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The different role of Cu^{++} and Zn^{++} ions in affecting the interaction of prion peptide PrP106-126 with model membranes

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Differential scanning calorimetric (DSC) experiments have shown that the ability of PrP106-126 to perturb 1,3-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) model membranes is differently affected by Cu^{++} and Zn^{++} ions.

Scrapie in sheep and goats, bovine spongiform encephalopathies in cattle, Creutzfeldt–Jakob disease, Gerstmann–Straussler–Scheinker syndrome and kuru in humans are transmissible neurodegenerative disorders characterized by progressive neuronal loss and protein aggregation/deposition in the brain.[1–4] An increasingly large body of evidence strongly suggests that these diseases are caused by infectious units named "prions".[5] According to the "protein-only hypothesis", the infectious agent consists entirely of a conformational isoform (PrP^{sc}) of the prion protein (PrP^{c}),[6] a protein normally found on the outer surface of neurons.[7–9] The conversion of PrP^{c} into PrP^{sc} occurs without any chemical modification of the protein molecule,[10–11] but the two conformers have remarkably different physico-chemical properties: in contrast with PrP^{c}, PrP^{sc} is resistant to protease digestion and has a marked tendency to form insoluble aggregates and amyloid fibrils.[12–14] It has been also shown that a synthetic peptide encompassing human prion residues 106–126 (PrP106-126) is highly fibrillogenic and toxic to neurons *in vitro*[15–18] and shares with PrP^{sc} many physico-chemical and biological properties *e.g.* resistance to protease digestion[19] and induced activation of astroglial and microglial cells *in vitro*.[15,20] Recently, it has been shown that Cu^{++} and Zn^{++} ions can influence PrP106-126 β-sheet content and aggregation and in particular that Cu^{++} enhances the peptide toxicity.[21] In addition, it has been shown that β-sheet and amyloid structure of PrP106-126 give rise to its toxicity and membrane binding affinity suggesting a possible relationship between these properties.[22] In a previous study, DSC experiments aimed at evaluating the effect of PrP106-126 on the thermotropic properties of DPPC model membranes showed that PrP106-126 can spontaneously transfer from water to the lipid bilayer, decreasing the enthalpy (ΔH) associated with the gel–liquid crystal transition.[23]

In the present study the different role of Cu^{++} and Zn^{++} ions in affecting the interaction of PrP106-126 with DPPC model membranes was investigated by DSC experiments. In particular DPPC–PrP106-126–Cu^{++} and DPPC–PrP106-126–Zn^{++} systems were investigated to evaluate how the ΔH and the temperature (T_m) of the gel–liquid crystal phase transition of the membrane are affected by the two peptide–metal complexes. In a control experiment a suitable amount of dry DPPC lipid film was vortexed with a PrP106-126 aqueous solution at a peptide/lipid molar fraction of 0.1. The lipid dispersion was then extruded to form large unilamellar vesicles (LUV) according to a procedure described elsewhere.[23,24] The final concentration of PrP106-126 was 300 μM. The system was then stepwise titrated with an excess of Cu^{++} performing a DSC scan (heating rate = 0.5 °C min^{-1}) after each Cu^{++} addition; the T_m and the ΔH of the lipid transition were not modified even in the presence of a large excess of copper (up to 1 : 7 peptide : copper molar ratio). In a second experiment, DPPC lipid films were vortexed with a PrP106-126 : Cu^{++} 1 : 1 aqueous solution under the same experimental conditions described above. The T_m (42.10 ± 0.05 °C) and the ΔH (26.5 ± 4 kJ mol^{-1}) of the lipid transition were similar to the control, within the limits of experimental error. This system was then stepwise titrated with an excess of Cu^{++} performing, after each addition, a DSC run (Fig. 1). Addition of Cu^{++} did not modify the T_m of the lipid transition, while the enthalpy decreased asymptotically to a final value of 13.8 ± 5 kJ mol^{-1} (see panel A of Fig. 2). This value was obtained at a PrP106-126 : Cu^{++} molar ratio of 1 : 4 and it was not modified by further addition of Cu^{++}.

In a third experiment aimed at testing the spontaneous transfer of the 1 : 1 PrP106-126–Cu^{++} complex from the aqueous environment to the membrane, the peptide–Cu^{++} solution was incubated with pure DPPC LUVs without modifying any of the other experimental conditions and then titrated with increasing amounts of Cu^{++}, performing a DSC run after each addition. A plot of the measured ΔH *vs* the Cu^{++} : PrP106-126 molar ratio (panel B of Fig. 2), has shown that the ΔH increases, beyond a peptide : Cu^{++} molar ratio of 1 : 1, up to a *plateau* value similar to the ΔH of pure DPPC LUVs. This suggests that Cu^{++} addition inhibits the ability of PrP106-126 to transfer from water to the hydrophobic core of the membrane at a peptide : Cu^{++} molar ratio ranging from 1 : 1 to 1 : 2 and that further addition of Cu^{++} does not modify appreciably its potential to interact with it. Whilst previously obtained results[23] have evidenced a spontaneous insertion of PrP106-126 into the

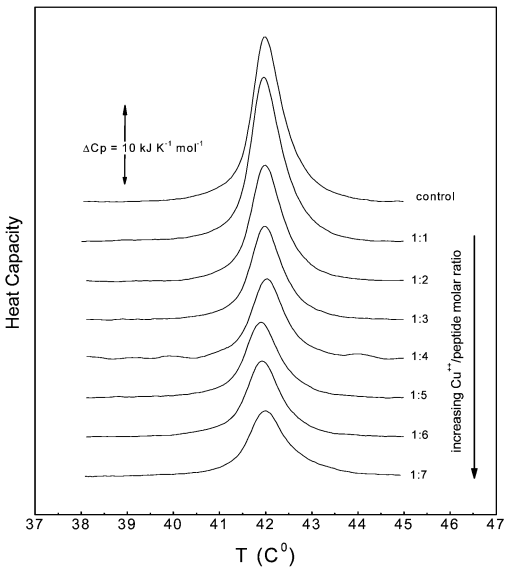

Fig. 1 DSC curves of DPPC–PrP106-126–Cu^{++} mixtures obtained at different PrP106-126/Cu^{++} molar ratios. The first curve represents the DSC profile of the DPPC–PrP106-126 system without metal, reported as a control. The second Cp curve (peptide/Cu^{++} molar ratio = 1 : 1) was obtained by vortexing the dry lipid film with a suitable amount of a freshly prepared Cu^{++}–PrP106-126 1 : 1 aqueous solution at a temperature above the temperature of the lipid transition. The final concentrations and experimental conditions are reported in the text. Cu^{++} was then stepwise added to this system up to a peptide : metal molar ratio of 1 : 7. A DSC heating run was carried out after each Cu^{++} addition and the obtained calorimetric profiles are reported. Each DSC run was repeated after 2 days to check for kinetic effects.

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lipid tails of DPPC LUVs, the present results show that Cu^{++}–PrP106-126 complexes incubated externally to DPPC LUVs do not perturb the hydrophobic core of the membrane, probably due to the copper-induced formation of large inert aggregates as reported elsewhere.[21]

The same experiments were carried out in parallel using Zn^{++} instead of Cu^{++} and the results are reported in Fig. 2. Differently from Cu^{++} complexes, when dry DPPC lipid films were vortexed with a PrP106-126 : Zn^{++} 1 : 1 solution, the ΔH of the lipid transition increases up to a value of 31.5 ± 0.5 kJ mol^{-1}; moreover, T_m is decreased of 0.55 ± 0.05 °C compared to the control. Further addition of excess Zn^{++} did not modify the T_m and ΔH values. Two factors may play a role in this remarkable difference in affecting the properties of DPPC observed for Zn^{++} complexes: (i) the different affinity of the two metals for the peptide; (ii) the different coordination geometries adopted by the two ions in solution. It should be noted that when a 1 : 1 PrP106-126 : Zn^{++} solution was incubated with pure DPPC LUVs and then titrated with increasing amounts of Zn^{++} (panel B of Fig. 2), no obvious differences from the experiments carried out with Cu^{++} were observed, thus showing that neither Cu^{++} nor Zn^{++} give rise to a PrP106-126–metal structure able to insert itself into the hydrocarbon core of DPPC when added to the exterior of the membrane.

The results here reported provide the first experimental evidence of the different roles played by Cu^{++} and Zn^{++} ions in affecting the interactions of PrP106-126 with DPPC model membranes. They are in agreement with previous findings suggesting a close relationship existing between Cu^{++} binding, peptide structure, membrane affinity, peptide-induced neuronal apoptosis for PrP106-126,[25] and the oligomerization state of the peptide aggregates.[26] More specifically, although aware of the limits that such a simplified model has in the understanding of the properties of PrP106-126 in cellular models, we suggest that the specific response of DPPC–PrP106-126 lipid bilayers to Cu^{++} addition reported here might help to clarify the Cu^{++} modulated activity of ion channels formed by PrP106-126 in model membranes recently observed,[27] and ultimately, the increased toxicity of PrP106-126 as a consequence of Cu^{++} addition.[21]

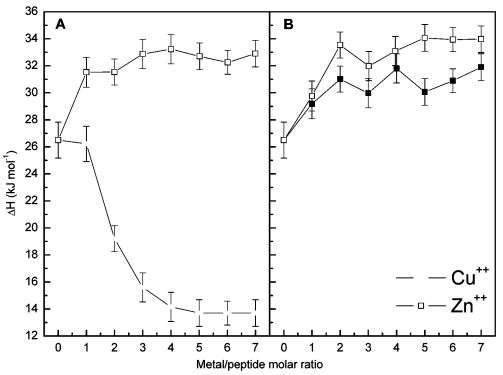

Fig. 2 Panel A). Effect of Cu^{++} additions (closed squares) on the enthalpy of the gel–liquid crystal lipid transition (ΔH) of DPPC–PrP106-126–Cu^{++} mixtures prepared as reported in the caption of Fig. 1. The ΔH values were obtained calculating the areas of the DSC peaks reported in Fig. 1; effect of Zn^{++} additions (open squares) on the ΔH of DPPC–PrP106-126–Zn^{++} mixtures prepared in parallel to the Cu^{++} titrations. Panel B). Effect of Cu^{++} additions (closed squares) on the ΔH of pure DPPC vesicles which have been previously incubated with PrP106-126–Cu^{++} 1 : 1 aqueous solutions; effect of Zn^{++} additions (open squares) in a parallel set of experiments.

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