Synthesis and Characterization of a Water-Soluble Metallopeptide That Resembles a Parallel β-Pleated Sheet

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The rational design of artificial proteins requires knowledge of the folding requirements for different structural motifs. Thus, the development of new model systems that mimic the properties of secondary protein structures has been the focus of much interest.[1] However, with but few notable exceptions, the succesful design of water soluble models for β-pleated sheets[2] and β-turns[3] has remained elusive due their tendency to form insoluble aggregates in aqueous solution. Thus, the majority of these compounds are only amenable to study in organic solvents.[4−7] Substitution-inert metal complexes have recently been used to help form such important protein structures as α-helices, multihelical bundles, and proline helices in aqueous solution.[8−12] Here, we explore the efficacy of applying this approach toward the design of water soluble β-sheet mimics. We report the properties of a new metallopeptide that consists of two divalinyl peptides attached to the carboxylate groups of the ruthenium polypyridyl complex $[Ru(bpy)_2L]^{2+}$, bpy = 2,2′-bipyridine, L = 3,5-dicarboxy-2,2′-bipyridine. This divalent ruthenium complex is indeed water soluble, and contains a parallel arrangement of interacting, extended peptide strands that is reminiscent of the parallel β-sheet structure. Circular dichroism results performed on a related compound support this assignment. The tendency of such a small metallopeptide to display β-sheetlike properties provides encouraging evidence to suggest that the use of substitution-inert metal complexes may provide a useful route toward the future design of artificial β-proteins.

Solution-phase methods were used to prepare the title compound. One equivalent of the ruthenium starting material, $[Ru(bpy)_2L]Cl_2$,[13] and two equivalents of the Val-Val−OMe were coupled using diisopropylcarbodiimide and 1-hydroxybenzotriazole in 1:1 CH_3CN/DMF. The reaction was monitored by reverse-phase HPLC using UV−vis diode array detection. Three product peaks were observed and separated by repetitive reverse-phase column chromatography using aqueous methanol eluents. The ^1H NMR spectra show that these compounds correspond to each of the two mono-substituted metallopeptides, having only one divalinyl chain attached to L (**I** and **II**), and the desired bis(divalinyl) complex (**III**). Semipreparative reverse-phase HPLC[14] and circular dichroism (CD) spectroscopy were used to separate and identify the two diastereomeric forms of **III**.[15] ^1H NMR measurements (400 MHz and 500 MHz) were performed in 9:1 H_2O/D_2O using presaturation solvent suppression. The 1-D spectrum of the Δ-*l* diastereomer consists of sharp, well-defined signals, indicating that this charged metallopeptide does not aggregate in aqueous solution. Individual residue assignments were made on the basis of 2-D TOCSY spectra which show four amide resonances appearing in the downfield region of the spectrum. NMR spectra could not be obtained for the uncomplexed ligand-peptide conjugate since it was found to be insoluble in aqueous solution at both neutral and acidic pH.

Table 1. Chemical Shifts (278 K), $^3J_{NH-C\alpha}$ Coupling Constants, and Temperature Coefficients for the Amide Protons of Δ-*l* **III**

residue	A	B	C	D
δ_{278K} (ppm)	9.870	9.086	8.957	8.702
$^3J_{NH-C\alpha H}$ (Hz)	7.9	7.7	8.2	7.2
$d\delta/dT$ (ppb/K)	−4.6	−7.5	−6.0	−9.1

Table 1 summarizes the ^1H NMR data acquired for Δ-*l* **III** in aqueous solution. Variable-temperature 1-D spectra were used to probe the conformational effects caused by coupling two peptide chains to $Ru(bpy)_2L$. All of the amide (N−H) signals for the two singly-substituted compounds, **I** and **II**, show large temperature coefficients in 9:1 H_2O/D_2O ($d\delta/dT \approx -9$ ppb/K), indicating that an unaggregated and unordered conformation exists in the cases where only one peptide chain is coupled to the metal complex. In contrast, the amide signals for Δ-*l* **III** show a more complicated behavior in which each amide proton experiences a different degree of solvent-shielding. Amides A and C are moderately solvent-shielded, whereas amides B and D are more strongly solvent-exposed. Thus, the two peptide chains of Δ-*l* **III** interact with one another in such a way as to profoundly affect the structure of the metallopeptide, perhaps inducing a more orderly conformation. While it is tempting to ascribe these solvent-shielding effects to the presence of intramolecular hydrogen-bonds, we note that the 2-D NOESY spectrum shows that the two N-terminal amide protons (A and B) are in close proximity to the bipyridyl ligand

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(1) See, for example: (a) Tuchscherer, G.; Mutter, M. *J. Pept. Sci.* **1995**, *1*, 3. (b) Choma, C. T.; Lear, J. D.; Nelson, M. J.; Dutton, P. L.; Robertson, D. E.; DeGrado, W. F. *J. Am. Chem. Soc.* **1994**, *116*, 856 and references therein. (c) Hecht, M. H. *Proc. Nat. Acad. Sci. U.S.A.* **1994**, *91*, 8729. (d) Hecht, M. H.; Richardson, J. S.; Richardson, D. C.; Ogden, R. C. *Science* **1990**, *249*, 884.
(2) (a) Schneider, J. P.; Kelly, J. W. *J. Am. Chem. Soc.* **1995**, *117*, 2533. (b) Tsang, K. Y.; Diaz, H.; Graciani, N.; Kelly, J. W. *J. Am. Chem. Soc.* **1994**, *116*, 3988. (c) Diaz, H.; Tsang, K. Y.; Choo, D.; Espina, J. R. ; Kelly, J. W. *J. Am. Chem. Soc.* **1993**, *115* 3790.
(3) Imperiali, B.; Kapoor, T. M. *Tetrahedron* **1993**, *49*, 3501.
(4) Kemp, D. S.; Bowen, B. R.; Muendel, C. C. *J. Org. Chem.* **1990**, *55*, 4650.
(5) Raj, P. A.; Soni, S. D.; Ramasubbu, N.; Bhandary, K. K.; Levine, M. J. *Biopolymers* **1990**, *30*, 73.
(6) Nowick, J. S.; Powell, N. A.; Martinez, E. J.; Smith, E. M.; Noronha, G. *J. Org. Chem.* **1992**, *57*, 3763.
(7) Balaram, H.; Uma, K.; Balaram, P. *Int. J. Pept. Protein Res.* **1990**, *35*, 495.
(8) Lieberman, M.; Sasaki, T. *J. Am. Chem. Soc.* **1991**, *113*, 1470.
(9) (a) Ghadiri, M. R.; Soares, C.; Choi, C. *J. Am. Chem. Soc.* **1992**, *114*, 4000. (b) Ghadiri, M. R.; Fernholz, K. A. *J. Am. Chem. Soc.* **1990**, *112*, 9633. (c) Ghadiri, M. R.; Choi, C. *J. Am. Chem. Soc.* **1990**, *112*, 1630.
(10) Ruan, F.; Chen, Y.; Hopkins, P. B. *J. Am. Chem. Soc.* **1990**, *112*, 9403.
(11) Mihara, H.; Nishino, N.; Hasegawa, R.; Fujimoto, T.; Usui, S.; Ishida, H.; Ohkubo, K. *Chem. Lett.* **1992**, 1813.
(12) Ogawa, M. Y.; Moreire, I; Wishart, J. F.; Isied, S. S. *Chem. Phys.* **1993**, *176*, 589.
(13) Fernando, R.; Maharoof, M.; Deshayes, K. D.; Kinstle, T. H.; Ogawa, M. Y. submitted for publication.
(14) The two diastereomers were separated using a Whatman Magnum 9 semipreparative C_{18} column (9 × 50 cm) using a binary elution gradient consisting of 10−40% methanol in 0.1% HTFA.
(15) Milder, S. J.; Gold, J. S.; Kliger, D. S. *Chem. Phys. Lett.* **1988**, *144*, 269.

0020-1669/95/1334-6423$09.00/0

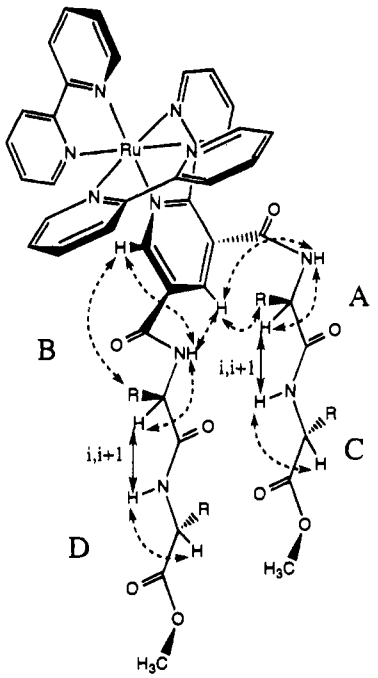

Figure 1. Schematic representation of Δ-*l* **III** showng the weak (- - -) and strong (—) NOE interactions observed for the peptide-based protons in 9:1 H_2O/D_2O (500 MHz, T = 278 K, τ_m = 500 ms, presaturation solvent suppression).

system (Figure 1). Thus, the degree of solvent-shielding experienced by these residues may result from steric interactions involving the peptide conformation and/or the bipyridyl ligands. Nonetheless, it is of interest to note that the two C-terminal amides (C and D), which should experience the smallest amount of interaction with the metal ligands, alternate between being solvent-shielded and solvent-exposed in a manner suggestive of a β-pleated sheet.

The 2-D NMR spectra of Δ-*l* **III** were used to more completely characterize its conformational properties. The amino acid residues of an idealized β-pleated sheet exist as extended peptide chains whose amide protons display large vicinal coupling constants ($^3J_{NH-C\alpha} \geq 7$ Hz).[16] The DQCOSY spectrum shows that all four amide protons have values of $^3J_{NH-C\alpha}$ which exceed this minimal criterion. The values for residues A–C fall within the range of $^3J_{NH-C\alpha} = 8.0 \pm 0.3$ Hz, similar to that reported for other acyclic β-sheet models.[2,7] Residue D exhibits a somewhat lower value (7.2 Hz) which indicates that it may experience an increased degree of conformational freedom, probably due to an "end-group" effect. This is consistent with the large temperature coefficient seen for its amide signal. Preliminary computer modeling studies suggest that the parallel alignment of two peptide chains attached to the carboxylate groups of L may result in their being translationally offset from one another (i.e. being slightly out-of-register) by approximately one-half amino acid (see Figure 1). This would result in having one C-terminal amino acid experience a larger degree of conformational lability than the other.

Within the β-sheet structure, sequential amino acids are expected to maintain close through-space contacts between their $C_i^{\alpha}H$ and $N_{i+1}H$ protons.[17] The NOESY spectrum of Δ-*l* **III** shows that, in addition to weak intraresidue NOE's, strong interresidue crosspeaks occur between the C^{α} protons of the two N-terminal residues (A and B) and the amide protons of the C-terminal residues (C and D, respectively).[18] These results show the existence of extended peptide strands in this metallopeptide and support the assignment of the β-sheet structure. However, we caution that an unequivocal conformational assignment requires the observation of close inter-strand contacts from the NOESY data. The absence of such data in our spectra suggests that Δ-*l* **III** probably experiences some degree of conformational flexibility, as would be expected for such a small, acyclic polypeptide. It is of interest to note that no unambiguous assignment of such cross-chain, interresidue NOE's has been reported for related β-sheet mimics.[2c,5,7] Nevertheless, analysis of the temperature coefficients, $^3J_{NH-C\alpha}$, and NOESY data presented here demonstrate that Δ-*l* **III** contains a parallel arrangement of interacting, extended peptide strands reminiscent of the β-sheet structure.

Circular dichroism spectroscopy offers a convenient method to determine the conformational properties of polypeptides. Unfortunately, CD analysis could not be performed on Δ-*l* **III** as its spectrum is dominated by the chiral ruthenium center.[15] Instead, measurements were performed on the related compound $[Ru^{III}(NH_3)_5L'(Val\text{-}Val{-}H)_2]^{3+}$ (**IV**), which contains peptide chains coupled to the carboxylate groups of L′ = 3,5-pyridinedicarboxylic acid. Thus, **IV** contains the essential steric features of Δ-*l* **III** but has the advantage of possessing an achiral metal center.[19] The CD spectrum of this compound consists of two strongly negative maxima at 225 nm (−22 120 mdeg cm^2 $dmol^{-1}$) and 197 nm (−18 850 mdeg cm^2 $dmol^{-1}$), which are consistent with the presence of the β-sheet and random coil conformations, respectively.[3,20] The latter feature may be due to the presence of disordered endgroups. A zero crossing occurs at 188 nm and a positive band is seen at 267 nm (3430 mdeg cm^2 $dmol^{-1}$), which probably arises from the properties of the pyridine ligand.[21]

In summary, the novel metallopeptide Δ-*l* **III** has been shown to display many characteristics of a β-pleated sheet including interacting peptide chains that display large vicinal coupling constants, short sequential $NH-C_{\alpha}H$ distances, and solvent-shielded amide protons. This conclusion is supported by CD measurements performed on the achiral analog, **IV**. The tendency of such small metallopeptides to display β-sheetlike properties provides encouraging evidence to suggest that the use of substitution-inert metal complexes may provide a new route toward the creation artificial β-proteins in aqueous solution.

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Supporting Information Available: Circular dichroism and 1-D and 2-D TOCSY, DQCOSY, and NOESY ^1H NMR spectra (5 pages). Ordering information is given on any current masthead page.

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(16) Pardi, A.; Billeter, M.; Wuthrich, K. *J. Mol. Biol.* **1984**, *180*, 741.
(17) Wuthrich, K.; Billeter, M.; Braun, W. *J. Mol. Biol.* **1984**, *180*, 715.
(18) Analysis of the 2-D NOESY data shows that peptides AC and BD are respectively coupled to the 3- and 5-carboxy postions of L, as shown in Figure 1.
(19) NMR measurements were not attempted for $[Ru^{III}(NH_3)_5L'(Val\text{-}Val{-}H)_2]^{3+}$ because of its paramagnetic ruthenium center.
(20) Jirgensons, B. *Optical Activity of Proteins and Other Macromolecules*; Springer-Verlag: New York, 1973.
(21) A reviewer correctly notes that the shorter wavelength CD bands may also experience contributions arising from an induced chirality of the pyridine ligand. This is the subject of current investigation.