Biosensors and Bioelectronics 26 (2010) 1399–1406

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Biosensing of lipid–prion interactions: Insights on charge effect, Cu(II)-ions binding and prion oligomerization

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ARTICLE INFO

Article history:
Received 6 April 2010
Received in revised form 2 July 2010
Accepted 17 July 2010
Available online 23 July 2010

Keywords:
Prion
Liposomes
Protein–lipid interaction
Rafts
SPR

ABSTRACT

The molecular mechanism involved in early stages of prion protein (PrP) conversion has been investigated using the chip based SPR technology, focusing on PrP interactions with membranes, either in its monomeric, oligomeric or Cu(II)-ions bound forms. We observed a strong interaction between PrP and cell membrane models of different lipid compositions. Circular dichroism tests show that membrane-bound, oligomerized or Cu(II)-complexed PrP may adopt a β-sheet-rich conformation. Moreover, upon PrP binding membrane vesicles may aggregate and/or be fragmented depending on vesicle net-charge and their lipid/raft composition. The whole study emphasizes the outstanding performance of the on-a-chip approach for the investigation of prion conversion and could be useful for developing sensor formats for prion assessments in biological samples.

1. Introduction

Prion proteins (PrPs) are cell membrane glycosylphosphatidylinositol (GPI)-anchored proteins directly involved in transmissible spongiform encephalopathies (TSEs) affecting a number of mammalian species (Caughey and Baron, 2006; Caughey et al., 2009). The disease leads to a progressive dementia, ataxia and finally death, probably following the conversion of the endogenous cellular prion protein (PrP^C) into the infectious pathological scrapie form (PrP^{Sc}). In contrast to PrP^C, which is a soluble α-helical protein with little β-sheet structure, PrP^{Sc} shows a high content of β-sheet structure with less α-helix structure and has a strong tendency to polymerize into amyloid fibrils (Fischer et al., 1996; Prusiner et al., 1983). This conversion is believed to be enhanced by prion interaction with various molecules such as nucleic acids, lipids, copper ions, chaperones (Hsp73, GroEL, Hsp70), some extracellular proteins (heparin, vitronectin), or protein located at membrane rafts (caveoline-1) (Noinville et al., 2008).

Cellular PrP^C can be anchored or attached to the outer layer of the plasma membrane (Eberl et al., 2004; Morillas et al., 1999). This suggests that conversion of prions may occur at the membrane surface (Caughey et al., 2009). Furthermore, some evidence suggests that the cellular plasma membrane plays a role in the mechanism by which PrP^{Sc} causes toxicity and neurodegeneration (Chesebro et al., 2005; Lashuel, 2005; Lashuel and Lansbury, 2006). The study of the effect of the GPI on conversion of PrP expressed in fibroblast cells suggests that lipid–prion interaction could yield the formation of anchorless intermediates playing a role in the conversion. As proposed for other pathologies caused by protein misassembling and amyloidosis, β-sheeted and amyloidic fibrillar structures of the prion peptides might increase their toxicity and affinity for membrane binding especially in cholesterol-rich membranes (Dobson, 2005; Rymer and Good, 2000).

In the present study, we have shown that recombinant PrP binds to small unilamellar liposomes immobilized on sensorchips. We characterized the binding of monomeric and oligomeric forms of ovine PrP variant ARQ, either in full-length or in N-terminal truncated forms (named ΔARQ), with liposomes of various lipid composition. Interestingly, we observed that upon membrane binding ARQ can switch from normal to β-sheet-rich structures. Our approach offers a simple and effective method to identify potential cellular molecules involved in prion conversion. Although our study has been done on unglycosylated and unglypiated recombinant PrP, findings obtained could be useful in the development of a new generation of biosensors that can be applied to detect PrP interactions in real biological samples.

2. Materials and methods

2.1. Purification of the recombinant prion protein

Full-length $A^{136}R^{154}Q^{171}$ variant ($ARQ^{23–234}$) of ovine PrP was expressed and purified as described previously (Rezaei et al., 2000). Briefly, the gene encoding ARQ (23–234) was cloned in pET 22b+

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doi:10.1016/j.bios.2010.07.064

and expressed by IPTG induction in the BL21 DE3 *Escherichia coli* strain. A high yield one-step method for the purification of the full-length PrP was performed on a Ni sepharose column by heterogeneous phase renaturation, based on the intrinsic affinity of the PrP N-terminal part for metal cations.

The C-terminal prion domain (ΔARQ) deprived of its unstructured N-terminal tail (103–234) was expressed in the BL21 DE3 *E. coli* strain by IPTG induction using its cloned cDNA in pET-28a plasmid (Novagen) as described previously (Eghiaian et al., 2004). The expressed truncated His_6-tagged ΔARQ was purified performing the same procedure as for the full-length variant (Rezaei et al., 2000). The His tag was cleaved using biotinylated thrombin (Novagen).

After purification, prion proteins were recovered in 0.5 g/L ammonium-acetate buffer at pH 5, and desalting was performed by elution on a G25 column (Hi Trap™, GE Healthcare) using Akta fast protein liquid chromatography (FPLC, Pharmacia). Then, samples were lyophilized and stored at −20 °C. Prior to analysis, lyophilized protein powder was solubilized in 10 mM sodium-acetate and then desalted in appropriate buffer, using a G25 column. Final protein concentrations were determined by measuring optical density at 280 nm and concentration calculus was made using extinction coefficients deduced from their compositions of 58,718 and 18,005 M^{-1} cm^{-1} for ARQ and ΔARQ, respectively. Both monomeric forms are stable for a week at 4 °C.

2.2. OvPrP 12mer purification

For the purification of the OvPrP oligomeric species O_3 and ΔO_3 (12mers) (Rezaei et al., 2002), the conversion of ARQ or ΔARQ to their oligomeric forms was performed by protein incubation in 20 mM citrate buffer, pH 3.35, at 50 °C for 10 min in a Perkin Elmer GenAmp2400 thermocycler. As shown previously, at pH 3.5 structurally distinct ovine prion protein soluble oligomers can be generated (Eghiaian et al., 2007). Reaction products were separated by size-exclusion chromatography (TSK 4000SW column, Tosoh Bio) using Akta FPLC allowing collection of homogeneous O_3 fractions. Typically oligomers were freshly prepared before use. Otherwise, their elution profiles were checked by size-exclusion chromatography. We found that at 4 °C, oligomers were stable for a week at pH 3.36; and just a few days when desalted against pH 7.

2.3. Liposome preparation

Soybean asolectine with an approximate phospholipid composition of phosphatidyl choline (PC), 29%; phosphatidyl ethanolamine (PE), 30%; phosphatidyl inositol (PI), 26%; phosphatidic acid (PA), 14%; phosphatidyl serine (PS), 1% (Meidleman, 1993) was purchased from Fluka. PC, PS, cholesterol and sphingomyelin were purchased from Sigma. Dry films of 10 mg lipids were prepared from a stock solution in chloroform evaporated under a stream of nitrogen and left under vacuum for at least 8 h to remove all traces of the organic solvent. The lipid film was suspended in either 20 mM citrate buffer, pH 3.35, or MOPS buffer pH 7, and gently vortexed and sonicated for a few minutes. Then, liposomes were freeze–thawed three times in liquid nitrogen. The liposome suspension obtained was extruded through a polycarbonate membrane with a pore diameter of 100 nm (Osmonics). The size of asolectine vesicles obtained was checked by dynamic light scattering (Malvern, UK).

2.4. SPR experiments

SPR experiments were conducted on a BIAcore 3000 (GE Healthcare). Experiments were carried out on L1 (GE Healthcare). Liposomes (1 mg/ml) were injected on sensor chips at a low flow rate (2 μl/min) for 20 min until a stable resonance unit (RU) level was obtained. After immobilization, the surface was washed with 10 mM NaOH to remove any multilamellar structure. The SPR signal was stable for at least 24 h indicating the stability of the immobilized membrane. Various OvPrP samples were run over liposome-coated flowcells. Each OvPrP sample was run on a freshly prepared and equilibrated liposome layer. All measurements were performed at 20 °C. RU levels were compared before and after each injection, to evaluate the amount of material grafted onto the sensor chip. Between two experiments, the sensor chip surface was regenerated with 8 M urea to denature and depolymerize PrP, and then with 20 mM CHAPS to desorb liposomes. Sensorgrams were analyzed using BIAevaluation Software.

2.5. Size measurements by dynamic light scattering (DLS)

The size measurements with the Zetasizer Nano serie (Malvern, UK), based on the principle of dynamic light scattering, were made at 20 °C using a helium–neon laser wavelength of 633 nm and detection angle of 90°. The results were analyzed by using either the mean values of size or the volume method for size distribution. For each size measurement, 10 runs of 11 individual measurements were done.

2.6. Circular dichroism spectroscopy

Circular dichroism (CD) measurements were performed on a JASCO J-810 spectropolarimeter equipped with a thermostatic cell holder, using a quartz cell of 0.5 mm path length. Spectra were collected over the wavelength range of 180–260 nm with a bandwidth of 1.0 nm and corrected for the contribution of the buffer. CD spectra of the PrP variants were recorded at 20 °C and at a final concentration of 20 μM. Each spectrum was an average of sixteen scans. For secondary structure estimation, CD spectra were analyzed and quantified using the DicroPro 2000 software.

2.7. Electron microscopy

Electron micrographs were acquired using a transmission electron microscope at 80 kV excitation voltage (Philips EM12). Liposomes of different lipid compositions at a concentration of 0.2 mg/ml in 10 mM MOPS, 100 mM KCl, pH 7.4 buffer were incubated with ARQ at various concentrations (0.1–5 μM) at room temperature for 5 min. After the incubation, 10 μl of the lipid–protein sample were adsorbed onto formvar/carbon-coated 200 mesh copper grids (Agar Scientific, Stansted, UK). The grids were blotted and, after drying, negatively stained by floating on 10 μl drops of 2% (w/v) uranyl acetate (Sigma).

3. Results

3.1. PrP preparations

Both recombinant ovine PrP (OvPrP) used in this study, ARQ (23–234) and ΔARQ (103–234), were purified under oxidizing conditions from inclusion bodies generated in *E. coli* and refolded to a α-helical conformations, to represent normal PrP^C. In addition 12mers of ARQ and ΔARQ (O_3 and ΔO_3 oligomers respectively) were tested in order to gain an insight into the mechanism of prion oligomer interactions with membranes.

Both monomeric variants showed the far-UV CD spectrums with typical features of a protein containing a large amount of α-helical structure with well defined minima at 208 and 222 nm (Fig. 1A and B). In contrast to their corresponding monomeric forms, 12mers have far-UV CD spectrums with a minimum at ~218 nm reflecting a β-sheet structure (Fig. 1A and B). Quantitative spectrum analyses