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Metal-dependent α-helix formation promoted by the glycine-rich octapeptide region of prion protein

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Abstract Prion diseases share a common feature in that the normal cellular prion protein (PrP^C) converts to a protease-resistant isoform PrP^{Sc}. The α-helix-rich C-terminal half of PrP^C is partly converted into β-sheet in PrP^{Sc}. We have examined by Raman spectroscopy the structure of an octapeptide PHGGGWGQ that appears in the N-terminal region of PrP^C and a longer peptide containing the octapeptide region. The peptides do not assume any regular structure without divalent metal ions, whereas Cu(II) binding to the HGGG segment induces formation of α-helical structure on the C-terminal side of the peptide chain. The N-terminal octapeptide of prion protein may be a novel structural motif that acts as a promoter of α-helix formation.

Key words: Prion protein; Secondary structure; Metal coordination; Raman spectroscopy

1. Introduction

The normal cellular prion protein (PrP^C) is a glycolipid-anchored membrane-surface protein expressed in neurons and glial cells [1,2]. Although little is known about the function of PrP^C, recent studies suggested that PrP^C may play a role in the maintenance of cerebellar Purkinje neurons [3] and in sleep regulation [4]. Mature human PrP^C after post-translational modifications spans residues 23–231 of the 253 amino acid residues coded by a single gene [5–7]. A molecular modeling study suggested that the C-terminal half of PrP^C may form a bundle of four α-helices at residues 109–122 (H1), 129–141 (H2), 178–191 (H3), and 202–218 (H4) [8] (Fig. 1). A recent NMR study on the three-dimensional structure of a peptide fragment (residues 121–231) of the mouse prion protein, which lacks the N-terminal half and H1 helix region, has confirmed the existence of the H3 and H4 helices at the predicted positions [9]. In the pathological process of prion diseases, PrP^C is converted into a β-sheet-rich form of prion protein (PrP^{Sc}), which aggregates into amyloid probably through intermolecular β-sheet formation [10]. The aggregated PrP^{Sc} acquires resistance to proteolytic degradation and causes fatal neurodegenerative diseases, including scrapie and bovine spongiform encephalopathy in animals and Creutzfeld-Jakob disease in humans [2]. Although the α-helix-to-β-sheet transition in the C-terminal half region may be related to the $PrP^C \rightarrow PrP^{Sc}$ transformation [8,11,12], the molecular mechanism of the conformational transition has not yet been clarified.

The N-terminal region of PrP^C, on the other hand, contains four tandem repeats of the octapeptide, Pro-His-Gly-Gly-Gly-Trp-Gly-Gln (PHGGGWGQ), at positions 60–91 with an analogous nonapeptide on the adjacent N-terminal side (positions 51–59, Fig. 1). Because of its glycine- and proline-rich amino acid sequence, neither α-helix nor β-sheet structure has been predicted in this region [8]. The protein found in the PrP^{Sc} amyloid, sometimes designated PrP 27–30, lacks the N-terminal region due to proteolytic truncation and it is considered that the N-terminal region is not directly related to the amyloid formation [13]. However, the octapeptide repeat is highly conserved among mammalian PrP^C proteins [14], implying some functional and structural roles of the octapeptide. A recent mass spectrometric study has shown that the octapeptide repeat provides a binding site for divalent metal ions, preferentially for Cu(II) [15]. The structure of a peptide consisting of four tandem repeats of the octapeptide has been investigated in the presence and absence of Cu(II) by using circular dichroism (CD) spectroscopy [16]. Although the CD spectrum was affected by the metal binding, the peptide secondary structure could not be fully revealed due to a strong distortion of the spectrum by the tryptophan residues. In this study, we have investigated the effect of Cu(II) binding on the secondary structure of peptides containing the octapeptide by using Raman bands sensitive to the peptide conformation.

2. Materials and methods

Copper(II) chloride was purchased from Nacalai Tesque, Inc. The oligopeptides containing amino acid sequence of human prion protein, PHGGGWGQ (NPr_1), HGGG, PHGGGWGQGGGTHSQWNKPS [PrP(84–103)], and three tandem repeats of NPr_1, were synthesized on an Applied Biosystems Model 431A automated peptide synthesizer from amino acid derivatives protected by the 9-fluorenylmethoxycarbonyl (Fmoc) group. The peptide was purified with a Jasco 880 HPLC on a reversed-phase column using a 0–50% linear gradient of acetonitrile in 0.1% (v/v) trifluoroacetic acid. The lyophilized powder of the peptide was dissolved in 0.1 M hydrochloric acid and again lyophilized to remove residual trifluoroacetic acid. The pH of the sample solution was adjusted by addition of KOH solution. The concentration of the peptide was determined from UV absorption of tryptophan ($\varepsilon_{280} = 5455$). Raman spectra were excited with the 514.5-nm line (5 mW at the sample) of a Coherent Inova 70 argon ion laser and were recorded on a Jasco NR-1800 triple spectrometer equipped with a liquid-nitrogen cooled CCD detector. Frequencies of Raman bands were reproducible to within ± 0.5 cm^{-1}. Samples were kept at 20°C during collection of Raman spectra.

3. Results and discussion

Raman spectra of H_2O and D_2O solutions of the NPr_1 octapeptide are shown in Fig. 2. In the absence of Cu(II), the peptide gives amide I (H_2O solution; Fig. 2A, top) and amide I′ (D_2O solution; Fig. 2B, top) bands at 1668 and 1658 cm^{-1}, respectively. These amide frequencies are characteristic of irregular structure of the peptide main-chain [17]. The cor-

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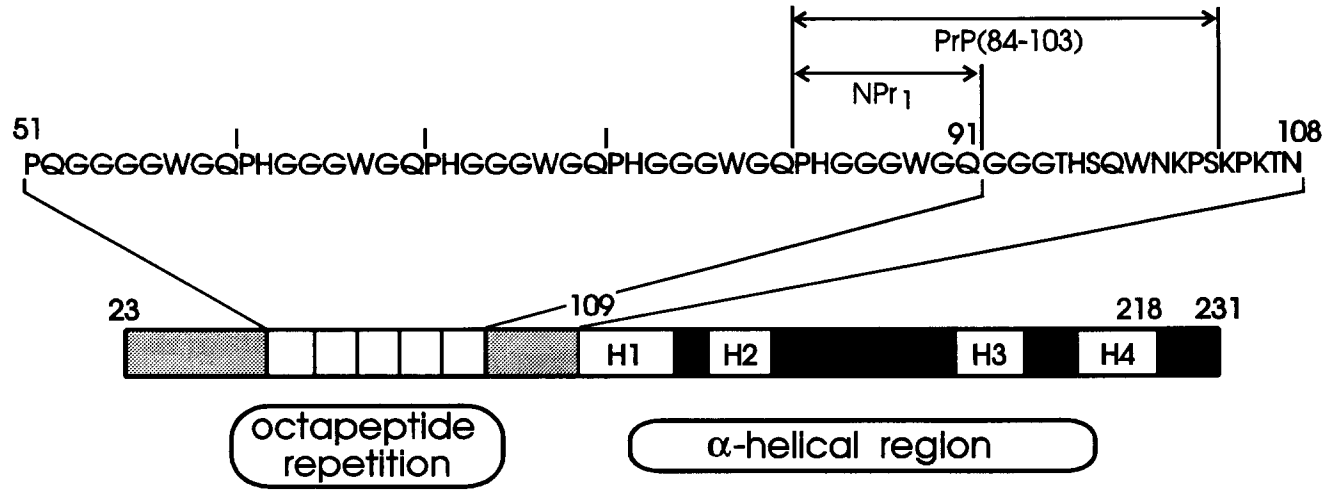

Fig. 1. Schematic representation of the human prion protein amino acid sequence [5–7]. The sequences of the oligopeptides, NPr_1 and PrP(84–103), are indicated at the top.

responding amide III band, which is expected around 1250 cm^{-1} for irregular structure, may be covered by a tryptophan band at 1254 cm^{-1}. The amide III band exhibits a drastic decrease in D_2O solution (Fig. 2B), reflecting the H→D exchange of amide protons. The deuterated peptide bonds result in amide III′ bands in the 990–940 cm^{-1} region (not shown). The 1271 cm^{-1} band remains in the D_2O solution spectrum and is assigned to histidine. A shoulder peak at 1237 cm^{-1} (Fig. 2A) is too weak to be assigned to an amide III mode of β-sheet structure and may be ascribed to tryptophan [17]. The Raman spectra clearly show that the NPr_1 peptide adopts an irregular structure in the absence of Cu(II).

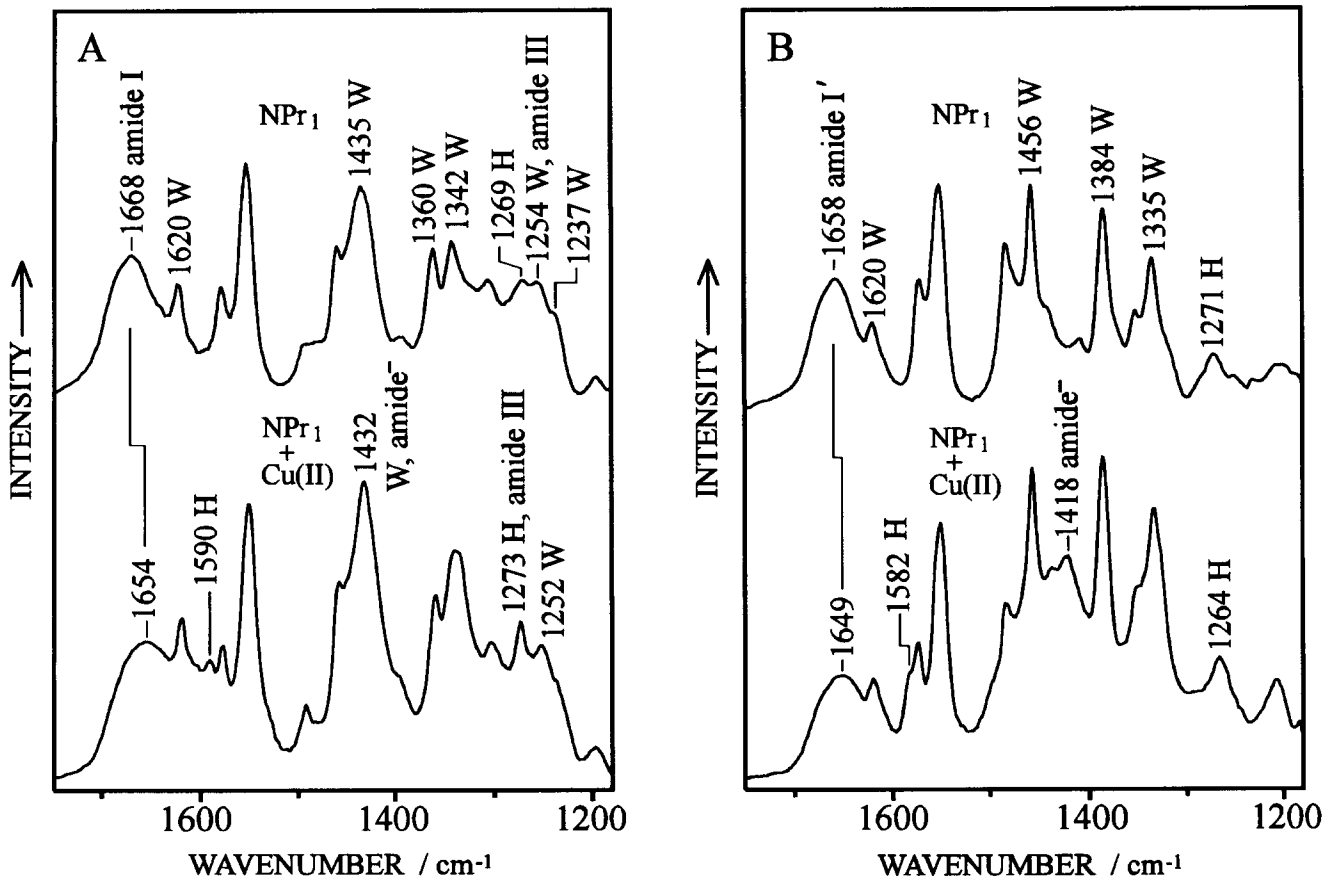

Fig. 2. Raman spectra (1750–1180 cm^{-1}) of H_2O (A) and D_2O (B) solutions of NPr_1, PHGGGWGQ, at 20 mM peptide concentration and pH (p*D*) 7.4; metal-free NPr_1 solution (top) and Cu(II)-NPr_1 complex in the presence of 40 mM $CuCl_2$ (bottom), respectively. Raman scattering of the solvents was subtracted from the solution spectra. Assignments of Raman bands are as indicated in the panels (H, histidine; W, tryptophan; amide$^-$, deprotonated main-chain amide).

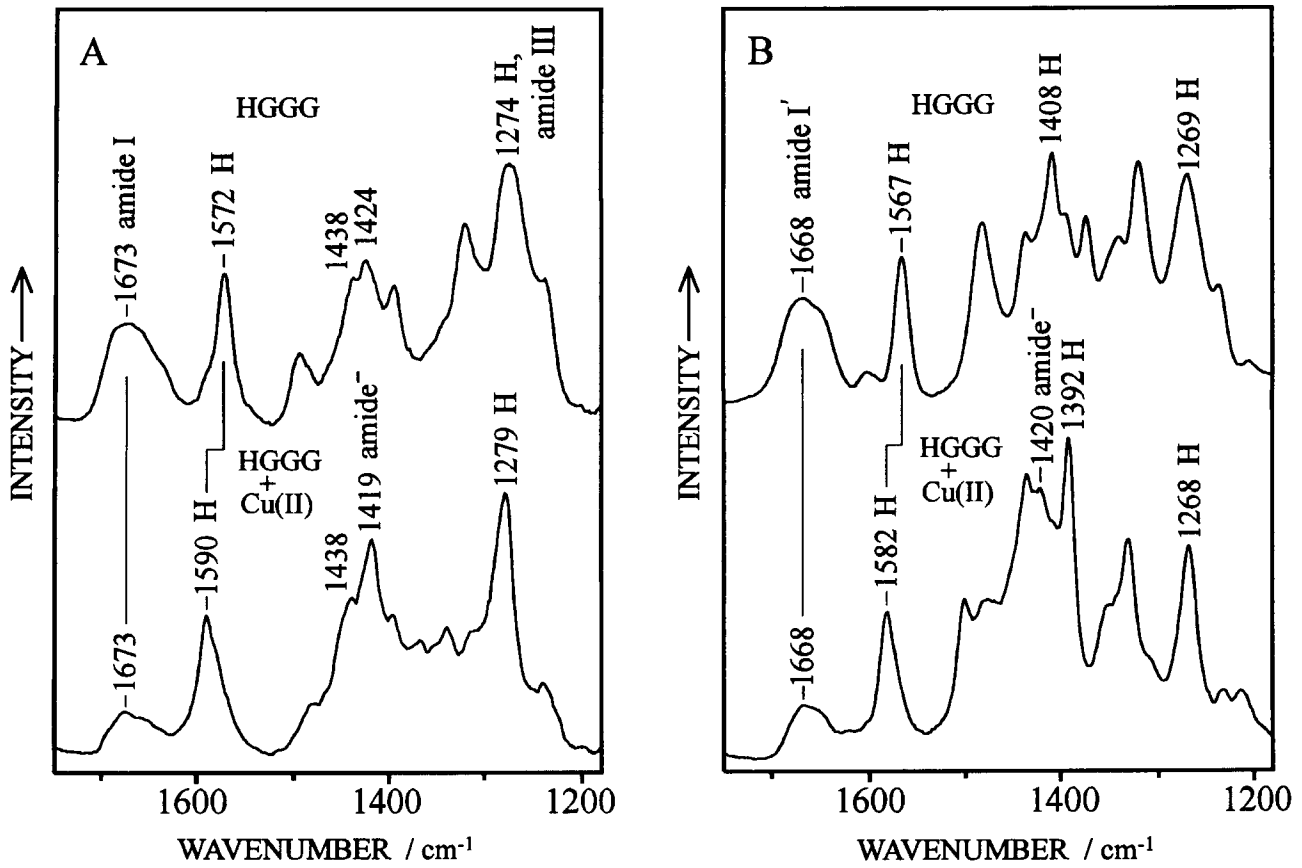

Fig. 3. Raman spectra (1750–1180 cm^{-1}) of H_2O (A) and D_2O (B) solutions of HGGG, at 20 mM peptide concentration and pH (p*D*) 7.4; metal-free peptide solution (top) and Cu(II)-peptide complex in the presence of 40 mM $CuCl_2$ (bottom), respectively.

In the presence of Cu(II), the amide I and I′ bands shift to 1654 and 1649 cm^{-1}, respectively, with significant intensity decreases (Fig. 2A,B; bottom). The diminution of amide I (I′) band intensity may be ascribed to deprotonation of amide nitrogens induced by metal coordination. In a deprotonated amide group, the amide I (I′) band (mainly C=O stretch) is replaced by two C=O/C-N^- stretching vibrations, i.e. an out-of-phase mode at ~1610 cm^{-1} (mainly observed in infrared spectra) and an in-phase mode at ~1420 cm^{-1} (strong in Raman spectra), owing to a significant coupling between the C=O and C-N^- stretching vibrations [18,19]. A new peak that appears at 1418 cm^{-1} in the D_2O solution spectrum (Fig. 2B, bottom) is assignable to the in-phase C=O/C-N^- stretching vibration of deprotonated amide groups. In H_2O solution, the corresponding C=O/C-N^- band is not clearly detected due to the strong overlap of a tryptophan band at 1435 cm^{-1} (Fig. 2A). However, the significant intensity increase at 1432 cm^{-1} in the presence of Cu(II) may be ascribed to the C=O/C-N^- band.

In order to specify the metal binding site of the octapeptide, we have examined the Cu(II) binding properties of a fragment of NPr_1, HGGG, which is analogous to a well-known Cu(II)-binding peptide, GGH, mimicking the Cu(II) transport site of human serum albumin [20]. As shown in the Raman spectra of HGGG (Fig. 3), a drastic intensity decrease of amide I (I′) band and concomitant intensity increase at the C=O/C-N^- stretching frequency occur upon addition of Cu(II). The other major spectral changes observed upon binding of Cu(II) are associated with the metal binding to the histidine side chain. The 1572 cm^{-1} band in the spectrum of metal-free HGGG (Fig. 3A, top) is the histidyl $C_\gamma=C_\delta$ stretching mode that is sensitive to the tautomerization of imidazole ring [21]. This band shifts to 1590 cm^{-1} upon complex formation (Fig. 3A, bottom), suggesting that the equilibrium between N_δ- and N_ε-protonated tautomeric forms is altered by the metal binding [22]. The corresponding 1567 cm^{-1} band of deuterated histidine also exhibits a high frequency shift upon metal binding (Fig. 3B). Although histidine bands are weak compared to very strong tryptophan bands in the Raman spectra of NPr_1, the 1590 cm^{-1} peak (Fig. 2A, bottom) and the 1582 cm^{-1} shoulder peak (Fig. 2B, bottom) appear in the spectrum of Cu(II)-NPr_1 mixture. On the basis of these results, we conclude that the nitrogens of deprotonated main-chain amides and the imidazole side chain of the HGGG segment are the metal-binding sites in the Cu(II)-NPr_1 complex.

As described above, main-chain amide groups involved in the Cu(II) coordination do not give normal amide I (I′) band. Thus, the remaining weak amide I (I′) band in the spectrum of Cu(II)-HGGG mixture is ascribed to a metal-unbound fraction of the peptide (Fig. 3). On the other hand, the remaining amide I (I′) band in the spectrum of Cu(II)-NPr_1 mixture cannot be ascribed to Cu(II)-unbound NPr_1, because its frequency (1654 cm^{-1} for amide I or 1649 cm^{-1} for amide I′) is significantly lower than that (1668 or 1658 cm^{-1}) of metal-unbound NPr_1 (Fig. 2). The amide I (I′) frequency of Cu(II)-NPr_1, together with the increased intensity at 1273

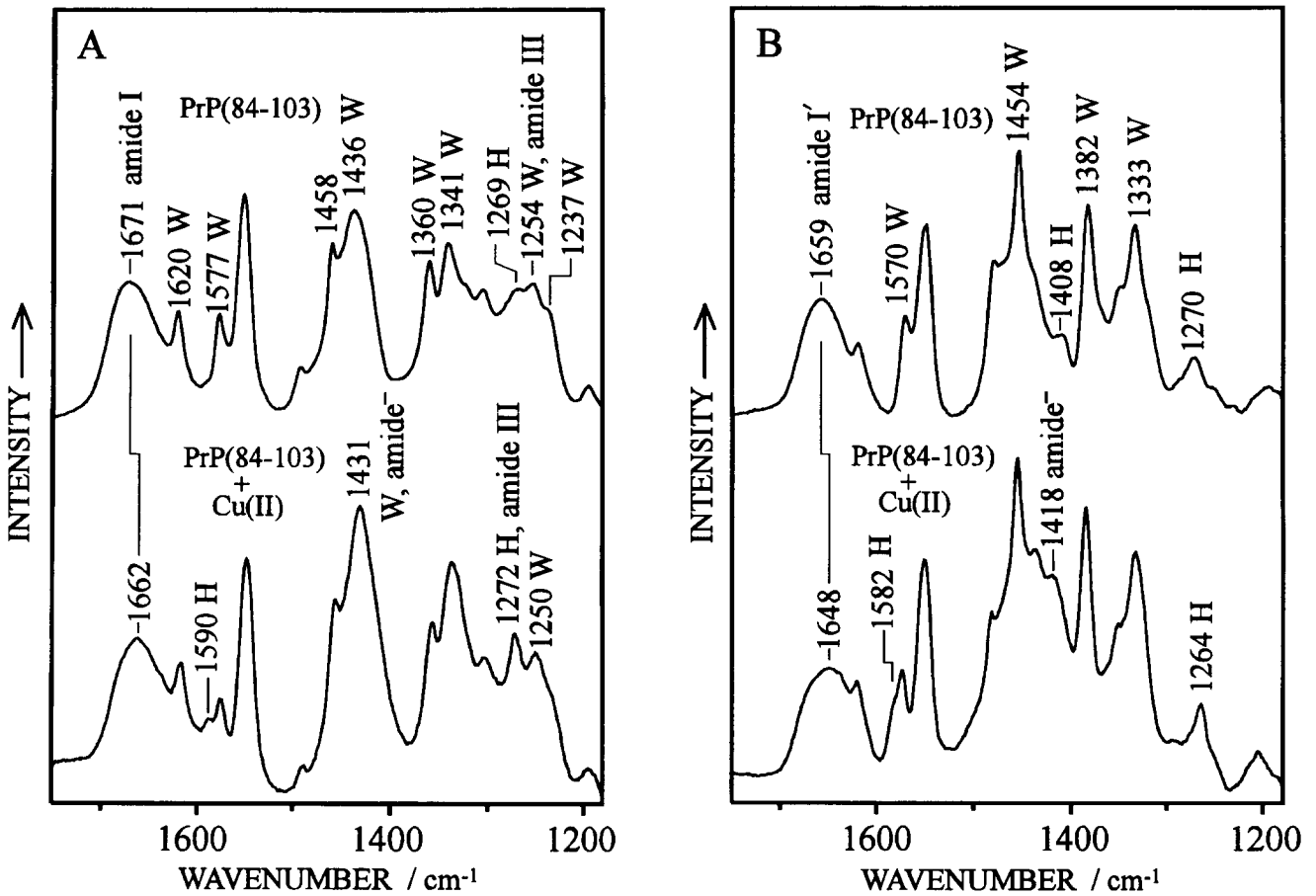

Fig. 4. Raman spectra (1750–1180 cm^{-1}) of H_2O (A) and D_2O (B) solutions of PrP(84–103), PHGGGWGQGGGTHSQWNKPS, at 20 mM peptide concentration and pH (p*D*) 7.4; metal-free PrP(84–103) solution (top) and Cu(II)-PrP(84–103) complex in the presence of 60 mM $CuCl_2$ (bottom), respectively.

cm^{-1} (amide III, Fig. 2A), is indicative of α-helical structures [17]. It is very likely that the Cu(II) binding to the HGGG segment of NPr_1 promotes conformational transition of the adjacent peptide segment, GWGQ, from an irregular loop to an α-helix.

Raman spectra of an oligopeptide composed of three successive copies of the octapeptide were also examined. Although the Cu(II) complex of the 24-mer peptide was soluble only at basic pH, the Raman spectrum at ~pH 10 also revealed α-helix formation of the peptide associated with the metal binding to histidine side chains and deprotonated amide groups (data not shown).

To address whether the α-helical conformation induced by Cu(II) binding in the octapeptide region affects the structure of adjacent peptide segment of PrP^C on the C-terminal side, we have examined Raman spectra of a peptide, PrP(84–103), composed of a single octapeptide unit (residues 84–91 of PrP^C) and the succeeding dodecapeptide (residues 92–103, Fig. 1). In the absence of Cu(II), PrP(84–103) gives strong amide I and I′ bands at 1671 and 1659 cm^{-1}, respectively, indicative of an irregular structure (Fig. 4A,B; top). In the presence of Cu(II), the 1418 cm^{-1} band arising from deprotonated amide groups and the 1590 cm^{-1} band of the metal-bound histidine becomes prominent (Fig. 4A,B; bottom). Concomitant with these spectral changes, the amide I (I′) band shifts down to the frequency region (~1650 cm^{-1}) of α-helical amide I (I′) vibration, as in the case of NPr_1. This apparent frequency shift of the amide I (I′) band cannot be explained only by the α-helix formation within the octapeptide unit in PrP(84–103). Another significant spectral change upon Cu(II) binding is observed in the frequency region of the main-chain skeletal C-C stretch (Fig. 5, lower two spectra). Both the intensity and frequency of this band are sensitive to the secondary structure of polypeptide [23]. A strong C-C stretching band appears in the 945–890 cm^{-1} region when the peptide chain forms an α-helix, whereas a very weak or no band is observed for β-sheet structure. Although an irregular peptide chain also gives a C-C stretching band, the frequency of this band is generally higher than that of the α-helical C-C stretching. For example, the C-C stretching band of poly(L-glutamic acid) exhibits a frequency shift from 926 to 938 cm^{-1} upon helix-to-coil transition [24]. In the absence of Cu(II), PrP(84–103) gives two Raman bands in the frequency region; a 936 cm^{-1} band and a very weak band near 920 cm^{-1} (Fig. 5). A drastic intensity increase of the 921 cm^{-1} band, which is accompanied by an intensity decrease of the 936 cm^{-1} band, occurs upon Cu(II) binding. Similar but slightly weaker spectral changes were also observed on metal binding to NPr_1 (Fig. 5, upper two spectra). These results, together with the spectral changes in the amide I (I′) region, provide evidence that the α-helical structure nucleated by Cu(II) binding in the octapeptide region extends over the

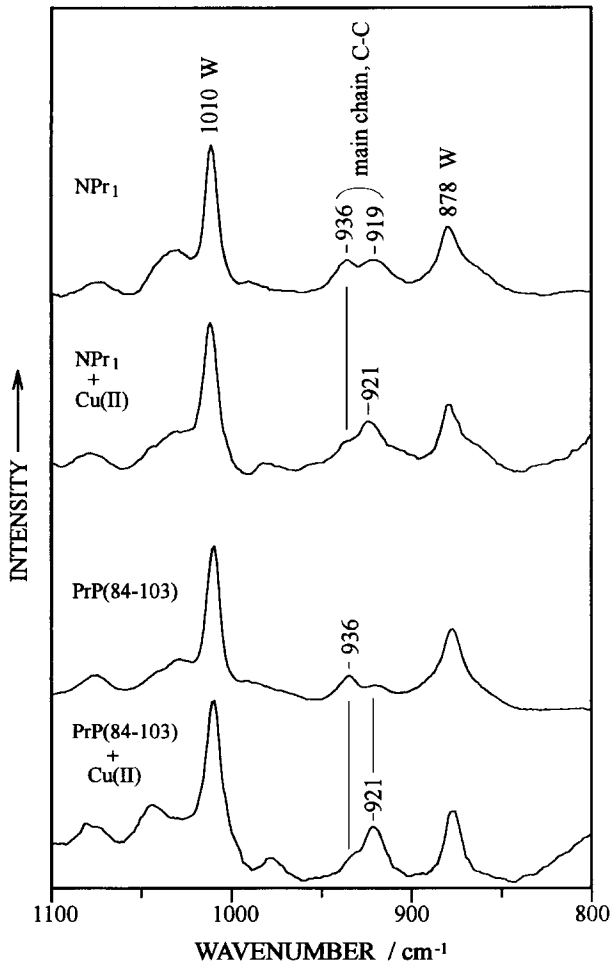

Fig. 5. Raman spectra (1100–800 cm^{-1}) of H_2O solutions of NPr_1 and PrP(84–103) at 20 mM peptide concentration and pH (*pD*) 7.4. From top to bottom: metal-free NPr_1, NPr_1 in 40 mM $CuCl_2$, metal-free PrP(84–103), and PrP(84–103) in 60 mM $CuCl_2$.

adjacent C-terminal segment of PrP(84–103). The octapeptide in PrP^C may be a novel structural motif that acts as a promoter of the α-helix formation.

The peptide segment corresponding to PrP(84–103) is in close proximity to the putative first helix region (residues 109–122) of a four-helix-bundle model proposed for the C-terminal region of PrP^C [8] (see Fig. 1). Studies on peptides containing putative helix regions have demonstrated that the helix regions can take not only α-helical but also β-sheet structures depending on the solution conditions [10,25,26]. A cooperative folding of the first and second helix regions has also been detected [26]. The nucleation of α-helical structure induced by binding of Cu(II) in the octapeptide motif and the subsequent propagation of the α-helical structure is likely to contribute to the folding of the first helix and then of the other helices through inter-helix interactions. A possible role of the octapeptide motif is to stabilize α-helical structure of PrP^C in the presence of Cu(II).

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