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$$(n\text{-}C_8H_{17})_2CHCH_2CH_2OMgBr \xrightarrow[i]{CH_2O} (n\text{-}C_8H_{17})_2CHCH_2CH_2OCH_2OMgBr \xrightarrow[ii]{CH_2O} (n\text{-}C_8H_{17})_2CHCH_2CH_2OCHO + CH_3OMgBr$$

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(11) Owing to a typographical error, Table I of ref 2b contains an erroneous shift value for the Et-br carbon resonance. The correct value is 39.69 ppm vs. Me_4Si.
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Notes

Low-Frequency Raman Spectra as a Conformational Probe for Polypeptides and Proteins

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The low-frequency domain of the vibrational spectra of biologically important molecules contains a wealth of information but has remained little explored due to difficulties in obtaining accurate data and their interpretation. Peticolas[1] recently reviewed the very low-frequency vibrational modes of polypeptides and proteins. Earlier, Painter et al.[2] had reexamined the low-frequency Raman modes of proteins but were unable to find a correlation of a strong band at ~28 cm^{-1} and its satellite at ~36 cm^{-1} with the secondary structure. The origin of low-frequency vibrational modes seems to be complex in nature and therefore has remained obscure. Nevertheless, interesting observations have been reported by a number of workers[3-6] in the frequency range 200–600 cm^{-1} for poly(α-amino acids) which have served as model systems for studies of proteins. Normal coordinate analysis has shown that the torsional modes about the single bonds in the polypeptide backbone occur in the low-frequency range,[6] and therefore we have begun an investigation of these bands as potential conformational probes for polypeptides and proteins. In this note, we discuss the low-frequency Raman spectra of the polypeptide poly(γ-benzyl glutamate), which occurs in the α-helical conformation,[7-10] and the polymer (Pro-Pro-Gly)$_{10}$ and the protein collagen, both of which exist in a triple-helical conformation.[11-13] Another objective of the present study is to explore whether low-frequency Raman modes will help distinguish between these two classes of polypeptide structures, since their vibrational modes in the "fingerprint" region of the vibrational spectra often overlap, precluding a clear-cut distinction.

Materials and Methods

Poly(γ-benzyl glutamate), M_r = 28000, was purchased from Sigma Chemical Co., St. Louis, MO. (lot 92F-5046), and (Pro-Pro-Gly)$_{10}$ was obtained from the Protein Research Foundation, Osaka, Japan. Chick skin type I collagen was a gift from Dr. J. Gross, Massachusetts General Hospital, Boston, MA.

The low-frequency Raman spectra of poly(γ-benzyl glutamate) and (Pro-Pro-Gly)$_{10}$ were obtained with a Spex Ramalog 4 double monochromator as previously described[14] at M.I.T., Cambridge, MA. The 488-nm line of an argon ion laser (Coherent Radiation CR-3) was used for the excitation. The spectra were obtained with a spectral slit width of 6 and 8 cm^{-1} respectively for poly(γ-benzyl glutamate) and (Pro-Pro-Gly)$_{10}$ at a scan speed of 0.2 cm^{-1}/s, a gain of 1 × 100K, and an integration time of 2 × 1K. The frequencies were calibrated by using acetonitrile as a standard and are accurate to about ±2 cm^{-1}. The back-scattering configuration was used to obtain the spectra.

The low-frequency Raman spectra of chick skin type I collagen were obtained with the 457.9-nm line of an argon ion laser (Spectra-Physics Model 164) with output power typically about 300 mW. A Spex Ramalog Model 1401 spectrophotometer at the University of Georgia, Athens, GA, was used. The spectral slit width was generally 8 cm^{-1}. The points in the spectrum were taken every 2 or 3 cm^{-1} with counts averaged at that point for 3 s. The spectra displayed are an overlay (ensemble average) of four or five such scans. The displayed spectra have also been subjected to a standard three-point smoothening, either once or twice. The frequencies were calibrated by using acetic acid as a standard and are accurate to about ±2–3 cm^{-1}. The spectrophotometer is controlled by a Digital PDP-11/34 dedicated computer which is used to store, manipulate, and display the spectra.[15] The spectrum

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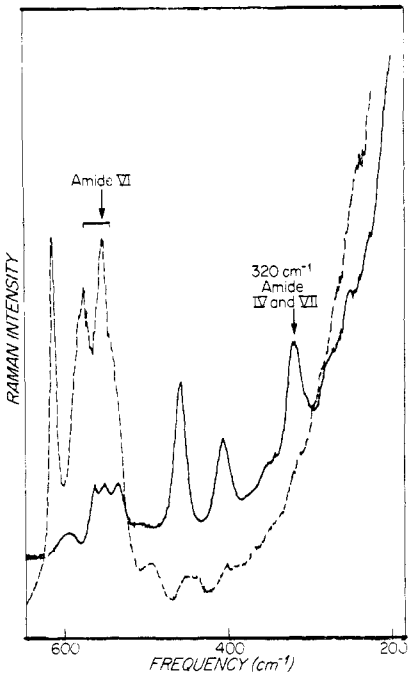

Figure 1. Laser Raman spectra of solid samples of poly(γ-benzyl glutamate) (---) and (Pro-Pro-Gly)$_{10}$ (—).

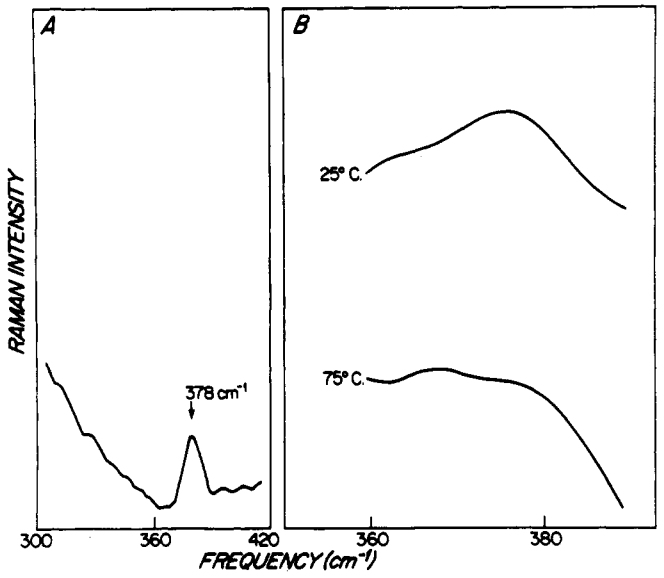

Figure 2. (A) Laser Raman spectrum of chick skin type I collagen in the 300–420-cm^{-1} range at 25 °C. (B) Laser Raman spectra of the same at 25 and 75 °C under experimental conditions identical with those in Figure 1A. The region from 360 to 390 cm^{-1} is shown.

of the solid collagen sample, shown in Figure 2A, was obtained with the sample outside of any container to verify the glass-free peak at 378 cm^{-1}. For the temperature study, the solid sample was placed in a standard melting point capillary that was sealed under low vacuum. The capillary was supported in a Harney-Miller cell, which was heated by passing N_2 first over a proportional heater onto the sample and then to a thermistor. The temperatures reported in Figure 2B are accurate to about 0.1 °C.

Results and Discussion

The low-frequency Raman spectra of poly(γ-benzyl glutamate) and (Pro-Pro-Gly)$_{10}$, free of glass background, are presented in Figure 1. The low-frequency Raman spectrum of solid chick skin type I collagen in the frequency range 300–420 cm^{-1} at 25 °C is presented in Figure 2A. Figure 2B shows the low-frequency Raman spectra of the same in the frequency range 360–390 cm^{-1} at 25 and 75 °C. Table I lists the frequency of the bands and their partial tentative assignments of the Raman spectra at 25 °C. The assignment of the bands for poly(γ-benzyl glutamate) are based on the earlier work of Miyazawa.[16] The bands observed in (Pro-Pro-Gly)$_{10}$ were assigned on the basis of Raman spectra of free proline,[17] glycylproline,[18] and poly(proline II)[17] and a recent Raman study of (Pro-Pro-Gly)$_{10}$ in aqueous solution.[13]

Table I
Frequencies (cm^{-1}) of Major Bands and Their Tentative Assignments in the Low-Frequency Raman Spectra of Poly(γ-benzyl glutamate) (PBG), (Pro-Pro-Gly)$_{10}$, and Collagen at Room Temperature

PBG	(Pro-Pro-Gly)$_{10}$	collagen	assigt
	320	378	amide IV–VII
	405		
	456		
	533		
	549		
554	562		amide VI
576			
611			

The Raman spectra of poly(γ-benzyl glutamate) and (Pro-Pro-Gly)$_{10}$ differ markedly. The spectrum of the α-helical polypeptide poly(γ-benzyl glutamate) does not manifest any major bands in the 300–550-cm^{-1} region but exhibits doublet peaks at 554 and 576 cm^{-1} with another intense band at 611 cm^{-1}. In contrast, (Pro-Pro-Gly)$_{10}$ exhibits intense bands at 320, 405, and 456 cm^{-1} and weak triplet peaks at 533, 549, and 562 cm^{-1}. At the level of the free amino acid proline, an intense band occurs at 448 cm^{-1} with two weaker bands at 369 and 573 cm^{-1}, whereas the dipeptide glycyl-proline exhibits three bands at 326, 393, and 447 cm^{-1} with an additional weak band at 403 cm^{-1}. The amide VI band occurs in the dipeptide as doublets at 541 and 559 cm^{-1}, while it is weak in free proline.[17]

The intense band at 320 cm^{-1} in (Pro-Pro-Gly)$_{10}$ (Figure 1 and Table I) corresponds to the 326-cm^{-1} band present in glycylproline[17] and is interestingly close to the 345-cm^{-1} band observed in the far-IR spectrum of rat tail tendon type I collagen reported by Gordon et al.[19] The above observations are further corroborated by the appearance of a band at 378 cm^{-1} in the low-frequency Raman spectrum of chick skin type I collagen at 25 °C (Figure 2A and Table I). Gordon et al.[19] attributed the 345-cm^{-1} band in the far-IR spectrum of collagen as being characteristic of its triple-helical structure based on the reduction in the intensity of this band on melting of collagen. In Figure 2B, the low-frequency Raman spectra of collagen at 25 and 75 °C are presented. It is interesting to observe that the 378-cm^{-1} band loses its intensity significantly at 75 °C when collagen exists in a "random" coil structure.[20] The results presented in Figure 2B provide convincing demonstration that the 378-cm^{-1} band in the Raman spectrum has its origin in the unique triple-helical structure of the polypeptide backbone in collagen.

Further support for the triple-helical origin of the 378- and 320-cm^{-1} bands in the Raman spectra of collagen and (Pro-Pro-Gly)$_{10}$ is derived from experimental and theoretical studies of polypeptide models of collagen, poly(glycine II), and poly(proline II). Poly(glycine II) exhibits an intense band at 365 cm^{-1} in its far-IR spectrum[21] which is close to the calculated E-species mode from phonon dispersion curves at 373 cm^{-1} by Small et al.[22] Singh and Gupta[23] have reported normal modes of vibration for poly(glycine II) and calculated a mode to occur at 367 cm^{-1} for $\delta = 0$ and at 340 cm^{-1} for $\delta = 2\pi/3$, which are in the range experimentally observed by Gordon et al.[19] Abe and Krimm[24] have also reported a Raman-active A-species

mode to occur at 345 cm^{-1}, which is in agreement with a Raman band at 335 cm^{-1} occurring in the Raman spectrum of poly(glycine II) and is reasonably close to the 378 cm^{-1} band observed in the present study of collagen. Miyazawa[16] has ascribed the band in the 300–400-cm^{-1} range to the internal torsional mode about the N–C^{α} bond, a mixed amide IV–VII mode with contributions from C–N–C^{α} bending and C=O stretching modes. The triple-helical "random coil" transition can a priori be expected to influence the secondary structure significantly and hence the Ramachandran angle ϕ[25] in particular and therefore it is not surprising that the amide IV–VII mode is temperature sensitive.

Conclusion

The low-frequency Raman bands of α-helical and triple-helical structures present in poly(γ-benzyl glutamate), $(Pro\text{-}Pro\text{-}Gly)_{10}$, and collagen are observed to be sensitive to their secondary structures. The 320-cm and 378-cm^{-1} bands occurring in $(Pro\text{-}Pro\text{-}Gly)_{10}$ and collagen are found to be unique to their triple-helical structures.

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Communications to the Editor

A New Polymeric Model for the Active Site in Artificial Photosynthesis

The primary photochemical step in photosynthesis is now generally recognized to be a one-electron transfer from the singlet excited state of a chlorophyll species to an electron acceptor. This reaction takes place within a reaction center protein that spans the thylakoid membrane of the chloroplast organelle of green leaves and algae. In the simplest photosynthetic system the electron acceptor contains the quinone moiety, such as an ubiquinone, menaquinone, or plastoquinone. An essential feature of this process is that the donation of an electron must lead to separation of the charged species $Chl^{+}\cdot$ and $Q^{-}\cdot$ so that they may undergo further reactive steps in the photosynthetic sequence. Recombination of this radical ion pair regenerates the original absorbing species with effective wastage of the photon energy as heat. It is believed that in natural photosynthesis, the thylakoid membrane plays an essential role in inducing charge separation and preventing the occurrence of this energy-wasting back-reaction.

Recent attempts to mimic the photosynthetic process have also used membranes in films, vesicles, and micelles with varying degrees of success. An alternative approach, due to Kong et al.[1], is to covalently link a porphyrin group P (similar to that in chlorophyll) to a quinone Q through a chain of methylene groups. By adjusting the length of the chain it is possible to obtain compounds soluble in organic solvents in which photoelectron transfer from P to Q can be observed and a reasonable number of charge-separated species identified by electron paramagnetic resonance (EPR) spectroscopy.[2]

We wish to report experiments which suggest an alternative approach to the synthesis of an effective reaction center for photosynthetic systems. It is now known that polyelectrolytes such as poly(acrylic acid) and poly(methacrylic acid), when copolymerized with relatively small amounts of hydrophobic monomers, will undergo "hypercoiling" when dissolved in aqueous media. That is to say the conformation of the polymer is neither that of a random coil nor that of an extended rod, but one in which the hydrophobic groups are much closer together than would be predicted from their relative concentrations using either of these two models. This was demonstrated by Holden et al.[3] and Guillet and Rendall,[4] who observed very high efficiences (~70%) of energy transfer from the naphthalene moieties to the anthracene in aqueous solutions in anthracene-terminated copolymers of acrylic acid and naphthyl methyl methacrylate (NMMA). Strong naphthalene excimer emission was also observed in polymers containing only a few mole percent NMMA, which was considered to be strong evidence for clustering of the naphthalene groups on the interior of the polymer coil.

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