Pd(en) as a Sequence-Selective Molecular Pinch for α-Helical Peptides

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A palladium(II)(ethylenediamine) complex was found to selectively stabilize α-helix conformation of peptides having two histidine (His) residues at i and i + (3 or 4) positions, whereas the helix conformation of the other peptides having one or two His at different positions is destabilized.

We and other groups recently developed artificial receptors which selectively bind a protein surface elaborately using multiple binding interactions such as hydrogen bonding, electrostatic, hydrophobic and coordination interactions.[1] For the aim to establish a rationale to design artificial receptors toward a specific protein surface, researches on the sequence-selective molecular recognition for natural peptides are essentially important. Although some successful examples were reported previously, these model studies for peptides were carried out limitedly in organic solvents or organic/aqueous solvent mixtures.[2] It is now needed to explore synthetic receptors available in pure water system. We describe herein that palladium(II) (ethylenediamine) (Pd(en)), a palladium-based organometallic compound, can selectively stabilize α-helix conformation of peptides having a specific pattern (H-i and i + (3 or 4)) in aqueous solution, whereas it destabilizes the helical conformation for the other peptides which are excluded out the pattern.

Palladium(II)-ethylenediamine dinitrate complex $[Pd(en)(NO_3)_2]$ is prepared according to the literature.[3] Since palladium cation shows high affinity for His, seven model peptides in which one or two His are positioned at the one side of the α-helix, are employed as target molecules (shown in Scheme 1). These peptide sequences were designed according to the previous literatures[4] and prepared using the typical solid phase peptide synthesis based on Fmoc chemistry, purified by reverse-phase HPLC, and characterized by MALDI-TOF mass spectroscopy.[5]

	5 10 15
peptide (H-16)	Ac-A E A A A K E A A A E E A A A ***H*** A-NH_2
peptide (H-13,16)	Ac-A E A A A K E A A K A A ***H*** A K ***H*** A-NH_2
peptide (H-12,16)	Ac-A E A A A K E A A A K ***H*** A A A ***H*** A-NH_2
peptide (H-5,9)	Ac-A E A A ***H*** K E A ***H*** A K E A A A K A-NH_2
peptide (H-11,16)	Ac-A E A A A K E A A K ***H*** A A A K ***H*** A-NH_2
peptide (H-9,16)	Ac-A E A A A K E A ***H*** A K E A A A ***H*** A-NH_2
peptide (H-5,16)	Ac-A E A A ***H*** K E A A A K E A A A ***H*** A-NH_2

A=Ala E=Glu K=Lys H=His (histidines are shown in bold)

Scheme 1. Peptide sequences employed in this study.

Interactions between Pd(en) and peptides are monitored by circular dichroism spectroscopy (CD, Jasco J-720W). Figure 1 shows two typical examples of the CD titration. In the case of a peptide (H-12,16), two negative Cotton peaks at 208 and 222 nm, characteristic of the α-helix conformation, were intensified by the addition of 1 equiv of Pd(en), and then lessened by the excess amount of Pd(en) (see Figure 1a). The α-helix content increased to the maximum value at a 1:1 ratio of the Pd(en):peptide and decreased by more than 1 equiv of Pd(en). In contrast, the Cotton peaks of the peptide (H-9,16) were monotonously lessened by the addition of Pd(en) (Figure 1b). Similar titration experiments were conducted for the other 5 peptides and the difference [θ] values at 1 equiv of Pd(en) addi-

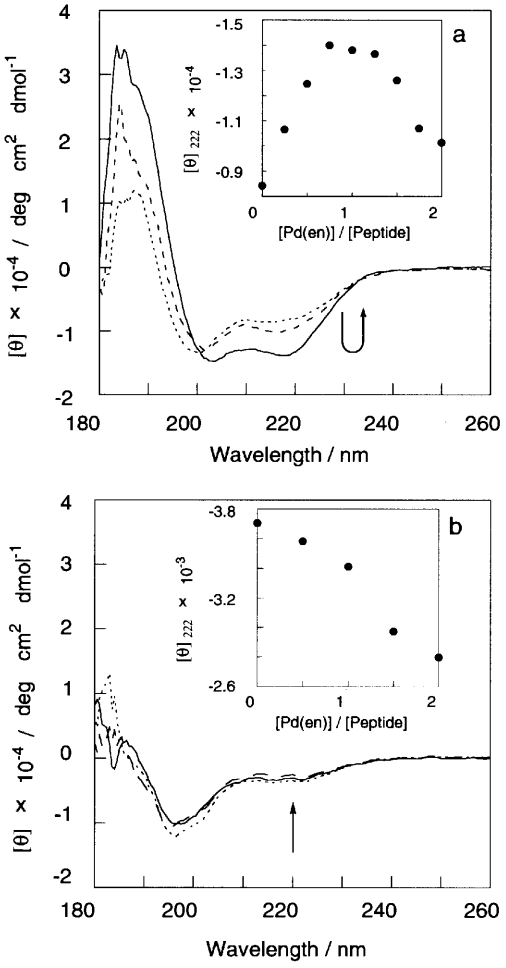

Figure 1. CD spectral change upon the addition of Pd(en) to (a) the peptide(H-12,16) , and (b) the peptide(H-9,16) . (dot line:0eq, solid line:1eq, broken line:2eq). Insets: titration curves plotting θ values at 222 nm against the concentration of Pd(en). [Peptide] = 50 μmol dm^{-3} in 10 mmol dm^{-3} borate buffer (pH 8.0), 25 °C.

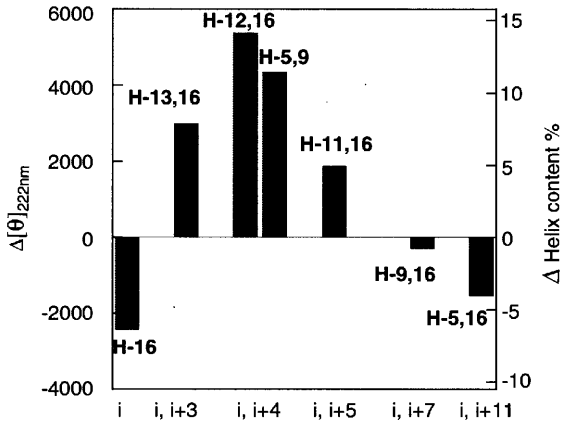

Figure 2. The difference θ values at 222 nm by the addition of 1equiv of Pd(en) to the various peptides.

tion were summarized in Figure 2. It is clear that the Pd(en)-induced CD spectral changes are greatly dependent on the distance between two His residues, that is, the α-helix conformation is stabilized for the peptides bearing two His at the distance of i and i + (3 or 4), whereas, it is destabilized upon the Pd(en) binding for the peptides bearing two His between i and i + (7 or 11) positions. In the case of i and i + 5, the helix content moderately increased. Thus, the order in the Pd(en)-assisted helix induction was as follows: H-12,16 (+14% [30%]) > H-5,9 (+12% [19%]) > H-13,16 (+8% [25%]) > H-11,16 (+5% [14%]) >> H-9,16 (–1% [18%]), H-5,16 (–4% [20%]), and H-16 (–6% [34%]).[6] These results may suggest that the Pd(en)-mediated cross-linking enhances the helix conformation, whereas the simple binding lessens the helix. It is also consistently explained that the helix decrease by the excess amount of Pd(en) is due to the suppression of the cross-linking by the second binding.

A distance between two His is estimated by the molecular modeling (Insight II, SGI WS) of a corresponding peptide in the α-helix conformation.[7] In the peptide(H-12,16), the distance between two N1 of the imidazole ring of His is roughly 2.8 Å, which fits suitably to the distance between two NO_3 of Pd(en). The distance in the peptide(H-13,16) is almost same (3 Å) as the peptide(H-12,16), whereas the distances are rather far in the other peptides (10 Å for the peptide(H-11,16), 9 Å for the peptide(H-9,16), 15 Å for the peptide(H-5,16)). These are in good agreement with the above-mentioned CD investigation.

The cooperative binding of Pd(en) to two histidines of the peptide(H-12,16) was demonstrated by ^{1}H-NMR titration (Figure 3).[8] Two singlet peaks due to ε-H of the imidazole ring of two histidines (both H-12 and H-16) which were observed at 8.58 ppm in the absence of Pd(en) completely disappeared upon addition of 1 equiv of Pd(en) and new peaks mainly appeared at 7.88 ppm by Pd(en) addition. Simultaneously, the peaks due to δ-H of two histidines at 7.28 ppm decreased and two peaks at 7.12 and 6.48 ppm newly appeared. These indicate that the two hisitidines are quantitatively bound to one Pd^{2+} center. These upfield shifts imply that NH protons are dissociated to form imidazolate anions.[8] The greater peak splitting suggests that the conformation of the two His residues are spatially fixed upon the complexation with Pd(en), so as to be distinguishable

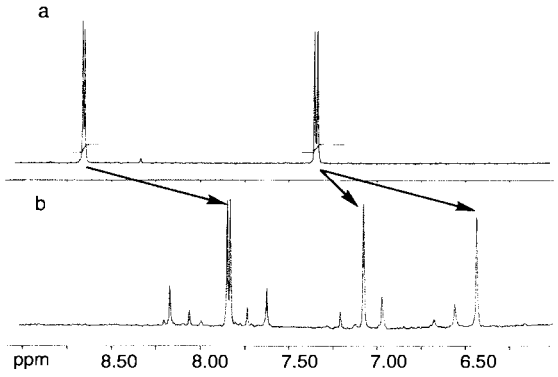

Figure 3. ^{1}H-NMR spectra of the His region of the peptide(H-12,16) in (a) the absence and (b) presence of Pd(en) (1 equiv).

in NMR. Furthermore, the NOE correlation between two δ-protons was detected upon the Pd(en) complexation, indicating that the distance between two histidines becomes close by the Pd(en)-mediated cross-linking. For other peptides, such main peaks were not clearly observed by the addition of Pd(en), and instead, many small peaks appeared, suggesting that various kinds of complexes exist as a mixture in these cases.

In conclusion, we established that Pd(en) is a versatile molecular unit for binding to peptides bearing two His at a specific pattern in aqueous solution. The ethylenediamine moiety in this organometallic receptor can be chemically modified with the combination to other binding interactions, so as to facilitate the more selective binding and modulation of a protein surface. This is sharply distinguished from the simple metal ions previously reported.[4] We are now under investigation in this line.

References and Notes

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6 The parentheses [ ] indicate the helix content in the absence of Pd(en). The helix contents are calculated according to the following paper: P. C. Lyu, L. A. Marky, N. R. Kallenbach, *J. Am. Chem. Soc.*, **111**, 2733 (1989).

7 The distances were estimated assuming that the conformation of the peptides is the typical α-helix in the region of the corresponding His residues.

8 Kostic and co-workers reported that the excess amount of Pd(en) can cleave a peptide bond of some His-appended peptides at higher temperature. a) T. N. Parac and N. M. Kostic, *J. Am. Chem. Soc.*, **118**, 5946 (1996). b) T. N. Parac and N. M. Kostic, *Inorg. Chem.*, **37**, 2141 (1998).