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Antioxidant properties of carotenoids: QSAR prediction of their redox potentials

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Abstract. There is a great need to predict the antioxidant properties of molecules such as carotenoids. These compounds are of great interest due to their contribution to various important biological and industrial processes, including toxicity and fate. In our study, redox potentials were compiled from several literature sources. Redox potential values ranged from 537.2 mV for zeaxanthin up to 691.5 mV for β-carotene; they correspond to the formation of cation radicals, using the standard calomel electrode (SCE). The redox potential values were measured using conventional electrochemical techniques, cyclic voltammetry and Osteryoung square-wave voltammetry. A quantitative structure-activity relationship (QSAR) was developed to model and consequently to predict the values of redox potential. The predicted values of redox potential for four external carotenoids, namely β-carotene, zeaxanthin, cantaxanthin and astaxanthin, are presented and discussed. They indicate the dependence of redox potential on structure, donor and acceptor groups and polarisability.

Key words: QSAR(s) — Carotenoids — Redox potential — Antioxidant properties

Introduction

Carotenoids are a very widespread group of natural pigments which are present in plants, fish and fruits, where they are responsible for their red, yellow and orange colouring. More than 600 carotenoids have been isolated and identified from natural sources, including 19 carotenoids in the human body (Packer 1992; Liu et al. 2000). Their long backbone chain of conjugated carbon-carbon double bonds creates a structure of conjugated polyenes. Carotenoids have many roles in mammalian metabolism, such as their conversion to retinal (vitamin A). In addition, they have been found to possess anticancer, anticardiovascular, anti-inflammatory and many others activities (Rice-Evans and Diplock 1993; Cadenas and Packer 1996; Rice-Evans and Packer 1998).

It is well known that reactive oxygen species (ROS) and reactive nitrogen species play a dual role in biological systems, since they can be either harmful or beneficial to living systems (Bauerova and Bezek 1999; Juranek and Bezek 2005). Under conditions of oxidative stress, low-molecular weight antioxidants are involved directly in the conversion of ROS to less reactive species. The protective effect of carotenoids is connected to their antioxidant ability, i.e. to scavenge toxic free radicals effectively. However, carotenoids are unstable and sensitive to light, oxygen, pH and temperature.

Carotenoids are active in electrotransfer processes in which cation radicals are formed. The presence of cation radicals can be measured by absorption spectroscopy or electron paramagnetic resonance techniques. Cyclic voltammetry and Osteryoung square-wave voltammetry are the most widely used techniques for the characterisation of redox systems (Bard and Faulkner 1980), with both techniques based on electrochemical electrode reactions. Due to their extended conjugation and ease of oxidation, carotenoids may be expected to undergo electron transfer reactions (O'Neil et al. 1991; Delasrivas et al. 1993; Gust et al. 1993). On the basis of these reactions we are able to perform measurements of the redox potential of carotenoids. Electrochemical studies show that during oxidation of carotenoid compounds, several reactive species are formed (Mairanovsky et al. 1975; Park 1978; Grant et al. 1988; Khaled et al. 1990):

$$Car \xleftrightarrow{E_1^0} Car^{\bullet +} + e^-$$

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$$Car^{\bullet+} \xleftrightarrow{E_2^0} Car^{2+} + e^-$$

$$^{\#}Car^{+} + e^- \xleftrightarrow{E_3^0} {}^{\#}Car^{\bullet}$$

where: *Car* – neutral species of the carotenoid, $Car^{\bullet+}$ – cation radical of the carotenoid, Car^{2+} – dication of the carotenoid, $^{\#}Car^{+}$ – deprotonated carotenoid cation, $^{\#}Car^{\bullet}$ – deprotonated carotenoid radical.

The study of oxidation products and their redox properties is necessary for a better understanding of the electrotransfer processes involving carotenoids. Many studies relating to carotenoids have been performed. Carotenoids have been studied by pulse radiolysis (Lafferty et al. 1977), laser ionisation (Mathis and Vermeglio 1972) and spectroscopically (Bors et al. 1984; Lee 1989; Chen 1991; Khaled et al. 1991; Khaled 1992; Jeevarajan et al. 1994a; van Breemen et al. 1996) to investigate their reactivity and chemical behaviour.

Quantitative structure-activity relationships (QSARs) can be used to investigate the mathematical relationship between a set of physico-chemical descriptors and chemical or biological activity. These descriptors describe the steric, electronic and hydrophobic nature of each compound and can be experimentally and computationally derived using a wide variety of available software. Using multiple linear regression analysis, one can extract a mathematical relationship between chemical descriptors and activity. The resultant QSAR model can then be used to predict the activity of new molecules, given only the values for the descriptors. Generally, to obtain a relationship with sufficient predictive power, one has to limit the molecules for which the QSAR will be applicable (classically tens of molecules) (Cronin and Livingstone 2004).

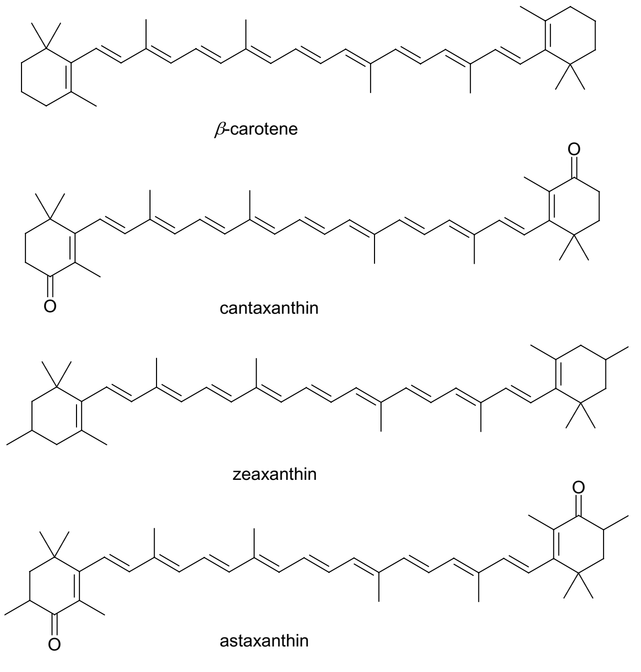

Figure 1. Structure of four external carotenoids.

In this study, in order to elucidate the contributions of various physicochemical parameters to the antioxidant activities of carotenoids, attempts have been made to correlate their antioxidant activities with their redox potentials. Lien's QSAR study (Lien et al. 1999) of phenolic antioxidants has been shown to be useful for predicting antioxidant activity. Consequently we have focused our attention on the use of QSAR to predict redox potential of carotenoids because redox potential is a precursor of chemical and biological activity.

Recently, it has been reported, that the polarizability effect of a molecule is closely linked with the number of valence electrons (Verma et al. 2005). Since the oxido-reduction properties of carotenoids (cation radicals, anion radicals, neutral radicals) are tightly linked with the number of valence electrons, the effect of polarizability can effectively be applied.

The aim of this study was to create a model for the prediction of redox potential of carotenoids and subsequently to use the model to predict redox potentials of four carotenoids in an external test set, namely *β*-carotene, zeaxanthin, cantaxanthin and astaxanthin (structures are shown in Figure 1). The quality of the model was assessed by external validation, comparing the predicted redox potentials to those obtained from literature (Jeevarajan et al. 1994b; Hoischen et al. 1999; Deng et al. 2000; Liu et al. 2000; Hapiot et al. 2001; Niedzwiedzki et al. 2005; Polyakov et al. 2006) for compounds not in the training set.

Experimental

Training set

A training set of 24 carotenoids was obtained from the literature, the structures of which are given in Figure 2. The redox potentials of all the carotenoids in the study were present in the literature and are shown in Table 1. These values were measured using the standard calomel electrode (SCE) at pH 7, room temperature and the solvent used was CH_2Cl_2 (Jeevarajan et al. 1994a; Deng et al. 2000; Hapiot et al. 2001; Liu et al. 2000; Niedzwiedzki et al. 2005; Polyakov et al. 2006).

Test set

For validation, a test set of four carotenoids, namely *β*-carotene, zeaxanthin, cantaxanthin and astaxanthin (not

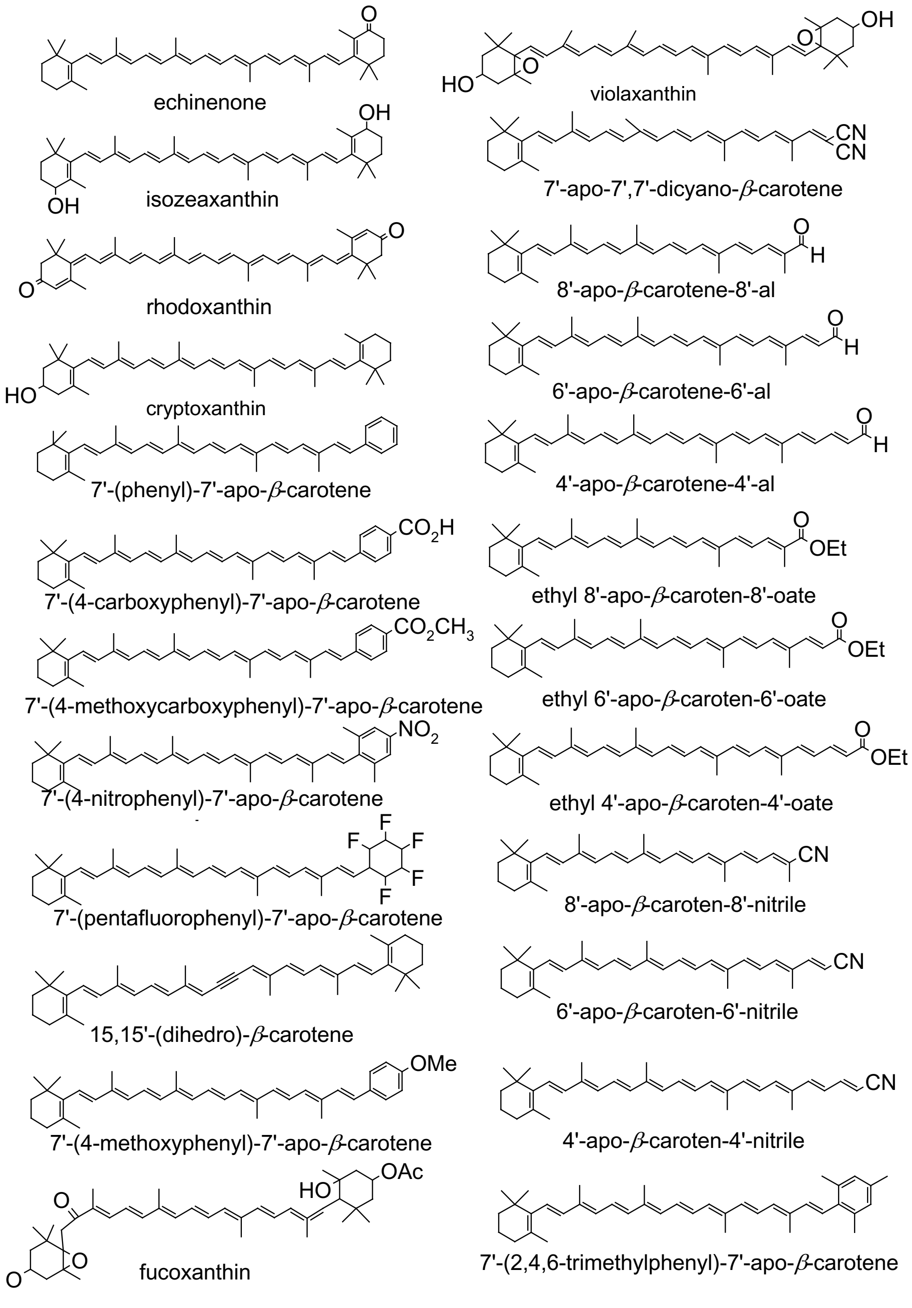

Figure 2. Structures of carotenoids used in training set.

included in the training set) was used. The redox potentials of these carotenoids were predicted using the QSAR model developed.

Descriptors

For all carotenoids included in our study, the 3D structures were generated. Simplified molecular input line entry system (SMILES) notations were entered into the TSAR for Windows version 3.3 software (Accelrys Inc., Oxford, England). More than one hundred descriptors were generated using TSAR (Accelrys Inc.), MOPAC 93 (Fujitsu Limited, 1993), HYBOT (MOLPRO version 2.1.0.706; Dr. Sergei V. Trepalin, 1997–2000), KOWWIN (U.S. Environmental Protection Agency, 2000) and selected descriptors were calculated using DRAGON professional, version 5.3 (R. Todeschini, Milano).

Table 1. Carotenoids used in training set and redox potentials obtained from the literature sources

Number	Name	E (mV) *vs.* SCE
1	echinenone	590.0
2	isozeaxanthin	550.0
3	rhodoxanthin	655.0
4	cryptoxanthin	560.0
5	fucoxanthin	790.0
6	violaxanthin	681.0
7	7’-apo-7’,7’-dicyano-β-carotene	720.0
8	ethyl 8’-apo-β-caroten-8’-oate	722.0
9	ethyl 6’- apo-β-caroten-6’-oate	692.0
10	ethyl 4’- apo-β-caroten-4’-oate	600.0
11	8’-apo-β-caroten-8’-al	720.0
12	6’-apo-β-caroten-6’-al	653.0
13	4’-apo-β-caroten-4’-al	625.0
14	8’-apo-β-caroten-8’-nitrile	722.0
15	6’-apo-β-caroten-6’-nitrile	672.0
16	4’-apo-β-caroten-4’-nitrile	660.0
17	7’-(2,4,6-trimethylphenyl)-7’-apo-β-carotene	560.0
18	7’-(4-methoxyphenyl)-7’-apo-β-carotene	550.0
19	7’-phenyl)-7’-apo-β-carotene	550.0
20	7’-(4-carboxyphenyl)-7’-apo-β-carotene	550.0
21	7’-(4-methoxycarbonylphenyl)-7’-apo-β-carotene	575.0
22	7’-(4-nitrophenyl)-7’-apo-β-carotene	605.0
23	7’-(pentafluorophenyl)-7’-apo-β-carotene	635.0
24	15,15’-didehydro-β-carotene	875.0

SCE, standard calomel electrode.

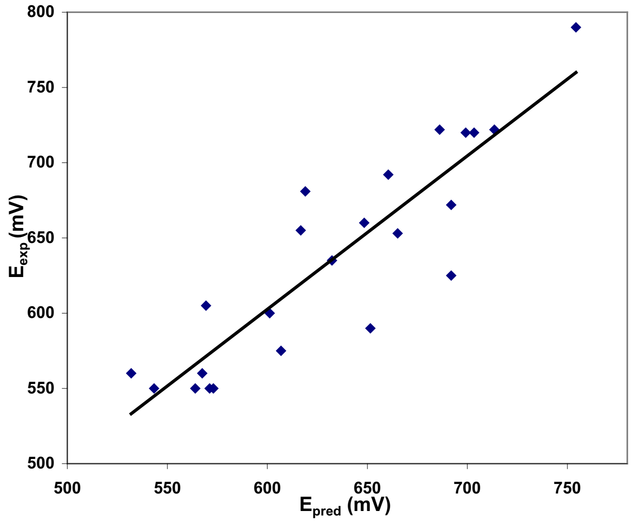

Figure 3. Plot of experimental redox potential (E_{exp}) *vs.* predicted redox potential (E_{pred}).

Statistics

The model was developed using a stepwise regression approach to identify significant descriptors using the MINITAB (version 14.12.0) statistical software. Equations were created using the multiple linear regression analysis.

The following statistical parameters are used to describe the regression analysis: s – the standard deviation, r^2 (adj) – the square of the correlation coefficient adjusted for degrees of freedom, F– the Fisher statistic, and n – the number of data points upon which the equation is based.

Results

In this study, a QSAR model was derived to predict the redox potential of carotenoids. Following stepwise regression, a significant correlation was obtained between the redox potential and the polarisability ZZ (α_{ZZ}) descriptor. No additional descriptors were identified as being significant during stepwise regression and the inclusion of further descriptors into the model increased model instability and decreased model interpretability. The derived model was:

$$E\ (\mathrm{mV}) = 1210 - 6.00\,\alpha_{ZZ} \qquad (1)$$

$n = 23$; $s = 33.1$; $r^2(\mathrm{adj}) = 0.774$; $F = 76.4$

This is a satisfactory model that allows redox potential to be predicted from the structure, without the need for experimental measurement. The relationship between E (mV) and α_{ZZ} is shown in Figure 3. (15,15’-dihydro-β-carotene appeared as an outlier in the initial model, possibly due to the presence of a triple bond in its structure and therefore has been removed from the Eq. (1)).

Stepwise regression revealed one parameter (α_{ZZ}) to be the best predictor of redox potentials for carotenoids. Polarisability (α) is the relative tendency of the electron cloud of an atom to be distorted from its normal shape by the presence of a nearby ion or dipole, that is, by an external electric field. Polarisability is defined as:

$$\alpha = \begin{pmatrix} \alpha_{XX} & \alpha_{XY} & \alpha_{XZ} \\ \alpha_{YX} & \alpha_{YY} & \alpha_{YZ} \\ \alpha_{ZX} & \alpha_{ZY} & \alpha_{ZZ} \end{pmatrix}$$

As carotenoids are cylindrical in shape (Figure 4), the electron cloud lies above the conjugated double bond chain. It is possible that this long chain is parallel to axis Z, therefore α_{ZZ} is the main contribution to polarisability and α_{ZZ} is the most significant descriptor. Polarisability has a negative correlation with the redox potential of carotenoids, suggesting that increasing polarisability reduces the antioxidant ability of the carotenoids.

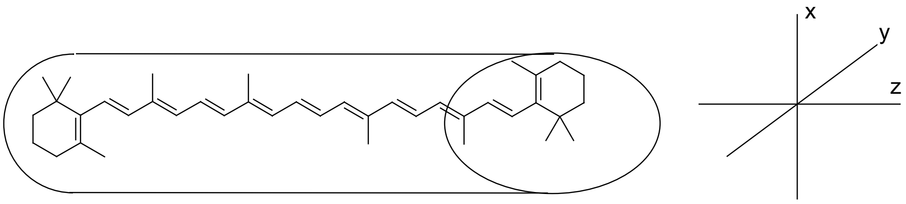

Figure 4. Cylindrical shape of a carotenoid with axes.

The redox potential (measured using SCE) for the carotenoids not included in the training set was calculated from Eq. (1). Their measured and calculated redox potentials are given in Table 2.

Discussion

As already discussed above, electrochemistry has proven to be a very convenient method for the production and study of cation radicals and other intermediates of carotenoids. The most widely used techniques for the characterization of redox systems are cyclic voltammetry and Osteryoung square-wave voltammetry (Liu et al. 2000). Measurement of redox potentials for chemically reversible couples is straightforward, however, direct determination of redox potentials of short-lived cation radicals and dications (i.e. chemically irreversible couples) is more difficult. This is documented in Table 2, showing the diversity of redox potential values of various carotenoids. In light of these results, the QSAR prediction of redox potentials of carotenoids may be beneficial.

The aim of this study was to create a model for the prediction of redox potential of carotenoids and use the model to predict redox potentials of further carotenoids. Table 2 shows the values of the experimentally determined redox potentials of β-carotene, zeaxanthin, cantaxanthin and astaxanthin, the literature source for these values, values of α_{ZZ} and values for the predicted redox potentials for these carotenoids. Redox potentials predicted in this study are in the range of literature data and the numbers highlighted with ◆ represent the closest values of redox potentials from literature.

For β-carotene, the predicted redox potential is 691.5 mV, which is consistent with the value of 634.0 mV from Hapiot et al. (2001). The redox potential for zeaxanthin is 537.2

Table 2. Measured *vs.* predicted values of E (E_{pred}) for the external validation set

Number	Name	E (mV)	Reference	α_{ZZ}	E_{pred} (mV)
1	β-carotene	567.0	Niedzwiedzki et al. (2005)	87.1	691.5
		540.0	Liu et al. (2000)		
		530.0	Liu et al. (2000)		
		570.0	Hoischen et al. (1999)		
		630.0	Kispert et al. (2004)		
		780.0	Grant et al. (1988)		
		634.0 ◆	Jeevarajan et al. (1994a)		
		530.0	Jeevarajan et al. (1994b)		
2	zeaxanthin	571.0	Niedzwiedzki et al. (2005)	112.6	537.2
			Liu et al. (2000)		
		560.0	Polyakov et al. (2006)		
3	cantaxanthin	689.0	Niedzwiedzki et al. (2005)	106.9	571.7
		705.0	Niedzwiedzki et al. (2005)		
		900.0	Khaled et al. (1991)		
		680.0 ◆	Polyakov et al. (2006)		
		775.0	Hapiot et al. (2001)		
4	astaxanthin	*	*	92.5	658.0

* values could not be obtained from literature; ◆ the closest value of E from literature.

mV and is very close to Chen's reported redox potential of 530.0 mV (Lien et al. 1999). For cantaxanthin, the value for the redox potential is poorly predicted by the model. The closest literature value is that reported by Polyakov et al. (2006), a value of 680.0 mV, compared to a value of 571.1 mV predicted by the model. The predicted redox potential of astaxanthin is 658.0 mV. A value for the redox potential of astaxanthin has not been found in literature.

This study demonstrates that the redox potential increases when polarisability decreases. Redox potential reflects the antioxidant activity of carotenoids, so antioxidant activity can be related to the polarisability of carotenoids. Poorer antioxidant activity is associated with greater polarisability for carotenoids.

Arranging the carotenoids according to increasing redox potential gives the following order: zeaxanthin < cantaxanthin < astaxanthin < β-carotene.

From basic chemistry it is known that compounds with lower redox potential are more easily oxidised. According to the ranking shown above, β-carotene is the most readily oxidised carotenoid and zeaxanthin is the most readily reduced one in this series. Combining these two results suggests that the greater the value of α_{ZZ}, the more readily the carotenoid is oxidised.

Conclusion

In this study, a QSAR model has been developed for the prediction of redox potentials for carotenoids using regression analysis. Since the best correlation between the redox potential of a series of carotenoids and the ZZ component of polarizability (α_{ZZ} descriptor) has been determined, no additional descriptors have been included into the model. The equation derived is applicable to predicting redox potentials of other carotenoids. A good correlation (r^2 (adj) = 77.4%) was obtained using α_{ZZ} as a single predictor.

The measurement of redox potential is difficult. As Table 2 shows, several redox potential values from different experimental measurements exist for an individual compound. Using the QSAR model described herein, redox potential of carotenoids can be readily predicted.

The model obtained indicates that molecular properties such as electron distribution and molecular topology (i.e. branching and other subtle effects) are important for controlling redox potential.

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