gation state in Li^+1b^- has also been found for an acyclic phosphonate[8] and is in contrast to the monomeric state found for sulfones[18] in THF solution. This disparate behavior may be ascribed to the enhanced basicity of the phosphonyl group[27] and the potential bidentate coordination of the lithium by the sulfonyl oxygens.

The intrinsic planarity and low rotational barrier for phosphonyl anions place stringent requirements on the design of chiral ligands for effective asymmetric reagents. The development of such chiral ligands along with further studies on the structure of related phosphorus-stabilized anions is in progress.

Acknowledgment. Financial support was provided by the Merck Sharp and Dohme Company in the form of a postdoctoral fellowship for P.C.M. We are deeply indebted to Prof. Gernot Boche and Friederike Haller (Phillips Universität Marburg) for their considerable efforts in obtaining the cryoscopic measurements and for stimulating discussions. We also acknowledge the assistance of Dr. Vera Mainz and Dr. M. F. Leopold for their assistance in obtaining ^{31}P-decoupled ^6Li NMR spectra and the Molecular Spectroscopy Laboratory funded by NIH Grant 1531957 and NSF Grant 85-14500. S.E.D. acknowledges support from the Alexander von Humboldt Foundation for a Senior Scientist Award (1990).

Supplementary Material Available: A listing of crystal and positional parameters, bond lengths and angles, and torsional angles for Li^+1b^- (24 pages). Ordering information is given on any current masthead page.

(27) Compare $-\Delta H°_{BF_3}$ (298 K): for tetramethylene sulfone, 12.3 kcal/mol; for trimethyl phosphate, 20.3 kcal/mol. Maria, P.-C.; Gal, J.-F. *J. Phys. Chem.* **1985**, *89*, 1296.

Iron(II) Organizes a Synthetic Peptide into Three-Helix Bundles

Marya Lieberman and Tomikazu Sasaki*

Department of Chemistry, University of Washington Seattle, Washington 98195

Received November 14, 1990

Considerable efforts have been directed[1] toward the design and synthesis of small model proteins to elucidate interactions involved in protein folding. We[2] and other groups[3] have devised a strategy in which a rigid molecule acts as a template to assemble secondary structures in simple model proteins. The X-ray structures of tris-bipyridine metal complexes suggest that covalent attachment of an amphiphilic α-helical segment to the 4-position of bipyridine would allow the formation of a three α-helix bundle protein on addition of a metal ion such as Fe^{2+}. Metal ions are known to play important roles in defining the three-dimensional structure of native metalloproteins such as DNA-binding zinc finger proteins.[4] Synthetic peptides bearing two metal binding sites at the i and $i + 4$ residues have been reported[5a-c] to form monomeric α-helices in the presence of heavy metal ions. Recently, several Zn^{2+} binding proteins derived from a four-α-helix motif were also synthesized.[5d] We report here the synthesis of a three-α-helix-bundle protein using a tris-bipyridine metal complex as a template as depicted in Scheme I.

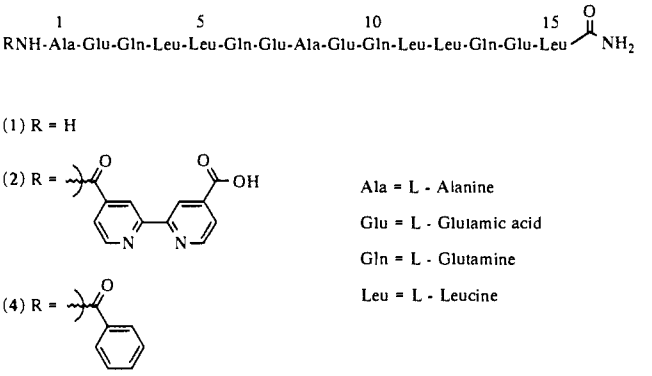


Figure 1. Structure of synthetic peptides **1**, **2** (pepy), and **4**.

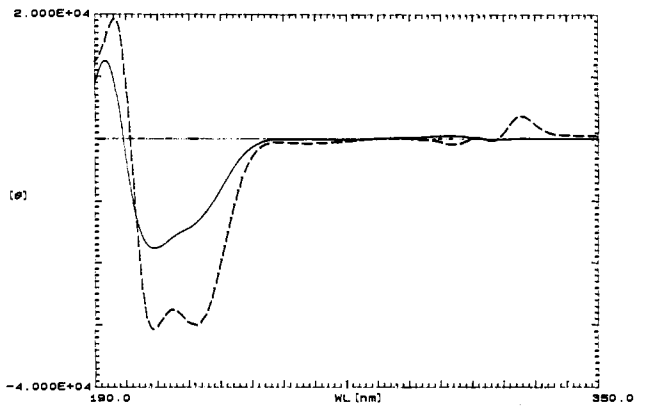
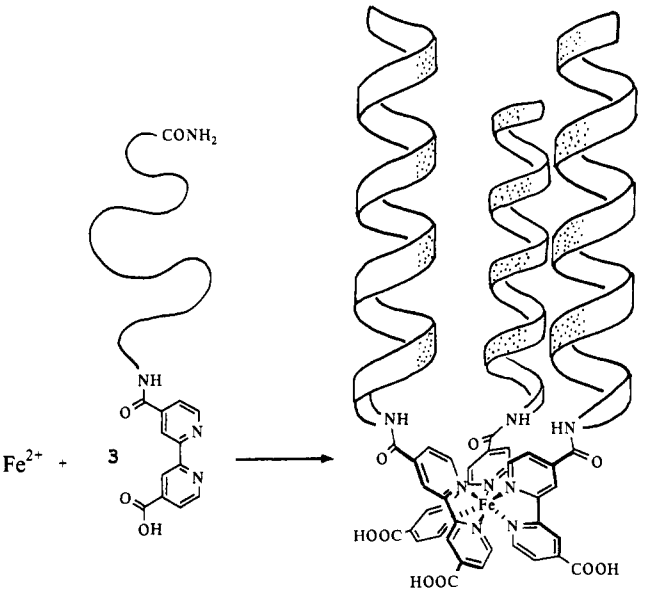


Figure 2. Circular dichroism spectra of pepy (—) and $Fe^{II}(pepy)_3$ (---). Obtained on a JASCO 700 CD spectrophotometer in 0.5-mm cells, 5×10^{-5} M solutions in 250 mM acetate buffer, pH = 4.8, 23 °C; x-axis, wavelength (nm); y-axis, molar ellipticity per residue (deg cm^2 dmol^{-1}).

Scheme I. Metal-Induced Formation of Three-α-Helix Bundle[a]



[a] The shaded regions of the helices represent hydrophobic residues.

* To whom correspondence should be addressed.

(1) (a) *Protein Engineering*; Oxender, D. L., Fox, C. F., Eds.; Alan R. Liss, Inc.: New York, 1987. (b) DeGrado, W. F. *Adv. Protein Chem.* **1988**, *39*, 51. (c) Mutter, M.; Vuilleumier, S. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 535. (d) Unson, C. B.; Erickson, B. W.; Richardson, D. C.; Richardson, J. S. *Fed. Proc.* **1984**, *43*, 1837. (e) Mutter, M.; Altmann, K.-H.; Vorherr, T. *Z. Naturforsch.* **1986**, *41b*, 1315. (f) Gutte, B.; Daumingen, M.; Wittschieber, E. *Nature* **1979**, *281*, 650.

(2) (a) Sasaki, T.; Kaiser, E. T. *J. Am. Chem. Soc.* **1989**, *111*, 380. (b) Sasaki, T.; Kaiser, E. T. *Biopolymers* **1990**, *29*, 79.

(3) (a) Mutter, M.; Altmann, E.; Altmann, K.-H. G.; Vuilleumier, S.; Gremlich, H.-U.; Muller, K. *Helv. Chim. Acta* **1988**, *71*, 835. (b) Mutter, M. *Trends Biochem. Sci.* **1988**, *151*, 260. (c) Montal, M.; Montal, M. S.; Tomich, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 6929. (d) Hahn, K.; Klis, W. A.; Stewart, J. M. *Science* **1990**, *248*, 1544. (e) Germann, W.; Heidemann, E. *Biopolymers* **1987**, *27*, 157.

(4) Parraga, G.; Horvath, S. J.; Eisen, A.; Taylor, W. E.; Hood, L.; Young, E. T.; Klevit, R. E. *Science* **1988**, *241*, 1489.

(5) (a) Ghadiri, M. R.; Choi, C. *J. Am. Chem. Soc.* **1990**, *112*, 1630. (b) Ghadiri, M. R.; Fernholz, A. K. *J. Am. Chem. Soc.* **1990**, *112*, 9633. (c) Ruan, F.-Q.; Chen, Y.-Q.; Hopkins, P. B. *J. Am. Chem. Soc.* **1990**, *112*, 9403. (d) Handel, T.; DeGrado, W. F. *J. Am. Chem. Soc.* **1990**, *112*, 6711.

0002-7863/91/1513-1470$02.50/0

Bipyridine-modified peptide **2**, "pepy", whose structure is shown in Figure 1, was synthesized via conventional solid-phase techniques.[6] Pepy reacted with $^1/_3$ equiv of iron(II) (as freshly prepared $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ solution) to form its iron complex **3**, $Fe^{II}(pepy)_3$. The complex is red; its absorption spectrum in water (λ_{max} (ϵ, M^{-1} cm^{-1}) = 313 (3.2×10^4) and 545 (7.0×10^3) nm) is comparable to that of the iron(II) tris-bipyridine complex[8] $[Fe(bipy)_3]^{2+}$. The stoichiometry of $Fe^{II}(pepy)_3$ was determined to be Fe:pepy = 1:2.7 by UV–vis titration of pepy with iron(II), confirming the formation of a tris-bipyridine type metal center.[9]

$Fe^{II}(pepy)_3$ appears to be monomeric by gel filtration chromatography on a Superose-12 column in pH 4.4 acetate buffer, in accordance with an intramolecular folded state. The 500-MHz ^1H NMR spectrum of a 5 mM solution of $Fe^{II}(pepy)_3$ in D_2O at 25 °C displayed only six resonances in the bipyridine region (δ 7–9 ppm), which suggests that the *fac* isomer of the iron tris-bipyridine core is favored.[10]

The circular dichroism spectra of pepy and $Fe^{II}(pepy)_3$ (Figure 2) are consistent with a random coil–α-helix equilibrium. $Fe^{II}(pepy)_3$ was found to be approximately 85% α-helical[11] in 250 mM acetate buffer, pH = 4.8. The metal-free peptide, pepy, showed only 35% α-helicity under the same conditions,[12] indicating that the spontaneous formation of an α-helical bundle[13] takes place upon the binding of Fe(II) to three pepy molecules.

The benzoic acid modified peptide **4** (Figure 1) did not form a colored complex with iron, nor was its α-helicity (28%) altered on addition of iron. This shows that the enhanced α-helicity of $Fe^{II}(pepy)_3$ over pepy is due to the interaction with the bipyridine ligand rather than to coordination of the peptide backbone or side chains.

In addition to the CD bands in the far UV region, $Fe^{II}(pepy)_3$ exhibited moderate CD absorptions at 304.5, 317, and 324 nm as shown in Figure 2. Pepy, on the other hand, showed no CD absorption in this region, suggesting that the observed CD bands in the 300–350-nm range are due to chirality at the metal center. In a strong denaturant, 6 M guanidine hydrochloride, the CD spectrum of $Fe^{II}(pepy)_3$ displayed less than 5% α-helicity, with complete loss of the bands between 300 and 350 nm. The folded protein structure rather than the mere proximity of L-amino acids must, therefore, be responsible for induction of chirality at the metal center. The metal center of $Fe^{II}(pepy)_3$ can assume either a Λ or a Δ configuration,[14a] but the α-helices are restricted to a right-handed twist and are predicted to have a left-handed supercoiling interaction.[16c] Thus, there are two distinct possible diastereomers of *fac*-$Fe^{II}(pepy)_3$. Since chiral $[Fe(bipy)_3]^{2+}$ complexes are known to racemize rapidly in solution,[14b,c] the chirality of the three-α-helix bundle appears to shift the racemization equilibrium of the metal center in favor of one of the diastereomers.

The four-α-helix bundle is a common motif in natural proteins[15] and has been successfully recreated in a number of designed proteins.[16] We have shown that, given a suitable framework such as $[Fe(bipy)_3]^{2+}$, three amphiphilic α-helices can form a stable structural entity. Furthermore, structural preferences of this moiety influence the stereochemistry at the metal center. We plan further modification of three-helix-bundle proteins to yield a variety of artifical metalloproteins which would provide a simple and well-defined model system to study the functions of metalloenzymes, such as electron transfer.

Acknowledgment. This work was supported in part by the donors of the Petroleum Research Fund, administered by the American Chemical Society. M.L. was supported by a fellowship from the Fannie and John Hertz Foundation. We thank Professors P. B. Hopkins and R. E. Klevit for valuable discussions and Mr. M. Tabet and Mr. M. Stahl for technical assistance in the synthesis of bipyridine-modified peptides.

(6) (a) Peptide **1** (Figure 1) was synthesized[6b] in a protected form on *p*-methylbenzhydrylamine (MBHA) resin. This peptide was designed to form an amphiphilic α-helix,[2a] and Sasaki and Kaiser[2b] showed that it forms a four-α-helix bundle when attached to a porphyrin template. While peptide **1** was bound to the resin, its N-terminus was covalently coupled with 2,2′-bipyridine-4,4′-dicarboxylic acid. 2,2′-Bipyridine-4,4′-dicarboxylic acid was prepared in 37% yield from 4,4′-dimethyl-2,2′-bipyridine (Aldrich) by oxidation with $KMnO_4$.[6c] The diacid (0.20 mmol) was dissolved in 3 mL of 5% diisopropylethylamine (DIEA) in CH_2Cl_2 in a flask equipped with a stir bar and drying tube. *N*-Hydroxybenzotriazole (HOBt) (0.50 mmol) and diisopropylcarbodiimide (0.50 mmol) were added to form the HOBt diester; after 45 min of stirring, the solution cleared and turned faint yellow. MBHA resin–$(aa)_{15}$–BOC (0.10 mmol) was deprotected with trifluoroacetic acid (TFA) and washed with CH_2Cl_2. The HOBt diester solution was added, and the mixture was shaken for 8 h. The resin was washed with CH_2Cl_2 and 33% EtOH/CH_2Cl_2. A negative Kaiser ninhydrin test[6d] for free NH_2 indicated that coupling was complete. Cleavage from the resin according to a published procedure[6e] using trimethylsilyl triflate in TFA as a deprotecting reagent in the presence of thioanisole and *m*-cresol at 0 °C for 2 h yielded crude peptide **2**, which was purified[7] (yield 19%) by gel permeation chromatography on a 1.2 cm × 20 cm Sephadex G-15 column, eluting with 2% NH_4HCO_3, followed by preparative reverse-phase HPLC (Vydak C_4 prep column, 20%–80% CH_3CN in water with 0.1% TFA over 20 min; **2** elutes at 54% CH_3CN). As a control compound, we also synthesized the benzamide-modified peptide **4** (Figure 1), using the same synthetic methodology.[7] (b) Stewart, J. M.; Young, J. D. *Solid Phase Peptide Synthesis*, Pierce Chemical Company: Rockford, IL 1984. (c) Case, H. F. *J. Am. Chem. Soc.* **1946**, *68*, 2574. (d) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595. (e) Fuji, N.; Otaka, A.; Ikemura, O.; Akagi, K.; Funakoshi, S.; Hayashi, Y.; Kuroda, Y.; Yajima, H. *J. Chem. Soc., Chem. Commun.* **1987**, 274.

(7) For **2**: amino acid analysis (expected values in parentheses) Glu/Gln 8.68 (8), Ala 2.00 (2), Leu 4.75 (5); FAB mass spectrum 2003, $(M + Na)^+$ = 2003, ΔM = 0; UV–vis (water) λ_{max} = 289 nm, ϵ = 11 170 M^{-1} cm^{-1}. For **4**: Glu/Gln 8.52 (8), Ala 2.00 (2), Leu 5.35 (5); time-of-flight MS (^{252}Cf fission fragment) 1880.7, $(M + Na)^+$ = 1881, ΔM = −0.3; UV–vis λ_{max} = 260 nm.

(8) $[Fe(bipy)_3]Cl_2$ has absorption maxima at 295 (sh), 300 ($\epsilon \sim 6.3 \times 10^4$ M^{-1} cm^{-1}),[8a] and 522 ($\epsilon = 8.65 \times 10^3$ M^{-1} cm^{-1}) nm.[8b] (a) Schläfer, H. L. *Z. Phys. Chem.* **1956**, *8*, 373–386. (b) Ford-Smith, M. H.; Sutin, N. *J. Am. Chem. Soc.* **1961**, *83*, 1830.

(9) Loss of bipyridine from $[Fe(bipy)_3]^{2+}$ is disfavored ($K_{eq} = 10^{-9.55}$) and accompanied by a transition from low spin ($S = 0$) to high spin ($S = 2$). See: Irving, H.; Mellor, D. H. *J. Chem. Soc.* **1962**, 5237. $Fe^{II}(pepy)_3$ is diamagnetic on the basis of its ^1H NMR spectrum.

(10) Peaks observed (ppm in D_2O/phosphate): 9.00 (br s), 8.93 (br s), 7.75 (br s), 7.70 (br s), 7.64 (m), 7.56 (m). The *mer* isomer has C_1 symmetry and should theoretically give 18 peaks in this region, unless fast exchange is occurring. For a spectrum of mixture of *fac* and *mer* isomers, see: Cook, M. J.; Lewis, A. P.; McAuliffe, G. S. G.; Thomson, A. J. *Inorg. Chim. Acta* **1982**, L25–L28.

(11) The degree of α-helicity of the synthetic peptides estimated[11a,b] by using the molar ellipticity per residue at 222 nm ($[\theta]$ = −33 500 deg cm^2 $dmol^{-1}$ for 100% helix). (a) Chang, C. T.; Wu, C.-S. C.; Yang, J. T. *Anal. Biochem.* **1978**, *91*, 13. (b) Johnson, C. *Proteins: Struct., Funct., Genet.* **1990**, *7*, 205.

(12) The CD spectrum of pepy did not change in the presence of 1×10^{-3} M ethylenediaminetetraacetic acid (EDTA).

(13) The observed *fac* preference of the metal center and the high α-helicity (85%) strongly suggest that the most stable folded state of $Fe^{II}(pepy)_3$ would be a symmetrical three-helix bundle.

(14) (a) On the basis of its CD spectrum, the preferred chirality of the metal center of $Fe^{II}(pepy)_3$ can be tentatively assigned to the Λ-configuration as shown in Scheme I. Λ-(−)-$[Fe(bipy)_3]^{2+}$ exhibits a negative peak at 285 nm ($[\theta] \leq -4.95 \times 10^5$ deg cm^2 $dmol^{-1}$) and a positive peak at 300 nm ($[\theta] \geq 1.34 \times 10^6$ deg cm^2 $dmol^{-1}$) in its CD spectrum.[4b–d] (b) Milder, S. J.; Gold, J. S.; Kliger, D. S. *J. Am. Chem. Soc.* **1986**, *108*, 8295. (c) Mason, S. F.; Peart, B. J. *J. Chem. Soc., Dalton Trans.* **1973**, 949. (d) Hidaka, J.; Douglas, B. E. *Inorg. Chem.* **1964**, *3*, 1180.

(15) Weber, P. C.; Salemme, F. R. *Nature* **1980**, *287*, 82.

(16) (a) Regan, L.; DeGrado, W. F. *Science* **1988**, *241*, 976. (b) Hecht, M. H.; Richardson, J. S.; Richardson, D. C.; Ogden, R. C. *Science* **1990**, *249*, 884. (c) Cohen, C.; Parry, D. A. D. *Proteins: Struct., Funct., Genet.* **1990**, *7*, 1. Also see refs 2 and 3a–d. (d) Ho and DeGrado reported that a 33-residue peptide with two amphiphilic helical regions designed to dimerize to a four-helix bundle trimerized by forming two three-helix bundles. Ho, S. P.; DeGrado, W. F. *J. Am. Chem. Soc.* **1987**, *109*, 6751.