The International Journal of Biochemistry & Cell Biology 47 (2014) 20–28

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Interaction between 14-3-3β and PrP influences the dimerization of 14-3-3 and fibrillization of PrP106–126

Jun Han[a], Qin-Qin Song[a], Peng Sun[a], Jin Zhang[a], Xu Wang[b], Juan Song[a], Gong-Qi Li[a], Ying-Hui Liu[a], Guo-Yong Mei[a], Qi Shi[a], Chan Tian[a], Cao Chen[a], Chen Gao[a], Bo Zhao[b], Xiao-Ping Dong[a,c,*]

[a] State Key Laboratory for Infectious Disease Prevention and Control, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases (Hangzhou), National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, 155 Changbai Road, Beijing 102206, People's Republic of China
[b] College of Chemistry and Materials Science, Nanjing Normal University, 122 Ninghai Road, Nanjing 210097, People's Republic of China
[c] Chinese Academy of Sciences Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, People's Republic of China

ARTICLE INFO

Article history:
Received 18 September 2013
Received in revised form 24 October 2013
Accepted 28 October 2013
Available online 22 November 2013

Keywords:
Prion disease
14-3-3
PrP
Fibril
Disaggregation

ABSTRACT

Proteins of the 14-3-3 family are universal participate in multiple cellular processes. However, their exact role in the pathogenesis of prion diseases remains unclear. In this study, we proposed that human PrP was able to form molecular complex with 14-3-3β. The domains responsible for the interactions between PrP and 14-3-3β were mapped at the segments of amino acid (aa) residues 106–126 within PrP and aa 1–38 within 14-3-3β. Homology modeling revealed that the key aa residues for molecular interaction were D22 and D23 in 14-3-3β as well as K110 in PrP. Mutations in these aa residues inhibited the interaction between the two proteins *in vitro*. Our results also showed that recombinant PrP encouraged 14-3-3β dimer formation, whereas PrP106–126 peptide inhibited it. Recombinant 14-3-3β disaggregated the mature PrP106–126 fibrils *in vitro*. Moreover, the PrP–14-3-3 protein complexes were observed in the brain tissues of normal and scrapie agent 263 K infected hamsters. Colocalization of PrP and 14-3-3 was seen in the cytoplasm of human neuroblastoma cell line SH-SY5Y, as well as human cervical cancer cell line HeLa transiently expressing full-length human PrP. Our current data suggest the neuroprotection of PrPC and neuron damage caused by PrPSc may be associated with their functions of 14-3-3 dimerization regulation.

1. Introduction

Prion diseases, also known as transmissible spongiform encephalopathies (TSEs), are fatal progressive neurodegenerative diseases in humans and animals and include Creutzfeldt–Jakob disease (CJD), Gerstmann–Sträussler–Scheinker syndrome (GSS), fatal familial insomnia (FFI), and kuru in humans, scrapie in sheep, and bovine spongiform encephalopathy (BSE) in cattle. All prion diseases have similar clinical symptoms and pathological changes, with a long latency period ranging from several months to decades (Venneti, 2010). A critical event in pathogenesis appears to be the conversion of protease-sensitive prion protein (PrP^C) to its protease-resistant isoform (PrP^{Sc}), which involves a conformational change in PrP from having high α-helix to high β-sheet content. A related peptide consisting of amino acids 106–126 exhibits a β-sheet structure in water and is able to form amyloid-like fibrils *in vitro* and displays neurotoxic activity in primary cultures of rat hippocampal neurons as well as many other cell lines (Henriques et al., 2009; Vassallo, 2009; Walsh et al., 2009a).

The 14-3-3 proteins comprise a family of abundant and widely expressed acidic polypeptides in all eukaryotic cells; they act as either homo- or heterodimers (Aitken, 2002; Berg et al., 2003). This highly conserved family of small (28–33 kDa) acidic dimeric proteins consists of at least seven distinct subunit isoforms (β, γ, ε, ζ, η, σ and τ/θ), of which α and δ are the phosphorylated forms of β and ζ (Berg et al., 2003). The distribution of the 14-3-3 protein family varies among tissues: isoforms of 14-3-3 including β, γ, ε, η, and ζ are present mainly in nervous tissues, 14-3-3τ appears mostly in T-lymphocytes and testicular tissue, and 14-3-3σ is present in epithelial cells. The 14-3-3 proteins are highly expressed in brain

* Corresponding author at: Chinese Academy of Sciences Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, People's Republic of China.
Tel.: +86 10 58900815; fax: +86 10 58900815.
E-mail addresses: zbchem@126.com (B. Zhao), dongxp238@sina.com (X.-P. Dong).

1357-2725/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biocel.2013.10.013

tissues, comprising roughly 1% of the total soluble proteins (Berg et al., 2003; Bridges and Moorhead, 2005). The 14-3-3 proteins form complexes with multiple protein ligands, *e.g.*, Raf-1, Bad, Bax, and Cdc25 (Gardino et al., 2006), and are involved in various cellular functions such as cell division, nuclear transport, apoptosis, and differentiation (Mackintosh, 2004; Yang et al., 2006).

In contrast to their ubiquitous distribution in the brain, 14-3-3 proteins are normally undetectable in cerebrospinal fluid (CSF). In fact, the release of 14-3-3 proteins into the CSF is generally associated with acute neuronal injury. In some cases, *e.g.*, acute cerebrovascular stroke and viral encephalitis, 14-3-3 protein levels may transiently increase in the CSF (Harris and Sadiq, 2009; Thomson and Bertram, 2001). In many neurodegenerative diseases such as Alzheimer disease and Parkinson disease, 14-3-3 proteins are generally undetectable in the CSF. However, the CSF 14-3-3 protein is frequently detected in patients with CJD, especially sporadic CJD (sCJD), and the sensitivity and specificity of detection are reported to be as high as 96% and 93–100%, respectively (Ladogana et al., 2009). Therefore, CSF 14-3-3 protein positivity Western blotting is accepted by the World Health Organization as a laboratory criterion for the diagnosis of sCJD. However, the exact role played by 14-3-3 proteins in the pathogenesis of prion diseases remains unclear.

In the current study, the possible interactions of 14-3-3 with PrP were analyzed to explore the roles that 14-3-3 proteins may play on PrP physiology and prion pathology. We found a reliable molecular interaction between PrP and 14-3-3β *in vitro* and *in vivo* by using co-immunoprecipitation and pull-down assays. The domains responsible for the interaction between the two proteins were mapped at amino acid (aa) residues 106–126 in PrP and aa 1–38 in 14-3-3β. Using homology modeling and further testing, we predicted and subsequently confirmed that the key amino acids for the interaction are D22 and D23 in the 14-3-3 protein and K110 in PrP. We also proposed that the process of 14-3-3β dimerization *in vitro* was promoted by full-length PrP (PrP23–231) but inhibited by the peptide PrP106–126, while 14-3-3β disaggregated PrP106–126 fibrils *in vitro*.

2. Methods

2.1. Ethics statement

All procedures were in accordance with the Institutes of Laboratory Animal sciences, CAMS&PUMC Guidelines for the Use and Care of Laboratory Animals in Research and the recommendations of the Welfare Report. All procedures were approved and supervised by the Research Ethics Committee, National Institutes for Viral Disease Control and Prevention, China CDC.

2.2. Plasmid construction, protein expression and purification

The bacterially expressed recombinant plasmids pHuPrP23–231, pHuPrP23–160, and pHuPrP-PG0, as well as the mammalian expression plasmid pcDNA-PrP1–253, are as described elsewhere (Han et al., 2006). Human PrP sequence encoding aa 106–126 was amplified by polymerase chain reaction (PCR) with primers 5′-GC*GGATCC*ATGACCAACATGAAGCACATGGCTG-3′ and 5′-CG*GAATTC*TTAGCCAAGGCCCCCCACCAC-3′ using pHuPrP23–231 as the template and inserted into vector pQE30. The bacterially expressed recombinant plasmid pGST-14-3-3β was generated as described previously (Han et al., 2006). To achieve different lengths of 14-3-3β fragments, serial PCR was conducted using pGST-14-3-3β as the template with different pairs of primers: 5′-CG*GGATCC*ATGACAATGGATAAAAGTG-3′ and 5′-CG*GAATTC*CTTAGCTGGAGATGACACGC-3′ for the sequence encoding aa 1–66, 5′-GC*GGATCC*ATGAACGAAGAGAGAAATCTG-3′ and 5′-GC*GGATCC*AAAGAAATGCAGCCTACACAC-3′ for aa 39–71, primers 5′-CG*GGATCC*ATGTCCTGGCGTGTCATCTC-3′ and 5′-CG*GAATTC*TTATTCAAATGCTTCCTGG-3′ for aa 60–156, primers 5′-CG*GGATCC*ATGTCTGAAGTGGCATCTGG-3′ and 5′-CG*GAATTC*TTAAGACTCTTCATTCAGCG-3′ for aa 132–213, and primers 5′-CG*GGATCC*ATGCGTCTTGGTCTGGCAC-3′ and 5′-CG*GAATTC*TTAGTTCTCTCCCTCC-3′ for aa 168–247. The cutting sites of the respective restriction enzymes are shown in italics. After verification using sequencing assays, various PCR products were inserted into vector pQE30. His-glutathione-S-transferase (GST) protein was purified as described previously (Han et al., 2006).

Various recombinant human PrP and 14-3-3 proteins were expressed in *Escherichia coli* JM109. The bacterially expressed recombinant His-tagged proteins were recovered from the urea-solubilized bacterial lysate after sonication using immobilized nickel-based affinity chromatography. The recombinant prokaryotic protein tagged with GST was expressed and purified using Glutathione Sepharose 4B Agarose (GE Health, USA). Protein concentrations were determined using a BCA kit (Qiagen, Germany).

2.3. Preparation of brain homogenates

Two-week-old hamster brains were inoculated with Scrapie strain 263K. Once the hamster has the typical clinical symptoms, hamsters were executed by ether anesthesia. Brain tissues were taken and stored at −80 °C. Normal and scrapie agent 263K-infected hamsters' brain tissues (10% w/v) were homogenized in lysis buffer (100 mM NaCl, 10 mM ethylenediaminetetraacetic acid, 0.5% Nonidet P-40, 0.5% sodium deoxycholate in 10 mM Tris–HCl, pH 7.4) as described previously (Han et al., 2006). The brain homogenates were centrifuged at 20,000 × *g* for 30 min and the supernatants were collected for further experiments.

2.4. Immunoprecipitation

Two μM various PrP and 2 μM various 14-3-3 were incubated in 500 μl binding buffer (50 mM Tris–Cl, 100 mM NaCl, 10 mM aprotinin, pH 8.0) at 4 °C for 2 h. After incubated with anti-14-3-3 pAb or mAb 3F4 for 2 h. 10 μl Protein G Sepharose (Roche, Switzerland) equilibrated by binding buffer was introduced into the reactions and incubated for further 2 h. The Sepharose beads were precipitated at 2000 rpm for 5 min and washed with 500 μl washing buffer (50 mM Tris–Cl, 500 mM NaCl, pH 8.0) for three times. Equal amount of GST protein was employed as negative control. The bound complexes were separated by 12% SDS-PAGE and transferred to nitrocellulose membranes. The bound 14-3-3β or PrP proteins were detected by anti-14-3-3β Ab or anti-PrP mAb 3F4 in Western blot, respectively.

Totally 0.2 ml of the supernatant of normal or scrapie-adapted hamster brain tissue homogenates were incubated with anti-14-3-3β pAb or anti-PrP mAb 3F4 at 4 °C for 2 h. The antigen–antibody complexes were collected by Protein G Sepharose as described above. The bound 14-3-3β or PrP proteins were detected by anti-14-3-3β pAb or anti-PrP mAb 3F4 in Western blot.

2.5. Pull-down assays

About 2 μM various PrP and 2 μM various 14-3-3β was incubated in 500 μl binding solution (20 mM Tris–Cl, 200 mM NaCl, 10 mM aprotinin, pH 8.0) at 4 °C for 4 h, while equal amount of His-GST protein was used as control. 10 μl of glutathione agarose beads or Ni-NTA agarose beads were added to the reaction solution and incubated at 37 °C for 30 min with end-over-end mixing. After centrifugation at 2000 rpm for 2 min, the supernatants were