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Water Soluble Complexes of Carotenoids with Arabinogalactan

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We present the first example of water soluble complexes of carotenoids. The stability and reactivity of carotenoids in the complexes with natural polysaccharide arabinogalactan were investigated by different physicochemical techniques: optical absorption, HPLC, and pulsed EPR spectroscopy. Compared to pure carotenoids, polysaccharide complexes of carotenoids showed enhanced photostability by a factor of 10 in water solutions. A significant decrease by a factor of 20 in the reactivity toward metal ions (Fe^{3+}) and reactive oxygen species in solution was detected. On the other hand, the yield and stability of carotenoid radical cations photoproduced on titanium dioxide (TiO_2) were greatly increased. EPR measurements demonstrated efficient charge separation on complex-modified TiO_2 nanoparticles (7 nm). Canthaxanthin radical cations are stable for ~ 10 days at room temperature in this system. The results are important for a variety of carotenoid applications, in the design of artificial light-harvesting, photoredox, and catalytic devices.

Introduction

Carotenoids are well-known as a class of pigments widespread in nature. These essential nutrients synthesized by plants and microorganisms are abundant in many vegetables, fruits, and fish. About 600 various carotenoids are known. However, only a few (about 20) have been found in human tissues. They are β -carotene, canthaxanthin, zeaxanthin, etc. The presence of a polyene chain and various terminal substituents in carotenoid molecules determines both their redox properties and the location inside the lipid layers in biological media. In most of the natural processes, including photosynthesis, their role is mostly related to the processes of energy and electron transfer.^{1,2} Photoinduced electron transfer in heterogeneous hosts attracts more and more interest in connection with the design of artificial light-harvesting, photoredox, and catalytic systems. In addition, recently more attention has been focused on the reactions between carotenoids and free radicals^{3–9} because of the ability of carotenoids to prevent the development of diseases caused by toxic free radicals.¹⁰ At the same time, wide practical application of carotenoids as antioxidants is substantially hampered by their hydrophobic properties, instability in the presence of oxygen and high photosensitivity. The application of carotenoids as components of the artificial solar cells is restricted by their instability toward reactive oxygen species and metal ions, especially in the presence of water.

The majority of carotenoids are lipophilic molecules with near zero inherent aqueous solubility. Moving carotenoids into a pharmaceutical application requires a chemical delivery system that overcomes the problems with parenteral administration of a highly lipophilic, low molecular weight compound. Many

different methods have been developed to make the carotenoids “water dispersible”, as true water solubility has not been described. Most of the attempts to increase solubility of carotenoids are related to the preparation of cyclodextrin inclusion complexes.^{11–19} Application of inclusion complexes was first related to an attempt to minimize the aforementioned disadvantages of carotenoids when these compounds are used in the food industry, cosmetology, and medicine.^{11–15,20,21} The main difficulty in practical applications of “host–guest” complexes is that the rigidly fixed volume of the cyclodextrin cavity prevents binding of either very small or very large molecules including many compounds that are of interest in medicine and pharmacology. To avoid these disadvantages, a search for complexing agents is being continued. The increased aqueous solubility of a number of carotenoids will likely find utility in their introduction into mammalian cell culture systems that have previously been dependent upon liposomes, or toxic organic solvents, for the introduction of carotenoids into aqueous solution.¹⁹ Also water soluble carotenoids display several technological applications that could be provided in food processing (colors and antioxidant capacity) as well as for production of therapeutic formulations, considering the better solubility and consequently higher bioavailability.^{11–13,20}

The present paper describes the complex formation between carotenoids and natural polysaccharide arabinogalactan (AG), a branched polymer with molecular mass 15 000–20 000. Arabinogalactans are found in a variety of plants but are more abundant in the *Larix* genus, primarily Western and Siberian Larch.^{22,23} Larch arabinogalactan is approved by the U.S. Food and Drug Administration (FDA) as a source of dietary fiber, but also has potential therapeutic benefits as an immune stimulating agent and cancer protocol adjunct. The immune-enhancing herb echinacea also contains AG, as do leeks, carrots, radishes, pears, wheat, red wine, and tomatoes. AG is an important source of dietary fiber. Research has shown that AG increases the production of short-chain fatty acids, principally

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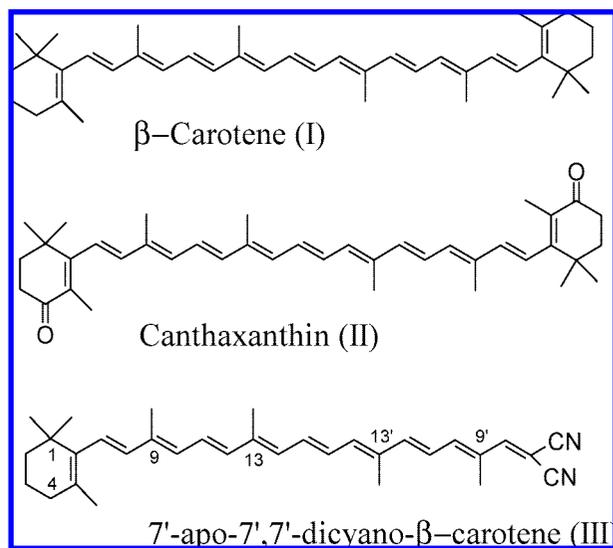
^{||} University of Alabama.

butyrate and propionate, which are essential for the health of the colon. AG also acts as a food supply for “friendly” bacteria, such as bifidobacteria and lactobacillus, while eliminating “bad” bacteria. AG has a beneficial effect upon the immune system as it increases the activity of natural killer cells and other immune system components, thus helping the body to fight infection.²³

In this paper, it will be shown that the carotenoid/AG complex formation results in an increase in solubility of carotenoids in water. In addition, the influence of AG on radical processes involving carotenoids and the reactivity in electron transfer reactions with metal ions and the reactions with free radicals are examined. These processes have been previously studied in detail in homogeneous solutions.^{24,25} Additional experiments are reported on the reactivity of this complex in the solid state and pulsed EPR measurements of the photoinduced charge separation on complex modified by TiO₂ nanoparticles.

Experimental Details

Carotenoids **I** and **II** were provided by Fluka, carotenoid **III** was synthesized at the University of Alabama.^{26,27} All carotenoids were kept at -18 °C in sealed ampules. The purity was assessed by ¹H NMR spectroscopy (360 MHz, CDCl₃) and TLC. The solvents, methanol and ethanol (Fisher, A.C.S.), were used as obtained.



Arabinogalactan was extracted from *Larix sibirica*.²⁸ Arabinogalactans are long, highly branched polysaccharides composed of galactose and arabinose molecules in a 6:1 ratio. Pharmaceutical-grade larch arabinogalactan is a fine, dry, off-white powder with a slightly sweet taste and mild pinelike odor. It is low in viscosity, dissolves completely in water or juice and, therefore, easy to administer, even to children. Two different methods of complex preparation were used. The first one is the addition of a small amount of methylene chloride to the solid mixture of carotenoid and arabinogalactan powder. After grinding this mixture and evaporating the solvent, it was dissolved in water. The absorption spectrum of this solution was analyzed. Although we have observed the carotenoid absorption peak in the absorption spectrum, the estimated concentration of the complex in solution was only several micromoles (complex of **I** was prepared in this way, β-carotene itself does not dissolve in water). The same result was obtained by mixing a water solution of arabinogalactan in an ethanol

solution of carotenoid. The second method of complex preparation used in the present work was a mechanochemical treatment of the solid mixture of carotenoid crystals with arabinogalactan powder. Typical mechanochemical reactions are those activated by cogrinding or milling of powder materials. These reactions are usually carried out either manually, in an agate mortar, or electromechanically, as in ball milling. In all these cases, the crystal lattice is destroyed and reformed through recrystallization. In such processes hydrogen bonds, π-stacking, van der Waals, ion pairing interactions, etc. are broken and formed through the reaction process leading to formation of supramolecular compounds or hybrid molecular crystals.^{29,30} In the present study, we used electromechanochemical equipment. Cogrinding of solid materials results in penetration of carotenoid molecules into the polymer of arabinogalactan without use of any organic solvents. This approach allowed us to prepare the water soluble composites of carotenoids **II** and **III** with arabinogalactan for the first time. The estimated solubility of these complexes prepared with molar stoichiometry 1:1, and estimated by HPLC analysis, is 5 mM in a water solution. X-ray diffraction analysis of solid complexes prepared mechanochemically was performed using the conventional X-ray diffractometer DRON-3 with Cu Kα radiation. Differential scanning calorimetry (DSC) of these samples was also performed using DSC-550 (Instrument Specialists Inc.) in an argon atmosphere (20 °C < *t* < 250 °C).

Photodegradation measurements were made by monitoring the change in the optical absorption of irradiated samples at room temperature (20 °C). UV-vis absorption spectra were recorded using a double-beam Shimadzu UV-visible 1601 spectrophotometer. Samples were irradiated in a quartz cell used for optical measurements. The irradiation source was a 250 W xenon lamp from the ILC Technology Company.

The reaction of carotenoids with the electron acceptor, ferric citrate complex (1:1), was initiated by mixing solutions of the acceptor and the complex in a water-alcohol mixture (30% ethanol). The reaction was monitored by changes in the absorption spectrum of the carotenoid. Ferric citrate was used also as a component for the photo-Fenton reaction.^{31,32}

A Vydac 201 TP54 polymeric C18 column (250 × 4.6 mm i.d.) packed with 5 mm particles (Hesperia, CA) and a Shimadzu LC-600 pump with a SPD-M10AVP PDA detector were used for the HPLC separation and detection. Acetonitrile was the mobile phase. The flow rate was 1 mL/min.

For EPR measurement the samples were prepared by adding 0.2 mL of carotenoid solution in CH₂Cl₂ or complex solution in H₂O to the same volume of TiO₂ powder (7 nm particles from Ishihara Sangyo Kaisha, Ltd., Japan) in a quartz EPR tube. The solvents were then evaporated under vacuum, and the tube was filled by argon. The samples were irradiated for 10 min with a 250 W xenon lamp through a water filter ($\lambda > 350$ nm) at 77 K. CW and pulsed EPR experiments were performed with an X-band Bruker E-580 spectrometer equipped with a MD5-W1 dielectric resonator, an Oxford instruments CF935 helium cryostat, and electrically controlled Oxford helium transfer line. The spectrometer is controlled through a Linux workstation with XEPR, the data acquisition and manipulation Bruker software.

Results and Discussion

Solubility of Carotenoids in Water As a Criterion of Complex Formation. It is well-known that carotenoids are highly hydrophobic, air- and light-sensitive compounds. Earlier there were several attempts to prepare water soluble complexes of carotenoids using water-oil emulsion or cyclodextrin

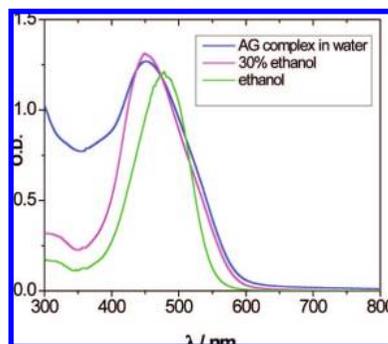


Figure 1. Absorption spectra of carotenoid **II** in different solvents. The increase of optical density at $\lambda < 400$ nm is due to arabinogalactan absorbance.

solutions.^{14,15} Cyclodextrins are well-known complexants widely used in pharmacology to increase the solubility of drugs.^{33–35} However, in the case of carotenoids, these complexes exist only as large aggregates with average size more than 100 nm.^{14,15} The solutions of all carotenoid–cyclodextrin complexes show a considerable change in color as compared to carotenoid solutions in organic solvents.¹⁸ For example, aqueous solution of the β -carotene–CD complex is an intense opalescent pink–orange, the CD complex of **III** is black, whereas its MeOH and CH_2Cl_2 solutions are violet.

In this study, we present the first example of water soluble composites of carotenoids. The estimated solubility of the complexes of carotenoids **II** and **III** with arabinogalactan (AG) prepared mechanochemically with molar stoichiometry 1:1 is 5 mM in a water solution. The carotenoid–arabinogalactan complexes maintain their original color and show insignificant changes in the absorption spectra. The UV–vis spectrum of aqueous solutions of a canthaxanthin–AG complex has the same absorption maximum as the spectrum of a canthaxanthin solution in 30% ethanol (Figure 1). As was mentioned in the Experimental Details, our attempts to prepare a concentrated solution of this complex using traditional methods for preparation of cyclodextrin complexes were not successful.

In addition, the complexes of carotenoids **II** and **III** with arabinogalactan prepared mechanochemically was investigated by X-ray diffraction phase analysis and differential scanning calorimetry (DSC) techniques. The results of these measurements are shown in Figure 2.

In the mixture of initial compounds before treatment, one can see the characteristic peaks of the crystal structure of the carotenoid (plot 1), which disappears after mechanochemical treatment (plot 3). We suggest that the absence of a crystal structure in this case is due to molecular penetration of the carotenoid into the arabinogalactan polymer matrix. We interpret this to mean that complex formation occurs in the solid state but not during further solubilization. We assume that deep penetration of carotenoid into arabinogalactan matrix does not occur when we try to prepare the complex by addition of a carotenoid solution to an arabinogalactan solution. This is confirmed by significant increase in solubility of the complex prepared mechanochemically as compared with traditional solvent-mediated methods. The important advantage of this method over solvent-mediated methods is also the possibility of complex preparation without using any toxic organic solvents.

Photostability of Carotenoids and Their Complexes in Water Solution. One of the main problems in the practical application of carotenoids is their photosensitivity and instability, especially in the presence of water. The photostability of drugs

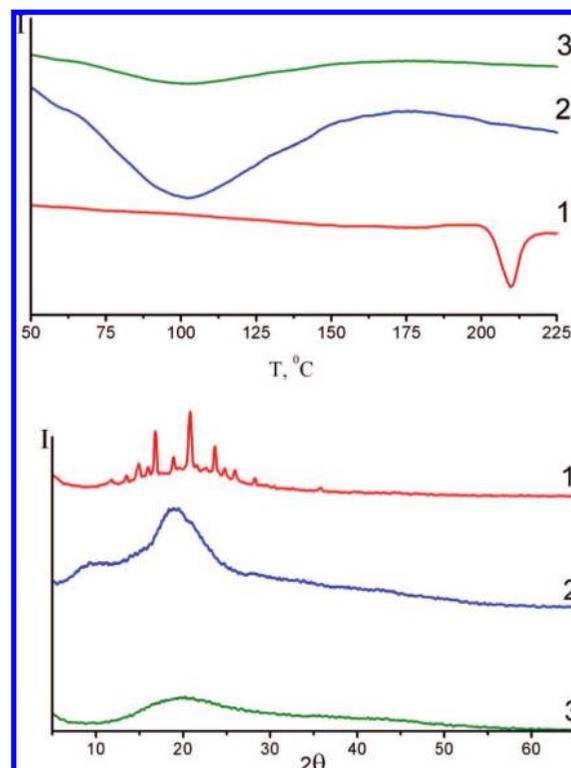


Figure 2. X-ray diffraction phase analysis (lower) and differential scanning calorimetry analysis (upper) of solid canthaxanthin–arabinogalactan complex prepared mechanochemically: (1) canthaxanthin; (2) arabinogalactan; (3) complex.

and vitamins has recently received increasing attention.³⁵ Serious toxic reactions produced by many pharmacologically important chemicals occur under sunlight irradiation. Photoallergic and photomutagenic effects are also of current concern. Intermediates and stable photoproducts, generated upon light absorption by drugs and vitamins, interact with cell components and lead to cell degeneration or death. Control of the drug photostability and preparation of protective strategies against the light-induced damage requires understanding the structural and environmental factors determining their photoreactivity.

In the present study, the relative photodegradation rate of canthaxanthin and its complex with AG was investigated during the photolysis in an aerated water–ethanol mixture (2:1) under the full light of a xenon lamp. The photodegradation rate was estimated from a decrease in the intensity of the carotenoid absorption maximum and was confirmed by HPLC measurement. Figure 3 demonstrates a significant increase in the photostability of canthaxanthin when incorporated into an AG complex. The estimated half-life decay is 200 s for the complex and 20 s for the pure carotenoid in 30% aqueous ethanol solution. Thus, the ratio of the photodegradation rates is ten times for the system under study.

We assume that the decreasing photostability of carotenoids in homogeneous aqueous solution is due to effect of water. Why the presence of water is so important? In various media, carotenoids can form carotenoid radical cations ($\text{Car}^{+\bullet}$) by electron transfer. This radical cation can be reduced by back electron transfer. However this reversible process is disrupted in the presence of H_2O which acts as a proton acceptor from the weak acid $\text{Car}^{+\bullet}$ to form neutral radical (Car^\bullet). This Car^\bullet is stabilized by the nearby proton acceptor, so a reversible electron transfer is prevented. Earlier it was demonstrated that the carotenoid neutral radicals are formed from corresponding

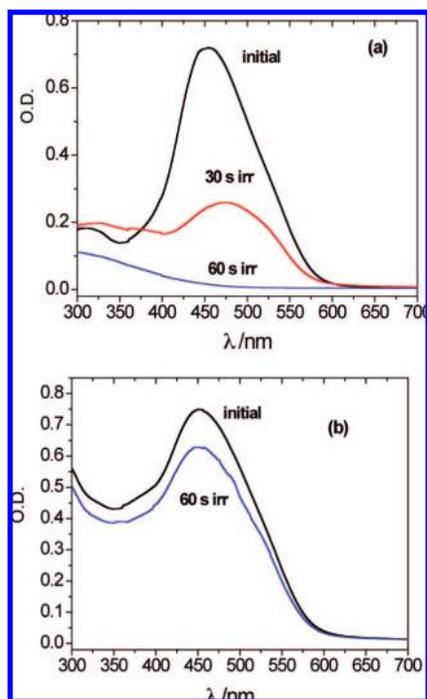
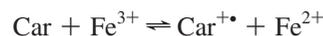


Figure 3. Photodegradation of canthaxanthin. Absorption spectra were recorded after different irradiation times in aerated 30% ethanol solution by the full light of a xenon lamp. (a) Pure canthaxanthin. (b) Canthaxanthin–AG complex.

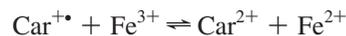
radical cations generated electrochemically or photochemically by proton loss.^{37–39} Although photoexcitation accelerates deprotonation of the radical cation, electrochemical measurements showed that the radical cations of a majority of carotenoids have pK values ranging between 4–7 and, therefore, can deprotonate spontaneously.^{40,41} We have suggested that incorporation of carotenoids into the hydrophobic polymer environment will reduce their interaction with water molecules. It was confirmed by the absence of a considerable blue shift in the absorption spectra of the AG complex of carotenoid containing a polar group (**III** for example) observed earlier in the presence of water in homogeneous solutions.¹⁸ To prove our suggestion that the presence of water accelerate the photodegradation rate of a carotenoid, we have repeated the same experiment using a pure ethanol solution of **III**. The result of this experiment was a significant increase in the carotenoid stability in the absence of water. The explanation of this fact is that the ethanol is less effective proton acceptor compare with water. Unfortunately we can not compare quantitatively the reactivity of carotenoids and their complexes in ethanol or other organic solvents due to insolubility of arabinogalactan in these solvents. These results confirm our suggestion that the main stabilizing factor appears to be the protection of carotenoids from water molecules by complexation.

Reactivity of the Complexes. Interaction of Carotenoids with Fe Ions in Solution. As shown in the Introduction, one of the most important natural processes involving carotenoids is the electron transfer from carotenoids to a variety of acceptors. In the present work, the influence of complexation with AG on the reactivity of carotenoids was studied using Fe^{3+} ion as an electron acceptor. The mechanism of the reaction between carotenoids and Fe^{3+} in organic solvents has been studied in detail.⁴² Note that the reaction with iron ions is considered by a number of authors to be one of the possible mechanisms of the pro-oxidant activity of β -carotene.^{7,25} It was shown that the

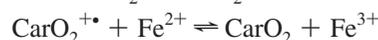
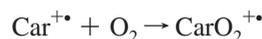
first step of the reaction is the electron transfer resulting in the formation of the carotenoid radical cation:



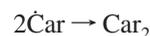
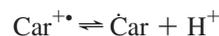
The latter also can react with Fe^{3+} :



In the presence of oxygen, the final oxidation products of carotenoids in this process was identified as a 5,8-epoxide.⁴²



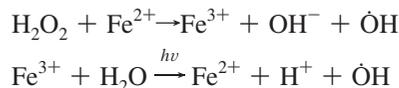
On the other hand, it was demonstrated³⁹ that in the presence of water, carotenoid radical cations can undergo deprotonation reactions resulting in neutral carotenoid radicals with further dimerization:



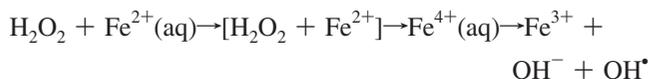
In the previous study, we have shown that complexation with natural oligosaccharide glycyrrhizin has a noticeable effect on the reactivity of carotenoids, such as a decrease in the electron transfer rates in the reaction with ferric chloride, and an increase in the lifetime of complexing radical cations.²⁴ To study a single electron transfer reaction of carotenoids in aqueous solution, we have chosen a ferric citrate complex ($Fe:cit$) as an electron acceptor.⁴³ It was found that in a 30% aqueous ethanol solution in the absence of AG, all carotenoids (**I–III**) react with $Fe:cit$ with the formation of carotenoid dimers. As mentioned above, dimer formation is possible by recombination of carotenoid neutral radicals formed by deprotonation of radical cations in the presence of water.³⁹ This reaction leads to the broadening of the carotenoid absorption spectra with the shift of absorption maxima to higher wavelength (see Figure 4a for example). In the absence of water, in pure ethanol, only β -carotene reacts with $Fe:cit$ resulting in a decrease in the absorption intensity (Figure 4b). All other carotenoids show no change in the absorption spectra in pure ethanol. In contrast to a homogeneous solution of carotenoids, their complexes with AG show no change in the absorption spectra during a 20 min observation period in 30% aqueous ethanol solution, as well as in water solution. We suggest that two factors play an important role in stabilization of carotenoids in AG complexes, namely, isolation from water molecules and isolation from metal ions. The absence of reaction for even β -carotene complex means that $Fe:cit$ does not penetrate into the polysaccharide matrix.

Interaction of Carotenoids with Free Radicals in Solution.

Interactions of carotenoids with active oxygen species is well studied and attract great attention in relation to antioxidant and pro-oxidant activities of carotenoids.^{1–6} In the present study, we used the photo-Fenton reaction^{43,44} listed below and $Fe(III):cit$ for generation of free radicals.



During irradiation of a water solution of Fe(III):cit by a xenon lamp through a water filter, only a decrease in the UV-vis spectrum of the Fe(III) absorption band was shown according to the last equation. Photolysis of the same solution in the presence of hydrogen peroxide results in the appearance of a new absorption peak near 500 nm (Figure 5). A modern interpretation of the Fenton (and photo-Fenton) mechanism⁴⁴ assumes that other oxidizing intermediates such as highly valent iron complexes (Fe^{4+}) are formed during oxidation of Fe^{2+} to Fe^{3+} .



One can assume that the long-lived species observed at 500 nm (lifetime more than 1 h) might be a citrate complex with a highly valent iron ion.

The Fenton reaction is widely used for generation of free radicals in solution for model experiments. As was shown by the EPR spin-trapping study, only peroxy radicals are detected in this system at high H_2O_2 concentration (more than 0.1 M).⁸ The interaction of OOH radicals with the carotenoid polyene chain leads to fast bleaching of the solution. In Figure 6a, the optical spectrum of Fe(III):cit (1) before exposure to 3 min of irradiation (2) is given. After addition of β -carotene (3), the spectrum shows the spectrum of β -carotene plus the residue of the spectrum from Fe(II):cit. Figure 6a (4) shows a rapid decrease of the β -carotene absorption after addition of H_2O_2 to the mixture of β -carotene with Fe(II):cit. The spectrum of Fe(II):cit (2) was obtained by irradiation of Fe(III):cit in the presence of water.

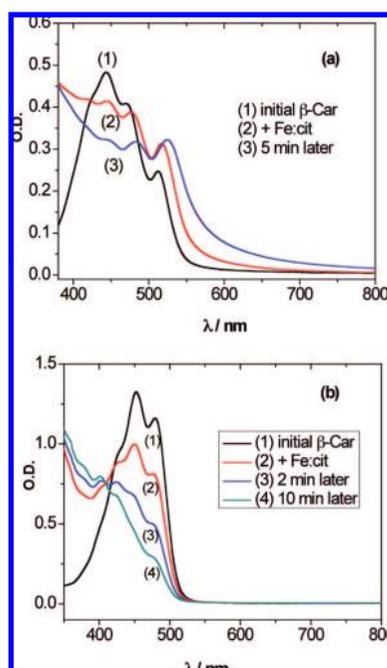


Figure 4. Absorption spectra of β -carotene in the presence of ferric citrate (0.5 mM) (a) in 30% ethanol. (b) in pure ethanol.

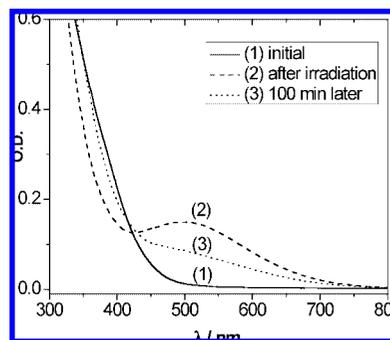


Figure 5. Absorption spectra of Fe:cit (0.5 mM) irradiated 1 min in the presence of H_2O_2 (0.25 M) in 30% ethanol.

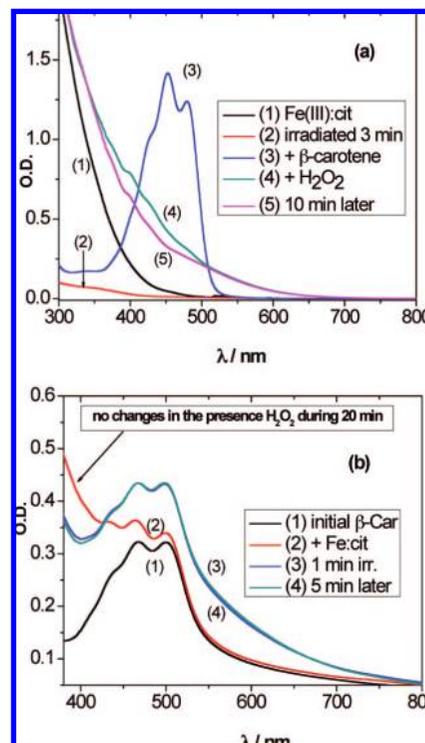


Figure 6. Absorption spectra of β -carotene in the presence of ferric citrate (0.5 mM) and H_2O_2 (0.25 M) (a) in ethanol and (b) in AG complex in 30% ethanol. The increase in optical density after irradiation of the sample is due to formation of Fe^{4+} .

The reaction of the OOH radical with the β -carotene-AG complex shows considerable effect of complexation (Figure 6b). Figure 6b demonstrates the stability of β -carotene (1) in the AG complex in the presence of Fe^{3+} ions (2) as well as in the presence of peroxy radicals generated by irradiation of the reaction mixture (3) and (4). A decrease in absorption at 300 nm and an increase of absorption at 500 nm after irradiation is due to formation of Fe^{4+} from Fe^{3+} during the reaction (see Figure 5). This dependence was also observed for the other two carotenoids, **II** and **III**. For example, Figure 7a and b show the difference in decay rates for carotenoid **II** and its AG complex respectively in the presence of a photo-Fenton reaction. In Figure 7a, the carotenoid absorption (1) decays in 20 min to that in (4). In Figure 7b, the decay is not as pronounced. The estimated ratio of decay rates equals 20 for this system. We propose that the stability of the carotenoids incorporated into the AG macromolecule might have practical applications. A decrease in reaction rate toward free radicals does not mean a decrease in antioxidant activity of a complex in living systems since polysaccharides are easily assimilated by living media.

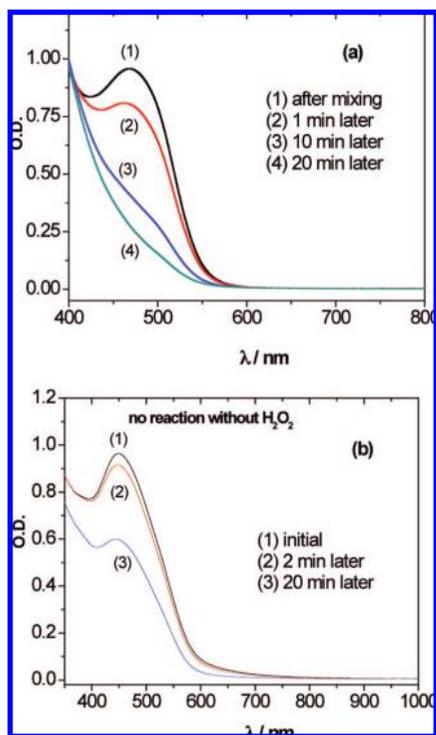


Figure 7. Absorption spectra of canthaxanthin in the presence of Fe(II): cit (0.5 mM) and H₂O₂ (0.25 M) (a) in ethanol and (b) in AG complex in water solution.

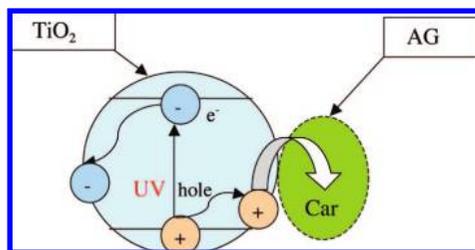


Figure 8. Schematic illustrations of the photocatalytic reaction processes of carotenoid (Car) adsorbed on the surfaces of TiO₂ nanoparticles.

Photo-induced Electron Transfer in the Solid State. The next experiment was carried out to investigate the efficiency of electron transfer from carotenoids to acceptors and the properties of the carotenoid radical cations in the solid state AG complex. These processes are intensively studied^{45–47} because of importance of carotenoids in photosynthesis and their possible use in artificial solar cells. In the present study, we have succeeded in detecting the canthaxanthin radical cation incorporated in a AG macromolecule during photoirradiation on TiO₂ nanoparticles. Among the semiconductors, titanium dioxide is the most suitable for many environmental applications. Titanium dioxide is widely used in photocatalysis and in artificial solar cells due to its ability to absorb light and to transfer negative or positive charge to a corresponding electron acceptor or donor^{48–52} (Figure 8).

Irradiation of the samples (see Experimental Details) was made under argon atmosphere at 77 K. Irradiation of TiO₂ nanoparticles in the absence of carotenoid results in the appearance of an EPR signal which we attributed to Ti³⁺ (Figure 9(1)a). Irradiation of pure canthaxanthin (without AG) adsorbed on the TiO₂ surface shows only a weak signal with $g = 2.0028 \pm 0.0002$, $\Delta H_{pp} = 13.0 \pm 0.5$ G, which is characteristic of carotenoid radical cations^{53–55} (Figure 9(1)b). In contrast, the carotenoid–AG complex when irradiated on TiO₂ shows a

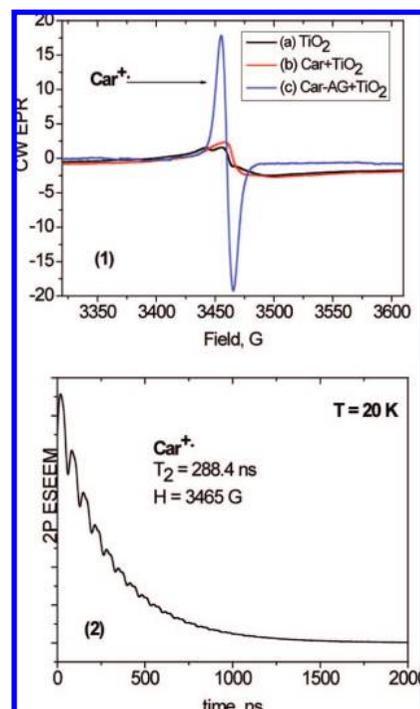


Figure 9. (1) EPR spectra detected after irradiation of TiO₂ powder (a), TiO₂ in the presence of canthaxanthin (b), and TiO₂ in the presence of canthaxanthin–AG complex in solid state at 20 K (c). (2) Two-pulse ESEEM spectrum of the canthaxanthin–AG complex: $B = 3465$ G, $\nu = 9.709885$ GHz, $\tau = 100$ ns, SRT = 999.6 μ s, $T = 20$ K.

significant increase in the EPR signal intensity (Figure 9(1)c) compared to that of the pure carotenoid radical. The two-pulse ESEEM (electron spin echo envelope modulation) spectrum of the Car radical–AG complex measured at the field position corresponding to the maximum EPR absorption exhibits proton modulation due to protons of carotenoid radical (Figure 9(2)). Phase memory time T_m for the carotenoid radical in a complex determined by fitting the echo decay curve as single exponential was found to be 288 ns. This is significantly shorter than that of carotenoid in frozen solution or on solid support (approximately 1000 ns). The observed increase in relaxation rate might be due to restricted mobility of carotenoid in the polysaccharide matrix as well as relaxation due to the titanium atoms.

We suggest that the low yield of charge separated state in the absence of AG might be due to the efficient back electron transfer on semiconductor materials. The “redox cycling”, where a product of the hole transfer acts, in turn, as scavenger for the photogenerated electrons, appears as a frequent cause of weak photocurrents.^{56,57} The isolation of the carotenoid radical cation from the TiO₂ surface by incorporation into the polysaccharide complex allows more efficient charge separation reducing the rate of back electron transfer. The same approach was used by Moore et al. for the design of molecular triads as a model for light harvesting complexes.^{58–60} Initial charge separation is accomplished by photoinduced electron transfer from the excited porphyrin to the attached quinone to generate the charge-separated species CP⁺Q[–]. In a second step, an electron is donated by the attached carotenoid moiety to the porphyrin to form the species C⁺PQ[–]. This species lives long enough to transfer an electron to a freely diffusing secondary quinone which act as a proton shuttle.^{58–60} The long lifetime of the triads or modified TiO₂ particles opens up wide possibilities for their use in molecular electronics as the nanosized means of communication and data processing and in sensors. Apparently, such

a way of light energy transformation is similar to the mechanism used by plants for utilization of solar energy in photosynthesis.

Previous studies on the use of a carotenoid-sensitized TiO₂ nanocrystalline mesoporous electrode in the preparation of a photovoltaic cell in the presence of water,⁶¹ indicated that the cell efficiency was partially limited by the recombination of the injected electron with the oxidized form of the carotenoid radical cation. The current results suggest the use of the polysaccharide complex in the preparation of a more efficient cell. Stabilization of the carotenoid radical cation would reduce the recombination rate.

As it was shown earlier, photogenerated EPR signals in carotenoid/TiO₂ systems are stable at 77 K, but they disappear when the temperature is increased above 250 K.⁵⁸ In the case of carotenoid-AG complex, the significant increase in stability of the radical cation was detected. Increasing the temperature up to room temperature does not lead to disappearance of the spectrum. The lifetime of canthaxanthin radical cation measured in the present study is approximately 10 days at room temperature.

Furthermore, the development of new, more effective drugs based on the complexes of tested medicines with natural compounds is now an intensively pursued area in medical chemistry, cosmetology, and the food industry. Fundamental studies on the nature of complexation and physicochemical properties of complexes substantially lag behind, although these investigations are of a great importance because of their predictive potential.^{15,24,62} In medicine, screening of new drugs is, as a rule, performed using animals. Therefore, the possibility of controlling the reactivity of chemical compounds by complexation and, equally importantly, of predicting the extent of an increase or decrease in their therapeutic activity would allow one to substantially reduce the number of in vivo experiments.

Conclusion

In the present work, we report the first example of water soluble carotenoid complexes. It was demonstrated that incorporation of carotenoids into polysaccharide arabinogalactan macromolecules result in significant change in their properties. It was demonstrated that the mechanochemical method of complex preparation has significant advantages as compared with traditional techniques. Primarily, the interest in solvent-free conditions stems from the possibility of obtaining the same product as that from solution without solvent because the process is cheaper, less time-consuming, and often more environmentally friendly.^{29,30} In the case of carotenoid chemistry, the solvent-free conditions open the possibility of obtaining products not otherwise accessible from solvents.

The increase of water solubility (5 mM), photostability (a factor of 10 increase), and stability by a factor of 20 toward metal ions in solution has significant importance in the practical application of carotenoids. An important result of this study is the increase in yield and stability of the radical cation of canthaxanthin in a solid state complex of AG. In a previous study, we have observed significant stabilization of the carotenoid radical cations in a glycyrrhizin complex in solution.²⁴ An increased stability of the carotenoid radical cation imbedded into a polysaccharide host opens wide possibilities for the application of these complexes in the design of artificial light-harvesting, photoredox, and catalytic devices.

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