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The carotenoid Bixin found to exhibit the highest measured carotenoid oxidation potential to date consistent with its practical protective use in cosmetics, drugs and food

Sefadzi Tay-Agbozo*, Shane Street, Lowell Kispert

Department of Chemistry, The University of Alabama, Box 870336, Tuscaloosa, AL 35487-0336, USA

ABSTRACT

The electrochemical oxidation potentials of *cis* bixin correspond to the production of the carotenoid radical cation, $Car^{\cdot+}$ and dication Car^{++}. The oxidation is a two-electron process with oxidation potentials at ~0.94 and ~1.14 V vs SCE (reference to ferrocene at 0.528 V) in THF. These potentials are higher than that of symmetrical canthaxanthin at 0.775 V and 0.972 V and for β-carotene at 0.634 V and 0.605 V respectively. The second oxidation potential for canthaxanthin is higher by 0.20 V than the first. Similar difference is observed for bixin. In contrast, the second oxidation potential for β-carotene is lower by 30 mV than that of the first. Reduction potentials were found to occur at ~ −0.69 and ~ −1.22 V vs SCE. The lifetime of the radical cation of *cis* bixin, $Car^{\cdot+}$, is short and decays rapidly at ambient temperature. The suggested scavenging ability of *cis* bixin towards reactive oxidative oxygen species is estimated to be 44. On the other hand, that of β-carotene, symmetrical canthaxanthin and the dicyano substituted carotenoid which exhibit oxidation potentials of 0.634 V, 0.775 V and 0.833 V vs SCE were measured to be 0.64, 1.96 and 23.60 respectively. The non-reversible electrochemical measurements suggest the tendency for bixin to react with trace amounts of reactive oxygen species ($\cdot$OH, $O_2^{\cdot-}$, $\cdot$OOH).

1. Introduction

1.1. Need for Electrochemical Measurements: Scavenging, Pro or Anti-oxidants

The carotenoid bixin shown in Fig. 1 is approved by the Food and Drug Administration (FDA) [1–2] as a suitable colorant for use in food, drugs and cosmetics (under authority granted by the Federal Food, Drug, and Cosmetic Act of 1938, and the 1960 Color Additive Amendments to the Act) [2]. For example, bixin is used in cosmetic compositions combined with a lipid soluble UV filter to protect the human epidermis against UV radiation [3]. Most carotenoids occur as the *trans* isomer hydrocarbon [4–8] and are water insoluble. However, bixin occur as the *cis* isomer and exhibits enhanced water solubility relative to the other carotenoids [9–11].

1.1.1. As a Carotenoid that Is Used as a Colorant in Many of the Products that we Use, What Other Properties might Bixin Exhibit that would Serve as an Advantage to those Using the Products when Bixin Is Added?

One of the most important biological properties of carotenoids is

their antioxidant activities which prevent damage to living cells when the carotenoids react with toxic free radicals [12–14]. Diseases such as infarction, cerebral thrombosis and tumors are partly as a result of the action of free radicals and reactive oxygen species (ROS) [15].

Most of the oxygen that is absorbed in the lungs is used for energy production. About 1–3% of this oxygen goes to make ROS [15]. These ROS include species such as hydrogen peroxide (H_2O_2) and the superoxide radical ($O_2^{\cdot-}$) which are not that reactive chemically on their own. They have the ability to produce very reactive free radical species such as the hydroxyl ($OH^{\cdot}$) radical when they make contact with transition metals, particularly iron and copper:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {\cdot}OH + OH^-$$
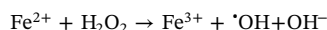
It is the $OH^{\cdot}$ which pose major threat as it is able to destroy almost every cell in the human body [15–16].

Published reports [17–18] have shown that carotenoids exhibit scavenging ability towards ROS such as $\cdot$OH, $\cdot$OOH, $O_2^{\cdot-}$ which increases nearly exponentially with increasing carotenoid oxidation potential (ox. pot.). For example, astaxanthin (~0.833 V) has a 2.6 scavenging ability. However, for carotenoids with low oxidation potential (ox. pot. ~0.60 V) as is observed for β-carotene, has a 0.64
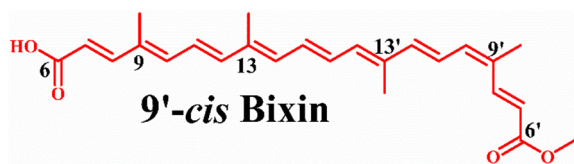
Fig. 1. The carotenoid 9′-*cis* bixin molecule.

scavenging ability and exhibits a prooxidant behavior [18]. When this occurs, Fe^{3+} is reduced to Fe^{2+} and the β-carotene radical is formed. The effectiveness of this carotenoid-driven Fenton reaction, increases with decreasing scavenging rates for free radicals and with decreasing ox. pot. of the carotenoids [17–18]. The shelf life of pharmaceuticals can be prolonged by carotenoids and depends on the ability of the carotenoids to scavenge free radicals [12, 17–18]. Like most carotenoids, bixin is an efficient quencher of singlet-state oxygen and of the triplet state of sensitizers [19] and has a great ability to scavenge reactive oxygen and nitrogen species [20].

1.2. Electrochemical Reactions of Carotenoids and Schemes

There has been extensive electrochemical [21–31], EPR [32–34] and optical [35–37] studies on carotenoid radicals including radical cations ($Car^{\bullet+}$), neutral radicals ($Car^{\bullet}$), monocations (Car^{+}), dications (Car^{2+}) and oxygenated radical species. Electrochemical studies of carotenoids including β-carotene [21, 24, 38] and synthetic derivatives with acceptor or donor terminal substituents [21, 25–30] have shown that, in aprotic solvents, oxidation of carotenoids results in at least three heterogeneous reactions (1, 2 and 3),

$$Car \overset{E_1^0}{\Leftrightarrow} Car^{\bullet+} + e^- \qquad (1)$$

$$Car^{\bullet+} \overset{E_2^0}{\Leftrightarrow} Car^{2+} + e^- \qquad (2)$$

$$[Car - H]^+ + e^- \overset{E_3^0}{\Leftrightarrow} [Car - H]^{\bullet} \qquad (3)$$

and three homogeneous reactions (4, 5 and 6),

$$Car^{2+} + Car \overset{K_{com}}{\Longleftrightarrow} 2Car^{\bullet+} \qquad (4)$$

$$Car^{2+} \overset{K_{dp}}{\Leftrightarrow} [Car - H]^+ + H^+ \qquad (5)$$

$$Car^{\bullet+} \overset{K_{dp}'}{\Leftrightarrow} [Car - H]^{\bullet} + H^+ \qquad (6)$$

where:

E_x^0 = potential (pot.) of reaction.
K_{com} = comproportination equilibrium constant.
K_{dp} = deprotonation equilibrium constant for dication.
K_{dp}' = deprotonation equilibrium constant for radical cation.

An electron is transferred from a carotenoid (Car, Eq. (1)), to form the radical cation at pot. E_1^0 ($Car^{\bullet+}$) which upon losing an extra electron (Eq. (2)) at pot. E_2^0 results in the formation of the dication (Car^{2+}). The radical cation being a weak acid, can lose a proton according to Eq. (6) at rate K_{dp}'. Mairanovsky et al. [24] suggested that, Car^{2+} (strong acid) can undergo deprotonation (Eq. (5)) at rate K_{dp} to form the proton loss cation ($[Car-H]^+$) which upon acceptance of an electron (Eq. 3) at red. pot. E_2^0 forms the proton loss neutral radical ($[Car-H]^{\bullet}$). The radical cation and dication exists in equilibrium (Eq. (4)) according to the magnitude of K_{com}.

In Fig. 2, is an example of a reversible CV for astaxanthin, astaxanthin monoester and diester astaxanthin. Only Eqs. (1) and (2) occur and not proton loss Eqs. (5) and (6). If a proton acceptor in the solvent is present, then Eqs. (5) and (6) become important and the reversible character is lost. The radical cation is formed at position 1 and the
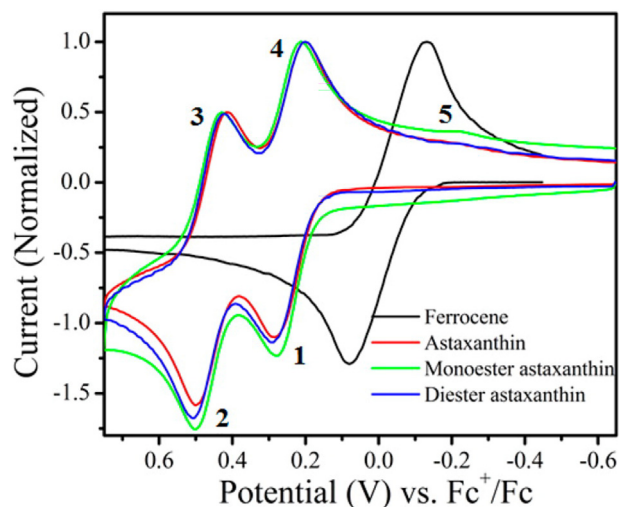

Fig. 2. Comparison of CVs at a scan rate of 500 mV/s for astaxanthin, monoester astaxanthin and diester astaxanthin in dichloromethane (DCM) respectively with ferrocene used as an internal reference label. The CV shows a typical reversible process with numbers 1 and 4, 2 and 3 indicating the reactions that occur in Eqs. (1) and (2) respectively, where radical cation at 1 and dication at 2 are formed. While at 3, the dication is reduced and at 4 the radical cation is reduced (Eqs. (1) and (2)). Position 5 indicates reaction occurring in Eq. (3) where the species formed from the loss of proton from the dication is reduced. Adapted from Focsan et al. J. of Phys. Chem. B 2014, 118 [9], 2331–2339.

dication at 2, while at 3, the dication is reduced and at 4, the radical cation is reduced forming the molecule. Position 5 indicates reaction occurring in Eq. (3) where the species formed from the loss of proton from the dication is reduced.

Published electrochemical measurements [23] (using 99.99% methylene chloride solvent contained in a Sure-Seal bottle) fitting the CV over a wide range of scan rate established the best ox. and red. pot. now available for astaxanthin, monoester astaxanthin and diester astaxanthin listed in Fig. 2.

Two reviews of the electrochemical properties of carotenoids have been published. One in 1999 on the studies [30] of 32 different carotenoids, comparing the oxidation potential as a function of donor/accepter substituents, conjugation chain length and symmetry. The following year, a review [29] of the oxidation potentials, reaction rate constants and kinetic equilibrium for naturally occurring carotenoids.

The carotenoid 15,15′-didehydro-β-carotene (Fig. 3) was studied [22] in 2001 to examine the dependence of ox. pot. and red. pot. on conjugation length relative to β-carotene. The substituted keto group added to β-carotene to form canthaxanthin (Fig. 3), permitted a study to establish the change in the ox. and red. pot. when oxygen atoms are added to carotene. In addition, the electrochemical facilities in Hapiot. et al. [22] lab allowed the recording of CVs over 5 orders of magnitude ($40–4 \times 10^6$ mV/s) of scan rates so very accurate ox. pot. and red. pot. could be deduced (all reference to Ferrocene at 0.528 V) by simulating the peak positions as a function of scan rate. These potentials are listed in Table 1.

1.3. Complications with Choice of Solvents

Grant et al. [21] in 1988 studied the electrochemical oxidation of three all *trans* carotenoids: β-carotene, β-apo-8-carotenal and canthaxanthin (Fig. 3) in various solvents. They observed that the electro-oxidation pathway is through a two or one-electron process, and it was dependent on the nature of the solvent. While β-apo-8-carotenal and canthaxanthin (Fig. 3) proceeded via two-electron irreversible processes in THF as shown in Fig. 4, they proceed via a one-electron