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Solubilization and stabilization of macular carotenoids by water soluble oligosaccharides and polysaccharides

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ABSTRACT

Xanthophyll carotenoids zeaxanthin and lutein play a special role in the prevention and treatment of visual diseases. These carotenoids are not produced by the human body and must be consumed in the diet. On the other hand, extremely low water solubility of these carotenoids and their instability restrict their practical application as components of food or medicinal formulations. Preparation of supramolecular complexes of zeaxanthin and lutein with glycyrrhizic acid, its disodium salt and the natural polysaccharide arabinogalactan allows one to minimize the aforementioned disadvantages when carotenoids are used in food processing as well as for production of therapeutic formulations with enhanced solubility and stability. In the present study, the formation of supramolecular complexes was investigated by NMR relaxation, surface plasmon resonance (SPR) and optical absorption techniques. The complexes increase carotenoid solubility more than 1000-fold. The kinetics of carotenoid decay in reactions with ozone molecules, hydroperoxyl radicals and metal ions were measured in water and organic solutions, and significant increases in oxidation stability of lutein and zeaxanthin in arabinogalactan and glycyrrhizin complexes were detected.

Introduction

Xanthophylls are the special group of carotenoids which contain oxygen functionalities at one or both ends of the molecule, providing them with unique biological, chemical and physical properties. In particular, hydroxyl groups are responsible for the xanthophylls' abilities to orient within cell membranes in ways other carotenoids cannot [1–4]. The present study is devoted to two representatives of xanthophyll carotenoids, lutein and zeaxanthin (Fig. 1), which play a special role in the prevention and treatment of visual diseases. These carotenoids are not produced by the human body and must be consumed in the diet.

Lutein and zeaxanthin selectively accumulate at an extremely high concentration in the macula of the primate eye retina through the action of specific high-affinity binding proteins [5], potentially slowing the onset of age-related macular degeneration [6,7], and they have been recently added to the list of beneficial nutrients provided by leafy greens. It is suggested that lutein and zeaxanthin play a role in blue light filtration and antioxidant function [8,9]. The higher-energy, blue wavelengths of visible light are 100 times more effective at inducing free radical formation in the cells of the retina than the lower-energy, red wavelengths of visible light [10]. Reacting as antioxidants with free radicals and reactive oxygen species, macular carotenoids protect the retina against peroxidation and photo-damage [11–15].

The important feature of xanthophyll carotenoids is their ability to form J- and H-type of self-assembled complexes in aqueous media and even in lipid membranes [16–20]. Molecular self-assembly in biological systems attracts considerable attention, since it is important for the functioning of living organisms. It has been shown that lutein and zeaxanthin form aggregates when dissolved in hydrated polar solvents and that this aggregation is characterized by dramatic changes in their absorption spectra and photo-physical properties [16,18,20]. The H-aggregates, in which the molecules are tightly stacked with the conjugated chains oriented more or less parallel to each other, show a large blue shift of the absorption spectrum and loss of vibrational structure of the S_2 excited state. The blue shift of the absorption spectrum is explained in terms of excitonic interaction between the

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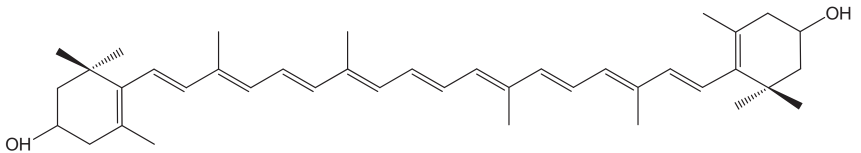
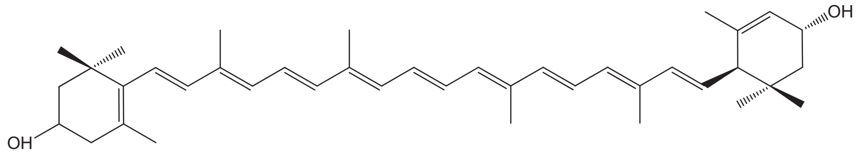


Zeaxanthin



Lutein

Fig. 1. Structural formulas of zeaxanthin and lutein.

closely packed carotenoid chromophores [18–20]. The second aggregation type, characterized by a red shift of the absorption spectrum where the resolution of vibrational bands is preserved, is attributed to J-type aggregation, in which there is a more head-to-tail organization of the conjugated chains. The self-assembly of xanthophylls leads to new photo-physical properties which can have an impact on various applications, particularly in relation to solar energy conversion [21]. One photo-physical mechanism that generally requires the proximity of two chromophores is singlet fission. In this mechanism, a chromophore is photoexcited to its singlet excited state and subsequently partitions its energy over two neighboring chromophores that both remain in triplet excited states. The chromophore of zeaxanthin is favorable for the production of triplet excited states via fission, and a high yield of triplet excited states via singlet fission was found for its aggregate [22]. On the other hand, only very restricted data exist on the influence of aggregation on the chemical properties of carotenoids. Recently it was demonstrated by optical and EPR spin trapping techniques that aggregation of xanthophyll carotenoids results in an increase in their photostability in aqueous solution, but it significantly reduces their antioxidant ability [16].

The practical use of lutein, zeaxanthin and other carotenoids is restricted by their insolubility in water and their poor chemical stability. A successful approach to improve the effectiveness and safety of poorly soluble drugs is to increase their solubility and dissolution rate by the formation of "host–guest" complexes with synthetic or natural water-soluble polymers or oligomers [23–34]. Most of the earlier attempts to increase the solubility of carotenoids were based on the preparation of cyclodextrin inclusion complexes [23,35–40]; however, cyclodextrin complexes demonstrate low solubility and fast aggregation in aqueous solution.

Recently, we have described the synthesis of novel carotenoid complexes with unique physicochemical properties [16,24–28]. In these studies we used two "host" molecules derived from plants: the triterpene glycoside glycyrrhizic acid (GA),[1] a natural compound extracted from the licorice root [41,42], and arabinogalactan (AG), a natural water soluble polysaccharide extracted from Siberian larch [43–45].

Glycyrrhizic acid (Fig. 2) self-associates into dimers containing a hydrophobic and hydrophilic component in a donut like shape [25,46]. The polyene chains of carotenoids can reside within the hydrophobic area while allowing the hydrophilic terminal rings to stick out on each end. As a result, complexation with GA does not reduce scavenging ability of carotenoids with low oxidation potential toward oxygen radicals, and it may even enhance this ability considerably for carotenoids with high oxidation ability (canthaxanthin, for example) [26]. Arabinogalactan is a highly branched polysaccharide polymer composed of galactose and arabinose fragments in a 6:1 ratio. It has previously been reported to function as a complexing agent to make carotenoids water dispersible with increased photostability [27] and photocatalytic activity [28]. The unique properties of GA and AG complexes were demonstrated for two natural carotenoids: β-carotene and canthaxanthin [23–28].

The influence of arabinogalactan and glycyrrhizin on the reactivity of lutein and zeaxanthin and their H-aggregates in some practically important processes, such as prevention of photo-degradation and improvement of antioxidant activity, was investigated in our previous study [16]. A strong influence of complexation on the reactivity of carotenoid monomers and aggregates was found. In particular, an increase of photostability of both monomers and aggregates in aqueous solutions was detected [16]. The increase of scavenging ability toward peroxyl radicals was also detected by EPR and explained by the decreasing aggregation rate in the presence of GA and AG [16].

The present study is devoted to the investigation of oxidation stability of lutein and zeaxanthin in solution and in their inclusion complexes with AG, GA and its disodium salt (sGA). The kinetics of carotenoid decay in reactions with ozone molecules, hydroperoxyl radicals and metal ions was measured in water and organic solutions. We then compared the behavior of the complexes prepared by using two different approaches, namely, liquid-phase synthesis and solid-state mechanochemical association. The latter approach permits solid-state preparation of complexes of insoluble drugs in one technological step without use of any organic solvents [27,31–33]. In addition, complex formation with sGA was demonstrated by NMR relaxation methods, and complexation with arabinogalactan was analyzed by surface plasmon resonance (SPR) techniques [47].

Experimental

Lutein (>90%, the rest amount is zeaxanthin) was provided by Kemin Health (Des Moines, Iowa), and zeaxanthin (>95%) came from Kalsec (Kalamazoo, Michigan). As the "host" molecules we used arabinogalactan from Siberian larch wood [44], glycyrrhizic acid from Ural licorice root [48] and disodium salt of glycyrrhizic acid (CFS, 98%) from Shaanxi Sciphar Biotechnology Co., Ltd (Xi'an, China). Amine-coupling reagents (N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride

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[1] *Abbreviations used:* SPR, surface plasmon resonance; GA, glycyrrhizic acid; AG, arabinogalactan; NHS, N-hydroxysuccinimide; EDC, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride; CPMG, Carr–Purcell–Meiboom–Gill sequence; PBS, phosphate buffered saline; DSC, differential scanning calorimetry.

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