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Copper-induced conformational change in a marsupial prion protein repeat peptide probed using FTIR spectroscopy

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Abstract We report the first Fourier transform infrared analysis of prion protein (PrP) repeats and the first study of PrP repeats of marsupial origin. Large changes in the secondary structure and an increase in hydrogen bonding within the peptide groups were evident from a red shift of the amide I band by >7 cm^{-1} and an approximately five-fold reduction in amide hydrogen–deuterium exchange for peptide interacting with Cu^{2+} ions. Changes in the tertiary structure upon copper binding were also evident from the appearance of a new band at 1564 cm^{-1}, which arises from the ring vibration of histidine. The copper-induced conformational change is pH dependent, and occurs at pH >7.

Key words: Fourier transform infrared spectroscopy; Repeat peptide; Prion protein; Copper binding; Marsupial protein; Secondary structure

1. Introduction

Prion diseases are fatal neurodegenerative disorders associated with abnormal metabolism of prion protein (PrP) [1]. PrP is a cell-surface protein with unclear functions, which is normally present in a soluble form, PrP^{C}. In diseased animals, an infectious aggregated form, PrP^{Sc}, is observed, which is composed largely, if not entirely, of an alternative conformer of PrP [1]. This identification with conformational diseases [2,3] has lead to intensive investigation of the properties of PrP using structural [4–7] and biophysical approaches [3,8].

Although relatively short ($\sim$253 residues, mammalian), the sequence of PrP is unusual in that it contains a number of regions with quite different amino acid compositions [9] and apparent structure. Solution nuclear magnetic resonance (NMR) structures of several full-length mammalian PrPs show that the N-terminal region (residues 23–125), including the PGH-rich tandem repeats (residues 60–91), are unstructured, while the C-terminal region (residues 126–231) adopts a loosely folded structure containing three α-helices and two short β-strands [4–7]. Mammalian PrPs have five tandem octapeptide repeats with the consensus sequence PHGGGWGQ [9].

Of the several functions ascribed to PrP, some findings suggest a role in copper metabolism [10–12] associated with binding to the N-terminal region at physiological copper concentrations [13,14]. Interestingly, although this region of PrP is not essential for pathogenesis, it modulates the extent and timing of disease presentation [15], and it has been suggested that copper may also have a role in the disease [16]. Recently, copper binding to the C-terminal domain at pHs <6 only [17], and to the region between the repeats and C-terminal region (92–125) [18,19] has been shown. Studies using recombinant full-length protein, as well as synthetic peptides containing the N-terminal repeats, show preferential binding of copper over other metals [14,19–21].

Various biophysical methods have been applied to study the structure of the repeat region of PrP in the presence and absence of copper [14,19–29]. These have used a range of synthetic and cloned peptides containing different numbers of repeats with either consensus [20–22,24,26–28] or actual [19,22,23,25,29] sequences from mammals or chicken, or full-length mammalian protein [14]. The possibility of an effect on repeat-region conformation from peptide length and sequence, particularly from use of designed (multiple-consensus) sequences or authentic sequences covering only part of the N-terminal region of PrP, needs to be considered. However, the fact that there is considerable natural variability in repeat sequence from the consensus, suggests that critical features of the conformational changes may be relatively insensitive to the exact repeat sequence or its environment. The biophysical studies have yielded variable results for the structural changes, as well as the stoichiometry and strength of copper binding, which may be due to a variety of experimental factors apart from peptide differences: pH dependence, buffer artifacts, solubility problems for both protein and copper and their complexes at pH >7, and different concentration ranges for the analytical methods [17]. In the absence of copper, CD [21,23–25] and Raman [22,26] spectroscopies, as well as NMR [4–7] have shown that the repeat region is unstructured. However, another CD study reports the presence of non-random structure similar to the poly-L-proline type II left-handed helix [27], while a recent NMR study suggests certain residues within the repeats adopt loop and β-turn structure [28]. Also, copper binding to octarepeat peptides

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Abbreviations: FTIR, Fourier transform infrared; PrP, prion protein; TFA, trifluoroacetic acid

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has been reported to induce the formation of α-helical structure as probed by Raman spectroscopy [22,26], in disagreement with one CD study which suggests no structural changes upon addition of copper [20]. Yet other CD experiments suggested the presence of turns and structured loop resulting from copper addition to repeat peptides [23–25].

Of available methods, Fourier transform infrared (FTIR) spectroscopy has not so far been used to probe the conformational changes of PrP repeats upon interaction with copper. FTIR spectroscopy is a well-established technique for the conformational analysis of peptides and proteins [30]. Information on the secondary structure of peptides can be derived from analysis of the strong amide I band, which arises primarily from the C=O stretching vibration of the amide group. Here we report the first FTIR spectroscopic analysis of the tandem repeats of a PrP. This is also the first biophysical characterization of a region of a marsupial PrP. Only one amino acid sequence of a marsupial PrP has been reported, that for brush-tailed possum [31]. It contains the four-repeat sequence PQGGGTNWGQ PHPGGSNWGQ PHPGGSSWGQ PHGGSNWGQ within the N-terminal repeat region, i.e. all with different 9- or 10-residue sequences. In the absence of other marsupial sequences which might reveal a dominant marsupial consensus, we chose to synthesize a peptide containing three copies of the second-repeat sequence [(PHPGGSNWGQ)$_3$G; Msp3]. This contains the apparently dominant 10-residue marsupial repeat and, as for the third repeat, most resembles the eight-residue consensus motif of mammals (PHGGGWGQ). But, interestingly, it has two additional polar residues (SN) in the middle of the repeat which are similarly present in the other three repeats of possum PrP. We report the conformational properties of Msp3 in the presence and absence of copper, probed using FTIR spectroscopy. As copper binding has been proposed to involve histidine residues of the PrP repeat [14,18,23,24,29], we have also investigated the copper binding properties of the model di-histidine peptide using FTIR.

2. Materials and methods

2.1. Chemicals

Copper (II) chloride was analytical grade and purchased from Sigma.

2.2. Peptide synthesis

Synthetic peptide (PHPGGSNWGQ)$_3$G (Msp3) was supplied by the Biomolecular Resources Facility at the John Curtin School of Medical Research, the Australian National University, Canberra, ACT, Australia. The peptide was synthesized using the Applied Biosystems 430A Peptide Synthesizer with the 9-fluorenyl methoxycarbonyl method. Cleavage of the peptide from the resin was performed in a mixture of 10 ml trifluoroacetic acid (TFA), 0.5 ml H_2O, 0.75 g phenol, 0.5 ml thioanisole, and 0.25 ml ethanedithiol. The crude peptide was purified by Bio-Rad high-performance liquid chromatography on a C_{18} column, using a 4–40% linear gradient of acetonitrile in 0.09% (v/v) TFA. The peptide was then freeze dried.

2.3. Sample preparation

In order to remove residual TFA, the peptide was further purified by mini-column chromatography, using anion exchange resin (amberlite IR-45). The column was pre-equilibrated with bidistilled water. The peptide was dissolved in 1 ml of bidistilled water and transferred to the column, which was then eluted with bidistilled water. Collected fractions were freeze dried [32]. UV/vis spectrophotometry was used to check the peptide concentration based on tryptophan absorption at 280 nm ($\varepsilon_{280} = 5500\ M^{-1}\ cm^{-1}$). In order to avoid copper precipitation as copper hydroxide at pHs above 6 [10], the sample was prepared according to the method of Takeuchi (personal communication), with some modifications. The Msp3 solution was basified to about pH 12 by addition of NaOH, and then $CuCl_2$ solution was added. The pH was adjusted to the desired pH by addition of HCl. The solution was freeze dried and redissolved in ^2H_2O. In the final solution, the concentrations of Msp3 and $CuCl_2$ were 5 and 20 mM, respectively. For the di-histidine peptide, the concentrations of di-histidine and $CuCl_2$ were 40 and 10 mM, respectively.

2.4. Infrared spectroscopic measurements and analysis

Infrared spectra were recorded on a Perkin Elmer Spectrum One Fourier Transform Infrared spectrometer. Samples were placed in a micro cell fitted with CaF_2 windows and a 50 μm teflon spacer. 400 scans at resolution 4 cm^{-1} were taken and signal averaged. FTIR spectra were recorded in ^2H_2O and not H_2O to avoid the strong interference of water absorbance in the amide I region. Use of ^2H_2O also allows use of longer-path-length cells to obtain higher signal to noise ratio. Also, measurement in ^2H_2O allows monitoring of the hydrogen–deuterium (H–D) exchange process [30,33,34] in copper-free and copper-bound forms of the peptide. Spectra were recorded at 25°C and the sample compartment was continuously purged with nitrogen gas to suppress water vapor absorbance in the spectral region of interest. Data were analyzed using the Spectrum v.3.00 software from Perkin Elmer. Subtraction of the ^2H_2O spectrum from the sample spectrum was carried out digitally to give a straight baseline in the region 2000–1800 cm^{-1} (see [33]). The resulting absorbance spectrum of the peptide was analyzed using the second-derivative procedure with a 13 data point Savitzky-Golay smoothing window [33]. H–D exchange was measured by monitoring the change in the intensity of the amide II band as a function of time [33,34].

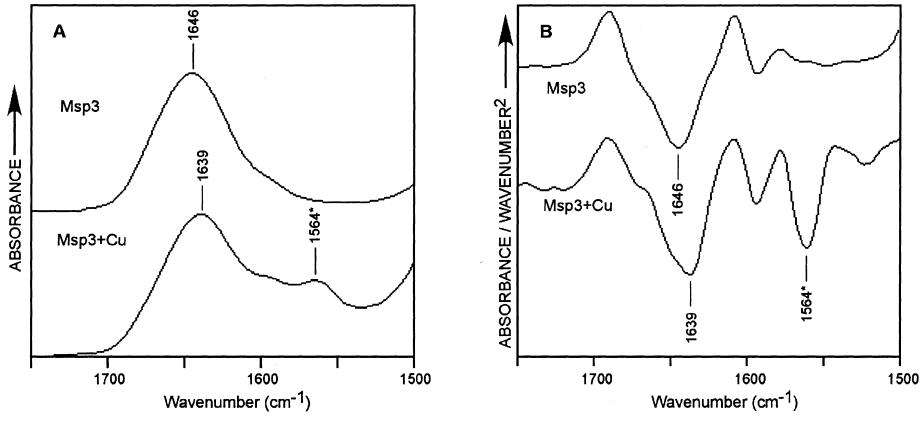

Fig. 1. Effect of copper addition on the FTIR spectrum of the Msp3 peptide. Absorbance (A) and second-derivative (B) spectra of the Msp3 (PHPGGSNWGQ)$_3$G peptide in ^2H_2O at pH 8, recorded in the absence (top) and presence (bottom) of 20 mM $CuCl_2$. The peptide concentration used for these measurements is 5 mM. The asterisks indicate the appearance of a new band upon addition of copper.

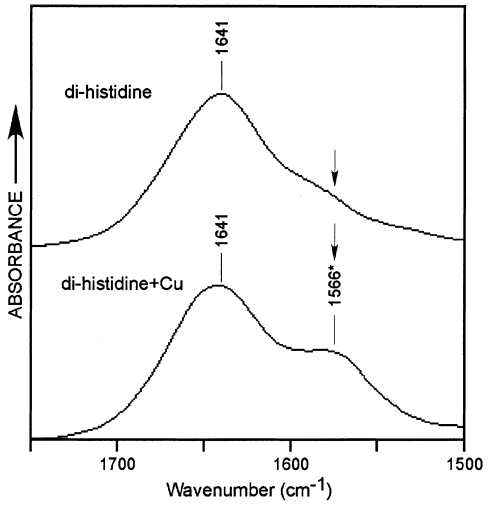

Fig. 2. Effect of copper addition on the FTIR spectrum of the di-histidine peptide. Absorbance spectra of di-histidine at 40 mM concentration recorded in ^2H_2O at pH 8 in the absence (top) and presence (bottom) of 10 mM $CuCl_2$. The asterisk indicates the appearance of a new band upon addition of copper.

3. Results and discussion

Fig. 1 compares the FTIR spectra of the Msp3 peptide recorded in the presence and absence of copper at pH 8. Analysis of the absorbance spectra (Fig. 1A) reveals that the amide I maximum shifts from 1646 cm^{-1} to 1639 cm^{-1} upon addition of copper. An additional difference between the two spectra is the appearance of a new band at 1564 cm^{-1} for the peptide in the presence of copper. Detailed analysis using the second-derivative procedure was also performed (Fig. 1B). Other than the appearance of a shoulder near 1670 cm^{-1}, the second-derivative analysis (Fig. 1B) confirms what can be seen from the absorbance spectra (Fig. 1A).

The amide I maximum at 1646 cm^{-1} for the copper-free form of the peptide in ^2H_2O can be attributed to random-coil structure. The presence of random-coil structure has been reported for other PrP repeat peptides, in the absence of copper, using various biophysical techniques including Raman spectroscopy [21–26]. The assignment of the band at 1639 cm^{-1} in the copper-bound form of the peptide is complicated as absorbance in this region can arise from β-sheet and β-turn structures, as well as from solvent-exposed α-helices [30]. However, an increase in ordered secondary structure, in the presence of copper, is indicated by the fact that the amide I band shifts toward lower frequency, reflecting an increase in hydrogen bonding. Although the assignment of this band is not straightforward, it is possible that it reflects solvent-exposed α-helical structure. This assignment would be in good agreement with the Raman spectroscopic measurements of Miura et al. [22,26], who detected an increase in helix content upon interaction of copper ions with a human PrP repeat peptide.

Besides the large change in the amide I band frequency, the appearance of an additional band near 1564 cm^{-1} suggests a change in peptide tertiary structure upon copper binding. In order to identify the origin of this band, FTIR measurements were performed with di-histidine peptides in the presence and absence of copper (Fig. 2). The major difference between the two spectra is the appearance of a new band near 1566 cm^{-1}, in the presence of copper. This band occurs at a similar band frequency to that observed with the Msp3 peptide in the presence of copper. As the 1566 cm^{-1} band appears upon addition of copper, it can be ascribed to the copper-bound form of histidine. This suggests the band observed at a virtually identical position in the spectrum of the copper-bound form of the Msp3 peptide can also be attributed to the ring vibration of the histidine residue when it is bound to copper.

In order to investigate the effect of pH on the peptide structure, FTIR spectra were recorded at different pH values, ranging from 6 to 10, in the presence and absence of copper. The results show no significant pH-induced changes in the structure of the peptide in the absence of copper (Fig. 3). The amide I band frequency is centered at 1644 cm^{-1} throughout the pH range studied. However, in the presence of copper, the spectrum of the peptide recorded at acidic pH displays a band frequency of 1646 cm^{-1} which shifts to 1639 cm^{-1} at alkaline pH (>7). Furthermore, a new band at 1564 cm^{-1}, as discussed above, is also observed. This change is associated with interaction of copper ions with the peptide, which appears to occur at alkaline pH.

It has been suggested previously that at pH <6 no binding between the peptide and copper ions occurs [23,24,26]. It is,

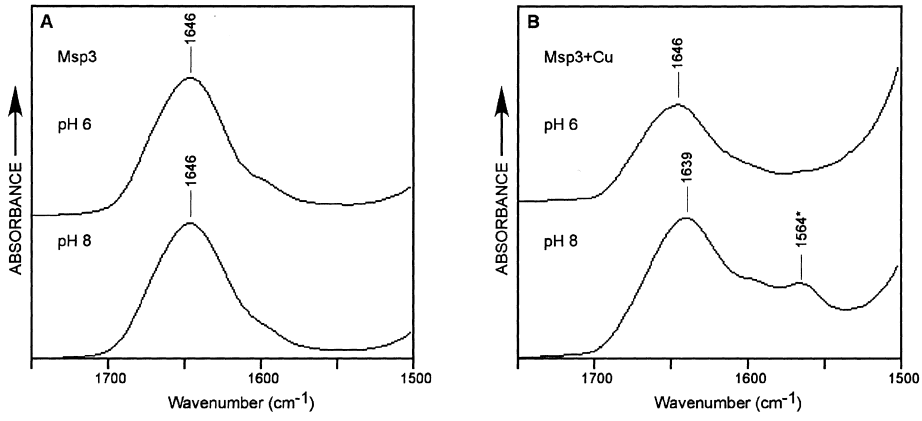

Fig. 3. Effect of pH on the FTIR spectrum of Msp3 and the copper-complex of Msp3. Absorbance spectra of Msp3, $(PHPGGSNWGQ)_3G$ peptide in ^2H_2O at 5 mM peptide concentration in the absence (A) and presence (B) of 20 mM $CuCl_2$, at pH 6 (top) and pH 8 (bottom). The asterisk indicates the appearance of a new band upon addition of copper.

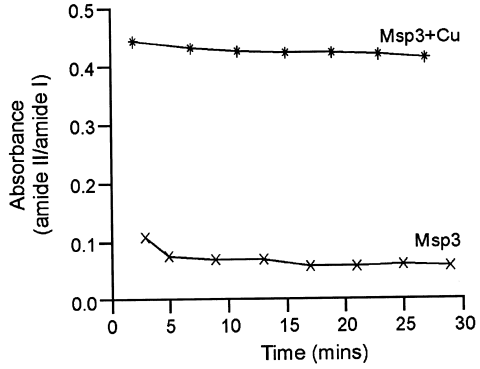

Fig. 4. H–D exchange of Msp3 and the copper-complex of Msp3. Comparison of the H–D exchange process of Msp3 (×) and Msp3+Cu (*) at pH 8, expressed as a ratio of the absorbance of the amide II (~1550 cm^{-1}) to amide I bands, as a function of time after addition of ^2H_2O. Greater reduction in the intensity of the amide II band relative to the amide I band reflects a greater extent of H–D exchange.

therefore, not surprising that the amide I band for the peptide at pH 6 is observed at 1646 cm^{-1} (a frequency similar to that observed for the metal-free peptide), even though $CuCl_2$ is present. Furthermore, the 1564 cm^{-1} band is absent at this pH, which links the appearance of this band to the binding of copper to the peptide. These observations additionally confirm that the spectral changes observed at alkaline pH values reflect conformational changes associated with binding of copper. The amide I band positions for copper-bound peptide at pHs 10, 9, and 8 are virtually identical, suggesting that increasing the pH has no effect on the structure of the copper-bound form of the peptide. This is also evident from the fact that the peptide in the absence of copper shows virtually no change in the amide I band position as the pH is raised from 6 to 10.

FTIR spectroscopy is a particularly valuable tool for monitoring H–D exchange properties of peptides and proteins [30,33,34]. This can be done through monitoring the reduction in the intensity of the amide II band as a function of time in ^2H_2O [30,33,34]. In this study we compared the H–D exchange rate of the Msp3 peptide in the presence and absence of copper (Fig. 4). From these measurements we find that in the presence of copper the H–D exchange rate is reduced by approximately five-fold compared with that for the peptide in the absence of copper. This suggests that the copper-bound peptide has a more rigid, solvent inaccessible structure. This is highly likely as we have observed that copper binding results in a more strongly H-bonded structure, reflected by a shift in the frequency of the amide I band towards lower frequency. The combination of the amide I band analysis and H–D exchange measurement further strengthens our suggestion that copper binding results in an increase in ordered secondary structure.

To conclude, the results of our FTIR spectroscopic analysis reveal large changes in the secondary and tertiary structure of a marsupial PrP repeat peptide associated with the binding of copper. Copper binding results in a more strongly H-bonded structure that has a significantly reduced solvent accessibility compared with the copper-free form of the peptide.

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