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Host−Guest Complexes of Carotenoids with β-Glycyrrhizic Acid

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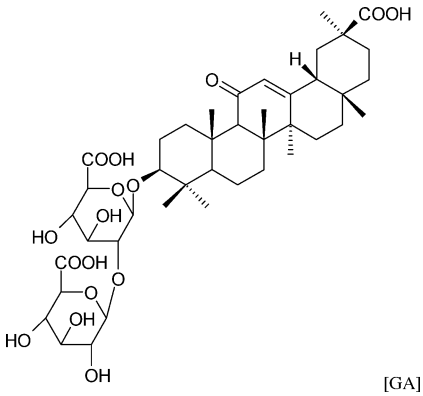
The structure, stability, and reactivity of the host−guest complexes between a set of carotenoids and the triterpene glycoside, β-glycyrrhizic acid (GA), were investigated by different physicochemical techniques: high-performance liquid chromatography, optical absorption, and fluorescence spectroscopy. It has been demonstrated recently that the molecular complexes of GA with a number of drugs are characterized by reduced toxicity and increased therapeutic activity of these drugs. In the present work it was found that carotenoids form 1:2 complexes with GA in aqueous solutions as well as in polar organic solvents, methanol, acetonitrile, and dimethylsulfoxide. We assume that the structure of the complex is a cycliclike dimer of GA encapsulating a carotenoid molecule. The stability constants in all solvents are near 10^4 M^{-1}. In addition, GA forms inclusion complexes with carotenoid radical cations, which results in their stabilization. Complex formation (a) decreases the rate of electron transfer from carotenoids to electron acceptors (Fe^{3+} or quinone) and (b) considerably increases the lifetime of the carotenoid−quinone charge-transfer complex and the yield of the major product (a carotenoid−quinone adduct). A thermodynamic study shows that hydrophobic interactions are the main driving force of the carotenoid−GA complex formation. These results are important for understanding both the nature of GA complexes and the influence of GA on the therapeutic activity of some drugs. Furthermore, carotenoid−GA complexes could be used for the design of artificial light-harvesting, photoredox, and catalytic systems.

Introduction

At present, of particular interest are investigations performed in the area of physical organic chemistry concerning the mechanisms of chemical reactions in organized media. In particular, this refers to a study of supramolecular inclusion complexes of active compounds that can be of interest in medicine, pharmacology, artifical light-harvesting, and other areas. Most studies concern the inclusion complexes of cyclodextrins that are widely used in practice as agents for transporting and conserving drugs.[1−3] 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. Therefore, the search is being continued for complexing agents devoid of these disadvantages. One of these compounds is glycyrrhizic acid (or glycyrrhizin). β-Glycyrrhizic acid (GA) is a compound that belongs to the triterpene glycoside family and contains both hydrophilic (glucuronic acid) and hydrophobic (glycyrrhetic acid) regions. GA is extracted from the Ural licorice root (*Glyzyrrhiza glabra L*).

There is evidence in the literature that glycyrrhizic acid in solution can create cyclic structures that can form inclusion complexes with various organic compounds.[4−5] GA is of considerable interest to pharmacologists because of its unique



[GA]

physiological activity. Its preparations are especially popular in connection with AIDS treatment.[6] This compound is particularly attractive for three main reasons. First, GA in contrast to the cyclodextrins has an open chain structure, and thus, for complex formation, there are no rigorous restrictions on the size of a "guest" molecule. Second, the authors who measure some parameters of GA complexes with various drugs indicate their unusual stability.[4,5,7,8] The stability constant of GA complexes is in the range of 10^5 M^{-1}, which is 2 orders of magnitude higher than a mean stability constant of cyclodextrin complexes.[9] Third, it was demonstrated also that application of glycyrrhizic acid together with other medicines strengthens their therapeutic action (sometimes, by orders of magnitude) and reduces side effects, e.g., the toxic action on the alimentary canal.[7,8,10,11] Studies on the mechanisms of glycyrrhizic acid complexation are of practical and theoretical interest because physicochemical data

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on the structure and properties of its complexes are scarce. It is worth noting that at present progress in developing novel forms of medicines has been related not only to a search for new active substances but also to regulating the effect of already available preparations. Complexation is one method for regulating this effect.

Carotenoids are a class of pigments widely found in nature. These essential nutrients are synthesized by plants and microorganisms and exist in many foods including vegetables, fruits, and fish. About 600 various carotenoids are known. However, only a few (about 20) have been found in human tissues. These include β-carotene, canthaxanthin, zeaxanthin, etc. The presence of a polyene chain and various terminal substituents in carotenoid molecules determines both their redox properties and their location inside the lipid layers in biological media. In most of natural processes, including photosynthesis, their role is most often related to the reactions of energy and electron transfer.[12−13] Photoinduced electron transfer in heterogeneous hosts has recently attracted more and more interest in connection with the design of artificial light-harvesting, photoredox, and catalytic systems. In addition, recently much attention has been focused on the reactions between carotenoids and free radicals[14−19] because of the ability of carotenoids to prevent the development of diseases caused by toxic free radicals.[20] 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.

Fundamental and applied research has recently been devoted to the inclusion complexes of carotenoids with natural compounds, specifically the cyclodextrins,[21−28] which are assumed to possess protective properties and to decrease the hydrophobic behavior of the included molecules. Application of inclusion complexes was first related to an attempt to minimize the aforementioned disadvantages of carotenoids when used in the food industry, cosmetology, and medicine.

The present paper concerns the complexation of carotenoids with β-glycyrrhizic acid. The complexation was detected by changes in absorption and fluorescence intensity of carotenoids in the presence of GA. In addition, we studied the influence of GA on radical processes involving carotenoids. The reactivity was studied using electron transfer with acceptors, quinones, and iron ions. These processes have been previously studied in detail in homogeneous solutions.[29−30]

Experimental Section

Glycyrrhizic acid is extracted from the Ural licorice root. The methods of GA purification are described elsewhere.[31] Carotenoids **I** and **III** (Figure 1) were provided by Fluka, carotenoid **IV** by Roche Vitamins and Fine Chemicals, Nutley, NJ, and carotenoid **II** by Professor Molnár, the University of Pécs, Hungary. The others (**V** and **VI**) were synthesized at the University of Alabama.[32−33] All carotenoids were kept at −18 °C in sealed ampules. The purity was assessed by ^{1}H NMR spectroscopy (360 MHz, $CDCl_3$) and thin-layer chromatography (TLC). The solvents, acetonitrile (Fisher, HPLC grade), methanol, ethanol (Fisher, ACS grade), and dimethylsulfoxide (DMSO, 99.5%, Aldrich, ACS grade) were used as obtained.

The stoichiometry and stability of complexes were measured by conventional methods[34,35] and were detected by changes in either absorption spectra or fluorescence of carotenoids depending on GA concentration. Absorption spectra were recorded of aqueous solutions using a Shimadzu UV−vis 1601 spectrophotometer, and the fluorescence spectra were recorded of DMSO solutions with a FluoroMax-2 (SPEX Industries) fluorimeter.

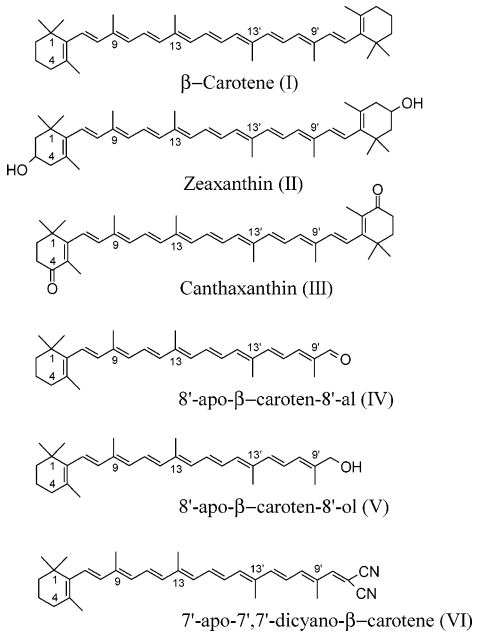

Figure 1. Structural formulas of carotenoids.

The reaction of carotenoids with the electron acceptors, $FeCl_3$, and dichlorodicyanobenzoquinone (DDQ) was initiated by adding the acceptor solution to the solution of the complex in either acetonitrile or methanol. The reaction was monitored by changes in the absorption spectrum of the carotenoid and its radical cation. To analyze the products of the reaction between carotenoids and quinone using high-performance liquid chromatography (HPLC), the reaction was carried out in acetonitrile at reagent concentrations in the range of 0.01−0.1 mM at room temperature. For details see ref 36. A Vydac 201 TP54 polymeric C18 column (250 mm × 4.6 mm i.d.) packed with 5 mcm 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.

Results and Discussion

In the present work, conclusions about the formation of inclusion complexes of carotenoids with glycyrrhizic acid are based on changes in both the absorption and the fluorescence spectra of carotenoids in the presence of GA and their reactivity. Control measurements (the addition of acetic acid with $pK_a \approx 4.6$ to a carotenoid solution that is close to that of GA[5]) indicate that none of the observations can be attributed to changes in *medium* acidity.

Measurement of the Stability and Stoichiometry of GA Complexes in Aqueous Solutions. Since carotenoids are insoluble in water and GA is also poorly soluble in pure aqueous solutions ($<10^{-4}$ M), it is impossible to measure the concentration dependences over the necessary concentration range (10^{-5}−10^{-3} M) in pure water solution. For this reason in model experiments we used water−ethanol mixtures. Previous investigations on the complexes of lappaconitine with GA[8] and β-ionone with cyclodextrin[37] showed that the addition of small amounts of alcohol can slightly decrease complex stability but has no effect on its stoichiometry. Using carotenoids as inclusion compounds for studying the structure of GA complexes was highly convenient. First, the presence of a linear hydrophobic conjugated chain in the carotenoids (Figure 1) implies structural similarity of the complexes. Second, the large extinction coefficients of carotenoids ($\sim 10^5$) make it possible to use optical

methods and thus to work with very low concentrations. Note that the use of optical methods for inclusion complex analysis is more convenient compared to NMR techniques in the case of carotenoids. This is due to two reasons. First is the very low solubility of carotenoids in water and even in alcohol solutions. Second, the sensitivity of NMR spectra to complex formation decreases considerably in the presence of alcohol or other organic solvents. NMR techniques are widely used for studying the stability and stoichiometry of cyclodextrin inclusion complexes,[9,37] but in the case of GA complexes the changes in chemical shifts are much lower even in aqueous solutions.[8]

To determine the stability and stoichiometry of complexes, we chose the conventional, well-tested methods for analyzing the concentration dependence of absorption and fluorescence spectra.[34−35] Experimentally, all carotenoids showed nearly the same change in extinction coefficient at a fixed wavelength in the presence of GA (∼10%). The stoichiometry of the complexes was calculated using Job's plot of the dependence of the optical density of the solution with mole fraction of carotenoid at a constant total concentration of reagents: [Car] + [GA] = constant. Since this experiment requires variations over a wide concentration range of both GA and carotenoid, the measurements were performed with only the two carotenoids **IV** and **V** that displayed the best solubility in alcohols. Both carotenoids have the same Job's plot. The position of a maximum of the curve at $R = [\mathrm{Car}]/([\mathrm{Car}] + [\mathrm{GA}]) = 0.33$ corresponds to the 1:2 ratio between carotenoid and GA molecules in the complex. In the literature it was suggested that in aqueous solutions GA molecules form cyclic dimers of either the torus[4] or the podant[5] type.

To estimate the complex stability constant, the change in optical density of the solution was measured at a fixed concentration of carotenoids with varying GA concentration (Figure 2).

Under certain experimental conditions ([Car] ≪ [GA]) the Benesi−Hildebrand plot (eq 1) can be used to estimate both the complex stability constant and the order of complexation from a single experiment

$$A/\Delta A - 1 = 1/[\mathrm{GA}]^n \times 1/K \qquad (1)$$

Here $\Delta A = \Delta\epsilon \times [\mathrm{Car}]$, and K is the stability constant of the complex for the reaction

$$\mathrm{Car} + n\mathrm{GA} \overset{K}{\rightleftharpoons} \mathrm{CarGA}_n$$

$$K = \frac{[\mathrm{CarGA}_n]}{[\mathrm{Car}] \times [\mathrm{GA}]^n} \qquad (2)$$

The plot of $A/\Delta A$ versus 1/[GA] provides the linear dependence for a second-order reaction ($n = 1$). For a third-order reaction ($n = 2$), the linear dependence would be obtained in the coordinates of $A/\Delta A$ versus $1/[\mathrm{GA}]^2$. In our experiments, in all cases, the linear dependence was obtained for $n = 1$ only. Taking into account the result of the analysis of Job's plot, it was concluded that the reaction of complexation is second-order between one carotenoid molecule and one dimer of glycyrrhizic acid

$$\mathrm{Car} + \mathrm{GA}_2 \overset{K}{\rightleftharpoons} \mathrm{CarGA}_2$$

Computer simulation of the experimental concentration dependence in Figure 2a as well as the estimation of the complex stability constant from eq 1 provide the same value $K = 10^4$ ($\pm 10^3$) M^{-1} for the β-carotene−GA complex. Carotenoids **IV** and **V**, whose solubility in water−alcohol solutions is greater than that of β-carotene, form less stable complexes ($K \approx 10^3$ M^{-1}), as expected from the hypothesis that hydrophobic interactions are essential for complex formation with GA.

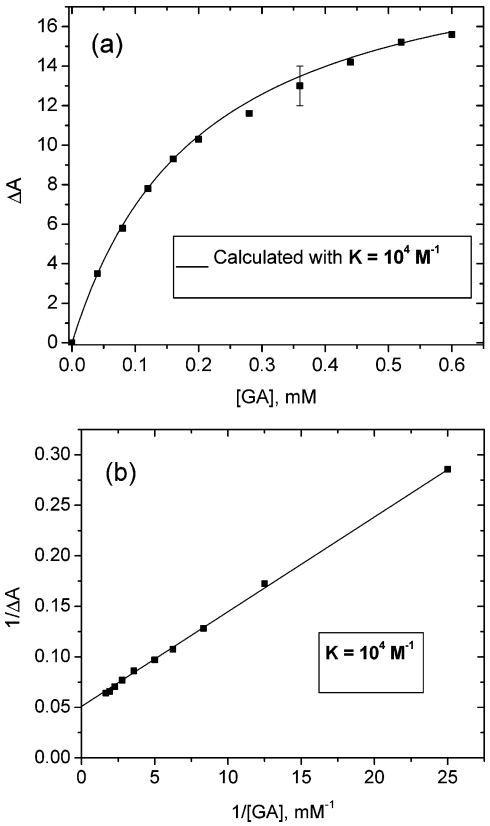

Figure 2. (a) Optical density (A) of carotenoid **I** (2.5 μM) at 440 nm vs GA concentration in 20% ethanol solution. (b) Benesi−Hildebrand plot.

To prove the hypothesis that hydrophobic interaction is the main driving force of the carotenoid−GA complex formation, a thermodynamics study was carried out for carotenoid **IV** in the temperature interval 293−306 K. Through the use of the relevant equations, $-\Delta G = RT \ln K$, $\Delta G = \Delta H - T\Delta S$, and $\ln(K_2K_1) = -(\Delta H/R)(1/T_2 - 1/T_1)$, the following parameters were obtained. The enthalpy of this complex formation was determined to equal +42.9 kJ/mol, $\Delta G = -19.9$ kJ/mol, and $\Delta S = +211$ J/(mol K) at $T = 293$ K. The experimental error in this study was about 25%. Positive values of both enthalpy and entropy contributions point out that hydrophobic interaction is an important factor in the binding, and the complex formation follows considerable desolvaion of ligand molecules. This feature is characteristic for inclusion complexes. There are several examples of thermodynamics studies of molecular complex formation of β-glycyrrhizic acid with various organic molecules.[5,7] It was found that β-glycyrrhizic acid forms 1:2 complexes for these "guest" molecules, most frequently values characteristic of hydrogen-bond formation. It should be noted that the heat of formation of molecular complexes is generally dependent on the electronegativity of the donor group in the "host" molecule. A linear correlation was found between the formation enthalpies of GA complexes and the Hammett constants of the substituents in the nitro derivatives.[5] Another study[7] shows the presence of both enthalpy and entropy contributions to the complex formation of GA with two drugs, 8-hydroxy-5-nitroquinoline and nitroglycerin. Indeed, the presence of a number of carboxy and hydroxy groups in the GA

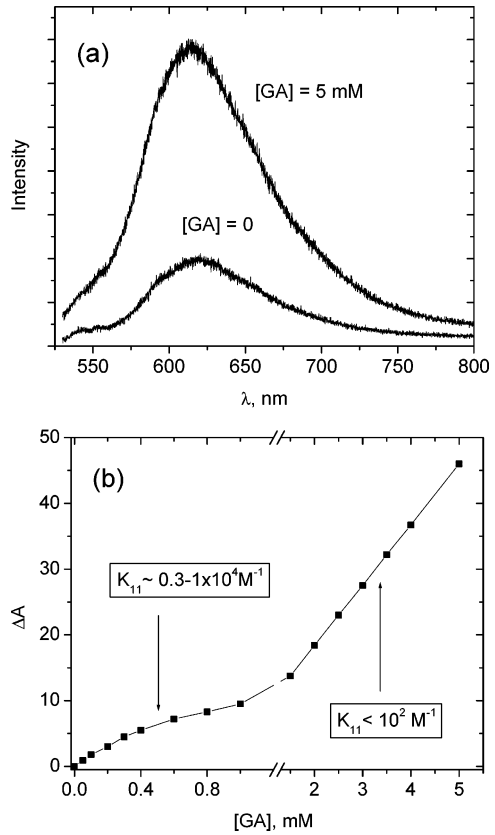

Figure 3. (a) Fluorescence spectrum of canthaxanthin (**III**) solution, 0.02 mM with and without GA in DMSO containing 5% of water. The excitation wavelength is 470 nm; the detection wavelength is 620 nm. (b) Dependence of the fluorescence intensity of carotenoid **III** on GA concentration in solution.

and guest molecules is favorable for intermolecular hydrogen-bond formation in these cases. All these data demonstrate that the high stability constants of the complexes of β-glycyrrhizic acid can be attributed to the mutual effects of both enthalpy and entropy contributions to the complex formation.

Measurement of the Stability and Stoichiometry of GA Complexes in Nonaqueous Solutions. In the present work, we investigated the possibility of complex formation between carotenoids and GA in organic solvents. Since the optical absorption spectra of carotenoids are insensitive to the presence of GA in nonaqueous media, an attempt was made to study the influence of GA on the fluorescence spectrum of carotenoids. Canthaxanthin (**III**) was used in this investigation. The fluorescence method is widely used to study inclusion complexes of the "guest−host" type. There are many examples of an increase in fluorescence intensity for cyclodextrin complexes.[38−40] The luminescence of molecules imbedded in the cavity of cyclodextrin is strengthened by the protection against quenching and other processes occurring in solution. The usual form of the concentration dependence of the fluorescence intensity of the included compounds is similar to the changes in optical density of the solution (see, for example, Figure 2a), which reaches a plateau at $K \times [GA] \gg 1$. In the present work (Figure 3), the increase of fluorescence intensity of carotenoid **III** in the presence of 1 mM GA in pure DMSO was about 15% from the value in the absence of GA (Figure 3b). The addition of a small amount of water (5%) to DMSO had no influence on the fluorescence intensity in the absence of GA but leads to an increase of the effect up to 50% in the presence of 1 mM of GA. The complex stability constant in DMSO was estimated by the methods of analysis applied to aqueous solutions. Over this concentration range (0−1 mM of GA), the calculation provides the value $K = 0.3-1 \times 10^4$ M^{-1}. The large error in the calculation of the stability constant is due to the superposition of two processes. One can see that in the plot of the concentration dependence the fluorescence intensity does not reach the plateau but starts to increase linearly when the GA concentration exceeds 1 mM (Figure 3).

Similar changes in the properties of GA solutions at the same concentration (1 mM) have been observed previously.[41] The authors established that when the GA concentration is about 1 mM in a water−ethanol solution (5−10% ethanol), the viscosity of the solution changes precipitously. It was assumed that at this concentration in aqueous solutions GA forms structures of the micellar type. This hypothesis for micelle formation was confirmed by studying the processes of micelle formation of water-soluble GA derivatives, in particular, their sodium sulfates.[6] We failed to find data on micelle formation in other solvents in the literature. Coincidence of the values of the GA concentration at which the peculiarities are observed in the concentration dependence in DMSO measured in the present work and the critical concentration of micelle formation in water[6] indicates that both hydrophobic and dipole−dipole interactions contribute to the process of complexation.

It is worth noting that a study of complexation should not be limited to stabilization and an increase in the bioaccessibility of inclusion compounds. It is also possible that an increase in carotenoid stability in complexes can lead to a loss of some desirable properties, e.g., antioxidant and light-protecting activity. A previous paper has shown that blocking of a cyclohexene ring by cyclodextrin causes a substantial decrease in the rate of peroxide radical scavenging by carotenoids,[28] in accord with the earlier hypothesis for binding of peroxide radicals to the terminal double bonds of carotenoids, resulting in the formation of corresponding adducts.[15,18] Therefore, an important aspect of the present work is a study of carotenoid reactivity in a complex.

Interaction of Carotenoids and Their Complexes with $FeCl_3$ in Polar Media. As shown in the Introduction, one of the most important natural processes involving carotenoids is electron transfer from carotenoids to acceptors. In the present work, the influence of GA on the reactivity of carotenoids was studied using Fe^{3+} ion as an acceptor. The mechanism of the reaction between carotenoids and $FeCl_3$ has been studied in detail.[30] It was shown that the first step of the reaction is the electron transfer resulting in formation of the carotenoid radical cation

$$\mathrm{Car} + \mathrm{Fe}^{3+} \rightleftharpoons \mathrm{Car}^{+\bullet} + \mathrm{Fe}^{2+}$$

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

$$\mathrm{Car}^{+\bullet} + \mathrm{Fe}^{3+} \rightleftharpoons \mathrm{Car}^{2+} + \mathrm{Fe}^{2+}$$

Carotenoid radical cation and dication are subject to cis−trans isomerization. In the presence of oxygen, the oxidation of carotenoids leads to a stable product, a 5,8-epoxide. Note also 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.[42−43]

In the present work, this process was studied using acetonitrile as the solvent, because the carotenoid radical cations are unstable in aqueous solutions and are not detectable in stationary absorption spectra. In acetonitrile, all carotenoids form relatively stable radical cations according to their absorption spectra. The strongest radical cation signal was observed for β-carotene,

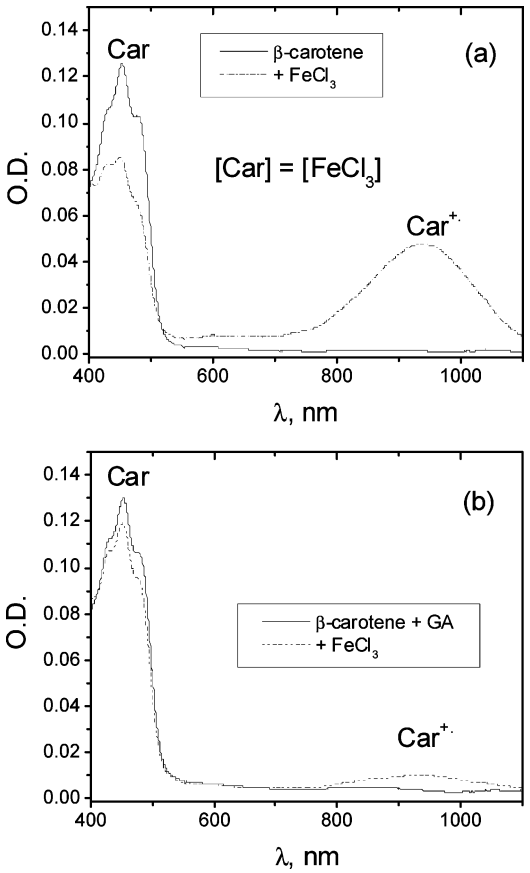

Figure 4. Absorption spectra of β-carotene, 1.3 μM, recorded before and after mixing with $FeCl_3$, 1.3 μM, in acetonitrile at room temperature: (a) in the absence of GA; (b) in the presence of 0.1 mM GA.

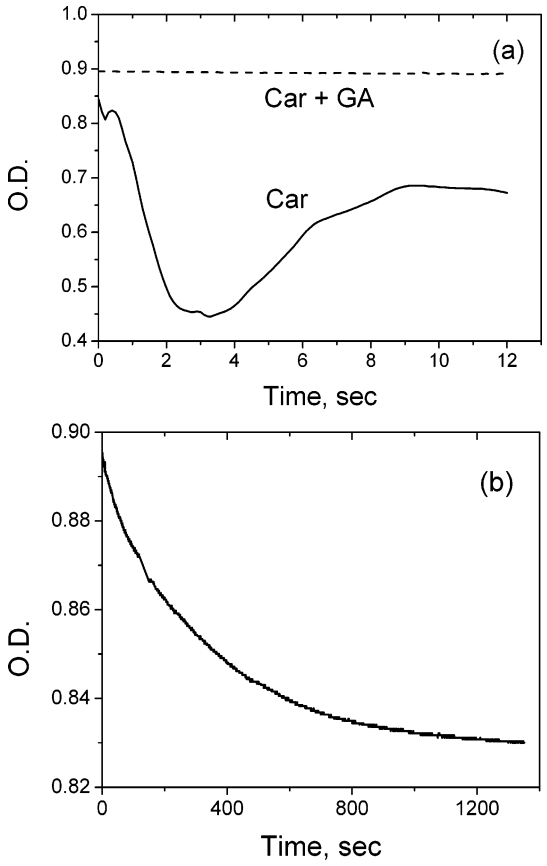

Figure 5. (a) Signal decay kinetics at a maximum of carotenoid **IV** absorption with and without 0.1 mM GA at 455 nm in acetonitrile at −30 °C. (b) Signal decay kinetics of the complex at 455 nm in acetonitrile at long reaction times. [Car] = [$FeCl_3$].

which has the lowest redox potential, $E^{ox}_{1/2} = 0.54$ V vs a saturated calomel electrode (SCE)[44] (Figure 4). For other carotenoids, the signal intensity at room temperature was ~10% of that recorded for β-carotene. Remember that in organic solvents complexation of carotenoids cannot be followed by classical methods (absorption or NMR spectra). Therefore, in this case, the conclusions about the interaction between carotenoids and GA are based on the changes in the reactivity of carotenoids in the presence of GA.

Figure 4b shows the absorption spectra of the β-carotene/GA complex recorded before and after mixing with $FeCl_3$. The absorption of radical cation resulting from the reaction is observed at 935 nm. As follows from comparison of Figures 4a and 4b, in the presence of GA, there is a considerable decrease in the yield of β-carotene radical cation, which can be attributed to a decrease in the electron-transfer rate in the case of complexation. The conclusion about the influence of GA on the reaction kinetics can be derived from the time dependence of the carotenoid signal. Since at room temperature the reaction of direct electron transfer for β-carotene is very fast, kinetic measurements were performed at low temperatures for carotenoids with redox potentials exceeding that of β-carotene. Thus, for carotenoid **IV** ($E^{ox}_{1/2} = 0.72$ V vs SCE[44]), we measured the signal decay kinetics at 455 nm and −30 °C (Figure 5).

The rapid decay during the first 2 s of the reaction can be assigned to the reaction of direct electron transfer resulting in formation of the corresponding radical cation. As mentioned above, the major products of a given reaction are carotenoid isomers formed during the radical cation lifetime.[30] It is assumed that the subsequent increase in absorption at 455 nm is due to the absorption of the neutral molecules of carotenoid cis and trans isomers resulting from back electron transfer. As follows from Figure 5a, the reaction between carotenoid−GA complex and ferric chloride (1 equiv) is substantially slower. At longer reaction times (Figure 5b), a further decrease in absorption at 455 nm can be ascribed to decay and slower redox processes (the reaction with oxygen[30]).

With excess $FeCl_3$, all carotenoid is relatively rapidly transformed into the radical cation both with and without GA (Figure 6).

The first and second redox potentials of β-carotene nearly coincide.[44] Thus, the three species, neutral, radical cation, and dication, are present when oxidation occurs and are in comproportionation equilibrium

$$2Car^{+\bullet} \rightleftharpoons Car^{2+} + Car$$

The behavior of β-carotene radical cation is quite different in the absence or presence of GA (Figure 6). Whereas a free radical cation transforms rapidly into dication (absorption at 889 nm), in the complex the radical cation is stabilized; i.e., it can be observed for a much longer period (tens of minutes). Since the rates of electron transfer (both the first and the second) decrease significantly, the charged forms of carotenoids (cations and dications) do not leave the complex after the reaction and are also stabilized inside the cavity. Recall that control measurements with the addition of acetic acid indicate that all observed effects are not due to a change in medium acidity.

Interaction of Carotenoids and Their Complexes with DDQ in Polar Media. Another example of the influence of GA on the reactivity of carotenoids is the electron transfer from

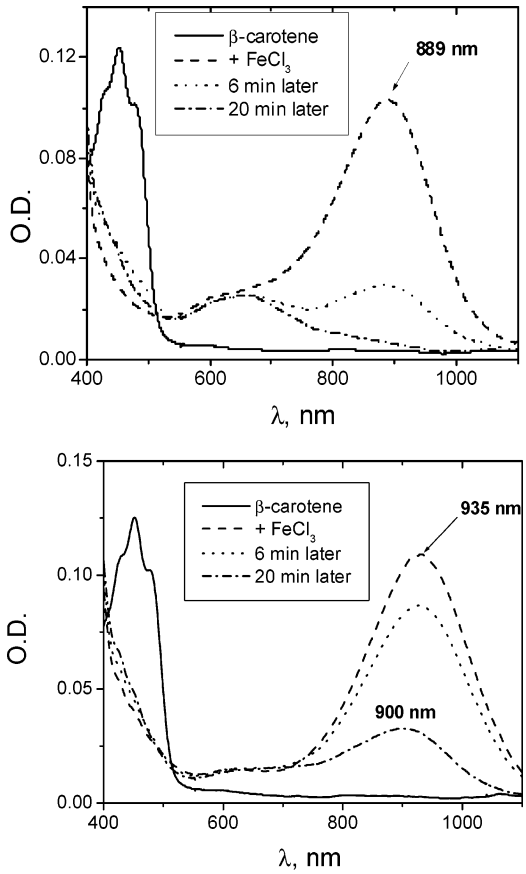

Figure 6. Absorption spectra of β-carotene, 1.3 μM, recorded before and after the mixing with $FeCl_3$, 7 μM, in acetonitrile at room temperature: top, in the absence of GA; bottom, in the presence of 0.1 mM GA. The signal at 935 nm is due to the β-carotene radical cation, and that at 889 nm the dication of β-carotene.

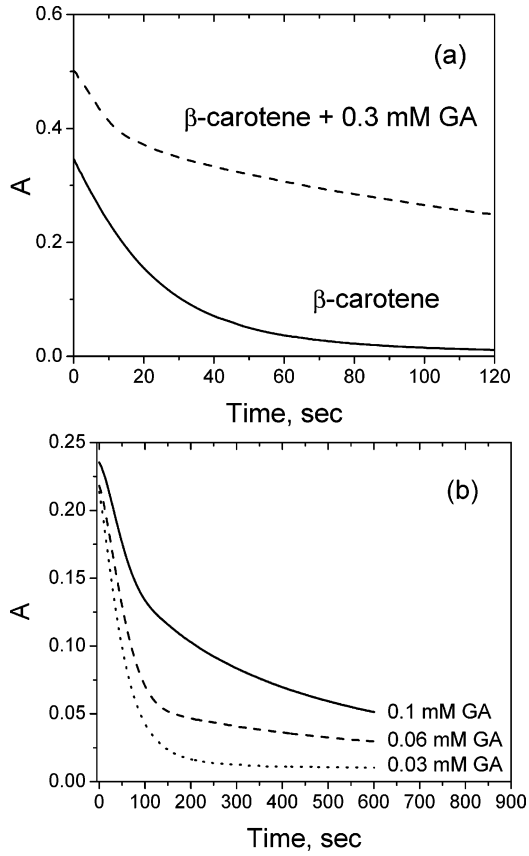

Figure 7. Kinetics of the decay of β-carotene radical cation at 935 nm: (a) 4 μM β-carotene + 4 μM DDQ with and without GA; (b) 2 μM β-carotene + 5 μM DDQ for various GA concentrations.

carotenoid to DDQ. Quinones are known to be important natural electron acceptors in photosynthetic centers.[45−46] As a model system for studying the reactivity of inclusion compounds, we used DDQ, which, owing to its low reduction potential, reacts with carotenoids without additional initiation by either light or temperature. It has been shown that in this reaction a radical ion pair (RIP) is formed via an intermediate charge-transfer complex (CTC)[29]

$$\mathrm{Car} + \mathrm{Q} \rightleftharpoons \mathrm{CTC} \rightleftharpoons \mathrm{Car}^{+\bullet} + \mathrm{Q}^{-\bullet} \rightarrow \text{cis isomers} + \mathrm{Car{-}Q}$$

The product of RIP recombination is the quinone−carotenoid adduct, whereas the radical cations escaping recombination are subject to cis−trans isomerization.[36,47]

As in the reaction with $FeCl_3$, radical cation formation was observed by absorption spectroscopy after the addition of quinone to the carotenoid solution. The peculiarity of a given reaction is the difference in the form of the kinetic curves describing the carotenoid radical cation decay with and without GA (Figure 7a). Whereas for a free carotenoid the radical cation signal decays exponentially with a half-life τ of ~20 s, in the presence of GA there are two processes, i.e., the fast exponential process ($\tau \approx 20$ s) and the slow process $I = a/(1 + t/\tau)$ ($\tau \approx 1000$ s).

The kinetic curves of the signal decay at 935 nm at various GA concentrations show that the contribution of a slow component of the kinetic increases nonlinearly with increasing GA concentration (Figure 7b). It was suggested that the slow decay component is due to the radical cation in the complex. The observed ratio of free carotenoid radical cation and its complex in solution is proportional to the square of the GA concentration, so we can estimate the stability constant of the GA complex with radical cation in acetonitrile from the ratio of slow and fast kinetics components using eq 3

$$K_{12} \times [\mathrm{GA}]^2 = \frac{[\mathrm{CarGA_2}^{+\bullet}]}{[\mathrm{Car}^{+\bullet}]} \qquad (3)$$

Taking into account that this ratio at a GA concentration of 0.1 mM is about unity (Figure 7b) we can estimate the complex stability constant in acetonitrile to be near 10^8 M^{-2}. The two-component kinetics of the radical cation signal decay indicates that the carotenoid radical cations are present in solution in equilibrium with both the free and the complex forms. Note that the contributions of these states can be observed separately only in the absence of a fast exchange between these states.

Thus, as in the previous example, we conclude that the carotenoid radical cations are bound in the complex. An increase in the lifetime of the radical cation is expected to cause an increase in the yield of cis isomers. If, however, the CTC is imbedded in GA, the yield of the "cage" product, i.e., the carotenoid−quinone adduct, should increase. To elucidate this problem, we analyzed the composition of the reaction products for several carotenoids (**I**, **III**, and **VI**) by means of HPLC. In all cases, the result was the same, i.e., a substantial increase in the yield of the adduct.

Figure 8, the chromatogram of the products of the reaction between carotenoid **III** and quinone with and without 0.2 mM GA, shows the decrease of the amount of initial compounds (DDQ and carotenoid) in the presence of GA as well as

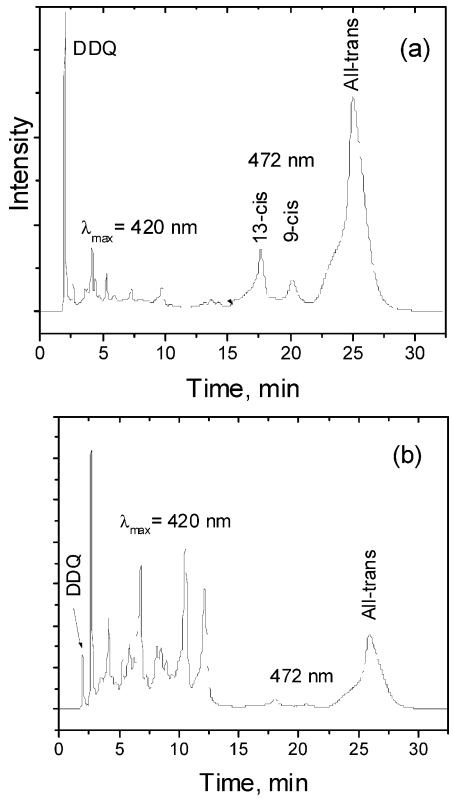

Figure 8. HPLC chromatogram detected at 420 nm in acetonitrile of the products of the reaction between carotenoid **III** and dichlorodicyanobenzoquinone (concentrations of 0.06 mM): (a) without and (b) with 0.2 mM GA.

carotenoid isomers (17−27 min). Simultaneously an increase of the yield of adducts (3−10 min) was detected. A large number of adducts are due to the possibility of the addition of quinone at the various double bonds of the conjugated canthaxanthin chain.

This result allows us to conclude that GA can form stable structures not only with individual compounds and their ions but also with charge-transfer complexes. As has been shown, in the case of carotenoids, this leads to a change in both the reaction direction and the ratio of products. The present work is a first example of inclusion complexes of GA with unstable radical intermediates. Only a few examples exist of inclusion complexes of cyclodextrins with organic anion and cation radicals.[48−49]

Conclusion

In the present work, we have verified the formation of host−guest complexes of carotenoids with GA and measured the stoichiometry and stability constants of these complexes. Our data indicate that glycyrrhizic acid forms complexes with carotenoids of a 1:2 composition, $CarGA_2$, in both aqueous and organic polar solvents: DMSO, acetonitrile, and alcohols. In all solvents, the complex stability constant is about 10^4 M^{-1}. The complex structure is suggested to consist of a carotenoid molecule located within a torus formed by the glycyrrhizic acid dimer (Figure 9).

It was established that complexation 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 quinone, an increase in the lifetime of radical cations in complexes, and a change in the ratio of reaction products. High stability of the carotenoid radical cation imbedded into GA host opens wide possibilities for the application these carotenoid−GA complexes for the design of artificial light-harvesting, photoredoxm and catalytic systems.

Finally, we would like to point out a potential practical application of the data. 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 great importance because of their predictive potential. 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 important, 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.

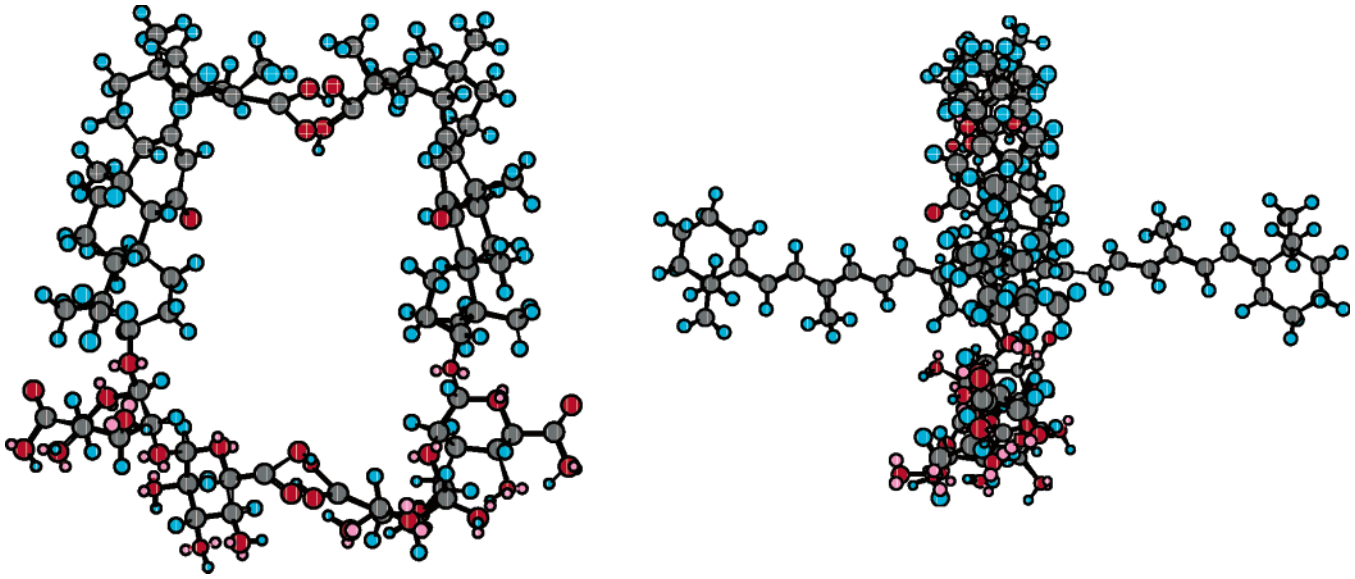

Figure 9. Schematic Chem3D Pro (Cambridge Software, Cambridge, MA) presentation of the suggested structures of the GA dimer and their inclusion complex with the carotenoid.

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