Water soluble biocompatible vesicles based on polysaccharides and oligosaccharides inclusion complexes for carotenoid delivery

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Abstract

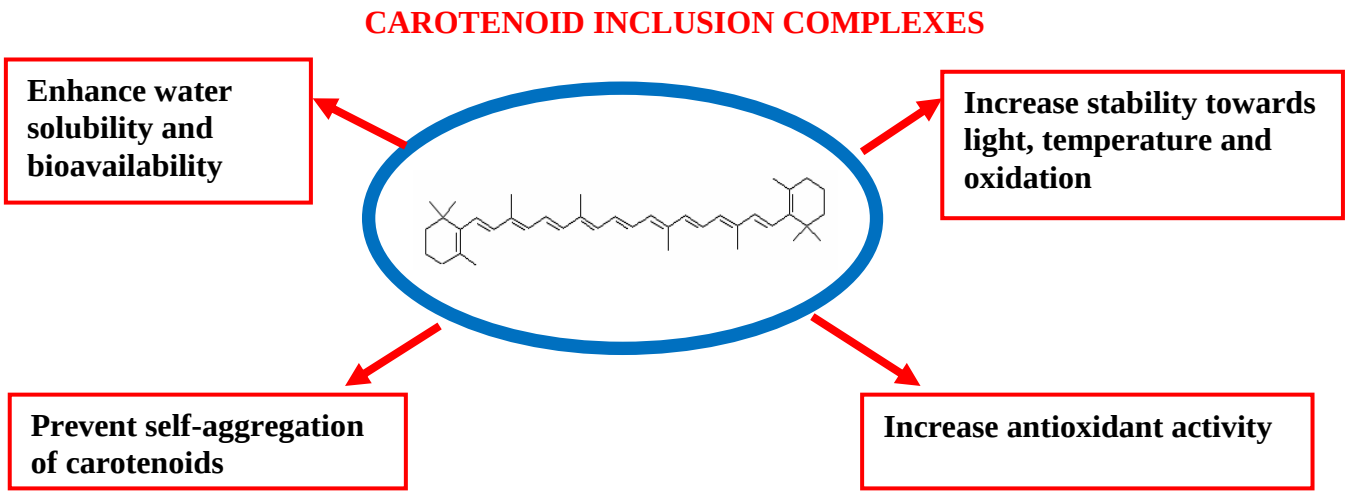
Since carotenoids are highly hydrophobic, air- and light-sensitive hydrocarbon compounds, developing methods for increasing their bioavailability and stability towards irradiation and reactive oxygen species is an important goal. Application of inclusion complexes of "host-guest" type with polysaccharides and oligosaccharides such as arabinogalactan, cyclodextrins and glycyrrhizin minimizes the disadvantages of carotenoids when these compounds are used in food processing (colors and antioxidant capacity) as well as for production of therapeutic formulations. Cyclodextrin complexes which have been used demonstrated enhanced storage stability but suffered from poor solubility. Polysaccharide and oligosaccharide based inclusion complexes play an important role in pharmacology by providing increased solubility and stability of lipophilic drugs. In addition they are used as drug delivery systems to increase absorption rate and bioavailability of the drugs. **In this review we summarize the existing data on preparation methods, analysis, and chemical reactivity of carotenoids in inclusion complexes with cyclodextrin, arabinogalactan and glycyrrhizin**. It was demonstrated that incorporation of carotenoids into the "host" macromolecule results in significant changes in their physical and chemical properties. In particular, polysaccharide complexes show enhanced photostability of carotenoids in water solutions. A significant decrease in the reactivity towards metal ions and reactive oxygen species in solution was also detected.

Keywords: polysaccharides; oligosaccharides; arabinogalactan; glycyrrhizin; cyclodextrins; carotenoids; inclusion complexes; water solubility; oxidation stability; photostability; free radicals; antioxidant activity.

Chemical compounds studied in this article:

Astaxanthin (PubChem CID: 441651); Lutein (PubChem CID: 5281243); Zeaxanthin (PubChem CID: 5280899); Canthaxanthin (PubChem CID: 14523227); beta-carotene (PubChem CID: 5280489); Glycyrrhizic acid (PubChem CID: 14982); Arabinogalactan (PubChem CID: 24847856); beta-Ionone (PubChem CID: 638014)

Graphical Abstract



1. Introduction

Carotenoids are a class of hydrocarbon 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 [Landrum, 2009]. However, only a few (about 20) have been found in human tissues. These include β-carotene, canthaxanthin, zeaxanthin, etc (Figure 1).

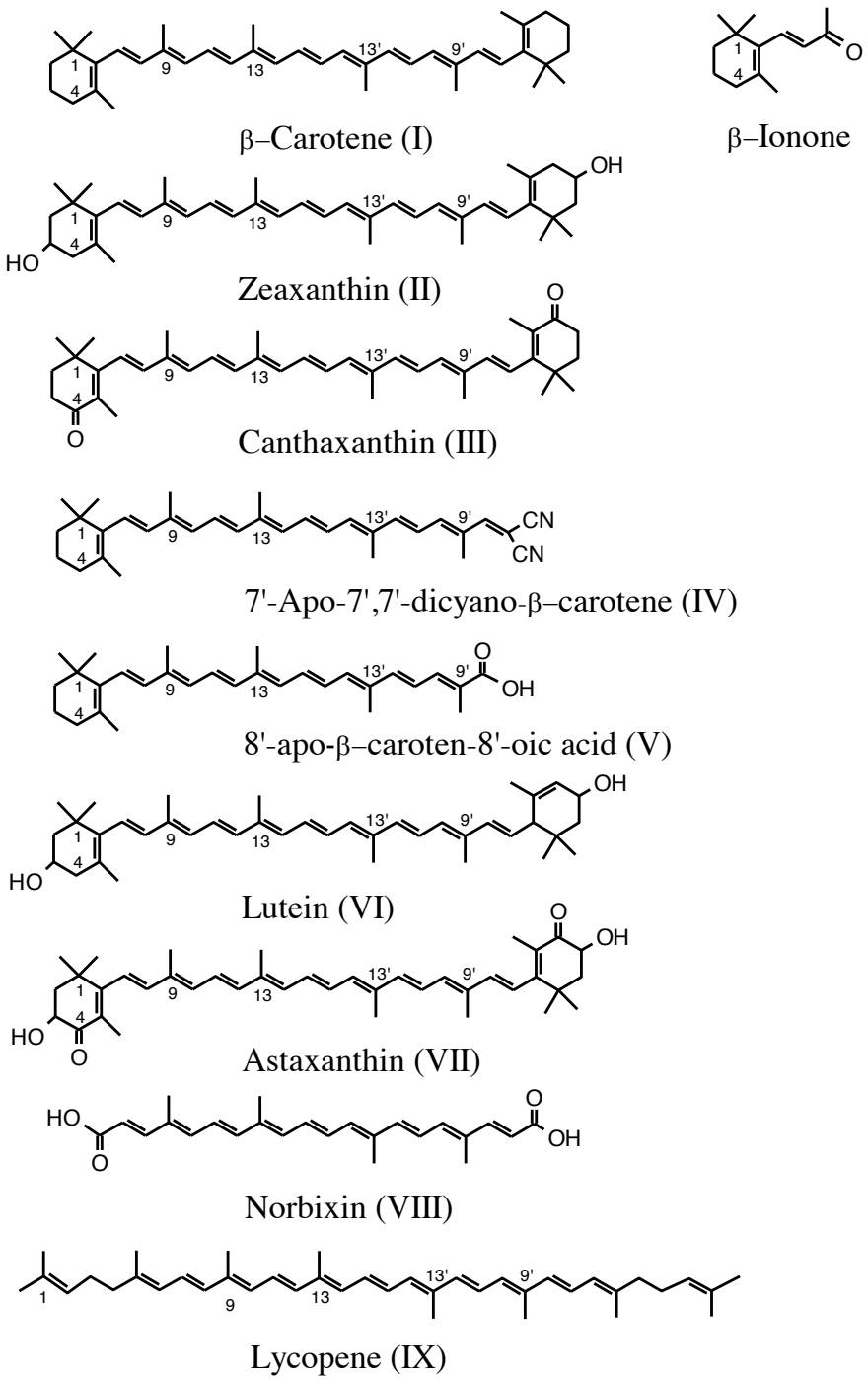


Fig. 1. The structures of β-Ionone, and selected natural and synthetic carotenoids (**I**-**IX**).

The presence of a polyene chain and various terminal substituents in carotenoid molecules determines their color, redox properties and location inside the lipid layers in biological media. For example, lutein and zeaxanthin, which belong to the group of oxygenated carotenoids, are accumulated at the highest concentration in the macula of the human eye, and perform protective functions from UV radiation and free radicals [Mares, 2004]. The reactions between carotenoids and free radicals [Kispert & Polyakov, 2010; Polyakov & Kispert, 2009; Polyakov et al., 2001a; Edge et al., 1997; Woodall et al., 1997; Al-Agamey et al., 2004; Palozza et al., 2001] also attract significant attention because of the ability of carotenoids to prevent the development of diseases caused by toxic free radicals, including cardiovascular disease, cerebral thrombosis, arteriosclerosis, vision disease, tumors and other age-related diseases [Lancrajan et al., 2001; Krinsky et al., 2005; Lemire et al., 2013; Tanumihardjo, 2013]. Carotenoids are assumed to protect cells by scavenging either free radicals or excited oxygen that have a severe impact on cells. Of no less significance are the membrane-stabilizing and immune stimulating functions of carotenoids as well as their provitamin A activity. Vitamin A and carotenoids favor normal metabolism, enhance the resistance of an organism against infections, provide normal operation of the organ of vision, exert beneficial effect on the performance of skin and mucous membranes and are involved in redox processes. At the same time, wide practical application of carotenoids as antioxidants or food colorants is substantially hampered by their hydrophobic properties, instability in the presence of oxygen and metal ions, and high photosensitivity.

One of the promising ways to overcome these problems is the preparation of nanosized carriers for carotenoid stabilization and delivery as well as supramolecular inclusion complexes. Nanosized carriers, such as oil drops, polymers, lipoproteins, liposomes and micelles allow one to prepare water dispersions of carotenoids with enhanced stability [Hou et al., 2014; Toniazzo et al., 2014; Tan et al., 2014; Ascenso et al., 2013; Luxsuwong et al., 2014; Donhowe et al., 2014; Acevedo et al., 2014; Bustos-Garza et al., 2013].

Inclusion complexes are widely used in medicine to improve the solubility and stability of low soluble drugs, for drug delivery, and to decrease their toxicity and side effects [Buschmann & Schollmayer, 2002; Davis & Brewster, 2005; Tolstikova et al., 2009; Polyakov & Leshina, 2011]. 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 (Figure 2).

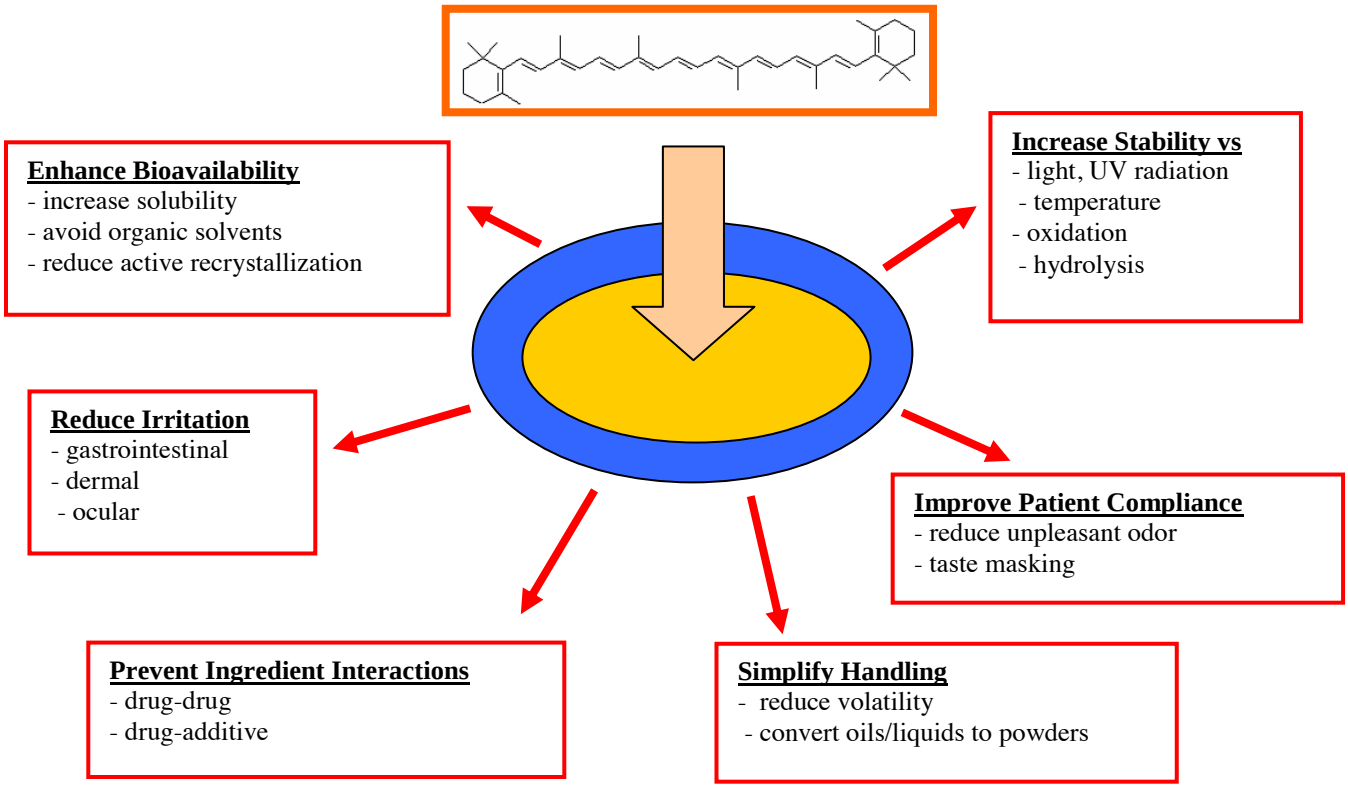


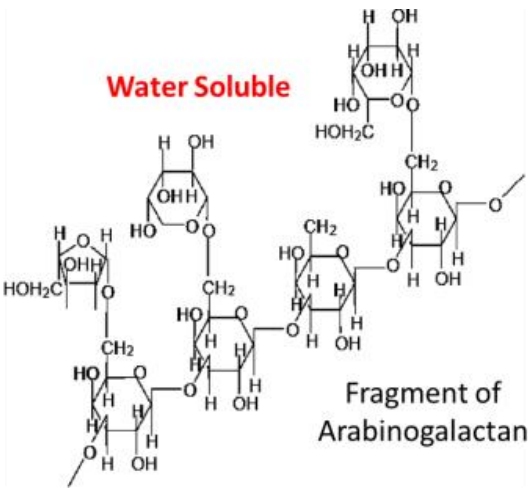
Fig. 2. Diagram of the uses of inclusion complexes in medicine.

Fundamental and applied research has recently been devoted to the inclusion complexes of carotenoids with natural oligosaccharides and polysaccharides, which are assumed to possess protective properties and to decrease the hydrophobic behavior of the included molecules [Polyakov et al., 2004a; 2006a; 2006b; 2009; 2010; 2013; Apanasenko et al., 2015; Gomes et al., 2014; Pinho et al., 2014; Gharibzahedi et al., 2014; Lopez-Nicolas et al., 2014; Yuan et al., 2013; 2012; De Oliveira et al., 2011; Stancanelli et al., 2012; Tachaprutinun et al., 2009]. For example, astaxanthin encapsulated into poly(ethylene oxide)-4-methoxycinnamoylphthaloyl chitosan showed minimal heat degradation after a two-hour heating at 70 °C in an aqueous environment,

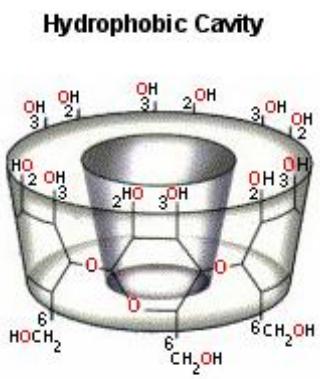
104 in contrast to that of unencapsulated carotenoid molecules which were almost completely
105 destroyed [Tachaprutinun et al., 2009].

106 Most earlier studies involved the inclusion complexes of cyclodextrins (CD, Figure 3B) that
107 were widely used as agents for transporting and conserving drugs [Polyakov et al., 2004a; Davis
108 & Brewster, 2005; Mele et al., 1998; 2002; Szente et al., 1998; Lancrajan et al., 2001]. Although
109 carotenoid-cyclodextrin complexes demonstrate enhanced storage stability [Yuan et al., 2013],
110 an important problem with the use of CD complexes is their poor solubility. In reality, these
111 complexes form water dispersions, rather than solutions. According to the studies of Mele and
112 coauthors [Mele et al., 1998; 2002], carotenoid-cyclodextrin complexes in water form large
113 aggregates with size of 100-200 nm. This results in weakly colored opalescent solutions
114 [Polyakov et al., 2004a]. The reduced color intensity significantly decreases the use of
115 carotenoids, in particular as food colorants.

116 Therefore, the search is being continued for complexing agents devoid of these
117 disadvantages. Recently the first example of water soluble complexes of β-carotene and some
118 other carotenoids with natural oligosaccharide and polysaccharide were reported [Polyakov et
119 al., 2006a; 2006b; 2009; 2010; 2013; Apanasenko et al., 2015]. One of the compounds that
120 appear to be promising is β-glycyrrhizic acid (or glycyrrhizin, GA, Figure 3C). GA belongs to
121 the triterpene glycosides and contains both hydrophilic (glucuronic acid) and hydrophobic
122 (glycyrrhetic acid) regions. GA is extracted from the licorice root (*Glyzyrrhiza glabra L*).



(A)



(B)

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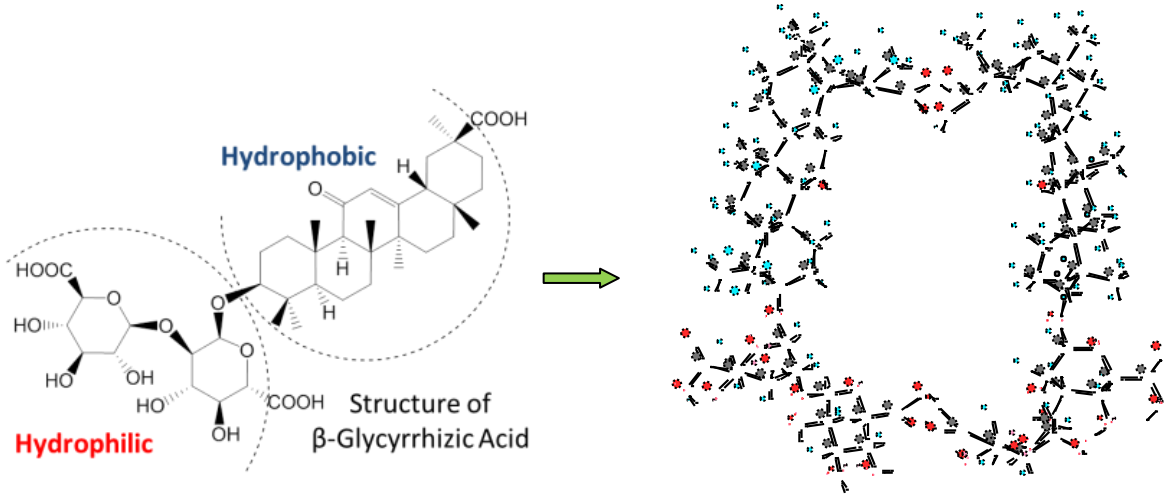


(C) (D, Dimer of glycyrrhizic acid)

Fig. 3. The structures of arabinogalactan (A, fragment), cyclodextrin (B), β-glycyrrhizic acid (C) and Schematic Chem3D Pro presentation of the suggested structures of the GA dimer (D). Arabinogalactan from the western and Siberian Larch produce low-viscosity solutions, and are approved by US FDA as a source of dietary fiber, cancer protocol adjunct and immune stimulating agent. GA complexes demonstrate high stability, reduce side effects and strengthen the therapeutic efficiency of various drugs. The GA "donut" forms a ring around the carotenoid "stick".

It was suggested in many studies that glycyrrhizic acid in solution can create cyclic structures (Figure 3D) that can form inclusion complexes with various organic compounds [Polyakov et al., 2006a; Polyakov et al., 2008; Polyakov & Leshina, 2011; Borisenko et al., 2013], as well as micelles at high concentration [Kornievskaya et al., 2007]. This compound is particularly attractive for many reasons. The first reason is the unusually high stability of GA complexes with various drugs [Maistrenko et al., 1994; Gusakov et al., 2001; Polyakov et al., 2005]. The stability constants of GA complexes are in the range of 10^5 M^{-1}, which are two orders of magnitude higher than the average stability constant for cyclodextrin complexes [Connors, 1996]. However, the most important reason was that the application of glycyrrhizic acid together with other drugs strengthens their therapeutic efficiency by orders of magnitude and exhibits reduced side effects [Tolstikova et al., 2009; Polyakov & Leshina, 2011].

The second "host molecule" is the natural polysaccharide arabinogalactan (AG, Figure 3A), a branched polymer with molecular mass of 13000-16000, consisting of arabinose and galactose

monosaccharides. It is 100-percent water-soluble and produces low-viscosity solutions. Arabinogalactans are found in a variety of plants but are more abundant in the Larix genus, primarily Western and Siberian Larch [Odonmazig et al., 1994; D'Adamo, 1996]. Larch arabinogalactan is approved by the U.S. Food and Drug Administration (FDA) as an important source of dietary fiber, and 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. It is known that AG increases the production of short-chain fatty acids, principally butyrate and proprionate, 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 [D'Adamo, 1996].

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. Also water-soluble carotenoid compositions display several technological applications that could be used in food processing to enhance color and antioxidant capacity. They could also be used for the production of therapeutic formulations given the better solubility and consequently higher bioavailability. It is worth noting that 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. From our opinion, preparation of inclusion complexes with natural polysaccharides and oligosaccharides is one method for regulating this effect.

The present review summarizes the existing data on preparation methods, analysis, and chemical reactivity of a number of natural and synthetic carotenoids with natural oligosaccharides and polysaccharides, namely with cyclodextrins, glycyrrhizin and arabinogalactan. We will focus our attention on the stability and reactivity of carotenoid

complexes in important electron transfer reactions with metal ions and quinones as well as in the reactions with free radicals and reactive oxygen species. These processes have been previously studied in detail in homogeneous solutions [Polyakov et al., 2001b; Gao & Kispert, 2003]. Carotenoid radicals, that are intermediate products in these reactions, attract significant attention since their properties are extremely important for understanding the molecular mechanisms of carotenoid activity [Kispert & Polyakov, 2010; Polyakov & Kispert, 2009; Konovalova et al., 2001; Focsan et al., 2008]. Another fundamental property of the carotenoids is their ability to form aggregates in the presence of water. In particular, xanthophyll carotenoids form J- or H-types of dimer aggregates that significantly changed their photophysical and chemical properties [Polyakov et al., 2013; Ruban et al., 1993; Billsten et al., 2005; Wang et al., 2012; McHale, 2012; Wang & Tauber, 2010]. It has been demonstrated that inclusion complexes protect carotenoids from aggregation and this property increases their antioxidant activity [Polyakov et al., 2013] for GA complexes.

2. Cyclodextrin inclusion complexes.

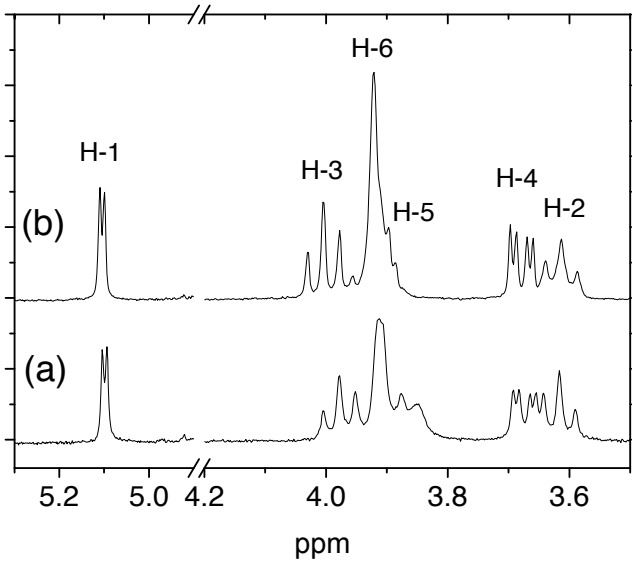
2.1. Preparation and NMR evidence of CD complex formation in aqueous solution.

One can find several practical examples of use the carotenoid-cyclodextrin complexes in the food, cosmetics and pharmaceutical industry [Bartlett et al., 2010; Gomes et al., 2014; Pinho et al., 2014; Gharibzahedi et al., 2014; Lopez-Nicolas et al., 2014; Yuan et al., 2013; 2012; de Oliveira et al., 2011]. In the food industry, carotenoids are mainly used as food colorants and antioxidants. The use of CD complexes instead of pure carotenoids results in increasing stability of the colorants under storage and simplicity in using without first solublization in organic solvents. For cosmetics, carotenoids are used as antioxidants, but limited in application by the intense color of carotenoids. Incorporation of carotenoids into a cyclodextrin cavity reduces significantly their color intensity. In particular, the cosmetic cream with β-carotene-cyclodextrin complex has a nice pink color instead of the saturated red color for pure carotene.

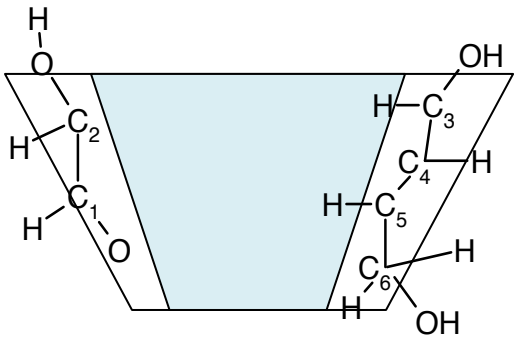
Usually two methods of CD complex preparation are used. In the first method, solid carotenoid and the appropriate amounts of CD are ground together until a homogeneous powder is obtained. Grinding is continued after adding a small amount of water to give a paste, which is then stored overnight under nitrogen, treated with water, and the suspension stirred for several hours. In the second method, the solution of carotenoid in an organic solvent is added to the aqueous CD solution. The precipitation is filtered, dried and then dissolved in water.

NMR spectroscopy is used to detect the formation of a cyclodextrin inclusion complex. For instance, ^{1}H-NMR experiments can provide information about the stoichiometry, stability, and the structure of CD complexes [Connors, 1996; Szejtli, 1996; Yamamoto & Inoue, 1989]. In particular, Job's plot, which correlates the chemical shift and the host/guest ratio, has been widely used to determine complex stoichiometry [Greatbanks & Pickford, 1987; Casy et al., 1991]. The possibility of detecting inclusion complexes by NMR spectroscopy is based on the expectation that if a guest molecule is incorporated into the CD cavity, the screening constants of the CD protons inside the cavity (H_3 and H_5) should be sensitive to the changed environment, but the outside protons (H_1, H_2, and H_4), should not (Figure 4A,B).

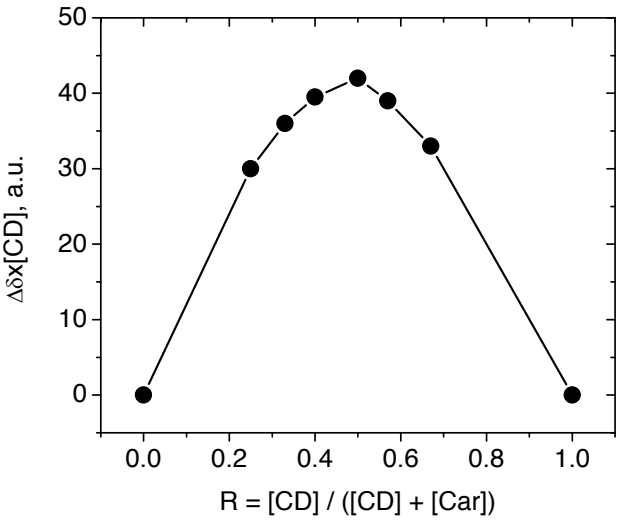
In the case of carotenoids, since sufficiently high concentrations of carotenoid-CD complex (~1 mM) are needed for NMR measurements, the NMR evidence for the inclusion of the cyclohexene ring into the CD cavity was obtained only for β-ionone as a model system containing the same cyclohexene ring as β-carotene [Polyakov et al., 2004b] and partly water soluble sodium salt of apo-carotenoic acid (**V**, Figure 1) [Polyakov et al., 2004a]. It was shown that the terminal cyclohexene fragment of β-ionone, which is present in most carotenoids, has the requisite size for incorporation into the CD cavity. To calculate the inclusion complex stoichiometry and its association constant, the variation of NMR chemical shifts of the CD protons was measured as a function of carotenoid concentration.


H-6
H-1
H-3
H-4
H-5
H-2
(b)
(a)
5.2
5.0
4.2
4.0
3.8
3.6
ppm

(A)


H
O
OH
H
C2
C3
H
C4
H
H
C1
C5
H
O
H
C6
H
OH

(B)


50
40
30
20
10
0
Δδx[CD], a.u.
0.0
0.2
0.4
0.6
0.8
1.0
R = [CD] / ([CD] + [Car])

(C)

Fig. 4. (A) ^{1}H-NMR (360 MHz) spectra of aqueous solution of β-CD in the presence (a) and absence (b) of **V**; B) Schematic illustration of CD structure. CD protons inside the cavity (H_3 and H_5) should be sensitive to the changed environment, but the outside protons (H_1, H_2, and H_4), should not; (C) Job's plot corresponding to the chemical shift displacement of 3-H protons of β-CD in the presence of **V** (adopted from [Polyakov et al., 2004a]).

The stoichiometry of the CD complexes of β-ionone and carotenoid **V** was obtained by the continuous variation technique (Job's plot) [Yamamoto & Inoue, 1989]. The position of the maximum at R = 0.5 on the Job's plot indicates that both compounds form a 1:1 complex with β-CD (Figure 4C). The value of the association constant (K_{11} = 1536 ± 75 M^{-1}) was obtained for the carotenoid **V** CD complex [Polyakov et al., 2004a]. This example was the first direct NMR evidence of the inclusion complex formation of a carotenoid. The inclusion of the cyclohexene ring into the CD cavity was later confirmed by molecular dynamic calculations of the astaxanthin complex with hydroxypropyl-beta-cyclodextrin [Yuan et al., 2012]. On the other hand, the analysis of the solid β-CD complex of β-carotene, astaxanthin, lycopene, and norbixin (Figure 1) by means of Raman spectroscopy and quantum mechanics calculations shows that the polyene chain of the carotenoid is located inside the CD macro-cycle structure (Figure 3B) [De Oliveira et al., 2011].

2.2. UV-Vis absorption study.

CD complexes with a number of natural and synthetic carotenoids show a considerable change in color compared to carotenoid solutions in organic solvents [Polyakov et al., 2004a]. Highly water soluble HP-β-CD and HP-γ-CD were used in the UV-Vis experiments. For example, the β-carotene CD complexes are opalescent pink-orange in aqueous solution. These complexes have a broad absorption band up to 1100 nm with reduced intensity (about one order of magnitude). We suggest that the broadening of the absorption band is due to the aggregation of complexes in aqueous solution. Such aggregate formation was previously detected by light

scattering spectroscopy [Mele et al., 1998; 2002]. Aggregate formation was directly observed by changes occurring in the UV-Vis spectra after mixing separately prepared solutions of CD in H_2O and carotenoid in ethanol [Polyakov et al., 2004a].

2.3. EPR study of the scavenging ability of carotenoids towards peroxyl radicals.

Since the antioxidant activity is very important for the practical application of carotenoids, the influence of complexation on the scavenging ability of carotenoids towards free radicals was investigated in a number of physicochemical studies [Polyakov, et al., 2004a; 2006b; 2010; Yuan et al., 2013]. The EPR spin trapping technique was employed [Polyakov et al., 2004a; 2006b; 2013]; to investigate the scavenging ability towards peroxyl radicals, and the Fenton reaction in DMSO solvent was used to generate the radicals (Eqn. 1-3) [Walling, 1998].

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \quad (1)$$

$$^{\bullet}OH + DMSO \longrightarrow {}^{\bullet}CH_3 + CH_3(OH)SO \quad (2)$$

$$^{\bullet}CH_3 + H_2O_2 \longrightarrow {}^{\bullet}OOH + CH_3 \quad (3)$$

Note that the Fenton reaction has been suggested as one of the possible sources of reactive species in living cells [Welch et al., 2001; Lemire et al., 2013].

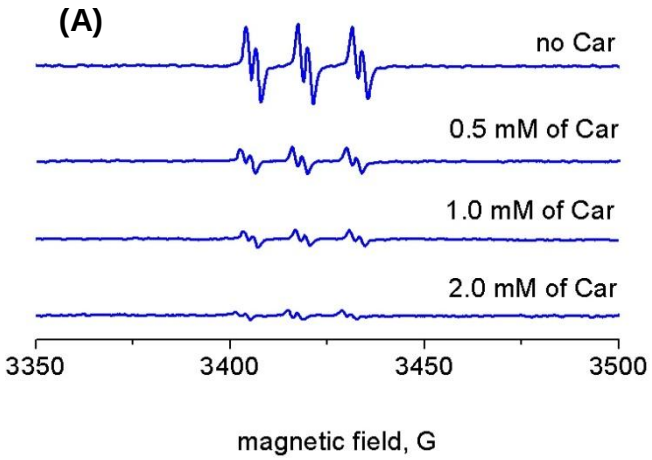
The scavenging ability was measured as the relative scavenging rate of the carotenoid (Car) to that of the spin trap (ST) rate [Polyakov et al., 2001a; 2001c]. These values were determined from the concentration dependence of the spin adduct yield (A) by using equation 4.

$$A(Car) = \frac{k_{ST}[ST]}{k_{ST}[ST] + k_{Car}[Car] + W} \quad (4)$$

Here k_{Car} and k_{ST} are the reaction rate constants of the carotenoid and the spin trap with a free radical, and W is the sum of other decay processes. It was observed that k_{Car} values depend on the redox properties of carotenoid and increase with increasing oxidation potentials [Polyakov et al., 2001a]. According to this result, β-carotene shows the least antioxidant ability among the carotenoids under study. The values of k_{Car}/k_{ST} change from 0.6 for β-carotene to 24 for

carotenoid **IV**. Even higher value of k_{Car}/k_{ST} = 40 was later observed for carotenoid **V** [Polyakov et al., 2004a].

As an example, Figure 5A demonstrates the decrease of the PBN-OOH spin adduct yield with increasing carotenoid **V** concentration in DMSO solution as a result of the scavenging process.



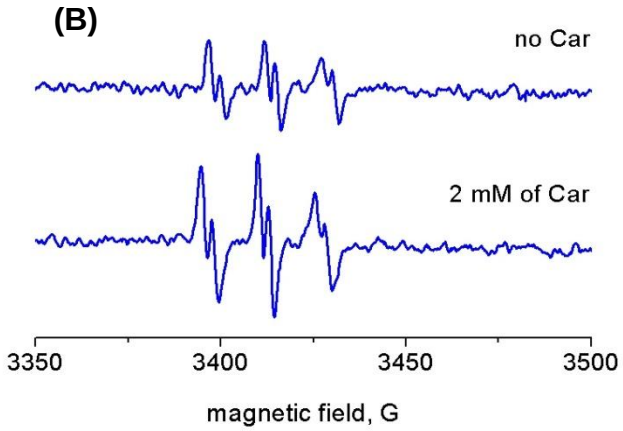


Fig. 5. (A) Variation of PBN-OOH spin adduct (a_N = 13.8 G, a_H = 2.4 G) EPR spectrum in the presence of carotenoid **V** in DMSO. Concentration of PBN = 10 mM; Fe^{2+} = 1 mM; H_2O_2 = 500 mM. (B) Variation of PBN-OOH spin adduct (a_N = 15.2 G, a_H = 2.9 G) EPR spectrum in the presence of **V**-HP-β-CD complex in H_2O showing a pro-oxidant effect (an increase in spin adduct yield). Concentration of PBN = 10 mM; Fe^{2+} = 1 mM; H_2O_2 = 500 mM; HP-β-CD = 4 mM. (adopted from [Polyakov et al., 2004a])

The same technique was applied to study the scavenging ability of the CD inclusion complex of **V** in water [Polyakov et al., 2004a]. In contrast with Figure 5A, no decrease in spin adduct yield was observed for the CD complex of this carotenoid (see Figure 5B). Moreover, one can see the appearance of the pro-oxidant effect (increase of spin adduct yield) in the presence of the

311 carotenoid. It was suggested [Polyakov et al., 2004a] that the absence of the antioxidant effect
312 (decrease in spin adduct yield (Figure 5B) is due to the protection of the radical sensitive site of
313 the carotenoid (cyclohexene ring) by CD. This result confirms our assignment that peroxyl
314 radicals attack mainly the cyclohexene ring of the carotenoid. The occurrence of the pro-oxidant
315 effect for the Car-CD complex was attributed to the reaction of the carotenoid with Fe^{3+} ions.
316 Fe^{3+} ions, present in solution as the product of the Fenton reaction, oxidizes the carotenoid and
317 regenerates Fe^{2+}: (Eqn. 5).

318 $$Car + Fe^{3+} \rightarrow Car^{+\bullet} + Fe^{2+} \quad (5)$$

319
320 The radical cation of carotenoid, $Car^{+\bullet}$, was detected as a product of this reaction. In the
321 presence of excess of H_2O_2 this reaction will results in a repetition of the redox cycle of the
322 Fenton process and result in additional free radicals (Scheme 1, pro-oxidant activity)). The role
323 of the Fenton-like processes in *in vivo* generation of toxic free radicals is now being widely
324 discussed [Timoshnikov et al., 2015; Lemire et al., 2013].

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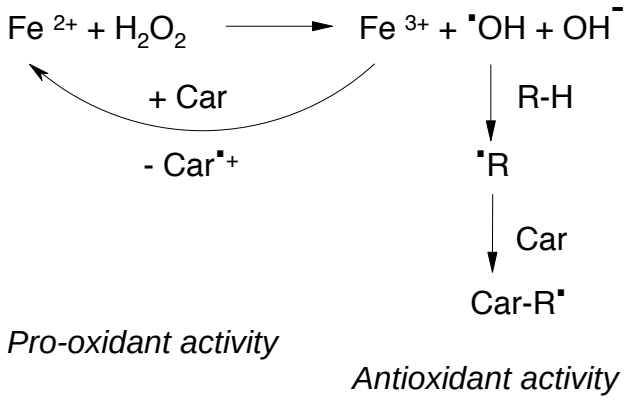


326
327
328 **Scheme 1**. The suggested mechanism of the pro-oxidant activity of carotenoids in the
329 presence of Fe ions [Polyakov et al., 2001c]. The antioxidant activity requires addition of free
330 radicals to the polyene chain of the carotenoid, or hydrogen abstraction from the cyclohexene
331 ring of the carotenoid by the OH radical, generating $[Car–H]^{\bullet}$ + H_2O.
332

333 Summarizing these results, we can conclude that complexation with CD protects the
334 carotenoid during storage and transportation to the target. Recent *in vivo* and *in vitro*

335 experimental data demonstrated that cyclodextrins stabilize carotenoids and allow efficient
336 cellular uptake [Szente et al., 1998; Pfitzner et al., 2000; Lancrajan et al., 2001; Francz et al.,
337 2000; Gharibzahedi et al., 2014; Lopez-Nicolas et al., 2014; Yuan et al., 2013]. At the same
338 time, carotenoids encapsulated in the CD cavity show no photoprotection of human skin
339 fibroblasts against UV irradiation [Offord et al., 2002].
340
341 **3. Glycyrrhizin inclusion complexes.**
342 *3.1. Preparation and analysis of GA complexes of carotenoids.*
343 Glycyrrhizic acid (GA, Figure 3C) is a unique natural compound of considerable interest to
344 pharmacologists not only due to its physiological activity, but also due to its ability to enhance
345 the activity of some drugs by non-covalent complex formation. A number of studies were
346 performed to investigate the structure of these complexes as well as the influence of GA on
347 radical processes involving carotenoids [Kornievskaya et al., 2007; Polyakov et al., 2006a;
348 2006b; 2008; 2013; Borisenko et al., 2013]. Special attention was paid to the antioxidant activity
349 of carotenoids in the complexes. It was found that GA forms cyclic dimers at low concentration
350 in aqueous solutions (0.01-1 mM), and micelle like aggregates at high concentration (> 1 mM)
351 [Polyakov et al., 2006a; Kornievskaya et al., 2007]. The formation of cyclic dimers was
352 confirmed by quantum-chemical calculation and MS analysis [Borisenko et al., 2013]. In
353 contrast to CD complexes where stability constant decreases significantly with addition of
354 organic solvent to the water solution, GA complexes are stable even in pure organic solvents
355 (ethanol, methanol, acetonitrile, DMSO) [Polyakov et al., 2006a; 2006b].
356 Since glycyrrhizic acid is soluble both in water and organic solvents, a solution of carotenoid
357 in an organic solvent was added to the aqueous or organic GA solution and then stirred for
358 several hours [Polyakov et al., 2006a; 2006b] for complex preparation.
359 To understand the behavior of inclusion complexes in living systems, a very important
360 question is their stability in non-aqueous media. For this purpose the possibility of complex

formation between carotenoids and GA was investigated in several organic solvents, namely in alcohols, acetonitrile and DMSO. Complex formation between canthaxanthin and GA in a DMSO solution was investigated by fluorescence techniques [Polyakov et al., 2006a]. Due to the high sensitivity of the fluorescence intensity to the media properties, this approach can be used to study inclusion complexes of the "guest-host" type [Lopez et al., 2003; Rekharsky & Inoue, 1998]. The luminescence of molecules imbedded in the complex is increased due to the protection provided against quenching and other processes occurring in solution (Figure 6). The complex stability constant in DMSO was estimated as $K \sim 10^4$ M^{-1} at a GA concentration below 1 mM [Polyakov et al., 2006a].

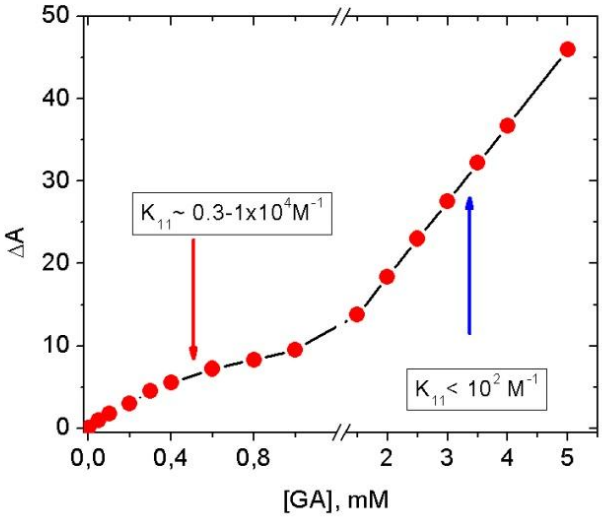


Fig. 6. Dependence of fluorescence intensity of carotenoid **III** (0.02 mM in DMSO solution containing 5% of water) with GA (mM) (adopted from [Polyakov et al., 2006a], Fig. 3).

The change in the properties of the GA solutions at the 1 mM concentration point has been observed in several studies, and this effect was explained by the formation of GA micelles [Polyakov et al., 2008; Kornievskaya et al., 2007]. Since there is no evidence of GA micelle formation in organic solvents at this time, we can assume the formation of larger aggregates occurs due to H-bonding interaction. The increase of the observed effect in the presence of water indicates the participation of water molecules in the GA aggregates formation.

3.2. *Interaction of carotenoids and their GA complexes with Fe ions, quinone and ozone molecules.*

An important question that is widely discussed is the influence of complex formation on carotenoid reactivity and stability [Yuan et al., 2013; Polyakov et al., 2010; 2013; Apanasenko et al., 2015; Tachaprutinun et al., 2009]. The reactivity of carotenoids towards metal ions, quinones and free radicals is closely related to their antioxidant activity as well as their stability in living systems, food and medical preparations. One of the most important natural processes involving carotenoids is electron transfer from the carotenoids to acceptors. As it was mentioned above, the reaction with Fe ions is considered by a number of authors to be one of the possible mechanisms of the pro-oxidant activity of β-carotene. The first step of this reaction is the electron transfer from the carotenoid to the acceptor resulting in formation of the carotenoid radical cation (Eqn. 5). The carotenoid radical cation can also react with Fe^{3+}: (Eqn. 6).

$$Car^{+\cdot} + Fe^{3+} \rightleftharpoons Car^{2+} + Fe^{2+} \qquad (6)$$

Both the carotenoid radical cation and the dication can undergo *cis-trans* isomerization. In acetonitrile, all carotenoids form relatively stable radical cations according to their absorption spectra (Figure 7), but are not detectable in aqueous solutions [Polyakov et al., 2006a].

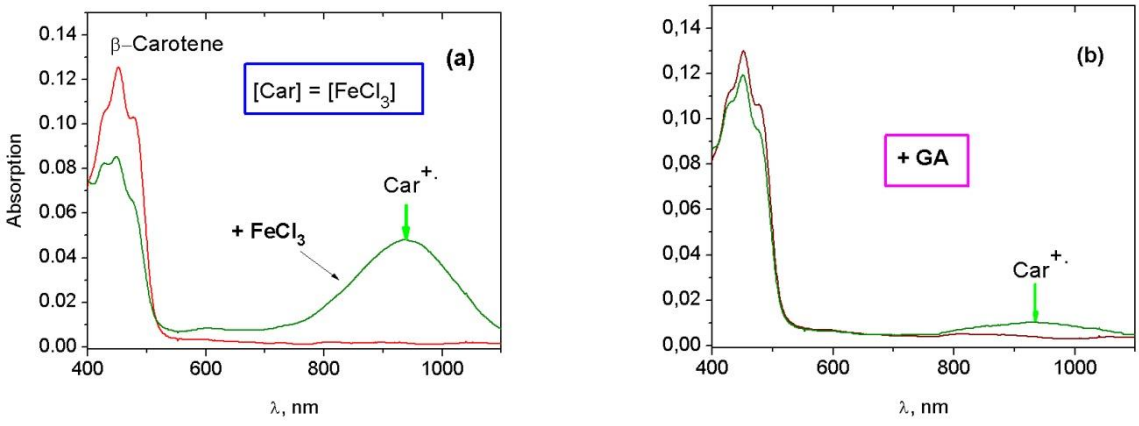


Fig. 7. Absorption spectra of β-carotene, 1.3 μM, recorded before and after mixing with $FeCl_3$, 1.3 μM, in acetonitrile at room temperature: (a) in the absence of GA; (b) in the presence of 0.1 mM GA (adopted from [Polyakov et al., 2006a], Fig. 4).

403 A considerable decrease in the yield of β-carotene radical cation was observed in the
404 presence of 0.1 mM GA. It can be attributed to a decrease in the electron transfer rate in the case
405 of complexation. With excess $FeCl_3$, all carotenoids are rapidly transformed into the radical
406 cations both with and without GA. Since the first and second redox potentials of β-carotene
407 nearly coincide, both radical cation and dication are present when oxidation occurs and are in
408 comproportionation equilibrium (K_{com}) (Eqn. 7).
409

410 $$2Car^{+\cdot} \underset{}{\overset{K_{com}}{\rightleftharpoons}} Car^{2+} + Car \qquad (7)$$

411
412 It has been demonstrated that the charged forms of carotenoids (cations and dications) do not
413 leave the complex after the reaction and are also stabilized.
414 Another example of the influence of GA on the reactivity of carotenoids is the electron
415 transfer from carotenoid to quinone [Polyakov et al., 2006a]. Quinones are known to be
416 important natural electron acceptors in photosynthetic centers [Lawlor, 1987]. Significant
417 stabilization of β-carotene radical cation by binding with GA was detected in the presence of
418 quinone in a polar solvent (acetonitrile) [Polyakov et al., 2006a]. The product of the radical ion
419 pair (RIP) recombination is the quinone–carotenoid adduct, whereas the radical cations escaping
420 recombination result in the formation of *cis*-isomers. How will the increase in the lifetime of the
421 radical cation influence the yield of main reaction products? To elucidate this question, the yields
422 of the reaction products for two carotenoids (**III, IV**) were compared using HPLC method. As a
423 result, a substantial increase in the yield of the carotenoid-quinone adduct was observed for these
424 carotenoids in the presence of GA. This observation allowed us to conclude that GA can form
425 stable complexes not only with neutral molecules, but also with their radical ions and charge
426 transfer complexes. In the case of carotenoids, this leads to a change in both the reaction
427 direction and the ratio of products. The important point is also the possibility to control the
428 lifetime of radical cations by the formation of "host-guest" complexes.

On the other hand, radical cations of carotenoids are unstable in aqueous solutions due to fast deprotonation and formation of neutral radicals [Gao et al., 2003], but no decrease in the reaction rate with Fe^{3+} ions was detected under these conditions in the presence of GA [Apanasenko et al., 2015].

However, it was demonstrated that complexation with GA or its disodium salt (sGA) reduce significantly the oxidation rate of the carotenoids lutein and zeaxanthin by ozone molecules (Table 1) [Apanasenko et al., 2015]. Even a greater influence was observed in the presence of the polysaccharide, arabinogalactan.

Table 1. Decay times (in sec) of the reaction of ozone with the carotenoids zeaxanthin and lutein in 75% water-ethanol solution as a function of GA concentration.

	Free carotenoid	1 mM GA	1 mM sGA	0.05 mM AG
Lut + O_3	150 ± 10	1290 ± 20	1310 ± 20	∞
Zea + O_3	200 ± 20	1100 ± 20	1140 ± 10	2150 ± 50

Ozone molecules are very reactive oxygen species that are able to break the double bonds of carotenoids with high efficiency [Henry et al., 2000]. Reaction occurs via addition of ozone to the double bond with formation of an ozonide, followed by breakage of the double bond with formation of a number of oxygenated products with reduced conjugated chains [Bailey, 1958]. As one can see from these results, significant reduction of the oxidation rate was detected for all systems under study. The most stable are the complexes with arabinogalactan. The stability of GA and sGA complexes depends on the concentration of the "host" molecules. Significant stabilization was detected for concentrations near 1 mM when GA exists mainly in the micellar form. Earlier a similar effect was detected for the canthaxanthin-GA complex [Gluschenko et al., 2011].

3.3. *Antioxidant and redox properties of supramolecular complexes of carotenoids with glycyrrhizic acid*.

Antioxidant activity is known to be one of the most important biological properties of carotenoids, because they react with toxic free radicals and thus prevent damage to living organism [Tanumihardjo, 2013; Al-Agamey, et al., 2004]. From a practical point of view, it is interesting to know how the complexation of carotenoids with glycyrrhizic acid will affect their ability to scavenge free radicals. As it was stated above, complexes of carotenoids with cyclodextrin is used to improve their solubility, and to increase bioavailability, however, it has failed to improve their antioxidant properties. As it was shown [Polyakov et al., 2004a], reaction can be almost totally inhibited in homogeneous solution by cyclodextrin due to embedding the cyclohexene fragment in the cyclodextrin cavity.

The antioxidant activity of carotenoids and their complexes with GA was studied by the EPR spin-trapping technique as described in 2.3. Comparison of the scavenging rates of peroxyl radicals by free carotenoids and their GA complexes in DMSO shows a strong dependence of the rate constants on the concentration of GA (Table 2).

Table 2. Relative rate constants of OOH radicals scavenging by carotenoids and their GA complexes (k/k_{ST}) in DMSO. $E_{1/2}$ is the redox potential of carotenoids (in Volts vs. SCE) [Polyakov et al., 2006b].

[GA] mM	**II** ($E_{1/2}$ = 0.56 V)	**III** ($E_{1/2}$ = 0.68 V)	**IV** ($E_{1/2}$ = 0.72 V)
0	4	2	7
0.5	4	59	133
1	4	46	116
2	4	6	38

Of importance is the absence of this dependence for zeaxanthin (**II**). Analyzing the oxidation potentials of these three carotenoids (see Table 2) and the dependence of the carotenoids scavenging rate [Polyakov et al., 2001a] on their $E_{1/2}$, points out that GA can affect the oxidation potential of the carotenoids. This hypothesis was verified by CV measurement of the oxidation

potential of two carotenoids **II** and **III** in the presence of GA. In both cases, an increase in $E_{1/2}$: by 0.05 V for canthaxanthin and by 0.03 V for zeaxanthin have been observed [Polyakov et al., 2006b]. Using this result and the diagram in Figure 8, the different behavior of carotenoids **II** - **IV** in the presence of GA can be explained. A negligible change in the oxidation potential for beta-carotene and zeaxanthin (<0.05 V) should cause no changes in their antioxidant activity. At the same time, this diagram predicts a substantial increase in the reaction rate for carotenoids with $E_{1/2} \sim 0.7$ V when their oxidation potential increases due to complexation.

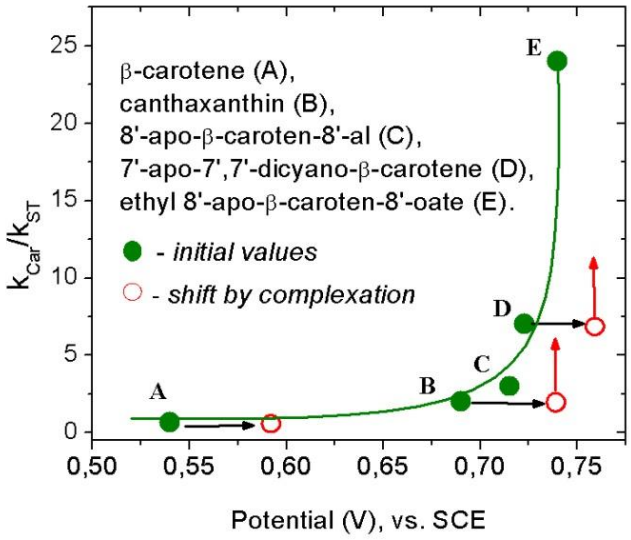


Fig. 8. The dependence of the carotenoid scavenging rate (k_{car}/k_{st})toward peroxyl radicals versus the oxidation potential of carotenoids. Arrows denote the shifts in oxidation potentials due to complexation with GA (adopted from [Polyakov et al., 2006b], Fig.5).

Note also, that the rate constants of peroxyl radical scavenging by carotenoids (Table 2) are different at various GA concentrations. The scavenging rates measured at low GA concentrations (0.5 мМ) considerably exceed those measured at high concentrations (2 мМ). This fact confirms the hypothesis for the dependence of the structure and properties of GA complexes on its concentration. It is assumed that interaction between carotenoids and peroxyl radicals occurs via hydrogen abstraction from the most acidic 4-H proton of carotenoids [Focsan et al., 2012; Polyakov et al., 2013]. GA forms a donut like dimer in which the hydrophobic polyene chain of carotenoids lies protected within the donut hole, permitting the hydrophilic ends and most acidic

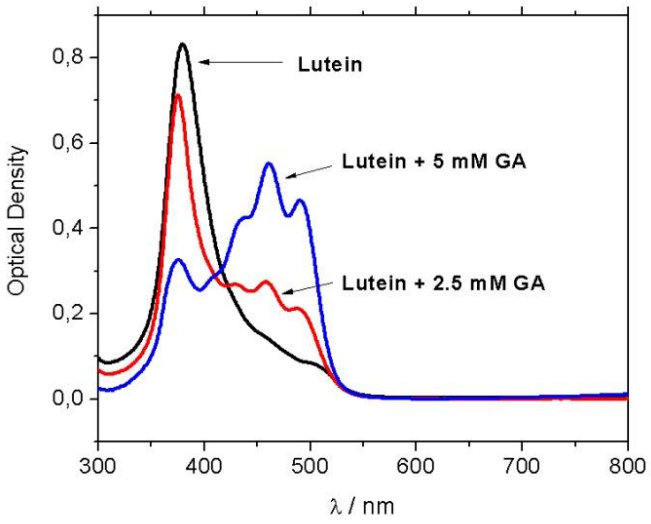
497 proton to be exposed to the surroundings. We assume that at high concentration of GA, the
498 terminal groups also become partly protected from the reaction with free radicals. Note, that in
499 the case of the CD complexes, the terminal group of the carotenoid is completely protected
500 which results in the inhibition of any antioxidant activity.

501 Although there is no data on the GA micelle formation in non-aqueous solvents, some x-ray
502 data exist on the important role of H-bond interaction in the formation of liquid crystal like
503 structures in some organic solvents [Tykarska et al., 2012]. Also NMR relaxation measurements
504 show significant decrease of the nuclear relaxation time of GA protons in the presence of
505 hydrophobic molecules in organic solvents [Gluschenko et al., 2011]. As it was described in the
506 previous paragraph, the stability constant of the GA complex with canthaxanthin in DMSO
507 estimated from fluorescence measurements is about 10^4 M^{-1} at low GA concentrations and $<10^2$
508 M^{-1} at high concentrations. Below a GA concentration of 1 mM, the GA-carotenoid complex
509 stoichiometry was determined to be 2:1.

510 *3.4. Glycyrrhizin complexes with xanthophyll carotenoids – prevention of aggregation.*

511 Xanthophyll carotenoids can self-assemble in aqueous solution and even in lipid membrane to
512 form J- and H-type aggregates [Wang et al., 2012]. This feature significantly changes the photo-
513 physical and optical properties of these carotenoids, and has an impact on solar energy
514 conversion and light induced oxidative damage. Molecular self-assembly in biological systems
515 attracts considerable attention, since it is important for the functioning of living organisms. It
516 was demonstrated by using EPR and optical absorption spectroscopy that complexation with GA
517 can reduce the aggregation ability of the xanthophyll carotenoids zeaxanthin, lutein, and
518 astaxanthin [Polyakov et al., 2013]. Figure 9 shows an example of the change in the absorption
519 spectrum of lutein in the presence of GA at 2.5 and 5 mM in a 25% ethanol solution. The
520 estimated stability constant of xanthophyll complex in this media is 1.7×10^7 M^{-2}. Increasing the
521 GA concentration shifts the equilibrium from the H-aggregate (λ_{max}= 380 nm) to the monomer
522 (λ_{max}= 460 nm). The large shift from 380 nm with the loss of vibrational structure of the S_2

523 excited state to 460 nm and the appearance of the vibrational bands indicates the presence of the
524 zeaxanthin monomers. From these experiments we can conclude that xanthophyll aggregation is
525 a reversible process, and complexation with GA prevents aggregation.



526

527 **Fig. 9**. Optical absorption spectra of lutein (6 μM) in 25% ethanol/water mixture at different GA
528 concentrations (adopted from [Polyakov et al., 2013]).
529

530 It was also found that the scavenging ability of xanthophyll carotenoids to trap hydroperoxyl
531 radicals decreases significantly in the presence of water in organic solvents due to aggregate
532 formation [Polyakov et al., 2013], but increases in the presence of GA as complexation prevents
533 aggregation of the xanthophylls allowing the cyclohexene ring to be exposed to the
534 surroundings. Considering the important role of these carotenoids in eye and skin health,
535 glycyrrhizin should be considered as a perspective delivery system to provide enhanced
536 solubility and activity of the xanthophyll carotenoids.

537

538 **4. Arabinogalactan inclusion complexes.**

539 The stability and reactivity of carotenoids in complexes with the natural polysaccharide
540 arabinogalactan (AG) were investigated by different physicochemical techniques: optical
541 absorption, HPLC, and pulsed EPR spectroscopy [Apanasenko et al., 2015; Polyakov et al.,
542 2013; 2010; 2009]. Polysaccharide complexes of carotenoids showed enhanced photostability

543 compared to pure carotenoids as well as reduced reactivity towards metal ions (Fe^{3+}) and reactive
544 oxygen species. On the other hand, the yield and stability of carotenoid radical cations produced
545 on titanium dioxide nanoparticles were greatly increased in the solid state complex of
546 arabinogalactan. We suggest that these results are important for a variety of carotenoid
547 applications.

548
549 *4.1. Preparation and analysis of inclusion complexes.*

550 Since arabinogalactan is a water soluble polymer, the methods of complex preparation are
551 similar to those for cyclodextrin complexes. In the solid state method, solid carotenoid and the
552 requisite amounts of "host" compound were ground together until a homogeneous powder was
553 obtained. Typical mechanical reactions are achieved by co-grinding or milling the powder
554 materials. These preparations are usually carried out either manually, in an agate mortar, or
555 electromechanically, as in ball milling [Dushkin, 2010]. In all these cases the crystal lattice is
556 destroyed and reformed through recrystallization. In such processes hydrogen bonds, π-stacking,
557 van der Waals, ion pairing interactions *etc.* are broken and formed leading to formation of
558 supramolecular compounds or hybrid molecular crystals [Chistyachenko et al., 2014; Dushkin et
559 al., 2008; Dushkin, 2010]. Co-grinding of the solid material results in penetration of the
560 carotenoid molecules into the "host" macromolecule without using any organic solvents. This
561 (mechanochemical) approach enabled water-soluble composites of carotenoids β-carotene and
562 cantaxanthin to be prepared for the first time [Polyakov et al., 2010; 2009]. The estimated
563 solubility of β-carotene and canthaxanthin complexes is 2-5 mM in a water solution. This value
564 is six orders higher than the characteristic solubility of free carotenoids in pure water, ~1 nM
565 [Stancanelli et al., 2012]. X-ray diffraction analysis and differential scanning calorimetry were
566 used to monitor the solid state complex formation. In a second method, a solution of carotenoid
567 in an organic solvent was added to the aqueous arabinogalactan solution. It was demonstrated
568 that the mechanochemical method for solid-state complex preparation has significant advantages
569 when compared with traditional techniques. Primarily, the interest in solvent-free conditions

570 stems from the possibility of obtaining the same product as that from solution without solvent
571 because the process is cheaper, less time consuming and often more environmentally friendly. In
572 the case of carotenoid chemistry the solvent-free conditions open the possibility of obtaining
573 products not otherwise accessible in solutions.

574 The carotenoid-arabinogalactan complexes maintain their original color and show
575 insignificant changes in absorption spectra. UV-Vis spectrum of aqueous solutions of the
576 canthaxanthin-AG complex has the same absorption maximum as that of the spectrum of the
577 canthaxanthin solution in 30% ethanol [Polyakov et al., 2009]. It was also found that
578 arabinogalactan prevents H-aggregate formation of xanthophyll carotenoids in the presence of
579 water in the same way as that for the glycyrrhizin complexes [Polyakov et al., 2013].

580
581 *4.2. Photostability of carotenoids and their AG complexes in a water solution.*

582 One of the main problems with the practical application of carotenoids is their
583 photosensitivity and instability, especially in the presence of oxygen and water. The
584 photostability of drugs and vitamins attracts increasing attention since serious toxic reactions
585 produced by many pharmacologically important chemicals occur under sunlight irradiation
586 [Tonnesen, 2004]. Photoallergic and photomutagenic effects are also of current concern.
587 Photogenerated intermediates can interact with cell components and lead to cell degeneration or
588 death. Control of the drug photostability and protective strategies against light-induced
589 preparation damage requires understanding the structural and environmental factors determining
590 their photoreactivity.

591 Although carotenoids are known as effective photoprotectors of living cells, in aerated
592 aqueous solution they are unstable under light irradiation, and would be unacceptable as
593 colorants and antioxidants in foods. The reason for this is that the UV irradiation of carotenoid
594 solutions results in a decrease of the absorption intensity with formation of reaction products that
595 absorb light at a lower wavelength. This effect can be explained by a decrease in the length of
596 the conjugated chain due to the addition of oxygen to the double bonds. It is known that under

irradiation, carotenoids can form radical cations in various media by electron transfer to the solvent molecules or to the appropriate electron acceptor. These radical cations can be reduced by back electron transfer. However this reversible process is disrupted in the presence of water molecules that act as a proton acceptor. It results in the formation of a carotenoid neutral radical that is stabilized by the nearby proton acceptor, so a reversible electron transfer is prevented. Earlier it was demonstrated that carotenoid neutral radicals are formed from the corresponding radical cations generated electrochemically or photochemically by proton loss [Gao et al., 2003]. Although photoexcitation accelerates deprotonation of the radical cation, electrochemical measurements showed that the radical cations of a majority of carotenoids have p*K* values ranging between 4 and 7 and, therefore, can deprotonate spontaneously [Liu et al., 2000; Kispert et al., 2004].

A significant increase (5-10 times) in photostability was detected for the AG complexes of various carotenoids [Polyakov et al., 2009; 2013]. As an example, Figure 10 demonstrates the difference in the photodegradation rate of canthaxanthin in the absence (a) and in the presence (b) of arabinogalactan.

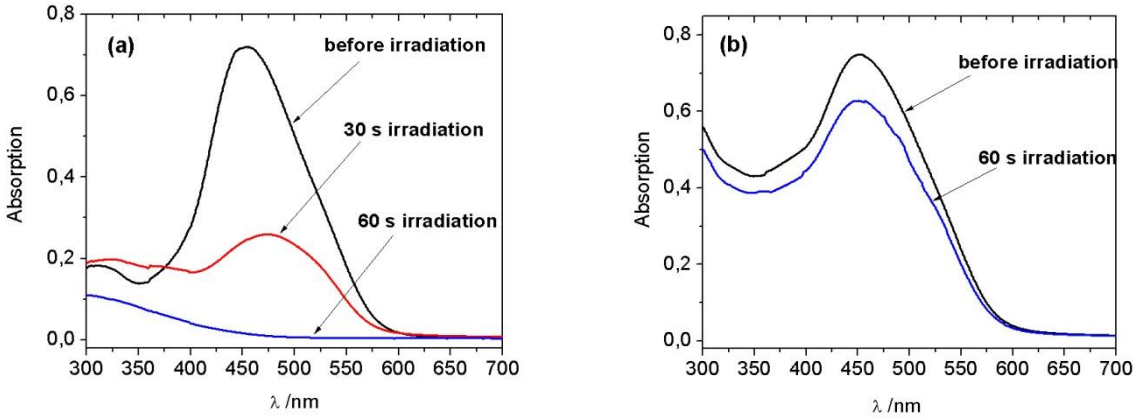


Fig. 10. Photodegradation of canthaxanthin. Absorption spectra were recorded after different irradiation times in aerated 30% ethanol solution by the full light of a xenon lamp. (a) Free canthaxanthin; (b) Canthaxanthin-AG complex (adopted from [Polyakov et al., 2009], Fig. 3).

618 We propose that the main mechanism of enhanced carotenoid stability in a polysaccharide
619 complex is isolation of the carotenoid from water by incorporation into the hydrophobic polymer
620 environment.

621 4.3. *Photo-induced electron transfer from carotenoids in the solid state*.

622 Electron transfer from carotenoids to a variety of acceptors is one of the most important
623 natural processes because of the importance of carotenoids in photosynthesis and their possible
624 use in artificial solar cells. Radical cations of canthaxanthin incorporated in solid
625 arabinogalactan was detected during photoirradiation of TiO_2 nanoparticles [Polyakov et al.,
626 2009]. Among the semiconductors, titanium dioxide is the most suitable for many environmental
627 applications. Due to its ability to absorb light, TiO_2 is widely used in photocatalysis and in
628 artificial solar cells [Katoh & Furube, 2014; Fenoll et al., 2012].

629 Irradiation of a carotenoid-AG complex adsorbed on the surface of TiO_2 ($T = 77°$ K, $\lambda > 350$
630 nm) shows that a significant increase occurs in the intensity of the EPR signal compared to that
631 of the free carotenoid or free TiO_2 nanoparticles [Polyakov et al., 2009]. It was suggested that the
632 low yield of the charge separated state on semiconductor materials in the absence of AG might
633 be due to efficient back electron transfer. The "redox cycling", where a product of the hole
634 transfer acts, in turn, as scavenger for the photogenerated electrons, appears as a frequent cause
635 of weak photocurrents [Solarska et al., 2006]. Isolation of the carotenoid radical cation from the
636 TiO_2 surface by incorporation into the polysaccharide matrix allows more efficient charge
637 separation, reducing the rate of back electron transfer. A number of authors used this same
638 approach for design of the "donor-bridge-acceptor" molecular triads as a model for the light-
639 harvesting complex [Kodis et al., 2004]. Apparently, such a way of light energy transformation
640 is similar to the mechanism used by plants for utilization of solar energy in photosynthesis.

641 An important result was published on the enhancement of the photocatalytic activity of TiO_2
642 nanoparticles covered by the carotenoid-AG complex in aqueous solution [Polyakov et al.,
643 2010]. Photoirradiation of TiO_2 nanoparticles by visible light in the presence of the β-carotene-

arabinogalactan complex leads to an enhanced yield of the reactive hydroxyl radicals as detected by the EPR spin-trapping technique. The observed enhancement of the photocatalytic efficiency for carotenoid complexes, as measured by the quantum yield of the desired spin-adducts, arises specifically from the decrease in the rate constant for the back electron transfer to the carotenoid radical cation. These results are important for a variety of TiO_2 applications, namely in photodynamic therapy, and in the design of artificial light-harvesting, photoredox, and catalytic devices.

4.4. *Reactivity of the carotenoid-arabinogalactan complexes in aqueous solution.*

The inhibition of electron transfer reaction in aqueous solution of the carotenoids β-carotene, lutein and zeaxanthin with Fe^{3+} ion as an electron acceptor was accomplished by complexation with AG [Polyakov et al., 2009; Apanasenko et al., 2015]. We suggest that two factors play an important role in the stabilization of carotenoids in the AG complexes, namely, isolation from water molecules and isolation from metal ions. The important conclusion that can be made from this result is that the Fe ions do not penetrate into the polysaccharide matrix.

Another important reaction of carotenoids in the presence of AG is the interaction with active oxygen species. Using the Fenton and photo-Fenton reaction [Silva et al., 2007; Bacardit et al., 2007] (Eqn. 8, 9), a significant stabilization of the carotenoids (**I, III, IV**, see Figure 1) in the AG complexes was detected [Polyakov et al., 2009].

$$Fe^{3+} + H_2O \xrightarrow{h\nu} Fe^{2+} + H^+ + \cdot OH \qquad (8)$$

$$H_2O_2 + Fe^{2+} \longrightarrow Fe^{3+} + OH^- + \cdot OH \qquad (9)$$

Complete inhibition of carotenoid oxidation by ozone molecules was also detected [Apanasenko et al., 2015]. It is proposed that the stability of the carotenoids incorporated into the AG macromolecule might have wide practical application. A decrease in reaction rate towards free radicals does not mean a decrease in antioxidant activity of a complex in living systems since polysaccharides are easily assimilated by living media.

673
674
675 **5. Conclusion**

676 In conclusion, incorporation of carotenoids into the oligosaccharide or polysaccharide
677 macromolecules results in significant changes in their physical and chemical properties. These
678 results provides new insight into the activity of carotenoids in living systems as well as opening
679 up new possibilities for their broad practical application in various fields. In this review we have
680 compared three types of carotenoid complexes, namely with cyclodextrins, glycyrrhizin and
681 arabinogalactan. All these complexes show very different physicochemical properties. CD
682 complexes enhance storage stability of carotenoids, but reduce their color intensity and don't
683 increase carotenoid solubility in water. In addition to the cyclodextrins inclusion complexes
684 which are already used in pharmacology, cosmetics and food industry, we propose two new
685 compounds, glycyrrhizin and arabinogalactan which form more stable complexes with
686 carotenoids and demonstrate some unique properties useful for many applications. In particular,
687 complexes of glycyrrhizin with some carotenoids show enhanced solubility and ability to
688 scavenge free radicals. In practice it means a significant reduction in the required dosage of
689 antioxidants in medical preparations and enriched drinks. Complexes with arabinogalactan are
690 unique water soluble compositions which provide enhanced stability of carotenoids at room
691 temperature in the presence of sunlight and water, and significantly reduce their interaction with
692 different additives including metal ions and active oxygen species. In addition, GA and AG
693 complexes maintain the original carotenoid color and prevent carotenoid aggregation.

694 The increased stability of carotenoid radical cations in solution (in GA complex) and in the
695 solid state (in AG complex) at room temperature may result in new discoveries in the design of
696 artificial light-harvesting, photoredox and catalytic systems.

697

698 **Acknowledgements**

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