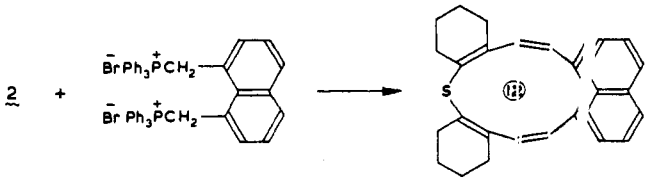
2.60–2.85 (m, 3 H) (benzenoid), an AB quartet at 3.20 and 3.38 (J_{AB} = 12 Hz, 2 H, *cis*-olefinic), an AB quartet at 3.65 and 3.82 (J_{AB} = 16.5 Hz, 2 H, *trans*-olefinic), 7.45–7.85 (broad s, 8 H, allylic methylene), and 7.95–8.35 (broad s, 8 H, nonallylic methylene).

1,8-Bis(triphenylphosphoniomethyl)naphthalene dibromide (**8**)[5b,12] was converted to the corresponding bisylide with sodamide in liquid ammonia,[12b] followed by treatment with the dialdehyde **2** in boiling ether–benzene. Chromatography on alumina gave 2% of *cis,cis*-naphtho[1,9,8-*fg*]dicyclohexeno[*b,k*]thiacyclododecapentaene (**9**) as colorless needles, mp 138–



140°;[9] mass spectrum, molecular ion at *m/e* 370; uv max (95% EtOH) 239 (ε 37,000), 322 (8800), and ~338 sh nm (7600); ir (KBr), only weak bands (at 948, 958, 978, and 988 cm^{-1}) in the 920–1000-cm^{-1} region. Assignment of the cis,cis stereochemistry is based on the ir spectrum and the nmr spectrum (100 MHz, $CDCl_3$), which showed signals at τ 2.30–2.55 (m, 2 H) and 2.60–2.90 (broad d, 4 H) (benzenoid), an AB quartet at 3.31 and 3.67 (J_{AB} = 12 Hz, 4 H, *cis*-olefinic), and 7.70–9.25 (m, 16 H, methylene).

The nmr spectra of **4**, **7**, and **9** indicate no appreciable ring current effects due to the macrocyclic rings, and presumably they are nonplanar molecules. The fact that the 13-membered ring compound **4** (type **1**, *m* = 6) shows no sign of aromaticity parallels the behavior of fully unsaturated nine-membered heterocycles (type **1**, *m* = 4) containing an S,[4,12] O,[13,14] or NCOOEt[15] grouping, but not that of 11*H*-azonine[16] which contains an NH grouping.

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Ordered Structures in Sequential Copolypeptides Containing L-Proline or 4-Hydroxy-L-proline[1]

Sir:

Ordered structures in sequential copolytripeptides and copolyhexapeptides containing L-proline and glycine have been detected in the solid state[2] and in solution.[3,4] Copolypeptides with glycine at every third residue, which is a strict steric requirement for the formation of the collagen triple helix,[5,6] have been most frequently studied. We have observed that sequential copolypeptides which do not contain glycine as every third residue may nevertheless develop order in dilute solution.

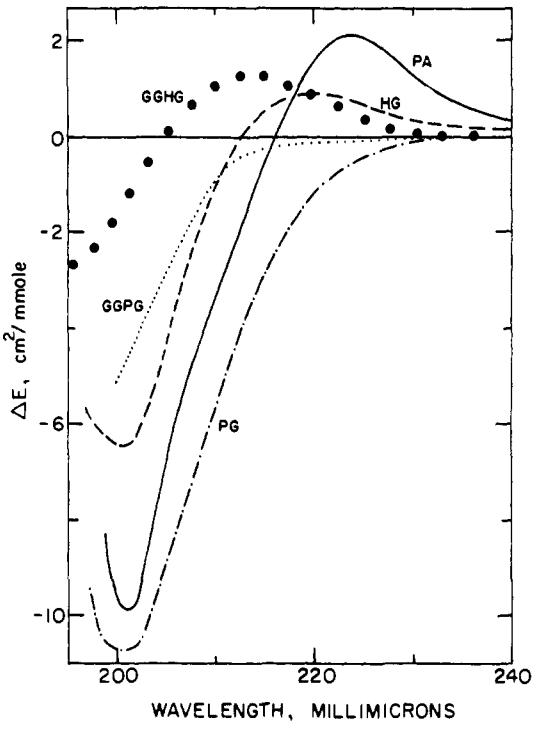

Figure 1. Circular dichroism of PA (——) at −42°, GGPG (. . . . . .) and GGHG (●●●) at −45°, and PG (–·–·–·) and HG (– – –) at −48° in 2:1 ethylene glycol–water.

Poly(L-prolyl-L-alanine) (PA), M_W = 3000, poly(L-prolylglycine) (PG), M_W = 13,200, poly(4-hydroxy-L-prolylglycine) (HG), M_W = 10,400, poly(glycylglycyl-L-prolylglycine) (GGPG), M_W = 6700, and poly(glycylglycyl-4-hydroxy-L-prolylglycine) (GGHG), M_N = 9400, exhibit only negative circular dichroism (CD) over the accessible spectral region in water at 75–85°. The minimum is at 196–198 mμ for GGHG, 204–205 mμ for PA, and close to 200 mμ for PG, HG, and GGPG, with an average CD per peptide bond of about −2 cm^2/mmol for GGPG and GGHG and about −6 cm^2/mmol for PA, PG, and HG. These spectra are qualitatively similar to that of heat-denatured collagen, which shows a broad minimum at 195–198 mμ with ΔE = −4 cm^2/mmol.[7] However, as is shown in Figure 1, major differences appear in the CD obtained at low temperatures in ethylene glycol–water mixtures. Although PG and GGPG still exhibit

(1) This work was supported by a contract with the Division of Biology and Medicine, Atomic Energy Commission.

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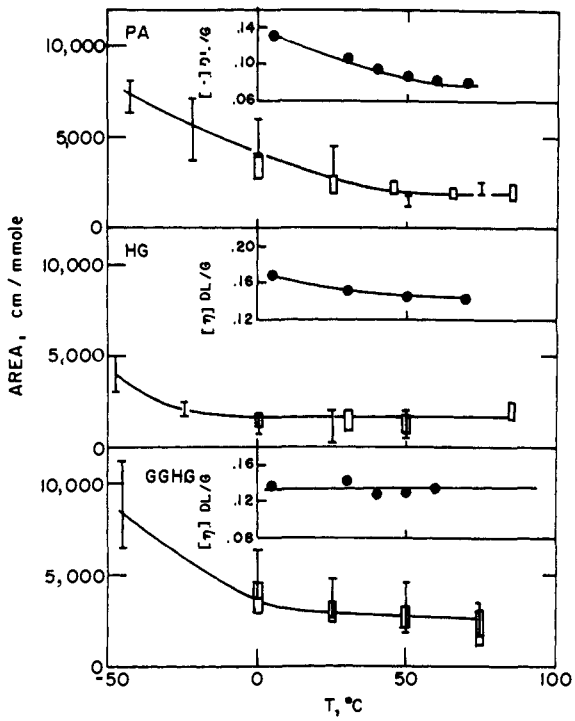

Figure 2. Area under the positive Gaussian CD band in water (rectangles) and 2:1 ethylene glycol–water (bars) for PA (top), HG (middle), and GGHG (bottom). The insets show the corresponding intrinsic viscosities in water. The temperature scale at the bottom applies to both the CD and viscosity data.

only negative CD, a positive band has appeared in the spectra obtained with PA, HG, and GGHG. As can be seen in Table I, the positive CD of PA and HG is similar in magnitude and location to that observed for collagen and the known ordered structures of proline and hydroxyproline containing polypeptides.[3,7-10] The positive CD of GGHG is shifted toward shorter wavelengths, and the minima are less intense than those of the previously known ordered structures.

Table I. Features in the CD of Ordered L-Proline and 4-Hydroxy-L-proline Containing Polypeptides in Order of Decreasing λ_{max}[a]

Polymer	λ_{max}	ΔE_{max}	λ_{crs}	λ_{min}	ΔE_{min}	Ref
Poly-L-proline	228	0.5	224	206	−13	*d*
Poly-L-proline[b]	228	1.4	222	206	−15	*e*
Poly(4-hydroxy-L-proline)	225	2.2	219	205	−14	*f*
PA, −42°[b]	224	2.1	216	201	−10	
Guinea pig skin collagen[b]	220	3.0	212	197	−17	*e*
HG, −48°[b]	220	0.9	213	201	−6	
Ichthyocol collagen[c]	220	1.8	212	198	−14	*g*
Rat tail collagen	219	1.3	214	197	−12	*h*
GGHG, −45°[b]	214	1.3	205	195	−3	

[a] The subscripts max, crs, and min refer to the observed maximum, crossover, and minimum. ΔE is the average CD per peptide bond and λ is the wavelength (mμ). [b] In 2:1 ethylene glycol–water (v/v). [c] Corrected for solvent refractive index. [d] Reference 9. [e] Reference 3. [f] Reference 10. [g] Reference 8. [h] Reference 7.

The magnitude of the positive Gaussian CD bands in poly-L-proline and poly(4-hydroxy-L-proline) has been found to correlate with the intrinsic viscosity when the polymers are converted to random coils by calcium chloride.[9,10] The magnitude of the positive CD band in PA, HG, and GGHG was detemined by resolution of the spectra into two Gaussian bands of opposite sign.[9] The area under the positive Gaussian bands, located at 218–220, 214–216, and 209–211 mμ in the CD of PA, HG, and GGHG, respectively, is shown as a function of temperature in water and 2:1 ethylene glycol–water in Figure 2. Similar results are obtained for either solvent system where corresponding measurements can be made. The area at the highest temperatures studied is similar to that observed with poly-L-proline under conditions where hydrodynamic properties demonstrate a random coil structure.[9]

The area for PA changes only slightly with temperature above 40°. Below this temperature, it begins to increase rapidly and at −42° it attains about the same value as was obtained for poly-L-proline[9] and poly(4-hydroxy-L-proline)[10] in aqueous solution. The intrinsic viscosity in water (inset) increases about 50% in this temperature interval. The marked increase in the area and its correlation with the intrinsic viscosity give evidence for the development of an extended ordered structure for PA below 40°. In their study of the low-temperature circular dichroism of poly(glycyl-L-prolyl-L-alanine) in 2:1 ethylene glycol–water, Blout and coworkers[3] employed a similar criterion as evidence for conformational ordering at low temperatures.

The CD of HG is qualitatively similar, but the area does not increase substantially until the temperature is below 0°. Even though GGHG contains more glycine than HG, the area under the positive Gaussian rises more rapidly at low temperatures. The areas attained at the lowest temperatures are comparable for PA and GGHG. The major conclusion can be drawn, from the data in Figure 2, that these three sequential copolypeptides are developing ordered conformations at the low temperatures.[3,9,10]

Theoretical conformational energy contour diagrams, based on interactions in a dipeptide unit, have been obtained by Flory and coworkers.[11,12] The conformational maps appropriate for PA suggest that this copolymer may attain an ordered conformation similar to that of poly-L-proline form II at sufficiently low temperatures. This conclusion is consistent with the reported hydrodynamic and optical properties. The relatively large area with low energy and the symmetrical nature of the conformational maps for a glycyl residue[11,12] would make the ordering of PG and GGPG more difficult unless interactions other than those between nearest neighbors occur. Since the conformational energy maps are assumed to be unchanged when 4-hydroxy-L-proline is substituted for L-proline,[13] the development of an ordered structure with HG and GGHG must result from either intermolecular interactions or specific long-range intramolecular interactions.

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Rare Earth Metal Ions as Probes of Electrostatic Binding Sites in Proteins

Sir:

One of the more important factors influencing the binding of metal ions to proteins has been suggested to be the size of the metal ion.[1] In the first-row transition series, however, where covalent interactions appear to be the dominant force, the ionic radius of the metal ion seems to bear no relationship to the strength of binding. This is shown by the following data for the binding of transition metal ions to conalbumin:[2] strength of binding, Fe(III) > Mn(II) > Co(II) > Cd(II) > Ni(II); ionic radius, 0.64, 0.80, 0.72, 0.97, and 0.69 Å, respectively.

In contrast, metal ions with closed electronic shells bind ligands through predominantly electrostatic interactions, and the strength of binding of these metal ions seems to be related to the size of the ion. Thus there is a direct relationship between the size of the ion and the strength of binding of metal ions with closed electronic shells to β-methylaspartase.[3]

Visible and ultraviolet absorption spectroscopy have been extensively employed to probe the binding sites of the first-row transition-metal ions.[4-6] Similar studies with metal ions such as calcium, which interact specifically with many proteins but which exhibit electrostatic binding, have not been feasible due to the experimental difficulty of observing electronic transitions in the vacuum ultraviolet region of the spectrum. As a result spectroscopic information about the protein binding sites of these metal ions is scarce.

The rare earth metal ions form complexes which are primarily electrostatic in nature and are analogous to those formed by the calcium ion. In contrast to the calcium ion, however, the rare earth ions exhibit sharp absorption bands in the visible and ultraviolet region of the spectrum due to Laporte forbidden f-f transitions.[7,8] These absorption bands are sensitive to both the symmetry of the complex environment and the strength of the binding of the ligand. The number of peaks observed in the absorption spectrum increases as the environment about the lanthanide ion is lowered in symmetry. The magnitude of the extinction coefficients and frequently the width of the absorption band are related to the strength of the complex formed.

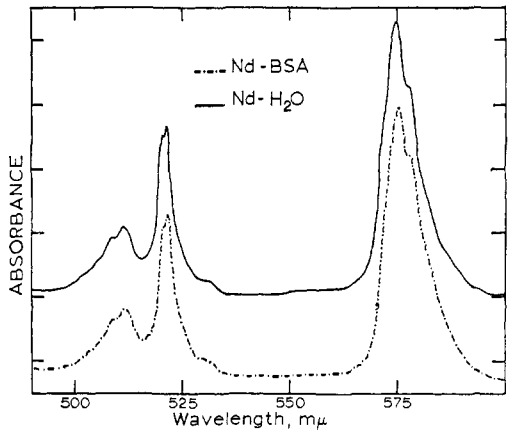

Figure 1. Absorption spectra of neodymium(III) ion (0.064 *M*) in water and in 2.11% bovine serum albumin solution (pH 5.6). These spectra were taken on a Cary 14 spectrophotometer using a 0–1.0 A slide wire. Each division corresponds to 0.1 absorbance unit.

Thus it is possible that rare earth metal ions may be used to probe calcium binding sites in proteins. This communication presents evidence that changes in the absorption spectrum of a rare earth metal ion upon binding to a protein can yield information about the protein ligands involved in complexation.

The protein which we have used in our initial experiments is bovine serum albumin (BSA). This protein was chosen because it is known to bind the calcium ion, presumably through electrostatic interactions with carboxyl groups of the protein.[9,10] The neodymium ion was the first lanthanide ion used since the ionic radii of calcium(II) and neodymium(III) are nearly identical (0.990 and 0.995 Å, respectively). Figure 1 shows the spectra obtained for the neodymium ion in water alone and in the presence of BSA at pH 5.6. These spectra are nearly identical.

The similarity of the absorption spectra of lanthanide complexes, regardless of the nature of the ligand involved, has long been a stumbling block preventing the use of these spectra in investigating complex systems. However, there are small changes in the 580- and 520-nm region of the absorption spectrum upon complexation and a difference spectrum (Figure 2) obtained using a 0–0.1 A slide wire presents a much more striking picture of these changes. The difference spectrum seems to be much more diagnostic of the type of complex formed than a simple perusal of the absorption spectrum. Because the changes seen in the 580-nm region of the spectrum are small, the sensitivity of observing complexation is limited. However, this is a region of the spectrum which is convenient for most biochemical studies.

All of the essential features of the BSA–neodymium difference spectrum have been reproduced with a variety of simple carboxylic acids. Acetate, propionate, and

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