapie disease may occur as a consequence of modulation of an interaction with this metal ion. Accordingly, we have compared the structural changes in M112ARQ and T112ARQ following exposure to Cu2+. Significantly, we have found that T112ARQ shows less β-sheet formation than does M112ARQ following exposure to Cu2+.

2. Materials and methods

2.1. Generation of ovine PrP plasmid constructs

DNA encoding ovine PrP-VRQ was amplified from genomic DNA that was isolated from spleen tissue of an ARR/VRQ sheep. The complete ORF of PRNP was inserted between the KpnI and the EcoRI restriction sites of pcDNA3 and the sequence verified. PCR was then carried out using primers that generated ovine PrP amino acid residues 25–232. The 5′ primer introduced sequences for an NdeI restriction site, a methionine translation initiation codon and a protective serine codon immediately following the methionine codon. The 3′ primer introduced a stop codon and an EcoRI restriction site. The sequence for ovine PrP 25–232 was subcloned into pET23b (Novagen) between the NdeI and EcoRI cloning sites. A plasmid containing pET23b-M112ARQ was generated by site-directed mutagenesis from the pET23b-VRQ plasmid using a commercial kit (Stratagene). In brief, a parental plasmid was subjected to PCR using complementary primers that forced nucleotide changes in a replication of the entire plasmid circle. Parental DNA was specifically digested by use of the restriction enzyme DpnI and the resultant mutagenized daughter plasmid was transformed into DH5α bacterial hosts for amplification and verification of the plasmid by DNA sequencing. Plasmid from correctly altered clones was then transformed into the BL21(DE3)pLysS expression host. DNA encoding PrP-T112ARQ was amplified from genomic DNA of an ARQ/ARQ PrP homozygous sheep, by PCR using primers that generated ovine PrP amino acid residues 25–232 as described above. The amplified sequences for ovine PrP-T112ARQ 25–232 was then subcloned into the pET23b vector between the NdeI and EcoRI cloning sites. Once the sequence was verified, plasmid from correctly altered clones was then transformed into DH5α and BL21(DE3)pLysS bacteria as above.

2.2. Expression and purification of ovine recombinant PrP

Recombinant PrP was purified from BL21(DE3)pLysS bacteria transformed with the prokaryotic expression vector pET23b that contained the open reading frame coding sequence of full-length, mature ovine T112ARQ or M112ARQ PrP (amino acid residues 25–232), in a method adapted from Hornemann et al. [11] and as described in detail elsewhere [27]. Oxidized and refolded recombinant PrP was stored at −80 °C.