Trisbipyridine Metal Ion's Nest in Three α-Helix Bundle Structure

Norikazu Nishino,* Tamaki Kato, Tomonori Murata, Hiroshi Nakayama, Toru Arai,
Tsutomu Fujimoto, Hitoshi Yamamoto,† and Susumu Yoshikawa†
Faculty of Engineering, Kyushu Institute of Technology, Tobata-ku, Kitakyushu 804
†*Osaka National Research Institute, AIST, Ikeda, Osaka 563*

(Received September 11, 1995)

A trisbipyridine nest of a three-helix bundle structure has been built in a pseudoprotein containing 5-bipyridylalanine in each peptide segment. The accommodation of Ni^{2+} ion in the nest that is situated in the hydrophobic region is demonstrated by the measurements of electronic and circular dichroism spectra.

In *de novo* design of artificial proteins, metal ion complexes have been employed to combine α-helical peptides into bundle structure.[1] The sites of complex formation were placed at the termini of peptide chains, and exposed to the aqueous exterior. In natural proteins, however, functionally important metal ions are usually complexed at the hydrophilic and hydrophobic boundary region. In order to mimic such metalloproteins, we attempted to settle the site of a metal ion complex in the hydrophobic core of an α-helix bundle structure.

Recently, 5-bipyridylalanine (Bpa) was developed as an ion binding non-natural amino acid by Imperiali et al.[2] and Yamamoto et al.[3] A pair of Bpas have been incorporated in model peptides to stabilize the conformation by metal complexation.[4] We also employed two Bpa residues to bridge the antiparallel β-strands in gramicidin S framework with various divalent metal ions.[5] On the other hand, the bipyridyl moiety of Bpa may form octahedral trisbipyridine complexes with metal ions. Therefore, in designing metalloprotein models for some artificial functions, it seems useful to make a metal ion's nest by incorporating three Bpa residues in an α-helix bundle structure.

We designed 3α-helix bundle structure on a cyclic pseudopeptide template[6,7] as illustrated in Figure 1. Two cyclohexylalanines (Cha) were introduced to the amphiphilic 13-peptide segment to enhance the hydrophobicity. The hydrophobic residues (Cha and Leu) are expected to form tight hydrophobic core to surround the hydrophilic metal ion's nest made of three Bpas. Three same segments were combined in parallel to *cyclo*(L-Lys-*m*-Abz)$_3$ (Abz = aminobenzoyl).[8] Synthesis of the helix peptide was carried out by the solid-phase synthesis using Kaiser's oxime resin and by the segment condensation in solution.[7] The condensation with the side chain of Lys in the cyclic pseudohexapeptide was carried out with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/N-hydroxybenzotriazole. The assembled polypeptide with protecting groups was treated with anhydrous HF, and the desired 3α-helix-on-template peptide (3αBpa) was purified by gel filtration with Sephadex G-75 column (40% acetic acid). The TOF-mass spectrum and the amino acid analysis confirmed the chemical structure.[9]

The binding of Ni^{2+} to the trisbipyridine nest was analyzed by change in absorption spectra with the addition of Ni^{2+} ion. By the addition of aqueous solution of $Ni(OAc)_2$ to 15 μmol•dm^{-3} of 3αBpa,[10] a band at 288 nm decreased in intensity with the concomitant appearance of a new band at 301 nm (Figure 2). The clear isosbethtic point indicated that only one kind of complex was formed in the reaction of 3αBpa and Ni^{2+} ion. The UV-titration experiment was executed to determine the stoichiometry of the complex between the pseudoprotein and Ni^{2+} ion.[11] The titration curve (Figure 3) indicates that the pseudoprotein 3αBpa forms 1 : 1 complex with Ni^{2+}.

Since we observed significantly slow spectral changes after the addition of Ni^{2+} ion, we analyzed the rate of complex formation by the change in absorbance intensity at 301 nm. It took over 3 h to settle Ni^{2+} to the trisbipyridine nest at concentrations 10 - 15 μmol•dm^{-3} of 3αBpa. The rate was about 100 times slower than that of complex formation of Ni^{2+} with three equivalent of 2,2'-bipyridine in the same condition. This is to be attributed to the repulsive effect of the hydrophobic core inside of the 3α-helix bundle conformation toward the approaching Ni^{2+} ion. To the solution of 3αBpa · Ni^{2+} complex, EDTA was added over 100 eq. to estimate the stability.

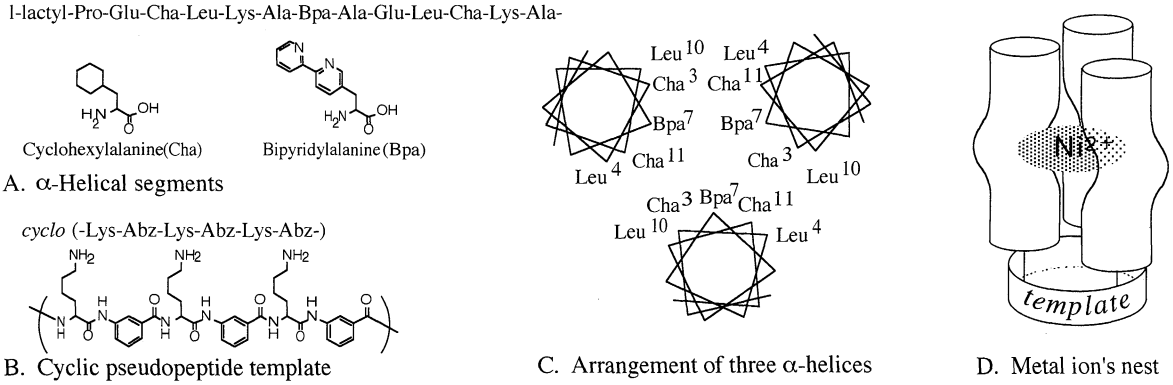

Figure 1. Design of metal ion's nest with three 5-bipyridylalanines.

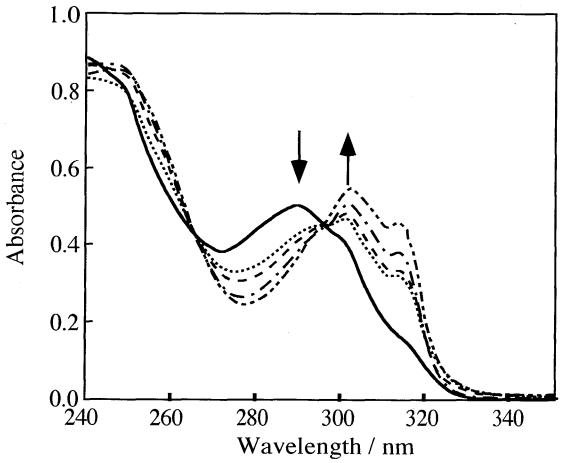

Figure 2. UV-VIS spectra of 3αBpa with $Ni(OAc)_2$ in H_2O (3% TFE). [3αBpa] = 15 μmol•dm^{-3} and the metal ions are (——) 0, (·····) 0.37, (- -) 0.63, (---) 0.88, and (----) 1.0 equiv. to 3αBpa.

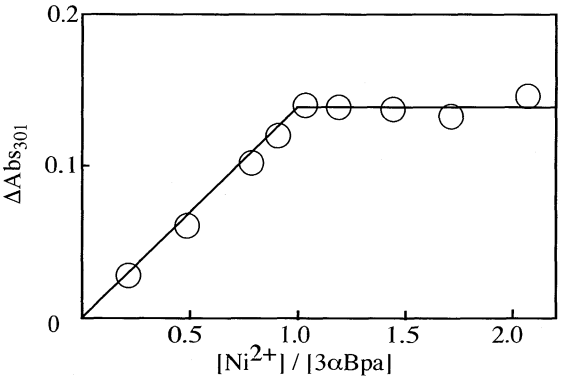

Figure 3. Plots of increase in absorption at 301 nm against the molar ratio of Ni^{2+} to 3αBpa.
[3αBpa] = 15 μmol•dm^{-3} in H_2O (3% TFE).

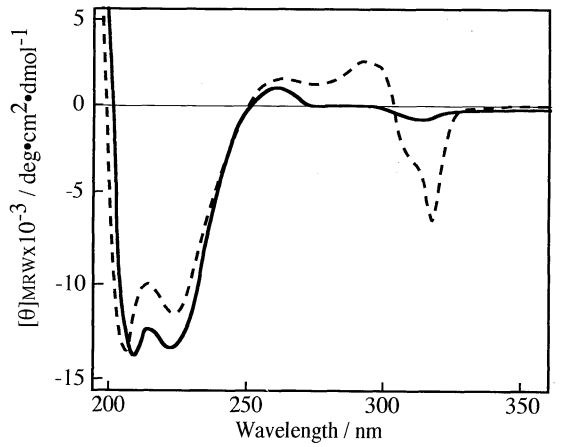

Figure 4. CD spectra of 3αBpa in the absence (——) and 2 days after the addition of $Ni(OAc)_2$ (1:1) (- -) in H_2O (3% TFE). [3αBpa] = 15 μmol•dm^{-3}.

Once settled in the trisbipyridine nest, the Ni^{2+} ion formed stable complex and was hardly exchanged by EDTA. This result also suggests that 3αBpa·Ni^{2+} complex has restricted conformation and the hydrophobic metal ion's nest of this pseudoprotein is highly shielded from the solvent.

Both of the conformations of polypeptide main chain and bipyridine moieties are examined by the measurements of circular dichroism (CD) spectra (Figure 4). The ellipticity at 222 nm in the CD spectrum of 3αBpa corresponds to 40% α-helicity.[12] Since the free 13-peptide took random coil structure in aqueous solution, such high α-helicity suggests the formation of the 3α-helix bundle structure induced by the aggregation of hydrophobic side chains. The CD was also observed at bipyridyl absorption band (280 - 320 nm). This fact suggests that the side chains of Bpa at the center of the α-helix rod are arranged in somewhat regular conformation. The addition of Ni^{2+} ion slightly decreased the main chain α-helical conformation (Figure 4). More interestingly, the intensity in CD of bipyridyl increased significantly after long incubation. It took overnight to reach to the equilibrium in complex formation judging from the CD incidents. Though the spectral data do not afford the definite evidence for the formation of exact 1 to 1 complex conformation (octahedral complex with Ni^{2+} and three bipyridyl moieties), the designed 3αBpa obviously accommodated a nickel ion and showed deeper CD suggesting the more efficient arrangement of bipyridyl groups by the metal ion.

Thus, as a simple model, we were able to demonstrate the synthesis of a 3α-helix bundle structure with a metal ion's nest in the hydrophobic core of a pseudoprotein. The further polishment in *de novo* design may give metalloproteins with more sophisticated function.

References and Notes

1 M. Lieberman and T. Sasaki, *J. Am. Chem. Soc.*, **113**, 1470 (1991); M. R. Ghadiri, C. Soares, and C. Choi, *J. Am. Chem. Soc.*, **114**, 825 (1992); M. R. Ghadiri and M. A. Case, *Angew. Chem., Int. Ed. Engl.*, **32**, 1594 (1993).

2 B. Imperiali and S. L. Fisher, *J. Am. Chem. Soc.*, **113**, 8527 (1991); B. Imperiali and S. L. Fisher, *J. Org. Chem.*, **57**, 757 (1992); B. Imperiali, T. J. Prins, and S. L. Fisher, *J. Org. Chem.*, **58**, 1613 (1993).

3 H. Yamamoto, T. Nishina, H. Nishio, N. Yumoto, Y. Tatsu, T. Taguchi, and S. Yoshikawa, *Peptide Chem.*, **1993**, 41 (1994).

4 H. Yamamoto, T. Nishina, T. Ando, T. Obata, N. Yumoto, T. Taguchi, Y. Tatsu, and S. Yoshikawa, *Peptide Chem.*, **1994**, 461 (1995).

5 N. Nishino, T. Arai, J. Hayashida, H. I. Ogawa, H. Yamamoto, and S. Yoshikawa, *Chem. Lett.*, **1994**, 2435.

6 M. Mutter and S. Vuilleumier, *Angew. Chem., Int. Ed. Engl.*, **28**, 535 (1989).

7 T. Arai, Y. Ide, Y. Tanaka, T. Fujimoto, and N. Nishino, *Chem. Lett.*, **1995**, 381.

8 The details in the synthesis of cyclic pseudopeptides including cyclo(L-Lys-*m*-Abz)3 will be reported elsewhere. FAB-MS m/z 742 $(M+H)^+$.

9 Shimadzu / Kratos MALDI II spectrometer was employed. m/z 5721 $(M+H_2O)^+$.

10 3αBpa was first dissolved in 2,2,2-trifluoroethanol (TFE) and diluted with H_2O to the appropriate concentration.

11 The solution mixed with Ni^{2+} was allowed to stand for 24 h at room temperature to complete the complex formation.

12 P. C. Lyu, J. C. Sherman, A. Chen, and N. R. Kallenbach, *Proc. Natl. Acad. Sci. U. S. A.*, **88**, 5317 (1991).