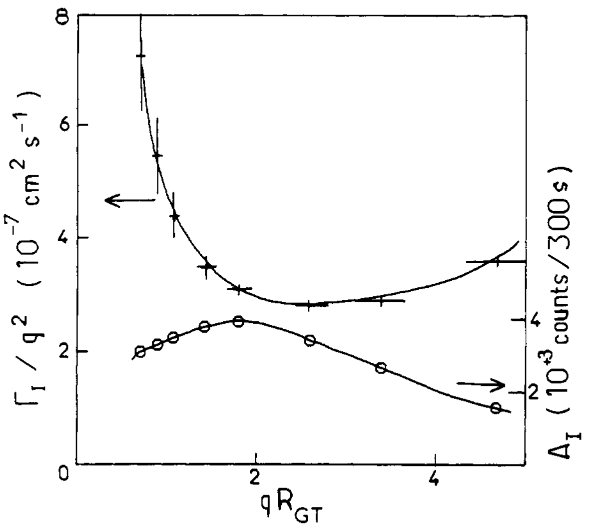

Figure 3. Experimental variation of the interdiffusion coefficient Γ_I/q^2 and of the observed scattering amplitude $A_I(q)$ as a function of qR_{GT} for the copolymer system.

where R_{GT} is the radius of gyration for a chain made of N monomers. If one writes the expansion of $\Gamma_{I,H}$ and $\Gamma_{I,C}$ introducing the translation diffusion coefficient $D_o = kT/N\zeta$, one obtains

$$\Gamma_{I,H} \simeq q^2 D_o\left[1 + q^2\frac{R_{GT}^2}{3}\right] \qquad (8)$$

$$\Gamma_{I,C} \simeq 6\frac{D_o}{R_{GT}^2}\left[1 + q^2\frac{3R_{GT}^2}{8}\right] \qquad (9)$$

From the intercept of the curves $\Gamma_{I,C}$ versus q^2 and $\Gamma_{I,H}$ versus q^2, one obtains for D_o 3.4 and 1.5 × 10^{-7} cm^2·s^{-1}, respectively. These values are in reasonable agreement since the exact molecular weight of the homopolymer and its polydispersity were not accurately determined. These equations predict also that the ratio of the slopes of the curves $\Gamma_{I,C}$ and $\Gamma_{I,H}$ as a function of q^2 should be equal to 9/4; experimentally, we found 1.5. This is qualitatively acceptable, and we think that a more precise analysis could explain these results.

Another problem which has to be solved is the position of the minimum of the curve $\Gamma_{I,C}/q^2$, which is obtained for $qR_{GT} = 2.5$ (see Figure 3). This minimum should coincide with the maximum of the scattering amplitude $A_I(q)$ at $qR_{GT} = 2$ (see Figure 3 and ref 8). At first sight, this difference cannot be explained by polydispersity and could be interpreted by taking into account the hydrodynamical interactions. This problem will be discussed in a forthcoming paper.

From these results, it seems that these experiments, made using the possibility offered by labeling a part of chain, show clearly the internal motion of a linear chain in a semidilute solution.

Acknowledgment. We express our gratitude to G. Jannink for his interest and his valuable comments on this work.

Registry No. (Deuteriostyrene)(styrene) (block copolymer), 108592-10-5; neutron, 12586-31-1.

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Bound Water in Collagen. Evidence from Fourier Transform Infrared and Fourier Transform Infrared Photoacoustic Spectroscopic Study

V. RENUGOPALAKRISHNAN,[*,†] G. CHANDRAKASAN,[‡,§] S. MOORE,[∥] T. B. HUTSON,[∥] C. V. BERNEY,[⊥] and RAJENDRA S. BHATNAGAR[‡]

Laboratory for the Study of Skeletal Disorders and Rehabilitation, Department of Orthopedic Surgery, Harvard Medical School and Children's Hospital, Boston, Massachusetts 02115, Laboratory of Connective Tissue Biochemistry, School of Dentistry, University of California, San Francisco, California 94143, National Center for Biomedical Infrared Spectroscopy, Battelle's Columbus Laboratories, Columbus, Ohio 43201, and Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.

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Introduction

The triple-helical conformation of collagen requires the presence of glycine in every third position in the amino acid sequence and consequently introduces steric constraints that restrict glycines to the inner core of the triple helix with side chains of the nonglycyl residues projecting from the surface of the triple helix.[1] The presence of intrachain hydrogen bonds stabilizes the triple helix and imposes additional constraints on the normal vibrations of the peptide moieties. Collagen participates in numerous highly specific biologically important interactions and therefore an understanding of the vibrational modes of collagen will pave the way for elucidating the nature of such interactions. Infrared spectroscopy has been extensively used to investigate the structure of collagen.[2-4] Synthetic polytripeptide models of collagen have been found to be useful systems for physicochemical investigations of collagen.[5] Infrared spectroscopy has been utilized to study polytripeptide models as films cast from aqueous solutions and from solutions in organic solvents.[6-9] In a recent study, FTIR spectroscopy was utilized to monitor intermolecular interactions in collagen self-assembly.[10] Despite numerous IR studies of collagen and collagen-like polypeptides,[6-10] the characteristic vibrations of the triple-helical structures remain poorly understood.

In the present study, FTIR photoacoustic spectroscopy is utilized to investigate collagen in the solid state. A resolution enhancement of the amide I band in collagen

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[*] To whom correspondence is to be addressed at the Laboratory for the Study of Skeletal Disorders, Children's Hospital, 300 Longwood Avenue, Enders 12, Boston, MA 02115.

[†] Harvard Medical School and Children's Hospital.

[‡] University of California.

[§] Senior Fulbright Scholar from Central Leather Research Institute, Council of Scientific and Industrial Research, Madras - 600 020, India.

[∥] Battelle's Columbus Laboratories.

[⊥] Massachusetts Institute of Technology.

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into components is presented here. Rockley et al.[11] demonstrated the agreement between FTIR photoacoustic spectra and conventional IR spectra of proteins. Recently, the application of FTIR photoacoustic spectroscopy to conformational analysis of polypeptides was discussed by Renugopalakrishnan and Bhatnagar.[12] It has been applied to the elucidation of the secondary structure of bone phosphoproteins[13] and a glycophosphoprotein, phosvitin.[14] Structural defects occurring in the application of pressure to compact the alkali halide–protein mixture into pellets[15-17] are avoided in FTIR photoacoustic spectroscopy. Alkali halide incorporation also causes hydration of the sample, and hence it is desirable to avoid such effects.

The present study is a part of a series of IR and Raman studies[18-20] of collagens and collagen-like polypeptides with state-of-the-art IR spectroscopy, which affords greater sensitivity in monitoring the triple helix and its interactions. A normal-coordinate analysis of the collagen triple helix has not been reported in the literature. Nevertheless, such an analysis has been performed for polyglycine,[21,22] which has served as a useful model for studying collagen. A complete assignment of the bands occurring in the FTIR photoacoustic spectra of the synthetic polytripeptides has to await the above important first step in the development of a theoretical analysis of vibrational spectra. The full potential of the wealth of information contained in the vibrational spectra of polypeptides and proteins is not presently realized since only a fraction of the vibrational spectra, principally the amide I–III frequencies, are utilized in deriving structural information.

Method

A sample of chick skin type I collagen was provided by Dr. J. Gross, Massachusetts General Hospital and Harvard Medical School, Boston, MA. The spectra were obtained from KBr pellets containing 0.5 mg of collagen. KBr pellets were made by pulverizing 1 mg of the sample with 199 mg of dried KBr. Approximately 100 mg of the mixture was placed in a die press to form the pellet. All spectra were obtained from 4000 to 500 cm^{-1} at a resolution of 4 cm^{-1}. A reference blank of pure KBr was used to create absorbance spectra. Any water vapor present was subtracted using the program VPSUB supplied by Digilab. The resolution enhancement of the amide I band in collagen was accomplished using the Kauppinin et al.[23] algorithm. The correct deconvolution parameter values, σ and K, were determined in the following manner: σ was varied until a slight negative dip appeared in the base line before and after the peaks. K was increased until the noise level in regions of the spectrum where no absorbance peaks occur was reduced to approximately that of the original spectrum. The optimal K and σ values are reported in the caption to Figure 2.

Calorimetric data were obtained on a differential scanning calorimeter (Perkin-Elmer Model DSC-4) in runs that spanned the region 30–140 °C. The basic principle of FTIR photoacoustic spectroscopy has been discussed by Krishnan.[24] FTIR photoacoustic spectra were recorded on a Digilab FTS 20E FTIR spectrometer at room temperature and represent the result of 512 scans. The photoacoustic interferogram, generated by the FTIR spectrometer by processing the photoacoustic signal produced when incident infrared light strikes the sample, is subjected to fast Fourier transform and then displayed as a spectrum. Milligram quantities of the synthetic polypeptide are placed in the sample compartment without the necessity of incorporation into an alkali halide matrix. The sample compartment is purged by dry N_2 gas. The spectra reported in this study were recorded at spectral resolution of 8 cm^{-1}, and measurement runs were approximately 8 min each.

Results and Discussion

Collagen. The frequencies of major bands in the FTIR spectrum of collagen are indicated in Figure 1. Similarly, the frequencies of major bands occurring in the FTIR PA spectrum of collagen are indicated in Figure 2. The spectra shown have been digitally subtracted from water spectrum. The frequencies of amide I and II bands observed in the FTIR spectrum of collagen (Figure 1) and in the FTIR PA (Figure 2) differ by only 1–2 cm^{-1}, and the FTIR PA spectrum of collagen (Figure 2) showed a shoulder at 1638 cm^{-1}, which was not observed in the FTIR spectrum of collagen (Figure 1). Bradbury et al.[25] observed the amide I band of collagen to consist of several components, the strongest having a frequency of 1660 cm^{-1}, which is remarkably close to the amide I frequency of 1659 cm^{-1} observed in this study. Fraser and McRae[3] reported the amide I band to be asymmetrical with a weaker band around 1635 cm^{-1}. We have analyzed the FTIR spectrum in the 1600–1700-cm^{-1} region using the resolution-enhancement method.[23] In the deconvoluted FTIR spectrum shown in Figure 3A (expanded view), the amide I band is observed to clearly split into a doublet with two components of frequencies 1659 and 1637 cm^{-1}, respectively.

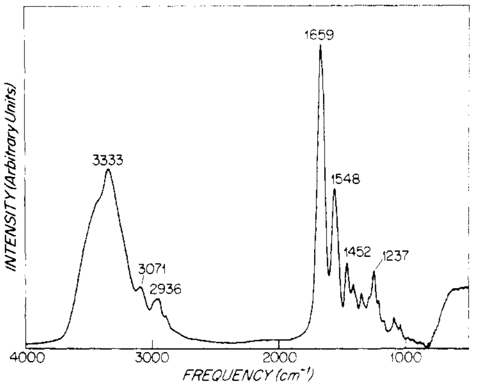

Figure 1. FTIR spectrum of chick skin type I collagen at room temperature.

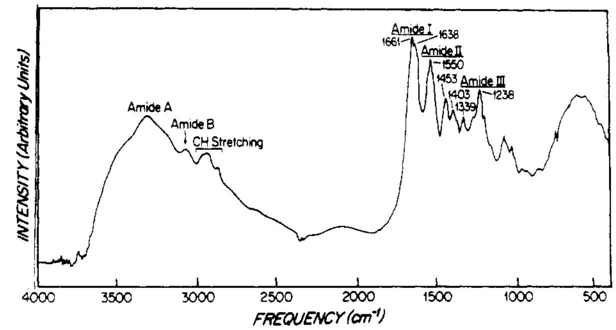

Figure 2. FTIR PA spectrum of chick skin type I collagen at room temperature.

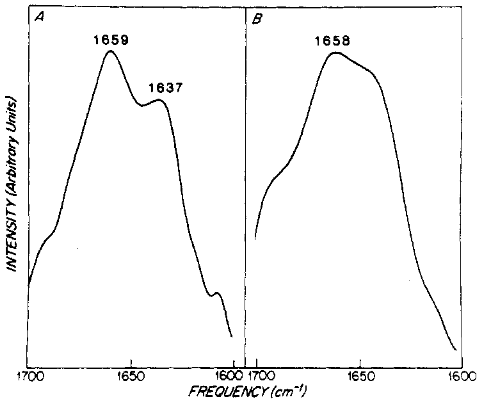

Figure 3. (A) Resolution-enhanced amide I region of the FTIR spectrum of chick type I collagen. The resolution-enhancement factor, K, and σ were 2.50 and 32 cm^{-1}, respectively, for the spectrum shown in Figure 2. (B) Resolution-enhanced amide I region of the FTIR spectrum of chick skin type I collagen at 75 °C.

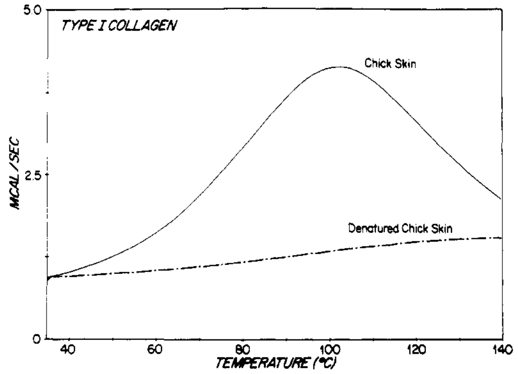

Figure 4. DSC curve for chick skin type I collagen.

Resolution enhancement of the amide I region was performed for a range of K and σ values, and the values corresponding to the expanded spectrum are shown in the caption to Figure 3A. The doublet structure observed is similar to the 1660- and 1663-cm^{-1} bands observed in the laser Raman spectrum of corneal collagen in water by Goheen et al.[26] In a recent FTIR study of collagen fibrillogenesis by Jakobsen et al.,[10] the formation of a shoulder at 1640 cm^{-1} was observed when fibrillogenesis was complete, and the above workers interpreted it as being indicative of conformational changes in the telopeptide region of collagen. It is interesting to speculate that the origin of the 1637-cm^{-1} band may well be in the bending mode of bound water in collagen. We have verified the presence of bound water by differential scanning calorimetry (DSC). The DSC curve for melting of chick skin type I collagen is shown in Figure 4. The data (Figure 4) show an endothermic event with an onset around 60 °C and a peak at 100 °C (the area under the curve implies an enthalpy change of around 45 cal/g). Heating to 140 °C reduced the sample weight from 7.53 to 6.66 mg, and subsequent runs on the same sample showed no further weight loss or endothermic events. We conclude that the loss of weight is due to the irreversible loss of bound water from the sample over the temperature interval 60–140 °C. The DSC curve of denatured collagen, also shown in Figure 4, is featureless. Resolution enhancement of the amide I band of collagen at 75 °C shown in Figure 3B clearly demonstrates the weakening of the intensity of the 1637 cm^{-1}, which is due to the gradual loss of bound water. Ramachandran and co-workers[27,28] proposed the involvement of water in the stabilization of the triple helix by forming interchain water bridges. Berendsen[29] has presented NMR evidence for the presence of bound water in collagen. The bending vibration of a free water molecule is usually observed around 1645 cm^{-1} (see ref 30 and 31), whereas it is low-frequency shifted in the case of collagen, indicating the presence of hydrogen bonds (see Pimentel and McClelland[32]). The proposal of hydrogen-bonded network in collagen has also been suggested by Lim,[33] which is in line with the speculation advanced here. Recently, Lazarev et al.[34] have discussed the amide I band in IR spectra of collagen and collagen-like polypeptides and presented a resolution of amide I band in collagen. The amide I band was observed to resolve into two components with frequencies of 1656 and 1637 cm^{-1}, respectively, which is in agreement with the results reported in the present study. Lazarev et al.[34] attributed the two components of the amide I band in collagen to imino and non-imino acid residues.

Conclusions

The presence of bound water, probably hydrogen bonded, in chick skin type I collagen has been shown from resolution enhancement of the amide I region of FTIR spectrum of collagen. However, as noted earlier, in the case of collagen,[20,35] the principle molecular vibrations of the polypeptide backbone are confined by stereochemical constraints to certain narrow regions in the spectra, viz., amide I, 1636–1661 cm^{-1}, amide II, 1549–1558 cm^{-1}, which differ from the characteristic vibrations for α-helical and β-sheet structures.

Note Added in Proof. Recently a FTIR study of lathrytic rat skin collagen with deconvolution of the amide I band has been reported by: Payne, K. J.; Veis, A. *Biopolymers* **1988**, *27*, 1749.

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Pulse Radiolysis Study on Electron-Transfer Reactions in Polymer Solutions at Low Temperatures

NORIYUKI KATO, TETSUO MIYAZAKI,* KENJI FUEKI, and TAKASHI YOKOI

Department of Synthetic Chemistry, Faculty of Engineering, Nagoya University, Chikusa-ku, Nagoya 464, Japan

TOSHIAKI KITANO

Research Center, Polyplastics Co. Ltd., Miyazima 973, Fuji, Shizuoka Pref. 416, Japan.

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Introduction

Intermolecular reactions of polymers with reactive chemical species, particularly polymer–polymer reactions, are of interest from the viewpoint of polymer science as well as chemical kinetics. A number of studies on diffusion-controlled intermolecular polymer–polymer reactions have been reported previously. These reactions of polymers are mainly concerned with free radicals and excited molecules.[1] The reactions of polymer anions have not been studied until recently. The formation of polymer anions has been investigated by the pulse radiolysis of the systems 2-methyltetrahydrofuran (MTHF)–poly(4-vinylbiphenyl) (PVB),[2,3] hexamethylphosphoramide–poly(methyl methacrylate),[4] and tetrahydrofuran–organopolysilane[5] systems.

Intermolecular polymer–polymer electron-transfer reactions, however, have not been reported as yet. It is generally difficult to separate clearly the electron-transfer reaction of anions from their neutralization reaction with cations. In the present work, we attempt to observe the electron-transfer reaction of polymer anions by pulse radiolysis at low temperature where the neutralization reaction of the anions are retarded remarkably.

Experimental Section

Poly(4-vinylbiphenyl) (PVB) was supplied by Aldrich Chemical Co., and poly(1-vinylpyrene) (PVPy) was synthesized by radical polymerization of 1-vinylpyrene, which was synthesized by Wittig's reaction from 1-pyrenecarboxyaldehyde.[6,7] The average molecular weights of PVB and PVPy were 1.35 × 10^5 obtained by viscosity measurement and 5 × 10^4 determined by gel permeation chromatography, respectively. The purification of the polymers was described in a previous paper.[2] Zone-refined biphenyl (Ph_2) and pyrene (Py) (Tokyo Kasei Chemical Co.) were used without further purification. A mixture of 2-methyltetrahydrofuran (MTHF)–tetrahydrofuran (THF) (1:1 in volume) was used as the solvent, since both PVB and PVPy are soluble in the mixture, which is transparent even at low temperature. The purification of the solvents was also described previously.[2] Pulse irradiations were made by generating electron beams with a Febetron 707 accelerator operated at an energy of 2 MeV, and the duration time of a pulse was 20 ns. The radiation dose delivered by one pulse is about 6 × 10^4 rad. The temperature of the sample can be controlled to a given temperature within ±0.1 K by use of a metal Dewar.[8]

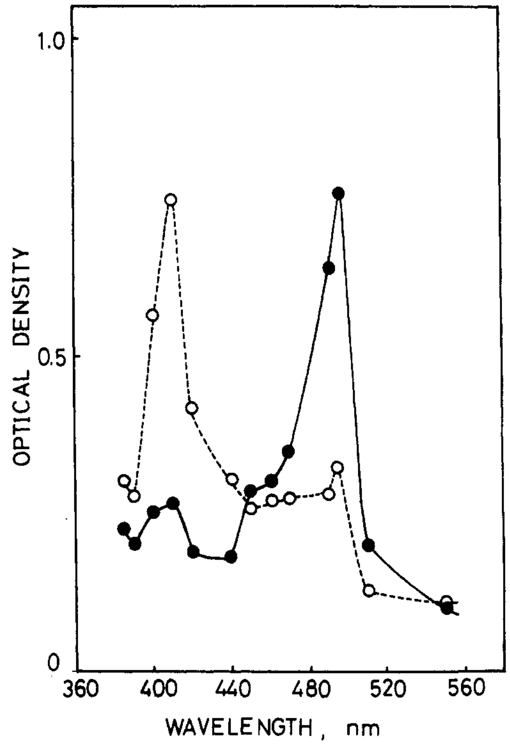

Figure 1. Optical absorption spectra for MTHF–THF–PVB (0.6 M)–PVPy (0.2 M) system at 140 K immediately after pulse irradiation (○) and 60 μs after pulse irradiation (●), respectively.

Results and Discussion

Figure 1 shows optical absorption spectra for the pulse-irradiated MTHF–THF mixtures containing two polymers, PVB and PVPy, as solutes at 140 K. The concentrations of PVB and PVPy are represented by biphenyl and pyrene monomer units (M; mol L^{-1}), respectively. A sharp absorption peak at 410 nm, denoted by open circles, is observed immediately after the pulse irradiation and decays almost to zero within 60 μs. Since the absorption peak at 410 nm is very similar to the absorption spectrum of biphenyl anions, which has a sharp absorption maximum at 408 nm,[9] the absorption peak at 410 nm is attributed to PVB anions (PVB^-),[2] formed by an electron-capture reaction of biphenyl molecules hanging to the polymer chain. A strong absorption band around 496 nm, denoted by filled circles, appears about 60 μs after the pulse irradiation. Since the absorption band around 496 nm coincides with the well-known absorption spectrum of Py anions at 492 nm,[10] the absorption band around 496 nm is ascribed to PVPy anions. Figure 2 shows that the absorption of PVPy anions at 496 nm increases complementarily with a decrease in that of PVB anions at 410 nm. The results demonstrate unambiguously that the electron-transfer reaction occurs from PVB anions to PVPy molecules. A similar electron-transfer reaction from biphenyl anions to PVPy molecules was also observed for the MTHF–THF–Ph_2–PVPy system. The mechanism of formation and decay of the anions in the radiolysis of MTHF–THF mixtures containing both PVB and PVPy is described by the following reaction scheme.

$$M \rightsquigarrow M^+ + e^- \qquad (1)$$

$$PVB + e^- \rightarrow PVB^- \qquad (2)$$

$$PVPy + e^- \rightarrow PVPy^- \qquad (3)$$

$$PVB^- + PVPy \rightarrow PVB + PVPy^- \qquad (4)$$

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* To whom correspondence should be addressed.

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