CHEMICAL AND BIOLOGICAL ASPECTS OF CAROTENOIDS

Adizova Shoira Toirovna
Bukhara Innovative Medical Institute, Assistant in biochemistry
Bukhara, Uzbekistan

The structure and biological functions of carotenoids are considered with an emphasis on xanthophylls, which have been of the greatest interest lately. The diverse biological activity of carotenoids determines the importance of these compounds in food design programs.

Key words: *carotenoids, xanthophylls, biological activity, food design.*

Carotenoids include more than 600 different fat-soluble pigments, which cause the coloring of a significant part of wildlife objects from yellow to red [1, 2]. As a rule, the carotenoid framework is constructed by eight isoprene (C5) fragments (therefore, carotenoids are tetraterpenoids) and can be graphically represented as a linear central part (for completely trans isomers, all-E) and two extreme structural fragments, X and X'

The existence of a large number of carotenoids is due to the difference in the structure of fragments X and X', but, strictly speaking, the presence of several geometric cis-trans isomers for each of the carotenoids should be taken into account, for example, three ordinary mono-cis (Z) isomers have a structure:

The number of possible structures of fragments X is large, but not all of them have the same human significance and frequency of detection in nature. The simplest one can be considered a substituent of an acyclic "linear" (as far as possible for the articulation of isoprenoid fragments) structure (I).

A carotenoid containing two fragments (I) on both sides is called lycopene, and is included in the initial part of the carotenoid metabolism schemes. Of great importance in nature are carotenoids containing a characteristic cyclohexene cycle in two isomeric forms – α- and β-ion structures, III and II, respectively. Substituents X and X' in carotenoid molecules can be different, for example, α-, γ- and δ-carotenes contain two different terminal fragments: II + III, I + II and I + III, respectively. Of these combinations, β-carotene, containing two β-ion (II) fragments, has the greatest biological activity. All such compounds are hydrocarbons and they belong to the group of carotenes. β-Carotene was first isolated by Wackenroder (1831) from carrots, which was reflected in the name of this substance (carot – carrot, English, and Daucus carota – wild carrot, Latin). Its empirical formula and structure were established, respectively, by Waldstetter (1906) and Zechmeister, Karrer and Kuhn (1928 – 1930) [3]. It was β-carotene that was the focus of attention of biologists and physicians due to its obvious property – by formally dividing into two equal parts, two molecules of retinal, or retinol (vitamin A), can be obtained from one molecule of this substance. However, the actual mechanism of conversion of β-carotene into retinal and retinol is quite complex, and involves oxidative degradation starting at one end of the molecule through the formation of apo-carotenals [3]. It should be noted that synthetic β-apo-8'-carotene, β-apo-12'-carotene and the ethyl ester of β-apo-8'-carotene acid are widely used as pigments in the food industry. The enzymatic conversion of beta-carotene supplied with the diet is important for animals and humans. It mainly occurs in the mucosa of the small intestine. β-carotene forms an unstable four-carbon heterocycle along the central double bond (15-15' by the carbohydrate atoms of the molecule) in the presence of molecular oxygen, which decays to form two retinal molecules. This process refers to a dioxygenase reaction, and the corresponding enzyme has been isolated from the intestinal mucosa of some mammals. It has been proved that carotene oxidase belongs to thiol enzymes (participation in the reaction of SH groups) and depends on iron ions. Also, a minimal presence of bile is necessary for this reaction [4].

A significant part of the retinal formed in the intestinal mucosa is then reduced to retinol, some of it can be oxidized to retinoleic acid. Retinol, as a rule, is esterified with higher fatty acid and the resulting ester is first a transport form (as part of chylomicrons), and then a reserve form (deposited in the liver). Since oxidative processes are usually associated with side reactions that are difficult to exclude, it is understandable why the vitamin activity of β-carotene is not twice as high, but twice as low as the activity of retinol. The conditional quantitative measure of this activity – 1 IU (international unit) corresponds to 0.6 mg of β-carotene.

As can be seen from the presented data, a decrease in the number of β-ion fragments halves the vitamin activity of carotenoids, and trans-cis isomerization has an even stronger effect on this property. Lycopene and δ-carotene have no vitamin activity at all. Animals are not able to carry out de novo biosynthesis of carotenoids, therefore they must receive these substances with food. The biosynthesis of carotenoids in the early stages involves lengthening the chain by attaching isoprenoid fragments to build a C20 framework, dimerization of which leads to the formation of phytoin (contains 4 fewer double bonds compared with lycopene).

Subsequent transformations can be combined into a simplified scheme, the following processes occur:

1) an increase in unsaturation – the formation of an unsaturated C=C bond;
2) cyclization of the terminal fragment (I II, I III);
3) hydroxylation to position 3 (or 3') of the cyclohexane fragment;
4) epoxidation (usually by double bond in position 5);
5) rearrangement of epoxy compounds into a furanoxide structure:

Hydroxylation of the terminal fragments synthesizes hydroxy derivatives, which, together with keto and various epoxy or furanoxy derivatives, form an extensive group of carotenoids called xanthophylls - substances of the terrestrial flora that are more common than carotenes. Accordingly, the introduction of oxygen into the cyclohexene fragment usually deprives the carotenoid of provitamin activity. In addition to plants, carotenoids are synthesized in the cells of some algae, phototrophic bacteria, in certain species of non-photosynthetic bacteria, lower fungi, yeast and actinomycetes [6]. In plant cells, carotenoids are localized in plastids in the form of globules, crystals, protein-carotenoid complexes included in the membrane structure [7]. Their fundamental importance is related to the process of photosynthesis. As auxiliary pigments of photosynthesis, carotenoids are components of photosystems I and II [8]. □-carotene protects the reaction centers of photosystems from photooxidation, and xanthophylls (lutein, violoxanthin, neoxanthin) perform a light-selective function [9]. In non-photosynthetic tissues and organs of plants, carotenoids stabilize cell membranes, forming relatively inactive peroxides, prevent chain oxidation reactions, regulate the transport and biosynthetic functions of membranes, participate in the processes of phototropism, phototaxis, reproduction in both plants and microorganisms. The accumulation of carotenoids in plants both qualitatively and quantitatively depends not only on the type of plant (genus, family) but also on the variety. There are known successes of genetic engineering methods that have made it possible to purposefully change the metabolism of carotenoids.

The maximum absorption of molecules is especially intensively shifted when keto groups are included in the conjugation chain, for example, when zeaxanthin is oxidized to capsanthin and capsorubin in peppers (Capsicum annuum, [5]) and the orange color is replaced by red. It is the presence of conjugated double bonds that causes the high lability of carotenoids, but also the easy oxidizability of these compounds with the formation, as noted above, of not too reactive epoxides – in some cases, it is possible to remove epoxy oxygen with the regeneration of the initial xanthophyll). This property of conjugated polyene systems determines the high antioxidant function of carotenoids and their corresponding importance for animals and humans in particular.

The common link for the vast majority of biochemical processes in which carotenoids participate is the free radical modification of molecules and components of biological membranes. In living systems, free radicals are formed in Red-Ox reactions carried out by single-electron transfer [13]. The reasons for the intensification of free radical processes in pro- and eukaryotic cells may be: ionizing radiation, products of macrophage activation (in animals), metabolic products of certain drugs, disorders in the body's antioxidant system. In this case, any organic substrates, including amino acids, proteins, lipids, carbohydrates, DNA and RNA, are subjected to oxidative action [14, 15]. Lipid molecules containing radicals of easily oxidized unsaturated fatty acids are most often targeted, therefore lipid peroxidation is one of the most common free radical processes of the body [16, 17]. Carotenoids neutralize peroxide radicals and prevent the peroxidation of lipid components of cell membranes [18]. A synergism of the antioxidant action of carotenoids was found in a mixture with other fat–soluble antioxidants -tocopherol and coenzyme Q10. [19] The antioxidant properties of many carotenoids cause their radioprotective, antimutagenic, immunomodulatory, anti-infective, anti-carcinogenic effects [20]. Recent studies have shown that astaxanthin, 3,3'-dihydroxy-β,β'-carotene-4,4'-dione synthesized by the marine microalgae Haematococcus pluvialis Flotow, turns out to be an antioxidant – more effective compared to such well-known free radical acceptors as α-tocopherol (vitamin E), β-carotene, lycopene, lutein et al. [21]. Another important discovery was the discovery of the special role of lutein and zeaxanthin (dihydroxy derivatives of α– and β–carotenes, respectively) in preventing age-related vision loss [20]. A series of studies on the biological activity of lycopene, which enters the body with tomatoes and their processed products, usually showed an inverse relationship between the consumption of this carotenoid and a number of cancers [20].

Thus, the high biological activity of carotenoids is an experimentally confirmed fact. Therefore, the task of modern society is to provide the population with high–quality food products, including purposefully enriched with carotenoids, which can be solved within the framework of "Food Design". It is in this direction that Belgorod State University has created a biologically active additive balanced in the ratio of xanthophylls (lutein and zeaxanthin) for the introduction of laying hens into the diet in order to obtain eggs with a high content of xanthophylls in the yolk.

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