Water Soluble Carotenoid Conjugates of with Oligo- and Polysaccharides. Synergy of Drug Transport and Efficacy.

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ABSTRACT

Water soluble carotenoid conjugates with β-glycyrrhizic acid (GA) and natural polysaccharide arabinogalactan (AG) were studied for the first time by various physicochemical techniques. Carotenoid conjugates exhibit several technological properties that could be used for food processing (food colorants and antioxidants) and for the production of therapeutic formulations (water soluble drugs and vitamins) with increased bioavailability. An important result for uses in photodynamic therapy was obtained after studying the generations of the photoinduced hydroxyl radical formed on the surface of TiO_2 nanoparticles in aqueous solution. A considerable increase in the yield of free radicals was detected in the presence of the carotene-AG complex. On the other hand, complexes of some carotenoids with glycyrrhizic acid show enhanced ability (in orders of magnitude) to scavenge free radicals. In addition, several examples of AG and GA conjugates with various drugs will be demonstrated.

Keywords: carotenoid conjugates, glycyrrhizic acid, arabinogalactan, water solubility, free radicals

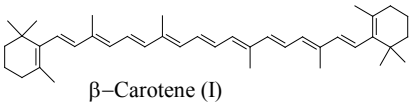
1 INTRODUCTION

Carotenoids are well known as a class of antioxidant pigments wide-spread in nature. 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 metal ions, and high photosensitivity. The preparation of supramolecular conjugates with natural oligosaccharides or polysaccharides minimizes the aforementioned disadvantages of carotenoids when these compounds are used in food processing (colors and antioxidant capacity) as well as for the production of therapeutic formulations considering the better solubility and consequently higher bioavailability. Here we report a study of the conjugates of carotenoids with a triterpene glycoside, β-glycyrrhizic acid (GA) and the natural polysaccharide arabinogalactan (AG), by various physicochemical techniques.

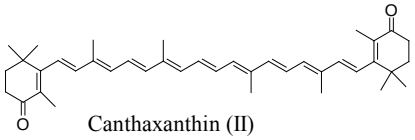
Both compounds are of considerable interest to pharmacologists because of their unique physiological activity. Also, there are no rigorous restrictions on the size of a "guest" molecule in contrast to the widely used cyclodextrin complexes. The stability constants of GA complexes are two orders of magnitude higher than the mean stability constant of cyclodextrin (CD) complexes. Finally, it was demonstrated that the application of glycyrrhizic acid strengthens the therapeutic efficiency of some drugs by orders of magnitude and reduces the side effects.

2 MATERIALS AND METHODS

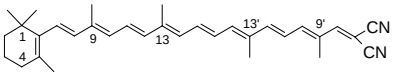
Carotenoids **I** and **II** were provided by Fluka, carotenoid **III** was synthesized at the University of Alabama, USA.



β–Carotene (I)



Canthaxanthin (II)



7'-apo-7',7'-dicyano-β–carotene (III)

Arabinogalactan was extracted from Larix sibirica. 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 pine-like odor. It exhibits a low viscosity, dissolves completely in water or juice, and therefore is easy to administer, even to children [1].

Glycyrrhizic acid is extracted from the Ural licorice root (*Glyzyrrhiza glabra L*). GA is of considerable interest to pharmacologists because of its unique physiological activity. In addition, application of glycyrrhizic acid together with other medicines enhances their therapeutic activity (sometimes, by orders of magnitude) and reduces side effects, e.g., the toxic action on the alimentary canal.

We use electro-mechanochemical equipment for the conjugates preparation. Typical mechanochemical reactions

are those activated by co-grinding or milling of powder materials. Co-grinding of solid materials results in penetration of carotenoid or drug molecules into the polymer of arabinogalactan without use of any organic solvents. This approach allowed us to prepare the water soluble composites of carotenoids **I** and **II** with arabinogalactan for the first time. The estimated solubility of these composites prepared with molar stoichiometry 1:1, and estimated by HPLC analysis, is up to 5 mM in a water solution. X-ray diffraction analysis of solid composites and differential scanning calorimetry were applied for control during sample preparation. NMR and HPLC analysis were used to control the appearance of side reactions during mechanochemical treatment.

See refs [2-9] for more experimental details.

3 RESULTS AND DISCUSSION

3.1 Water soluble conjugates of carotenoids with arabinogalactan

It was demonstrated that the incorporation of carotenoids **I** and **II** into arabinogalactan macromolecule increases the water solubility up to 5 mM and results in significant change in their properties [2].

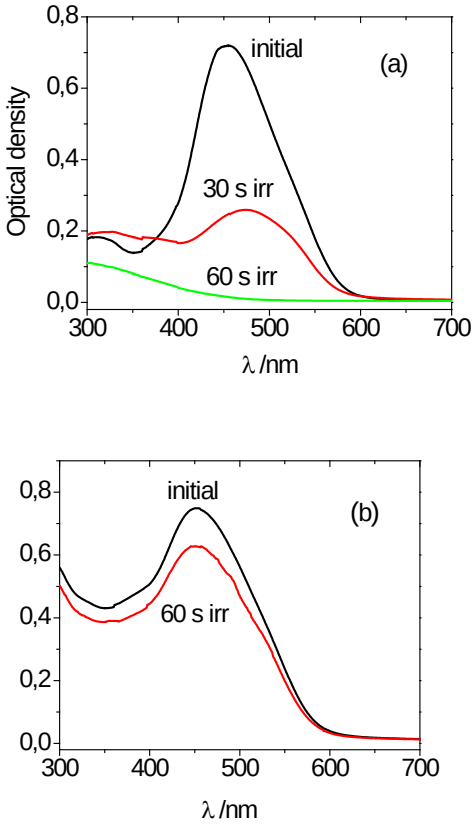


Figure 1: Photodegradation of canthaxanthin (a) in water-ethanol solution (2:1), (b) in arabinogalactan complex.

We have studied the relative photodegradation rate of carotenoids and their complexes with AG using canthaxanthin as an example (Figure 1). The photolysis of pure canthaxanthin and its complex in aerated water-ethanol mixture shows a significant increase in the photostability of canthaxanthin when incorporated into an AG complex. The estimated decrease in the photodegradation rate is ten times for this system. The increase of water solubility, photostability and stability by a factor of 20 when exposed to metal ions has significant importance in the practical application of carotenoids.

An important result for use in photodynamic therapy was deduced during the study of photoinduced hydroxyl radical generated on the surface of TiO_2 nanoparticles in aqueous solution [3]. A considerable increase in the yield of free radicals was detected in the presence of β-carotene-AG complex (Figure 2).

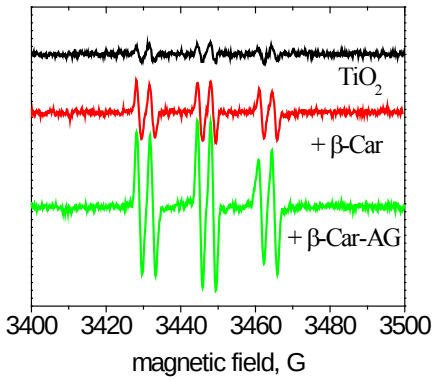


Figure 2: EPR spectra of α-phenyl-*N*-*tert*-butyl nitrone (PBN) spin adducts formed during the irradiation of a water suspension of TiO_2 nanoparticles in the absence (upper) and in the presence of β-carotene or β-carotene-AG complex (lower) with $\lambda > 350$ nm in aqueous solution.

Photosensitization of TiO_2 nanoparticles in aqueous solution by water soluble complexes of carotenoids results in the formation of hydroxyl and hydroperoxyl radicals on visible light irradiation. The electron paramagnetic resonance (EPR) spin-trapping technique has been used to detect free radical intermediates. An analysis of the results indicates that the observed enhancement of photocatalytic efficiency arises specifically from the decrease in the rate constant for recombination. Another important question is the stability of the photocatalyst. Usually carotenoids exhibit a high photodegradation rate in the presence of water. Here it has been shown that the supramolecular complexes of carotenoids with AG exhibit a significant increase in photostability in aqueous solutions as compared with pure carotenoids [2]. These results are important for a variety of TiO_2 applications, namely in photodynamic therapy, in the design of artificial light-harvesting, photoredox, and catalytic devices. Note that the high extinction coefficients of the carotenoid conjugates at

wavelengths 400-600 nm, allow one to extend the irradiation wave band from the UV to the visible range.

3.2 Conjugates of carotenoids with glycyrrhizic acid

The conjugates of carotenoids with glycyrrhizic acid don't show significant water solubility, but in contrast to CD and AG complexes they are soluble in some non-aqueous media (alcohols, acetonitrile, dimethyl sulfoxide), and show high stability in the solvents [4].

It was demonstrated that some conjugates have enhanced ability (in orders of magnitude, see Table 1) to scavenge free radicals [5].

[GA], мМ	I'	II	III
0	4	2	7
0.5	4	59	133
1	4	46	116
2	4	6	38

Table 1: Relative scavenging rates of carotenoids toward OOH radicals in the presence of GA. **I'**: zeaxanthin; **II**: canthaxanthin; **III**: 7',7'-dicyano-7'-apo-β-carotene.

The values given in Table 1 were obtained by subtracting the GA contribution from the total rate constant measured in the presence of glycyrrhizic acid. Thus, there is a synergetic GA effect observed by a several-fold increase in the rate of radical scavenging by carotenoids **II** and **III**. Of interest is the absence of this effect for zeaxanthin and non linear dependence of scavenging rate constants on GA concentration. It was determined from electrochemical measurements that the origin of this effect is the influence of conjugation on the oxidation potentials of carotenoids. The oxidation potentials of carotenoid conjugates showed an increase in $E_{1/2}$ by 0.05 V for canthaxanthin and by 0.03 V for zeaxanthin. Earlier we had shown that there is strong nonlinear dependence of the rate constant of the reaction with peroxyl radicals on the oxidation potential of carotenoids [6]. A change in the oxidation potential for β-carotene and zeaxanthin ($E_{1/2}$ ~ 0.55 V) causes no change in their antioxidant activity (Figure 3). At the same time, the diagram allows us to predict a substantial increase in the reaction rate for carotenoids with $E_{1/2}$ ~ 0.7 V when their oxidation potential increases due to conjugation.

The scavenging rates measured at low GA concentrations (0.5 mM) considerably exceed those measured at high concentrations (2 mM). This fact confirms the hypothesis for the dependence of the structure and properties of GA conjugates on their concentration. We have shown using NMR and gel-filtration chromatography that at concentrations above 1 mM GA forms micelle type aggregates in aqueous solution with about 90 monomers. At this moment we have no data on GA micelles formation in other organic solvents.

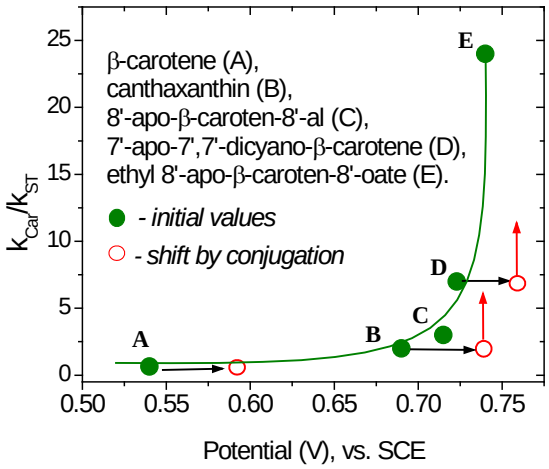


Figure 3: Diagram of the dependence of the carotenoid scavenging rate toward peroxyl radicals with the oxidation potential of carotenoids [6]. Arrows denote the shifts in oxidation potentials due to conjugation.

3.3 Water soluble AG and GA conjugates with insoluble drugs

In this part we provide several examples of AG and GA conjugates with other drugs to demonstrate some advantages of the presented approach.

The solubility of medicinal preparations in water is an important factor for their application. The solubility can be increased using methods based on the formation of their conjugates with water soluble synthetic or natural polymers or oligomers. A mechanochemical approach to the preparation of this kind of conjugates or solid disperse systems that form such complexes on dissolution in water has advantages in comparison with traditional liquid phase processes. First, this method is environmentally friendly, without using any toxic organic solvents; second, this approach results in more stable conjugates with higher solubility, and third, mechanochemically prepared conjugates demonstrate higher therapeutical activity [7].

In our opinion, the use of natural polysaccharides with low molecular weight (of the order of (1-3)•10^4 Da) for conjugate formation is very promising: they possess high water solubility over a wide range of pH, are not prone to form gels, and are easily metabolized. This type of compounds includes arabinogalactans, water soluble polysaccharides of the Siberian larch and the Gmelin larch. Their concentration in dry wood material reaches 15%, which makes AG one of the most accessible among natural and biosynthetic polysaccharides.

In the present work, a mechanochemical preparation of water soluble conjugates of AG and GA with the poorly soluble drugs (Sibazon (=Diazepam), Mezapam

(=Medazepam), Azaleptin (=Clozapine), Nifedipine and Indometacin) has been studied. HPLC and NMR analyses of ethanolic extracts of these conjugates have shown that under selected conditions no chemical reactions of the drugs under study occur during mechanical treatment.

Table 2 shows the increase in solubility of some drugs due to conjugation with arabinogalactan.

Sample	Method	Increase in solubility
Sibazon-AG	A	2.0
	B	3.0
Mezapam-AG	A	10.8
	B	46.8
Azaleptin-AG	A	14.3
	B	38.8
Indometacin-AG	A	1.7
	B	16.8

Table 2: Increase in water solubility of some drugs in arabinogalactan conjugates. A: without mechanical treatment, B: mechanically treated mixture.

In addition, the NMR relaxation method was used to characterize the drug conjugates. It is known that the spin-lattice (T_1) and the spin-spin (T_2) relaxation times are very sensitive to the intermolecular interaction and diffusion mobility of the molecules. Upon formation of a conjugate, the relaxation times of the protons considerably decrease due to slowing of the diffusion mobility. When molecules in a conjugate and in a solution exhibited fast exchange, the NMR signal varies monoexponentially with pulse delay. In the case of slow exchange (in comparison with the relaxation time), a biexponential dependence of the echo decay is observed (1).

$$A(t) = P_1 \exp(-\frac{t}{T_{21}}) + P_2 \exp(-\frac{t}{T_{22}}) \qquad (1)$$

The fast component P_1 corresponds to the fraction of molecules in the conjugate, whereas the slow one P_2 corresponds to the fraction of molecules in solution; T_{21} and T_{22} are the spin-spin relaxation times in the conjugate and in solution, respectively. Typical values of T_2 for the molecules in solution are 0.5—1 sec and 0.03—0.09 sec for the molecules in the conjugate. In all systems under study (excluding the AG—Sibazon conjugate), a biexponential decay of the NMR signal occurs, which is typical for so called "slow exchange". In the mechanically treated mixtures, the fraction of the drug bound with AG is higher than that in the non treated mixtures.

Pharmacological tests show that an increase in drugs activity occur for the AG conjugates as compared with free compounds [7].

The conjugates of the drugs under study with glycyrrhizic acid in water solution also demonstrate a significant increase in pharmacological activity: reduced therapeutic dose and decreased side effects. As an example, the conjugates of well known hypotensive and anti-arrhythmic drugs, Nifedipine and Lappaconitine with GA show an increase in activity by a factor of 300 and 20, respectively [8-9].

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