Carotenoids in Sol−Gels: Incorporation, Stability, and Sensitivity to Oxidant and Acid

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It is shown in this paper that carotenoids can be incorporated into sol−gels and can be evenly distributed by using appropriate amounts of organic solvents (e.g., EtOH and acetone). Some carotenoids (e.g., 8′-apo-β-caroten-8′-al) are relatively stable in sol−gels. The studied sol−gel matrix has no obvious effect on the stability of carotenoids. Carotenoids in sol−gels can still react with an oxidant and acid (FeCl$_3$ and H$_2$SO$_4$, respectively, in our experiments) that are present in the contacting aqueous solutions. This observation suggests that one may take advantage of the visible absorption of carotenoids and the solid and transparent character of sol−gels to build sensors for oxidants and acids using the carotenoid-containing sol−gels.

Introduction

Carotenoids play important photoprotection and light-harvesting roles in photosynthetic organisms.[1−3] They serve as photoprotection agents by quenching (bacterio)-chlorophyll [(B)chl] triplet states to prevent their reaction with molecular oxygen (which results in the formation of the damaging singlet oxygen) or directly reacting with singlet oxygen to detoxify it. Because carotenoids absorb light energy in the visible region of the spectrum, where (B)chls are not efficient absorbers, they serve as light-harvesting antennae. It has also been suggested that carotenoids can act as anticancer agents, based on the fact that carotenoids can function as antioxidants and free radical scavengers.[4−6]

The behavior of a variety of carotenoids in organic solvents has been extensively studied by electrochemical, optical absorption spectroscopic, electron paramagnetic resonance (EPR) and electron nuclear double resonance (ENDOR) methods.[7−12] These studies show that carotenoids can easily lose an electron by electrochemical or chemical oxidation (e.g., by FeCl$_3$). The resulting radical cations of some carotenoids are relatively stable in organic solvents (e.g., β-carotene and canthaxanthin in CH$_2$Cl$_2$), whereas in natural systems, radical cations of carotenoids are usually short-lived.

The electrode and homogeneous reactions that take place in electrochemical processes of carotenoids in organic solvents have been determined[7,8,13−16] and are shown in Scheme 1. Carotenoids can also react with acids in organic solvents to form carbocations.[17]

Sol−gels are prepared by hydrolysis of metal alkoxides [e.g., tetramethyl orthosilicate, Si(OCH$_3$)$_4$], followed by condensation to form a porous network. This porous network can be used as a matrix to incorporate biomolecules, such as proteins.[18] The incorporation of biomolecules into sol−gels has received considerable attention, because the doped sol−gels have potential applications in sensors, biosensors, biocatalysts, optical materials, electrodes, immunoadsorbents, and environment-related materials.[19] For example, glucose oxidase has been incorporated into sol−gels to detect glucose.[20,21] Yeast alcohol dehydrogenase incorporated into sol−gels has been employed as a sensor for alcohols and aldehydes.[22] Recently, it was shown that organically modified sol−gels can selectively take in and release proteins (ferricytochrome *c*, myoglobin, and hemoglobin), which is important in recognition, sensing, and separation.[23]

In this paper, we demonstrate that carotenoids can also be incorporated into and evenly distributed in sol−gels. The solid and transparent character of the matrix (sol−gel) may extend the application of carotenoids to
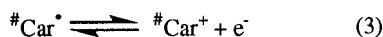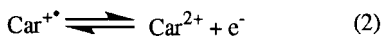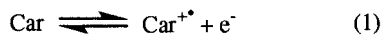
* To whom correspondence should be addressed.
(1) Goedheer, J. C. *Annu. Rev. Plant Physiol.* **1972**, *23*, 87.
(2) Koyama, Y. *J. Photochem. Photobiol.* **1991**, *139*, 265.
(3) Frank, H. A.; Cogdell, R. J. *Photochem. Photobiol.* **1996**, *63*, 257.
(4) Burton, G. W.; Ingold, K. U. *Science* **1984**, *224*, 569.
(5) Krinsky, N. I. *Clin. Nutr.* **1988**, *7*, 107.
(6) Ziegler, R. G. *Am. J. Clin. Nutr.* **1991**, *53*, 2515.
(7) Grant, J. L.; Kramer, V. J.; Ding, R.; Kispert, L. D. *J. Am. Chem. Soc.* **1988**, *110*, 2151.
(8) Jeevarajan, J. A.; Kispert, L. D. *J. Electroanal. Chem.* **1996**, *411*, 57.
(9) Piekara-Sady, L.; Jeevarajan, A. S.; Kispert, L. D. *Chem. Phys. Lett.* **1993**, *207*, 173.
(10) Wei, C. C.; Gao, G.; Kispert, L. D. *J. Chem. Soc., Perkin Trans. 2* **1997**, 783.
(11) Gao, G.; Deng, Y.; Kispert, L. D. *J. Phys. Chem.* **1997**, *101*, 7844.
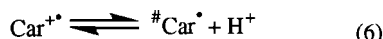(12) Khaled, M.; Hadjipetrou, A.; Kispert, L. D. *J. Phys. Chem.* **1990**, *94*, 5164.

(13) Mairanovsky, V. G.; Engovatov, A. A.; Ioffe, N. T.; Samokhvalov, G. I. *J. Electroanal. Chem.* **1975**, *66*, 123.
(14) Khaled, M.; Hadjipetrou, A.; Kispert, L. D.; Allendoerfer, R. D. *J. Phys. Chem.* **1991**, *95*, 2438.
(15) Jeevarajan, A. S.; Khaled, M.; Kispert, L. D. *J. Phys. Chem.* **1994**, *98*, 7777.
(16) Jeevarajan, A. S.; Khaled, M.; Kispert, L. D. *Chem. Phys. Lett.* **1994**, *225*, 340.
(17) Konovalov, V. V.; Kispert, L. D. *J. Chem. Soc., Perkin Trans. 2* **1999**, 901.
(18) Ellerby, L. M.; Nishida, C. R.; Nishida, F.; Yamanaka, S. A.; Dunn, B.; Valentine, J. S.; Zink, J. I. *Science* **1992**, *255*, 1113.
(19) Avnir, D.; Braun, S.; Lev, O.; Ottolenghi, M. *Chem. Mater.* **1994**, *6*, 1605.
(20) Tatsu, Y.; Yamashita, K.; Yamaguchi, M.; Yamamura, S.; Yamamoto, H.; Yoshikawa, S. *Chem. Lett.* **1992**, 1615.
(21) Glezer, V.; Lev, O. *J. Am. Chem. Soc.* **1993**, *115*, 2533.
(22) Williams, A. K.; Hupp, J. T. *J. Am. Chem. Soc.* **1998**, *120*, 4366.
(23) Rao, M. S.; Dave, B. C. *J. Am. Chem. Soc.* **1998**, *120*, 13270.

Scheme 1[a]

electrode reactions:

$$Car \rightleftharpoons Car^{+\bullet} + e^- \qquad (1)$$

$$Car^{+\bullet} \rightleftharpoons Car^{2+} + e^- \qquad (2)$$

$$^\#Car^\bullet \rightleftharpoons {}^\#Car^+ + e^- \qquad (3)$$

homogeneous reactions:

$$Car^{2+} + Car \rightleftharpoons 2Car^{+\bullet} \qquad (4)$$

$$Car^{2+} \rightleftharpoons {}^\#Car^+ + H^+ \qquad (5)$$

$$Car^{+\bullet} \rightleftharpoons {}^\#Car^\bullet + H^+ \qquad (6)$$

[a] # indicates that Car represents the carotenoid with one less proton.

sensors, optical materials, etc. Our study shows that carotenoids in sol–gels can sense oxidant and acid in contacting aqueous solutions. To our knowledge, the work reported here is the first attempt to study carotenoids in an artificial solid matrix. Following are the structures of the studied carotenoids.

8'-Apo-β–caroten-8'-al (I)

Canthaxanthin (II)

β-Carotene (III)

7'-Apo-7',7'-dicyano-β-carotene (IV)
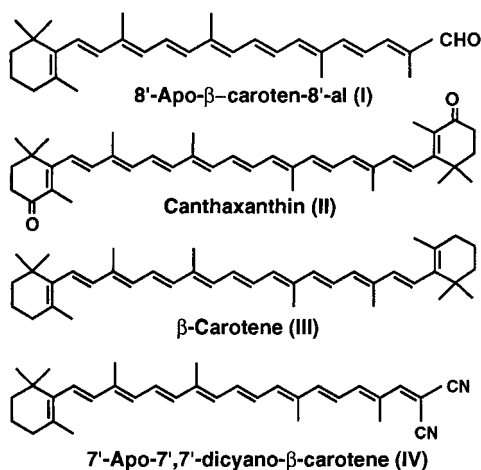
Experimental Section

Chemicals. 8'-Apo-β-caroten-8'-al (**I**) was obtained from Roche Vitamins and Fine Chemicals, Nutley, NJ; canthaxanthin (**II**) and β-carotene (**III**) were from Fluka. 7'-Apo-7',7'-dicyano-β-carotene (**IV**) was synthesized as previously described.[24] All carotenoids were all-trans and were kept at −16 °C, stored over Drierite, and wrapped with Parafilm and foil to avoid exposure to moisture and light. Just prior to use, they were allowed to warm to room temperature. Ethyl alcohol (absolute, >99.5%) and tetramethyl orthosilicate (>99%, TMOS) were from Aldrich. FeCl$_3$ was obtained from Sigma. H$_2$SO$_4$ was from Fisher. Deionized water (resistance >18 MΩ) was used in the preparation of sol–gels.

Preparation of Carotenoid-Containing Sol–Gels. TMOS (1.0 mL) was added into a 1.5 mL carotenoid/EtOH solution in a weighing bottle, followed by adding 1.0 mL of H$_2$O, stirring the solution with a stirring bar for 2 h, and then pouring the solution into 4-sided clear disposable polystyrene cells with 1 cm optical path length. The cells were sealed and kept at room temperature. Aluminum foil was used to avoid exposure to light. Optically transparent carotenoid-containing sol–gels were formed after a period of time (ca. 1 day).

Because some organic solvents (e.g., acetone) react with polystyrene, EtOH is used, although some carotenoids have

(24) Hand, E. S.; Belmore, K. A.; Kispert, L. D. *J. Chem. Soc., Perkin Trans. 2* **1993**, 659.
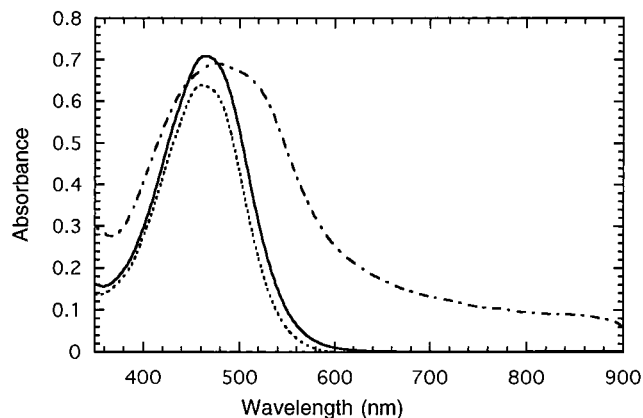
Figure 1. Optical absorption spectra of **I** in sol–gel without solvent evaporation (solid line), in sol–gel with partial solvent evaporation (dotted-dashed line), and in EtOH (dotted line).

poor solubility in EtOH and good solubility in other organic solvents. Because TMOS can also slowly react with polystyrene, hydrolysis of TMOS was carried out in a glass weighing bottle, rather than directly in the polystyrene cells.

The preparation of sol–gels without carotenoids followed the same procedure, except that 1.5 mL of EtOH instead of 1.5 mL of the carotenoid/EtOH solution was used.

Optical Absorption Spectroscopy and Photodegradation Experiments. Optical absorption spectra were recorded using a double-beam Shimadzu Model 1601 UVPC spectrophotometer. In the spectral measurements of carotenoids in sol–gels, a sol–gel without carotenoids served as the reference. Photodegradation experiments were carried out by monitoring the change of the optical absorption of irradiated samples at room temperature. The irradiation source was a 250 W xenon lamp from ILC Technology Company. Deionized water (10 cm path) was used as a filter to cut off infrared light, and a 340 nm (low-pass) optical filter cut off most of the UV light. The power reaching the samples was measured with a Newport Model 818-SL power meter. After irradiation, the optical absorption spectrum was recorded immediately.

AM1 Calculations. AM1 (Austin Model 1) semiempirical molecular orbital calculations[25] were carried out using Hyper-Chem software on a Gateway (Pentium III) computer.

Results and Discussion

1. Incorporating Carotenoids into Sol–Gels. The presence of an organic solvent is necessary to incorporate carotenoids into sol–gels without aggregation. Although carotenoids can be dissolved in TMOS, directly dissolving carotenoids in TMOS and then hydrolyzing TMOS will result in sol–gels with aggregates of carotenoids. The presence of an organic solvent can prevent the aggregation. In our preparation, EtOH was used. The ratio of the organic solvent, H$_2$O, and TMOS is also important to the even distribution of carotenoids in sol–gels. At the initial ratio of EtOH:H$_2$O:TMOS employed in our preparation (1.5:1:1 by volume), the final ratio of the alcohol (including EtOH and MeOH, which is generated in the hydrolysis) to H$_2$O is close to 3:1. At this ratio, carotenoids are evenly distributed in sol–gels. Figure 1 (solid line) shows the optical absorption spectrum of **I** in our sol–gels. When the initial ratio of EtOH:H$_2$O:TMOS equals 1:1:1, the carotenoids in sol–gels were unevenly distributed, as could be seen by the variations in color. In our preparation, carotenoids are actually dissolved in the mixture of H$_2$O and the alcohol,

(25) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.
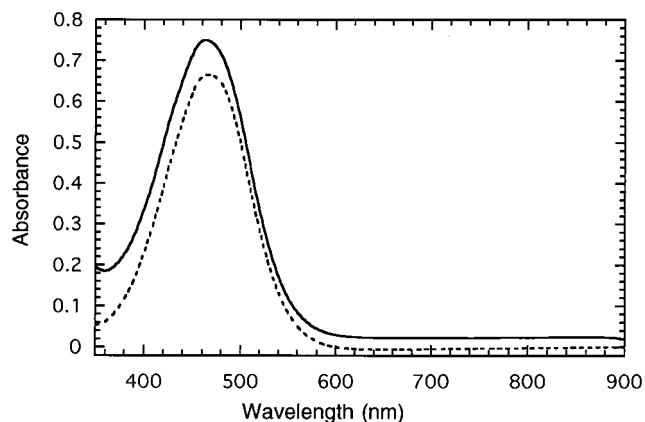
Figure 2. Optical absorption spectra of **I** (5.9 μM) in sol−gel just after preparation (dotted line) and 30 days after preparation (solid line).

Figure 3. Photodegradation of **I** (initial concentration 5.5 μM) in sol−gel. The sample was irradiated for 0, 5, 10, 15, or 20 min (from top to bottom). Irradiation power: 0.25 W cm^{-2}.

which is located in the pores of sol−gels. The polystyrene cells have to be sealed well to prevent the evaporation of solvents from sol−gels. If sol−gels are open to air, the evaporation of solvents causes shrinking of the sol−gels, and broadened and weakened absorption bands of carotenoids are obtained (see Figure 1, dotted-dashed line). The broadened and weakened absorption bands indicate aggregation of carotenoids.[26] When the solvents have almost totally evaporated, xerogels (dried sol−gels) are formed. Due to aggregation, the absorption spectra of carotenoids cannot be observed in xerogels, although xerogels are also transparent. Extracts from xerogels, by use of organic solvents, give characteristic absorption bands of carotenoids, which suggests that the broadened and weakened (finally disappeared) absorptions are not caused by decomposition of carotenoids.

The absorption maxima of carotenoids in sol−gels are very close to those in the mixture of EtOH and H$_2$O (3:1 by volume). For example, **I** has absorption maxima at 466 nm in sol−gels and 465 nm in the mixture of EtOH and H$_2$O (spectrum not shown). In EtOH, **I** has an absorption maximum at 460 nm (Figure 1, dotted line).

2. Stability of Carotenoids in Sol−Gels. Sol−gels provide solid, transparent matrixes for carotenoids and have no obvious effect on their stability, as compared to the cases in the mixture of EtOH and H$_2$O with the same ratio as in sol−gels. In sol−gels, **I** is quite stable, as shown in Figure 2. There is no obvious degradation of **I** after standing for 30 days at room temperature, although the sol−gels become slightly cloudy, which is the reason for the rise in the baseline of the spectrum. The small increase of absorbance of **I** is caused by the small volume decrease of the sol−gel with time, which is caused by some evaporation of solvents. Volume decrease in turn causes the concentration increase of **I**. Although the sol−gel-containing cells were sealed by Parafilm, it is difficult to completely prevent the evaporation of solvents at room temperature during a long period of time. After 30 days, the absorption band shape of **I** has no obvious change, either. **II** is also relatively stable in sol−gels at room temperature, although it is not as stable as **I**. After standing at room temperature for 19 days, ca. 44% of **II** decomposed (estimated from
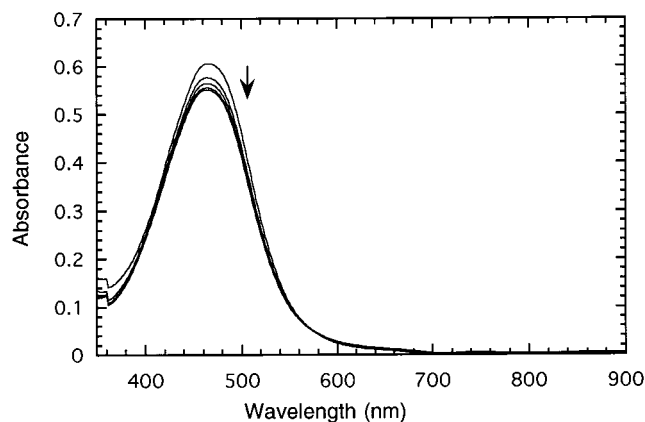
the absorbance, not considering the small volume decrease). **III** is not stable in sol−gels. Even before the formation of sol−gels, most of **III** decomposed, so it is impossible to observe the characteristic absorption band of **III** in sol−gels. After standing at room temperature for 4 days, ca. 53% of **IV** decomposed. Thus, the stability of these four carotenoids in sol−gels is **I** > **II** > **IV** > **III**. The stability of the four carotenoids in mixtures of EtOH and H$_2$O follows the same trend. In the mixture of EtOH and H$_2$O (3:1 by volume), no obvious decomposition of **I** was observed after 10 days at room temperature, but **III** showed a very large change in its band shape and a very large decrease in its absorbance after 3 days at room temperature.

Figure 3 shows the photodegradation of **I** in sol−gels. It is not fast under our irradiation intensity. Actually, the photodegradation rate of **I** in sol−gels is very similar to that in organic solvents (e.g., EtOH, CH$_2$Cl$_2$) at the same irradiation intensity. This similarity probably suggests that the vibrational deactivation of carotenoids is not suppressed in sol−gels, just as in the cases of organic solvents,[27,28] which is expected from the fact that carotenoids are dissolved in the mixture of the alcohol and H$_2$O. After absorbing light energy, carotenoids can rapidly transfer energy to the environment by vibrational deactivation; thus their photodegradation is slow.[27,28] In contrast, in liposome bilayers, the suppression of vibrational deactivation of carotenoids is suggested to be the reason for faster photodegradation than in organic solvents.[29]

3. Extraction of I from Sol−Gels and Xerogels. Because **I** is the most stable in sol−gels among the four studied carotenoids and extraction is time-consuming, **I** was chosen in the study of extracting carotenoids from sol−gels. EtOH is used as the extractant. Figure 4 shows (a) the decrease in the sol−gel phase and (b) increase in the EtOH phase of the concentration of **I** (indicated by the change in its absorbance) during extraction. When the extraction reaches equilibrium, **I** has a slightly higher concentration in the EtOH phase than in the sol−gel phase (absorbances of **I** are ca. 0.29

(26) Cudd, A.; Nicolau, C. In *Liposome Technology*; Gregoriadis, G., Ed.; CRC Press: Boca Raton, FL, 1984.

(27) Mehreteab, A.; Strauss, G. *Photochem. Photobiol.* **1978**, *28*, 369.
(28) Wasielewski, M. R.; Kispert, L. D. *Chem. Phys. Lett.* **1986**, *128*, 238.
(29) He, Z.; Kispert, L. D.; Metzger, R. M.; Gosztola, D.; Wasielewski, M. R. *J. Phys. Chem. B* **2000** *104*, 6302.
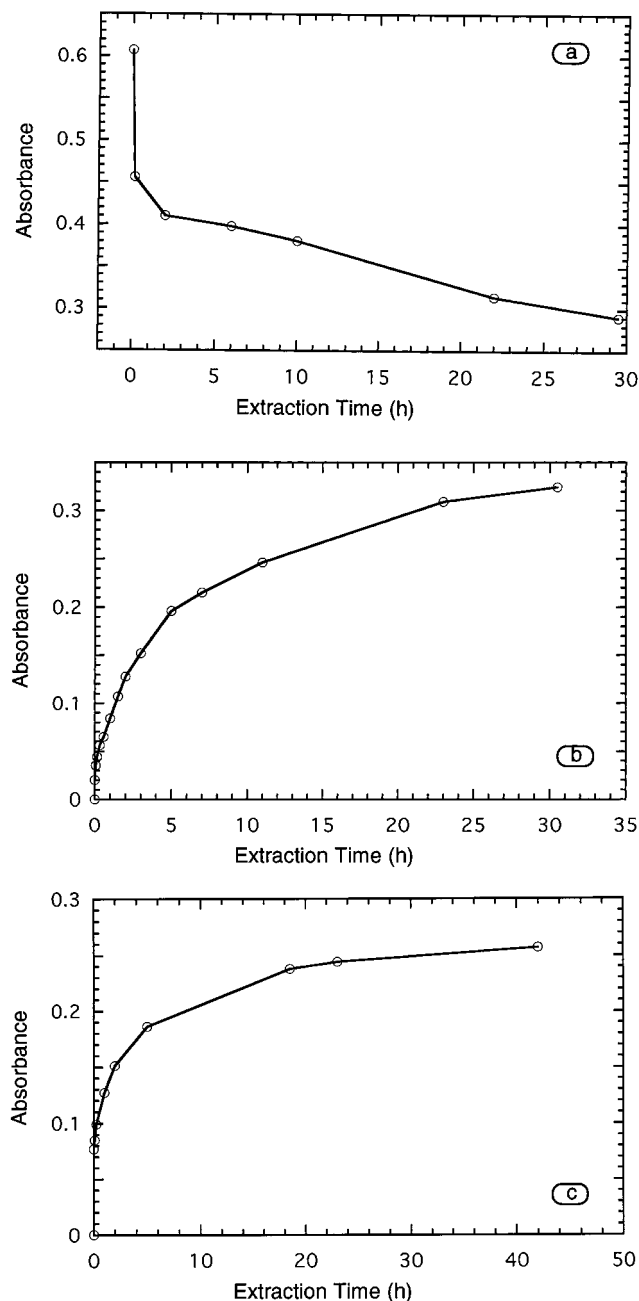
Figure 4. Absorbance changes of **I** with extraction time in (a) sol−gel phase (initial concentration 5.4 μM), (b) EtOH phase (from sol−gel), and (c) EtOH phase (from xerogel).

in sol−gel phase and ca. 0.32 in EtOH phase). The lower concentration of **I** in the sol−gel phase is probably due to the fact that the sol−gel network itself occupies some volume in the sol−gel phase. **I** can also be extracted from xerogels. Figure 4c shows the concentration increase of **I** in the EtOH phase during the extraction from xerogel. Because of the disappearance of the absorption band of **I** in the xerogel, the concentration decrease of **I** in the xerogel cannot be monitored by its absorbance. Since during the extraction the xerogel has no obvious increase in volume, the extraction of **I** from xerogel shows that EtOH can penetrate into the xerogel and, furthermore, the pores in the xerogel are large enough to allow the escape of **I**. From gas-phase AM1 calculation, **I** is ca. 25 Å in length and 6 Å in width at the widest part. If the pores in the xerogel were smaller
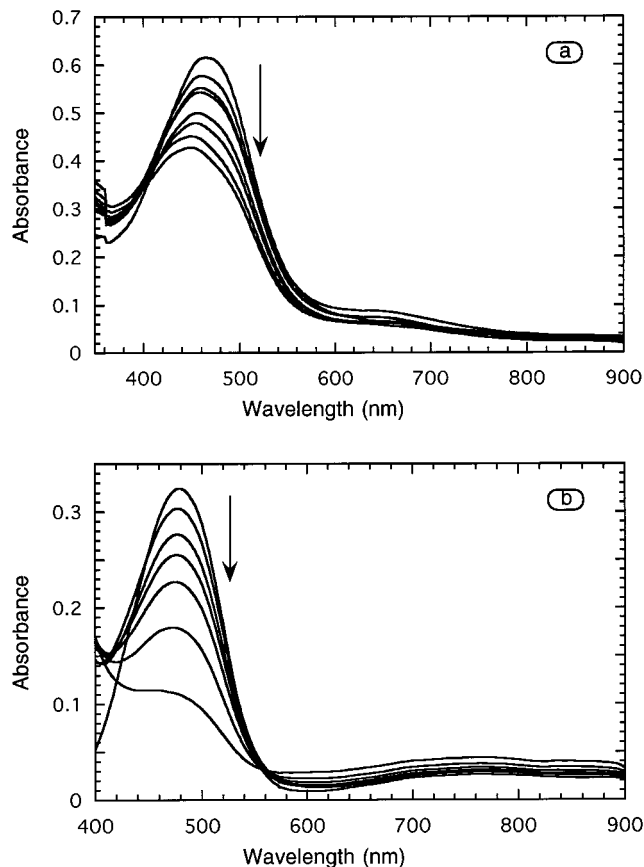
Figure 5. Optical absorption spectral changes of (a) **I** (initial concentration 5.5 μM) in sol−gel after adding ca. 10 mM FeCl$_3$/H$_2$O for 0, 10, 30, 60, 90, 120, 150, and 180 min (from top to bottom) and (b) **II** (initial concentration 2.8 μM) in sol−gel after adding ca. 10 mM FeCl$_3$/H$_2$O for 0, 17, 30, 50, 70, 90, and 120 min (from top to bottom).

than 25 Å in diameter, **I** might still escape from the pores. However, the extraction rate would be smaller than that from sol−gels that have much larger pore sizes. Thus, the pores in our xerogel should be larger than 25 Å in diameter, because the extraction rate from the xerogel is close to that from sol−gels. In the literature, it was reported that the pore size of xerogels is <100 Å.[18]

4. Sensitivity of Carotenoids in Sol−Gels to Oxidant and Acid. FeCl$_3$ was used to test the sensitivity of carotenoids in sol−gels to an oxidant in contacting aqueous solutions. It is known that FeCl$_3$ in CH$_2$Cl$_2$ can oxidize carotenoids to radical cations when the concentration of FeCl$_3$ is less than or equal to that of the carotenoid. Some resulting radical cations can exist for a fairly long time (e.g., the radical cation of **I** has a half-life of 149 s in CH$_2$Cl$_2$[30]). When more FeCl$_3$ is present, carotenoids can be further oxidized to dications.[31] The oxidization of carotenoids by FeCl$_3$ in CH$_2$Cl$_2$ is completed instantaneously.

Figure 5 shows the absorption spectrum changes of (a) **I** and (b) **II** in sol−gels with increasing periods of time after addition of FeCl$_3$/H$_2$O. From Figure 5, it is

(30) Deng, Y. Carotenoid Radical Cations and Dications Studied by Electrochemical, Optical, and Flow Injection Analysis: Lifetime, Extended Chain Conjugation, and Isomerization Properties. Ph.D. Dissertation, The University of Alabama, Tuscaloosa, Alabama, 1999.
(31) Jeevarajan, J. A.; Wei, C. C.; Jeevarajan, A. S.; Kispert, L. D. *J. Phys. Chem.* **1996**, *100*, 5637.
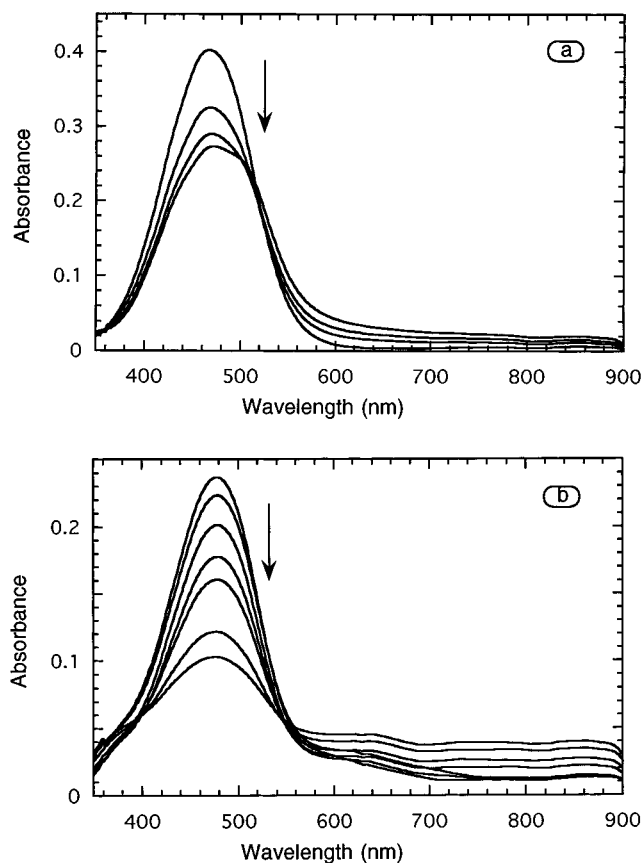
Figure 6. Optical absorption spectral changes of (a) **I** (initial concentration 3.6 μM) in sol–gel after adding H_2SO_4/H_2O for 0, 65, 120, and 195 min (from top to bottom) and (b) **II** (initial concentration 2.0 μM) in sol–gel after adding H_2SO_4/H_2O for 0, 10, 20, 45, 60, 90, and 120 min (from top to bottom).

obvious that **I** and **II** in sol–gels can still be oxidized by $FeCl_3$, as indicated by the decrease of their absorbance. In other words, carotenoids in sol–gels can sense oxidants in contacting aqueous solutions. This is understandable, because the small size of Fe^{3+} allows its diffusion into the pores of sol–gels. The oxidation rate of **I** and **II** depends on the diffusion rate of Fe^{3+} into sol–gels. During the oxidation, no absorption bands of radical cations of **I** and **II** [centered at 848 nm (**I**) and 887 nm (**II**) in CH_2Cl_2] were observed. Even when the oxidation rate is taken into account, the absence of radical cation absorptions of **I** and **II** indicates the instability of radical cations of **I** and **II** in sol–gels.

It has been suggested that acids react with carotenoids to form carotenoid carbocations.[17] Figure 6 shows the absorption spectrum changes of (a) **I** and (b) **II** with increasing periods of time after addition of H_2SO_4/H_2O (pH 3~3.5). The decrease of absorbance of **I** and **II** after addition of H_2SO_4 indicates that carotenoids in sol–gels can also react with the acid, which means that carotenoids in sol–gels can sense the presence of acids in aqueous solutions. The reaction rate also depends on the diffusion rate of the acid into sol–gels.

During the reaction of **I** and **II** with H_2SO_4, no obvious absorption bands of carbocations of **I** and **II** [centered at 844 nm (**I**) and 881 nm (**II**) in CH_2Cl_2[17]] were observed.

Concluding Remarks

Our preliminary studies demonstrate that carotenoids can easily be incorporated into and evenly distributed in sol–gels, and some carotenoids are relatively stable in sol–gels at room temperature. This stability of carotenoids and the solid, transparent character of sol–gels, along with the visible absorption of carotenoids, may allow carotenoid-containing sol–gels to have certain applications. For example, they may be used to build sensors for oxidants and acids in aqueous solutions, because the reaction of carotenoids with oxidants and acids results in a decrease of absorption in the visible region of the spectrum (bleaching), which can be used to indicate the existence of oxidants and/or acids. Because carotenoids are extremely insoluble in H_2O, they will not contaminate the tested aqueous solutions. Carotenoids also function as quenchers of damaging singlet oxygen and scavengers of free radicals. It is expected that the sensors built of carotenoid-containing sol–gels may also quench singlet oxygen and scavenge free radicals if the free radicals are not too large. In the future, the reactivity of carotenoids in sol–gels with singlet oxygen and free radicals will be investigated.

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