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Copper induces structural changes in N-terminus of human prion protein

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ABSTRACT

Copper ions reportedly bind to the cellular prion (PrP^C) and induce PrP proteinase K (PK) resistant from (PrP^{res}). PrP^C also plays a role in response to oxidative stress. By using purified human PrP23-98 containing octarepeats, we have found that Cu(II) induces PrP^{res} determined by Western blots and atomic force microscopy, and structural changes detected by hydrogen/deuterium exchange in the PrP N-terminus. Therefore, we have provided the evidence that copper ions play an important role in the change of N-terminus of human prion protein.

1. Introduction

The cellular prion (PrP^C) is a normal protein with unknown physiological functions. In some conditions, PrP^C converts into a conformationally altered isoform, the scrapie prion (PrP^{Sc}), that becomes infectious and causes transmitted spongiform encephalopathies (TSEs) or prion diseases [1]. PrP^C is highly conserved in species and ubiquitously expressed in different types of cells. The most documented feature of PrP^C is its ability to bind copper ions. PrP^C contains 5 to 6 copper binding sites in the N-terminal area with the octapeptide (PHGGGWGQ) repeats [2–4], specifically at the histidine (H) residue [5]. PrP^C reportedly acts as a copper transporter to promptly internalize copper in neuronal culture experiments [6,7], and also links to the influence of copper on depolarization of the synapse [8]. Cu(II)-binding converts PrP^C to proteinase K (PK)-resistant form (PrP^{res}) [9], forming PrP amyloid fibrils under the high pressure [10]. Disruption of copper homeostasis due to a mutation of Atp7a delays the onset of prion disease, suggesting that copper influences the pathogenesis of prion disease [11]. Cu(II)-induced PrP^{res} aggregates were usually happened in the full-length mouse and human PrP [9], although the Cu(II) induced PK-resistant form of PrP was reportedly distinct from the scrapie isoform [12]. Binding to Cu(II) may also induce PrP^C oligomerization resulting in neuroinflammation and neurodegeneration [13]. The N-terminal fragment, human PrP23-98 (HuPrP23-98), also binds to Cu(II) [14,15], and convert to PK-resistant aggregates after incubation with Cu(II) plus nicotinamide adenine dinucleotide phosphate (NADPH) [16]. The non-octarepeat Cu(II) binding site (H96 and H111) has reportedly been a key regulator of prion conversion [15]. Therefore, the N-terminal sequence of prion protein is the important area for PrP^C binding to copper and conformational conversion.

Hydrogen-deuterium (H/D) exchange in conjunction with mass spectrometry (MS) has been used for the structural characterization of the full-length mouse prion protein [17], prion amyloid fibrils [18], and brain-derived PrP^{Sc} [19]. Therefore, in the current study, we have used MS combined H/D and atomic force microscopy to determine Cu(II) induced PK-resistance and structural changes in the N-terminal sequence 23–98 of human prion protein (HuPrP23-98).

2. Methods

Purification and characterization of the N-terminus of human PrP23–98. To express HuPrP23-98, cells of *E. coli* containing pGEX-HuPrP23-98[3] was grown at 37 °C in 1 L of LB containing ampicillin (100 μg/ml). The glutathione S-transferase (GST)/HuPrP23-98 fusion protein was expressed by stimulation in the presence of IPTG and purified by standard procedures. Approximately 1 mg fusion protein was incubated with 1U thrombin (Sigma) for 20 min. GST and uncleaved fusion protein was removed by adding 10 ml of glutathione-Sepharose 4B (Sigma-Aldrich, St.

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Louis, MO, USA) to the cleavage reaction. The authenticity of HuPrP23–98 was verified by mass spectrometry and by Western blots using anti-PrP N-terminus monoclonal antibody (mAb) SAF-32.

Conversion of human PrP N-terminus into PK-resistant form. After incubation of HuPrP23-98 (15 μM) with or without 105 μM $CuCl_2$ in 100 μl of 25 mM *N*-ethylmorpholine, 30 mM KCl (NEMO-KCl) buffer, pH 7.4, at room temperature for 48 h, 20 μl of each sample was digested with PK (50 μg/ml) at 37 °C for 1 h, and then loaded to 10–20% tricine-SDS electrophoresis and subjected to Western blotting with anti-PrP mAb SAF-32 to observe PK-resistant fragments. The samples incubated without $CuCl_2$ will also loaded as controls.

Atomic Force Microscopy. Solution tapping mode atomic force microscopy imaging was performed using a combination contact-tapping mode liquid cell fitted to a Digital Instruments Nanoscope IIIA Multi-Mode scanning probe microscope (Digital Instruments, Santa Barbara, CA, USA). All images were acquired using 120-mm silicon nitride V-shaped cantilevers with integral oxide-sharpened pyramidal tips (type DNP-S). Prior to use, the AFM tips were exposed to UV irradiation to remove adventitious organic contaminants from the tip surface. The AFM images were acquired using the E scanning head, which has a maximum lateral scan area of 14.6 × 14.6 μm. Optimal tapping mode imaging was achieved at a cantilever drive frequency of ~8.9 kHz with lateral scan rates between 1 and 2 Hz. Under these conditions, the free amplitude of the tip is < 3 nm. In situ AFM imaging of the human PrP23-98 protein was achieved by transferring 5 μl of the sample solution onto freshly cleaved mica previously affixed to an AFM sample puck. The sample was immediately sealed in the AFM liquid cell, and the cell was filled with the sample buffer solution. Image analyses were performed using the Nanoscope software version 6.13 and ImageJ (NIH).

Hydrogen/deuterium (H/D) exchange. MALDI-TOF-MS was used to determine the amide hydrogen exchange[23,38]. The purified HuPrP23-98 protein (2 nmol) in 5 μl 25 mM PBS was incubated with 0.25 μl of 50 mM $CuCl_2$ at room temperature for 30 min, and then 2 μl protein/Cu(II) mixture will be added to 18 μl D_2O for H/D exchange for 5, 10 and 90 min. The HuPrP23-98 protein sample without incubation with Cu(II) were used as controls. After measurement of the mass [M+H] of peptide samples with different treatments, the hydrogen exchange rates were calculated. Calculation and comparison of the mass measurements of Cu(II)-treated and untreated samples, the hydrogen exchange rates of each peptide were finely be mapped and reveal the structural changes.

3. Results

Cu(II) induces protease-resistance of the PrP N-terminus. To prepare the starting materials, we expressed and purified the N-terminus of human PrP23-98 containing the entire octarepeat sequence and the related sequence GGGTHNQ (Fig. 1. A). The fusion proteins, GST-PrP23-98 was purified by GST pull-down procedures (M_r~34 kDa) (Fig. 1B, a1). HuPrP23-98 was purified after thrombin cleavage (Fig. 1B, a2). Since the sequence of octarepeats contains Cu(II)-binding sites, we incubated HuPrP23-98 with or without Cu(II) for 48 h and then one-half of each sample was digested with protease K (PK). As expected, HuPrP23-98 incubated with or without Cu(II) has shown its M_r of ~7.8 kDa (Fig. 1B, b1, b3). The HuPrP23-98 was completely digested by PK (Fig. 1B, b2). Unexpectedly, the PK-resistant form of HuPrP23-98 was present after incubation of Cu(II) for 48 h (Fig. 1B, b4). It is reported that incubation with a combination of Cu(II) and NADPH could generate the PK-resistant form of HuPrP23-98 in 30 min [1,16]. Our results demonstrate that, with a longer incubation, Cu(II) only could induce the PK resistant form in PrP N-terminus, indicating that the possible structural changes happen in this area after incubation with Cu(II).

Cu(II)-induced PK-resistant aggregates in MoPrP23–231 monitored by atomic force microscopy (AFM). The PK-resistant form of HuPrP23-98 induced by Cu(II) was also detected by the AFM technique. The AFM signals in Fig. 1C were presented as three-dimensional images. Whereas the small particles observed by AFM retained an ellipsoidal appearance (60–75 Å) (Fig. 1C, a, b), large aggregated structures (250 ± 45 Å) were observed (Fig. 1C, b). Further digestion with PK demolished almost all protein particles but some PK-resistant aggregates were still observed (Fig. 1C, c). In sum, these data suggest that Cu(II) produces an assembly of HuPrP23-98 complexes to form larger supermolecular aggregates that was protease-resistant.

Cu(II) induces structural changes in the PrP N-terminus measured by hydrogen/deuterium (H/D) exchange. In order to observe the "real" structural changes, we further used MALDI-TOF-MS to determine the amide hydrogen exchange, which is a useful tool for protein structure elucidation [2,3,20,21]. HuPrP 23–98 was treated with or without 3-fold molar excess of Cu(II) for 30 min and then incubated with or without D_2O for 0, 5, 10 and 90 min for H/D exchange. As shown in Fig. 2a, HuPrP23-98 has a mass [M+H] 7814.34 Da (cal. mass 7812.26 Da). Treatment with Cu(II) did not change the mass (7814.51 Da) (Fig. 2e). After incubation with D_2O for 5 min, HuPrP23-98 exchanged 19 protons (Fig. 2b), but HuPrP23-98 treated with Cu(II) exchanged 18 protons (Fig. 2f), without significant difference. After incubation with D_2O for 10 min, HuPrP23-98 exchanged 22 protons (Fig. 2c), while HuPrP23-98 treated with Cu(II) exchanged 38 protons (Fig. 2g), having 16 more protons exchanged. After incubation with D_2O for 90 min, HuPrP23-98 exchanged 18 protons (Fig. 2d) while HuPrP23-98 treated with Cu(II) exchanged 32 protons (Fig. 2h), having 14 more protons exchanged. The results demonstrate the higher H/D exchange rate of the PrP N-terminus after exposure to Cu(II). Our findings have firstly revealed that the structural changes happen in this so-called "unstructured" sequence of PrP^C during Cu(II)-binding process.

4. Discussion

In the field of the prion research, the physiological functions of PrP^C and pathological effects of PrP^{Sc}, have been concerned. As the cause of prion diseases, the fatal neurodegenerative disorders, PrP^{Sc} is a β-sheet enriched isoform that was converted from the α-helical PrP^C. PrP^C has long been known as a copper binding protein[2]. The copper binding sites are at histidine (H) residuesin the highly conserved octapeptide repeats and its neighboring area [3,15]. The binding to copper ions, Cu(II), induces the conformational changes of PrP^C, which forms proteinase K (PK)-resistant prion protein (PrP^{res}) [4,9] and PrP oligomerization [13]. The Cu(II)-induced PrP^{res} was reportedly distinct from the scrapie isoform [12] but with neurotoxicity [13]. In the present study, we have focused on the N-terminus (aa23-98) of human prion protein (HuPrP23-98) that contains four identical octapeptide (PHGGGWGQ) repeats and non-octarepeat Cu(II)-binding site at H96 [22]. In the present study, our first finding is that, by binding to Cu(II), HuPrP23-98 converts into its PK-resistant form detected by Western blots and atomic force microscopy (Fig. 1), indicating that Cu(II) only induces PK-resistance in the N-terminus of human PrP. By using hydrogen/deuterium (H/D) exchange analysis, we secondly found that the pre-binding to Cu(II) to HuPrP23-98 makes more protons exchanges after incubation with D_2O_2 (Fig. 2), indicating that Cu(II) induces the real structural changes in the N-terminus of human prion protein, which may introduce proteinase-K resistance.

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