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$^{\alpha}$ bond, a mixed amide IV–VII mode with contributions from C–N–C$^{\alpha}$ 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-Pro-Gly)$_{10}$, and collagen are observed to be sensitive to their secondary structures. The 320-cm and 378-cm^{-1} bands occurring in (Pro-Pro-Gly)$_{10}$ and collagen are found to be unique to their triple-helical structures.

Acknowledgment. This research was partially supported by research grants from the National Science Foundation. V.R. expresses his thanks to Professor M. J. Glimcher for encouragement and support.

References and Notes

(1) Peticolas, W. L. *Methods Enzymol.* **1979**, *61*, 425.
(2) Painter, P. C.; Mosher, E. E.; Rhoads, C. *Biopolymers* **1982**, *21*, 1469.
(3) Miyazawa, T.; Fukishima, K.; Sugano, S.; Masuda, Y. In: "Conformation of Biopolymers"; Ramachandran, G. N., Ed.; Academic Press: London, 1967; Vol. II, p 557.
(4) Koyama, Y.; Shimanouchi, T. *Biopolymers* **1968**, *6*, 1037.
(5) Itoh, K.; Nakahara, T.; Shimanouchi, T.; Oya, M.; Uno, K.; Iwakura, Y. *Biopolymers* **1968**, *6*, 1759.
(6) Fanconi, B. *Biopolymers* **1973**, *12*, 2579.
(7) Elliot, A.; Fraser, R. D. B.; McRae, T. P. *J. Mol. Biol.* **1965**, *11*, 821.
(8) Masuda, Y.; Miyazawa, T. *Makromol. Chem.* **1967**, *103*, 261 and references cited therein.
(9) Fasman, G. D.; Itoh, K.; Liu, C. S.; Lord, R. C. *Biopolymers* **1978**, *17*, 1729 and references cited within.
(10) Renugopalakrishnan, V.; Bhatnagar, R. S. *J. Am. Chem. Soc.* **1984**, *106*, 2217.
(11) Bhatnagar, R. S.; Rapaka, R. S. In "Biochemistry of Collagen"; Ramachandran, G. N., Reddi, A. H., Eds.; Plenum Press: New York, 1976; p 479.
(12) Okuyama, K.; Okuyama, K.; Arnott, S.; Takayanagi, M.; Kakudo, M. *J. Mol. Biol.* **1981**, *152*, 427.
(13) Diem, M.; Bhatnagar, R. S.; Druyan, M. E.; Renugopalakrishnan, V. *Biopolymers* **1984**, *23*, 2955.
(14) Renugopalakrishnan, V.; Kloumann, P. H. B.; Bhatnagar, R. S. *Biopolymers* **1984**, *23*, 627.
(15) Towns, T. G.; Carreira, L. A.; Irwin, R. M. *J. Raman Spectrosc.* **1981**, *11*, 487.
(16) Miyazawa, T. In: "Poly-Amino Acids"; Fasman, G. D., Ed.; Marcel Dekker: New York, 1967, p 69.
(17) Smith, M.; Walton, A. G.; Koenig, J. L. *Biopolymers* **1969**, *8*, 173.
(18) Lagant, P.; Loucheux-LeFebvre, M. H.; Huvenne, J. P.; Vergoten, G.; Fleury, G.; Legrand, P. *Biopolymers* **1983**, *22*, 1285.
(19) Gordon, P. L.; Huang, C.; Lord, R. C.; Yannas, I. V. *Macromolecules* **1974**, *7*, 954.
(20) Privalov, P. L.; Tiktopulo, E. I.; Tischenkov, V. M. *J. Mol. Biol.* **1979**, *127*, 203.
(21) Suzuki, S.; Iwashita, Y.; Shimanouchi, T. *Biopolymers* **1966**, *4*, 337.
(22) Small, E. W.; Fanconi, B.; Peticolas, W. L. *J. Chem. Phys.* **1970**, *52*, 4369.
(23) Singh, R. D.; Gupta, V. D. *Spectrochim. Acta, Part A* **1971**, *27A*, 385.
(24) Abe, Y.; Krimm, S. *Biopolymers* **1972**, *11*, 1841.
(25) Ramachandran, G. N.; Sasisekharan, V. *Adv. Protein Chem.* **1968**, *23*, 283.

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.

0024-9297/85/2218-1788$01.50/0

Table I
Properties of Acrylic Acid Copolymers

polymer	$\bar{M}_v$[a]	$\overline{DP}$	mol % P[b]	η_P	mol % Q[c]	η_Q
A	17.5×10^4	2430	0.11	2.7		0
B	5.5×10^4	764	0.14	1.0	0.40	3.0

[a] From intrinsic viscosity in dioxane using $K = 76 \times 10^{-5}$ and $a = 0.50$ (see: Sandler, S. R.; Karo, W. "Polymer Syntheses"; Academic Press: New York, 1977; Vol. II, Chapter 9. [b] From UV absorbance in methanol solution at 412 nm based on ϵ for model compound tetratolylporphine = 4.2×10^5. [c] From UV absorbance in MeOH at 255 nm based on ϵ for model compound 2-methylanthraquinone = 5.0×10^4.

Later, Herkstroeter et al.[5] confirmed the clustering of pyrene groups covalently attached to a polyelectrolyte by observations of excimer emission at high dilution in water solution.

In our proposed model reaction center we link one or more prophyrin groups to several quinones by copolymerization of small amounts of appropriate monomers with poly(acrylic acid). The resulting copolymer contains the P and Q groups at distances well spaced along the backbone of the polymer chain. In a strictly random coil, little interaction would be expected. However, in aqueous solution, the fluorescence of porphyrin is quenched due to the proximity of P and Q in the hypercoiled configuration. A particularly useful feature of this system is that the average distance between P and Q can be adjusted by changes in the pH or ionic strength of the solution, and as the experimental results demonstrate, the polyelectrolyte appears to assist in the generation and stabilization of charge-separated species.

Two polymers were used in these experiments: (A) a copolymer of acrylic acid (500 mg) and 10 mg of 5-(10,15,20-tritolylporphinyl)phenyl methacrylate (POMA, 0.2 mol %) and (B) a copolymer of acrylic acid (200 mg), 6.0 mg (0.3 mol %) of POMA, and 3.0 mg (0.5 mol %) of 2-isopropenylanthraquinone (IPAQ). The monomers were dissolved in dioxane, polymerized in a sealed ampule at 60 °C for 24 h using 1% 2,2′-azobis(isobutyronitrile) (AIBN) as catalyst, and isolated by threefold reprecipitation with hexane. Molecular weights were determined by viscometry in dioxane. The final composition of the porphyrin and anthraquinone (AQ) in the polymer was estimated by UV spectroscopy in methanol solution. Analytic details are summarized in Table I.

Polymer A contains about three porphyrin groups per chain, while polymer B contains one porphyrin and three AQ groups, on average. Since these polymers were prepared in solution at low conversion they are expected to have a normal distribution of molecular weights (i.e., $M_w/M_n = 2.0$). Thus, some of the low molecular weight components of polymer B may well contain no porphyrin groups. The effects observed will therefore tend to underestimate the true magnitude of the P–Q interactions.

Figure 1 shows the UV–visible spectra of the two polymers in methanol solution. The major differences are the peaks at 250 and 330 nm in polymer B attributable to the AQ moiety. There is also some broadening of the porphyrin absorptions at 500–650 nm, which might be due to intramolecular ground-state interactions with the AQ groups in the polymer solution. In aqueous solution, both polymers give bright green solutions at low pH and purple solutions above pH 8.

Electron paramagnetic resonance (EPR) measurements were conducted on a Varian E-12 EPR spectrometer equipped with a Varian E-257 temperature controller and interfaced with a Nicolet 1180 computer system for the acquisition of steady-state spectra. For EPR measurements at X-band (~9 GHz), solutions were transferred to 4-mm-o.d. quartz tubes, which were introduced into the Dewar of the temperature controller in the EPR cavity. The concentration of each solution was about 10 mg of polymer per millliter of water, which is equivalent to ~2 $\times 10^{-4}$ mol L^{-1} in chromophore concentration. The pH of each solution was adjusted, when necessary, by addition of sodium hydroxide to the system and measured by a pH meter. The solvent used was doubly distilled water, which formed a semitransparent solid matrix when cooled to −40 °C. No phase separation or precipitation of the polymer was observed under these conditions.

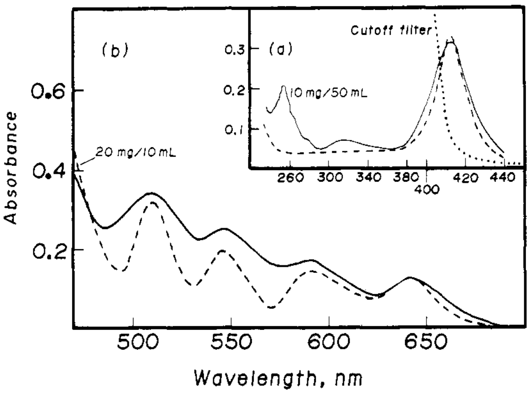

Figure 1. UV–visible absorbance of (– – –) poly(AA-POMA) and (—) poly(AA-POMA-AQ) with cutoff filter (methanol solution): (a) 220–450 nm; (b) visible absorbance.

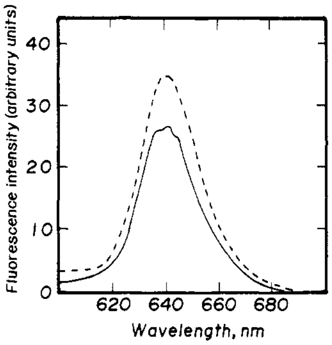

Figure 2. Quenching of porphyrin emission by bound AQ (pH 9): (– – –) poly(AA-POMA); (—) poly(AA-POMA-AQ).

The light source for broad-band visible light irradiation was a 150-W tungsten–halogen lamp mounted in a Cole-Parmer Model 9741-50 housing which was equipped with a focusing light pipe to channel the light beam to the EPR cavity. A Corning CS-373 filter was used ($\lambda > 400$ nm), the cutoff pattern of which is shown in Figure 1, along with the UV–vis spectra of our polymers. With this filter, light is exclusively absorbed by the porphyrin moiety in the polymer. The *g* factor of the light-induced EPR spectra was determined by measuring the magnetic-field displacements of the observed spectra from the middle two lines of the six-line EPR spectrum of solid SrO (containing Mn^{2+} as an impurity), present in the EPR cavity at the same time with the polymer samples.[6]

Evidence for the quenching of the singlet excited state of the porphyrin by the polymer-bound AQ unit was observed by fluorescence measurements of the polymers in aqueous solution. Polymer concentrations were about 8 $\times 10^{-7}$ mol L^{-1} in P concentrations. Figure 2 shows that about 20% of the porphyrin emission from polymer B at 640 nm is quenched at pH 9. At pH 4 the quenching is nearly 40%. However, although quenching occurs, it does not necessarily result in the generation of stabilized charge-separated ion radicals. Evidence for this important

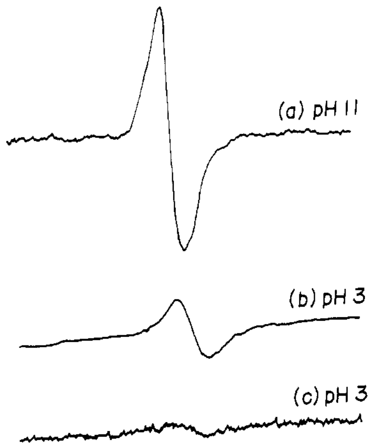

Figure 3. (a,b) EPR signal of poly(AA–POMA–AQ); (c) EPR signal of poly(AA–POMA). EPR operating conditions were 0.5-mT modulation amplitude at 100 kHz and 5-mW microwave power at 9.05 Hz; the temperature was 235 K. The spectral range displayed is 10 mT.

step is usually obtained by EPR measurements on irradiated systems, and polymer B does indeed show a strong photoinduced EPR signal in water solution. The strongest and most characteristic signal was obtained at pH 11 (Figure 3a). The asymmetry of the resonance has been observed previously in mixtures of porphyrin and quinone at low temperatures and in some cases for PQ molecules and can be attributed to the overlap of signals from $P^{+}\cdot$ and $Q^{-}\cdot$. A much less intense signal was observed in the same location from sample A, which contains no quinone moiety, and was attributed to a minor amount of electron transfer to impurities. No signal was observed for either polymer in the dark. The observed signal intensity increased with time of irradiation and decays slowly (over 5–10 min) after the light is turned off. Multiple irradiations gave similar rates of buildup and decay, with no apparent loss of intensity or irreversible effects. On the basis of similar experiments with other P–Q molecules, the quantum yield of stable radicals is estimated at 3%.

The g factor of the strong photodriven signal in sample B at pH 11 was 2.0037 ± 0.0002, in excellent agreement with the assumption that it is a spin-exchange average of $P^{+}\cdot$ ($g = 2.0025$) and $AQ^{-}\cdot$ ($g = 2.0047$). The yield of $P^{+}\cdot,Q^{-}\cdot$ radical pairs was ~3% based on a double integration of the EPR signal in Figure 3a. The g factor of the photodriven signal at pH 3 in Figure 3b is close to that of $P^{+}\cdot$ alone ($g = 2.0025$).

There is evidence from the visible absorption spectra of some ground-state interactions between P and AQ in methanol solution. This suggests the presence of a weak interaction between intramolecularly attached porphyrin and quinone at both high and low pH.

It can be concluded from the examination of the photoinduced signal and the g-factor measurements that photoinduced charge separation to form $P^{+}\cdot$ and $AQ^{-}\cdot$ is taking place following irradiation of polymer B in alkaline solution. This is surprising in view of the ground-state interactions between intramolecularly attached porphyrin and quinone in the polymer (detected by UV–vis spectra). These interactions normally tend to accelerate not only the forward but also the back-electron-transfer reaction. One model to account for the effective charge stabilization in the polymer system is an ionic domain effect provided by the carboxyl anions surrounding the porphyrin moiety. Thus, weak interactions (presumably hydrophobic and/or van der Waals) are nullified by the repulsive Coulombic interaction between an anionic radical of AQ ($AQ^{-}\cdot$) and the COO^{-} groups surrounding the porphyrin moiety. Similar Coulombic field applications to stabilize the charge-transfered species generated by the photoinduced electron transfer have been reported for the Ru(bpy)$_3^{2+}$–viologen combination.

At pH 3, although a clear photoinduced EPR signal was observed (Figure 3b), its g factor was very close to that of $P^{+}\cdot$ itself. This result probably implies that the $AQ^{-}\cdot$ undergoes secondary reactions at pH 3, leaving the $P^{+}\cdot$ behind. If either $AQ^{-}\cdot$ or $AQH\cdot$ were present in the same concentration as $P^{+}\cdot$, the observed g factor should have been significantly larger since both neutral and anionic semiquinones have g factors of ~2.0047.[8] One possibility is that the $AQ^{-}\cdot$ is protonated to $AQH\cdot$, which is unstable and possibly disproportionates. This process might also assist in preventing the back-electron-transfer step. In any case, the AQ is certainly important in promoting the photogeneration of an EPR signal at pH 3 since the control with no quinone (Figure 3c) showed little photoinduced EPR signal.

From these results we conclude that the inclusion of porphyrin and quinone moieties in polyelectrolyte molecules provides a novel model to study photoelectron-transfer processes involved in artificial photosynthesis. The use of such polymers not only permits experiments to be done in aqueous rather than organic media but also gives some stabilization of the resultant charge-separated ion pairs. Furthermore, variations in the efficiency of electron transfer and other important parameters in the process can be readily made by changing the pH or ionic strength of the medium.

Acknowledgment. We acknowledge the financial support of this research by the Natural Sciences and Engineering Research Council of Canada. Y.T. is grateful to the Oji Paper Co. Ltd. for financial support during his studies at the University of Toronto.

References and Notes

(1) (a) Kong, J. L. Y.; Loach, P. A. In "Frontiers of Biological Energetics—Electrons to Tissues"; Dutton, P. L., Leigh, J. S., Scarpa, A., Eds.; Academic Press: New York, 1978; Vol. 1, pp 73–82. (b) Kong, J. L. Y.; Loach, P. A. *J. Heterocycl. Chem.* **1980**, *17*, 737–744. (c) Kong, J. L. Y.; Spears, K. G.; Loach, P. A. *Photochem. Photobiol.* **1982**, *35*, 545–553.

(2) (a) McIntosh, A. R.; Siemiarczuk, A.; Bolton, J. R.; Stillman, M. J.; Ho, T.-F.; Weedon, A. C. *J. Am. Chem. Soc.* **1983**, *105*, 7215–7223. (b) Ho, T.-F.; McIntosh, A. R.; Bolton, J. R. *Nature (London)* **1980**, *286*, 254–256.

(3) Holden, D. A.; Rendall, W. A.; Guillet, J. E. *Ann. N.Y. Acad. Sci.* **1981**, *366*, 11–23.

(4) Guillet, J. E.; Rendall, W. A. *Macromolecules*, in press.

(5) Herkstroeter, W. G.; Martic, P. A.; Hartman, S. E.; Williams, J. L. R.; Farid, S. *J. Polym. Sci., Polym. Chem. Ed.* **1983**, *21*, 2473–2490.

(6) Wertz, J. E.; Bolton, J. R. "Electron Spin Resonance—Elementary Theory and Practical Applications"; McGraw-Hill: New York, 1972; Appendix D.

(7) Kaneko, M.; Yamada, A. *Adv. Polym. Sci.* **1984**, *55*, 1–47.

(8) Hales, B. J.; Case, E. E. *Biochem. Biophys. Acta* **1981**, *637*, 291–302.

(9) Permanent address: Oji Paper Co. Ltd., Research and Development Laboratory, 10-6 Shinonome 1-chome, Koto-ku, Japan.

J. E. Guillet* and Y. Takahashi[9]

Department of Chemistry, University of Toronto
Toronto, Ontario, Canada M5S 1A1

A. R. McIntosh and J. R. Bolton

Department of Chemistry
The University of Western Ontario
London, Ontario, Canada N6A 5B7

Received March 11, 1985