Theor Chem Acc (2010) 127:595–603
DOI 10.1007/s00214-010-0753-3

REGULAR ARTICLE

Theoretical study on the chemical fate of adducts formed through free radical addition reactions to carotenoids

Ana Martínez · Rubicelia Vargas · Annia Galano

Received: 4 February 2010 / Accepted: 27 March 2010 / Published online: 17 April 2010

Abstract It is well known that free radicals are responsible for oxidative stress and cause numerous health disorders. As a result, the study of molecules that can scavenge free radicals is significant. One of the most important classes of free radical scavengers are carotenoids (CAR). In this work, the effectiveness of the CAR in terms of the radical adduct formation (RAF) reaction is studied using density functional theory calculations (in polar and non-polar environments). The reactions between four CAR [β-carotene (BC), zeaxanthin (ZEA), canthaxanthin (CANTA) and astaxanthin (ASTA)] with eight different radicals ($^{\bullet}$OH, $^{\bullet}$OOH, $^{\bullet}CH_3$, $^{\bullet}O{-}CH_3$, $^{\bullet}OO{-}CH_3$, $^{\bullet}$SH, $^{\bullet}O{-}CH_2{-}CH{=}CH_2$, and $^{\bullet}OO{-}CH_2{-}CH{=}CH_2$), as well as substantial further reactions involved in the radical chain propagation, are analyzed. According to our results, the RAF reactions are controlled to a larger extent by the nature of the free radical than by the particular CAR they are reacting with. Thermochemistry calculations predict that each CAR molecule is able to scavenge at least two free radicals, which would lead to the termination of the radical chain process. Epoxy and diepoxy CAR species can be formed, being epoxy molecules as good free radical scavengers as their parent CAR. ASTA and CANTA are predicted to be less reactive, when reacting through RAF mechanism, than BC and ZEA.

Keywords Carotenoids · Radical reactions · Computational chemistry · Antioxidants · Thermochemistry

1 Introduction

Free radicals are short-lived very reactive species that can damage molecules of high biological importance like proteins and DNA. This chemical damage is commonly referred to as oxidative stress, and it has been held responsible for numerous health disorders like cancer [1–4], cardiovascular disorders [5–9], atherosclerosis [10–13], and Alzheimer's disease [14–18]. That is why the study of the compounds with free radical-scavenging activity is a current and important area of research. Carotenoids (CAR) are known for their efficiency as free radical scavengers (see for example [19, 20]), and three viable mechanisms are generally accepted for these processes [21]:

Electron transfer (ET):

$$R^{\bullet} + CAR \rightarrow R^{-} + CAR^{\bullet +} \qquad (I)$$

Radical adduct formation (RAF):

$$R^{\bullet} + CAR \rightarrow [R{-}CAR]^{\bullet} \qquad (II)$$

Hydrogen atom transfer (HAT):

$$R^{\bullet} + CAR \rightarrow RH + CAR(\text{-H})^{\bullet} \qquad (III)$$

The relative importance of these reaction channels depend on diverse factors, including the structural features of the reacting CAR [22], its location and orientation within the

---

Rubicelia Vargas on sabbatical leave at Instituto de Investigaciones en Materiales, UNAM.

A. Martínez · R. Vargas
Instituto de Investigaciones en Materiales, Universidad Nacional Autónoma de México, Circuito Exterior S. N.,
Ciudad Universitaria, 04510 Mexico DF, Mexico

R. Vargas · A. Galano (✉)
Departamento de Química, División de Ciencias Básicas e Ingeniería, Universidad Autónoma Metropolitana-Iztapalapa,
San Rafael Atlixco 186, Col. Vicentina, Iztapalapa.
A.P. 55-534, 09340 Mexico DF, Mexico
e-mail: agalano@prodigy.net.mx

membrane [23], and the nature of the reacting free radical [24–27]. The last aspect has also been reported to play a role on the reaction mechanism of other free radical scavengers [28]. In previous works from our group, we have studied the electron donor–acceptor capacity of different CAR as the starting point for the ET reactions [24–27], and also the electron transfer from [29] and to [30] different reacting free radicals. We have also studied the competition between HAT and RAF mechanisms [31, 32], and found that the HAT is a minor channel of reaction when peroxyl radicals are involved [31]. This finding is in agreement with previous works from other research groups. Burton and Ingold [21] suggested that β-carotene (BC) reacts with peroxyl radicals via an addition reaction; Liebler and McClure [33] identified β-carotene-radical adducts as oxidation products associated with antioxidant reactions; Mortensen [34] has shown that CAR scavenge peroxyl radicals (that are not very reactive) by adduct formation but not through the electron transfer mechanism.

Additionally, in a previous theoretical work, where all the possible sites of reaction were included, site 5 (see Fig. 1) of the polyene chain has been identified as the most reactive site toward peroxyl radicals [31]. This is in agreement with experimental product analysis studies for BC. Different products have been identified for the peroxyl oxidation of BC, in particular 5,6-epoxy-β-carotene; 5,6,5′,6′-diepoxy-β-carotene; 5,8-epoxy-β-carotene; and 5,8,5′,8′-diepoxy-β-carotene [35, 36]. Since epoxidation of olefins by different peroxyl radicals proceeds via addition reactions [37, 38], the above-mentioned products not only support the preponderance of RAF mechanism for the reactions of peroxyl radicals with BC but also that a significant proportion of the reaction involves site 5. Therefore, in the present work, we will focus our attention mainly on the RAF mechanism and, in particular, on additions to site 5 (and 5′).

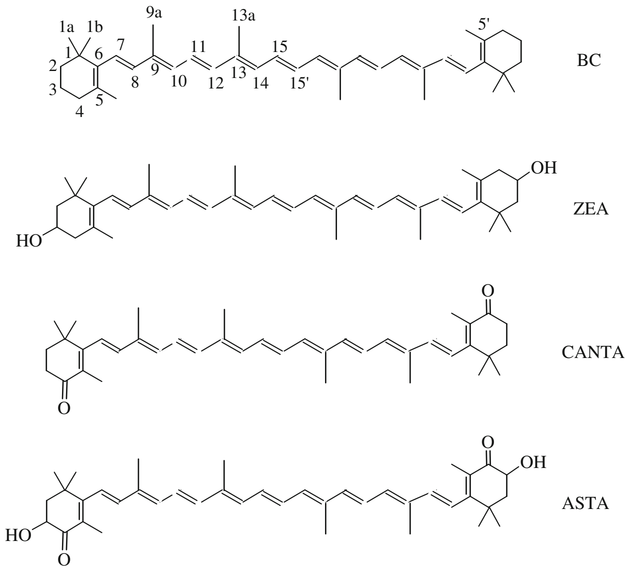


Fig. 1 Set of carotenoids (CAR) studied in this work

Most CARs are liposoluble molecules, and consequently, they remain in the fat of living organisms for rather long time. Therefore, CARs are expected to have more than one encounter with the free radicals since they enter to the body until they leave it. There are many previous studies on the reactions of the CAR with free radical; most of them had been focused on first possible reactions, but there is still very scarce information on the further reactions between the species that are formed in such processes and other free radicals. Therefore, it is the main goal of this work to investigate not only the first additions of free radicals of diverse nature to CAR but also further scavenge reactions of the formed products, which are not expected to be final products but intermediates susceptible of chemically evolving. With that purpose in mind, the reactions of β-carotene (BC), zeaxanthin (ZEA), canthaxanthin (CANTA), and astaxanthin (ASTA) with eight different radicals ($^{\bullet}$OH, $^{\bullet}$OOH, $^{\bullet}CH_3$, $^{\bullet}$O–CH_3, $^{\bullet}$OO–CH_3, $^{\bullet}$SH, $^{\bullet}$O–CH_2–CH=CH_2, and $^{\bullet}$OO–CH_2–CH=CH_2), as well as a substantial variety of possible further reactions involved in the radical chain propagation, have been analyzed. Such processes might be as important as the first reactions to the chemistry of CAR in the human body. The influence of the solvent polarity has also been investigated.

2 Computational details

All the electronic structure calculations were performed with the Gaussian 03 [39] package of programs. Full geometry optimizations and frequency calculations were carried out for all the stationary points using the density functional BPW91 and the LANL2DZ basis set, which is equivalent to D95V for the elements of the first row [40]. The BPW91 has been chosen since it has been proven to correctly reproduce the experimental evidences for the CAR reactions with free radicals while the much more widely used B3LYP functional fails [31]. Our results with LANL2DZ are in very good agreement with previously reported results on the addition reactions of $^{\bullet}$OOH to BC calculated at BPW91/6-31G(d, p) level of theory [31]. The agreement is concerning the structural information and also the relative energies, and supports the reliability of our results. Thermodynamic corrections at 298.15 K were included in the calculation of relative energies. Local minima were identified by the number of imaginary frequencies (NIMAG = 0). Solvent effects were included by single point calculations at the same level of theory using the polarisable continuum model, specifically the Integral Equation Formalism-Polarizable Continuum Model [41–44], with benzene ($\varepsilon = 2.247$) and water ($\varepsilon = 78.39$) as solvents for mimicking non-polar and polar environments, respectively, i.e., lipid and aqueous phases. The solute cavity was computed using atomic radii from the universal force field (RADII = UFF), which assigns individual spheres to H atoms (explicit hydrogen atoms). For the reasons presented in the introduction, we will focus on the addition to 5 and 5′ for the RAF mechanism.

3 Results and discussion

For this study, we used four different carotenoids (Fig. 1) and eight free radicals of different nature: $^{\bullet}OH$, $^{\bullet}OOH$, $^{\bullet}CH_3$, $^{\bullet}O{-}CH_3$, $^{\bullet}OO{-}CH_3$, $^{\bullet}SH$, $^{\bullet}O{-}CH_2{-}CH{=}CH_2$, and $^{\bullet}OO{-}CH_2{-}CH{=}CH_2$. This selection has been made with the purpose of studying the effect of the diverse nature of both reactants on the viability of the reactions.

Since it has been previously demonstrated that addition reactions between CAR and free radicals occur mainly at site 5 of the polyene chain [31], the first free radical addition to CAR has been modeled in the present work only for that site and the second at site 5′. In addition to the first and second RAF processes, the possible decomposition and epoxy formation of adducts when CAR react with peroxyl radicals, as well as the possible reaction between epoxy CAR and a second free radical, is analyzed. These processes are illustrated in Fig. 2 for BC and $^{\bullet}OOH$ as an example. Since the number of possible combinations of two free radicals is huge, in the present work we have studied only two variations for the second addition: $R_1^{\bullet} = R_2^{\bullet}$, and $R_1^{\bullet} = {}^{\bullet}OH$ with $R_2^{\bullet}$ = any of the other studied free radicals. We have chosen the second addition to the radical adduct $[CAR{-}OH]^{\bullet}$ since $^{\bullet}OH$ is the most reactive among the studied free radicals and in order to see if there is any differentiation when the second free radical is different than the first one.

To determine whether the reactions occur, it is necessary to follow the energy evolution associated with the first addition between different CAR and free radicals. It has been analyzed in terms of the adiabatic Gibbs free energy at 298 K. For the first addition to CAR, this quantity is calculated according to:

$$\mathrm{CAR} + R_1^{\bullet} \rightarrow [\mathrm{CAR}\text{-}\mathrm{R}_1]^{\bullet}$$

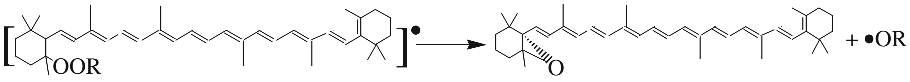
$$\Delta G^0_{\mathrm{add1}} = G_{[\mathrm{CAR-R_1}]^{\bullet}} - G_{\mathrm{CAR}} - G_{R_1^{\bullet}} \qquad (1)$$

where R_1 means that this is the first radical that is added to CAR. Table 1 reports the results for the first addition in water and benzene solutions. As these values show, most of the reactions are exergonic. However, the adiabatic Gibbs free energies for the reactions of CANTA and ASTA with $^{\bullet}OOH$ and $^{\bullet}SH$, as well as the reaction of $^{\bullet}OO{-}CH_3$ and $^{\bullet}OO{-}CH_2{-}CH{=}CH_2$ with the four studied CAR are very small, and they are in the limits of the calculations. As a consequence, the only conclusion that it is possible to derive is that these reactions are not as exergonic as the others.

According to the results of Table 1, it seems that the first addition is likely to occur in both environments for the other free radicals. Comparing the four studied CAR with respect to their free radical-scavenging activity, the reaction with BC and ZEA is slightly more exergonic than with ASTA and CANTA. The comparison among the reacting radicals presents a general tendency: the $^{\bullet}OH$ radical systematically gives the most negative values of $\Delta G^0_{\mathrm{add1}}$, followed by the $^{\bullet}CH_3$ and alcoxyl radicals, and the least exergonic ones are the reactions involving peroxyl radicals ($R{-}OO^{\bullet}$). This trend is the same regardless of the polarity of the environment. In any case, the differences between CARs neither seem to be critical nor does the variation on the polarity of the environment. From the values reported in Table 1, it is possible to establish that the addition reactions are controlled to a larger extent by the nature of the free radical than by the nature of the CAR. Something similar was reported before by our group for the electron transfer mechanism [24–27]. This is a logical finding based on the structure similarity of CAR, which are all long conjugated systems. The length of the conjugated chain is more important to the chemical reactivity of these species, through the RAF mechanism, than the presence of different groups at the end of the chain.

As it was mentioned before, the CAR reactions with peroxyl radicals, particularly $^{\bullet}OO{-}CH_3$ and $^{\bullet}OO{-}CH_2{-}CH{=}CH_2$, are the least energetically favored. A possible explanation for this result arises from the bond distances of the optimized structures. In all cases, the O–O bond distance changes from 1.40–1.41 Å in the isolated radical to 1.52–1.58 Å after the addition (Fig. 3). The RAF processes involving peroxyl radicals entail the weakness of the O–O bond, and for this reason, the reaction is not as exergonic as for other free radicals.

If the O–O bond is stretched out, it can also be dissociated. In order to see if this is energetically feasible, Gibbs free energies for such dissociation processes have also been computed. The reaction that was analyzed corresponds to the following scheme:



$$[CAR - OOR]^{\bullet} \rightarrow CAR > O + {}^{\bullet}OR$$

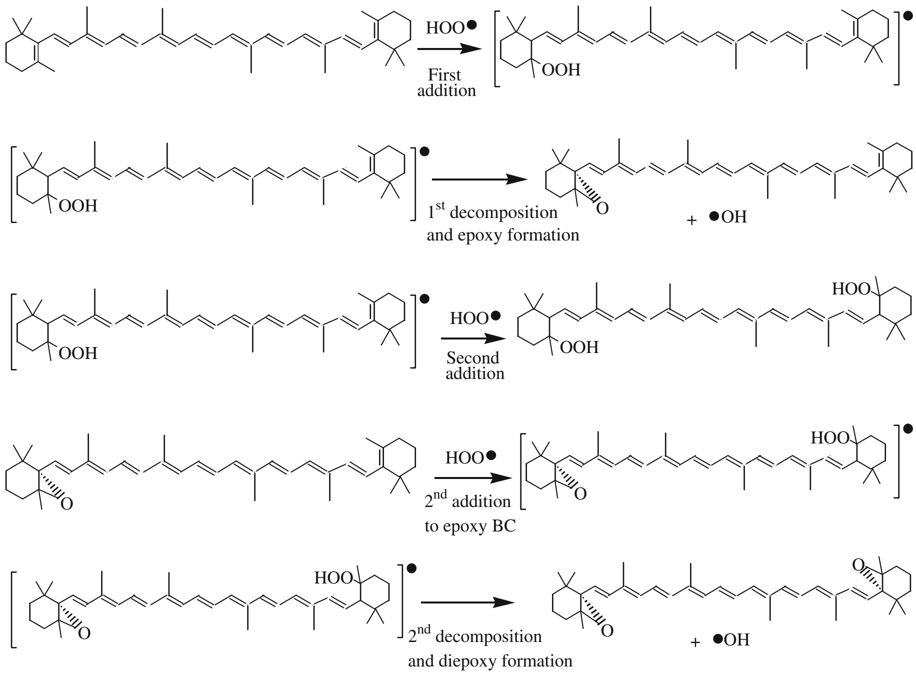


Fig. 2 Schematic representation of the studied reactions. *β*-carotene (BC) and $^\bullet$OOH are used as an example

Table 1 Adiabatic Gibbs free energy at 298 K (in kcal/mol) for the first radical addition to CAR ($\Delta G^0_{\mathrm{add1}}$)

$R_1^\bullet$	BC	ZEA	CANTA	ASTA
Water				
$^\bullet$OH	−34.00	−38.82	−33.86	−32.43
$^\bullet$OOH	−10.65	−9.09	−2.57	−1.91
$^\bullet$OCH$_3$	−17.15	−20.49	−13.69	−12.21
$^\bullet$OOCH$_3$	−5.41	−5.42	1.37	3.69
$^\bullet$CH$_3$	−24.46	−23.03	−20.97	−21.94
$^\bullet$SH	−10.51	−13.02	−4.40	−1.58
$^\bullet$OCH$_2$–CH=CH$_2$	−12.66	−18.41	−12.26	−11.63
$^\bullet$OOCH$_2$–CH–CH$_2$	−5.74	−4.95	0.33	2.91
Benzene				
$^\bullet$OH	−34.10	−40.43	−35.6	−39.81
$^\bullet$OOH	−10.74	−13.44	−6.0	−5.73
$^\bullet$OCH$_3$	−17.22	−23.75	−16.1	−15.69
$^\bullet$OOCH$_3$	−5.90	−7.79	−1.0	−0.03
$^\bullet$CH$_3$	−24.12	−25.07	−21.5	−23.79
$^\bullet$SH	−10.93	−14.64	−5.9	−7.24
$^\bullet$OCH$_2$–CH=CH$_2$	−14.60	−17.29	−13.7	−14.04
$^\bullet$OOCH$_2$–CH–CH$_2$	−5.06	−4.20	−2.8	−0.09

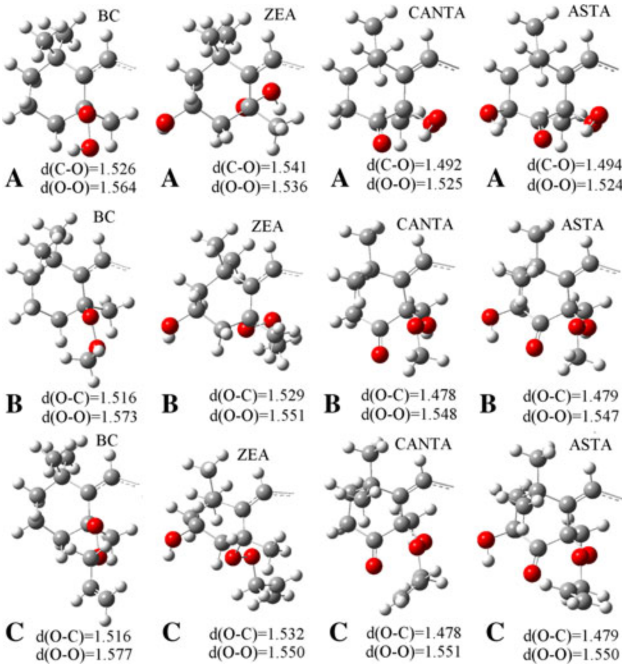


Fig. 3 Addition sites and their most relevant geometrical parameters (Å) for the adducts formed by addition of peroxyl radicals: **a** with $^\bullet$OOH radical, **b** with $^\bullet$OOCH$_3$ radical, and **c** with $^\bullet$OOCH$_2$–CH–CH$_2$ radical

and the change in Gibbs free energy associated to it was calculated according to

$$\Delta G^0_{\mathrm{dis1}} = G_{\mathrm{CAR>O}} + G_{^\bullet\mathrm{OR}} - G_{[\mathrm{CAR-OOR}]^\bullet} \qquad (2)$$

with R = H, CH$_3$ or CH$_2$CH=CH$_2$, and CAR>O representing the epoxy formed after dissociation. This process is in line with experimental works that report the formation of the epoxy CAR [35, 36]. The Gibbs free energies for these dissociation reactions are shown in Table 2.

The results reported in Table 2 indicate that the epoxy formation is exergonic for $^\bullet$OO–CH$_3$ and $^\bullet$OO–CH$_2$CHCH$_2$ but not in all cases for $^\bullet$OOH. In the latter case the values of $\Delta G^0_{\mathrm{dis1}}$ are small or close to zero. This can be explained by the formation of $^\bullet$OH radical as one of the dissociation

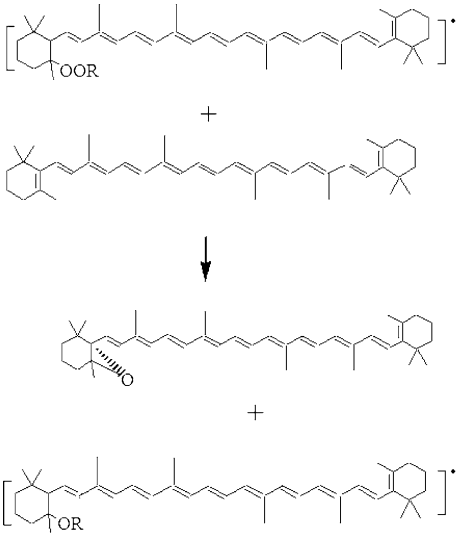
Table 2 Gibbs free energies for the dissociation processes ($\Delta G^0_{\mathrm{dis1}}$) at 298 K (in kcal/mol) $[\mathrm{CAR{-}OOR}]^\bullet \rightarrow \mathrm{CAR{>}O} + \mathrm{RO}^\bullet$

	BC	ZEA	CANTA	ASTA
Water				
R = H	−1.21	−5.66	−0.60	2.86
R = CH_3	−14.30	−17.18	−12.39	−10.58
R = $CH_2CH{=}CH_2$	−14.46	−18.14	−11.84	−10.29
Benzene				
R = H	0.13	−0.88	2.54	8.02
R = CH_3	−12.20	−14.03	−9.93	−5.17
R = $CH_2CH{=}CH_2$	−13.38	−17.96	−8.53	−5.45

products, which is a very reactive species. The presence of CH_3 and $CH_2CH{=}CH_2$ moieties on the other two studied peroxyl radicals help the O atom to cope with the electron deficiency on the formed alcoxyl radical ($^\bullet$OR) while there is no any electron donating group on the $^\bullet$OH radical to assist on that way. This is reflected on the structures of the $[\mathrm{CAR{-}OOR}]^\bullet$ adducts, where the elongation of the O–O bond, with respect to the isolated radicals, is ~0.11 Å for R = H and ~0.16 Å for R = $^\bullet$OO–CH_3 or $^\bullet$OO-CH_2CHCH_2. This difference indicates that the O–O bond in adduct with R = H is stronger than in the other two systems. According to our results, which are more negative in water than in benzene, a polar environment favors the O–O dissociation of adducts $[\mathrm{CAR{-}OOR}]^\bullet$ by stabilizing the $^\bullet$OR with respect to non-polar environments through solvation.

We have also considered that the vicinity of another CAR molecule can assist the dissociation of the O–O bond. In this case, instead of the formation of an alcoxyl radical, another CAR radical adduct is formed through a dissociation–addition reaction, as the following scheme indicates for BC as an example:

$$[CAR-OOR]^\bullet + CAR \rightarrow CAR > O + [CAR-OR]^\bullet$$



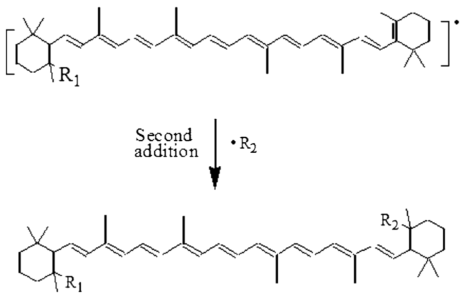
The corresponding Gibbs free energies were calculated according to

$$\Delta G^0_{\mathrm{dis1-add1}} = G_{\mathrm{CAR>O}} + G_{[\mathrm{CAR-OR}]^\bullet} - G_{[\mathrm{CAR-OOR}]^\bullet} - G_{\mathrm{CAR}} \quad (3)$$

and are reported in Table 3. All the dissociation–addition reactions were found to be exergonic in both polar and non-polar environments. Since $^\bullet$OH is not one of the products of the reaction in this case, and moreover, the reaction involving $[\mathrm{CAR{-}OOH}]^\bullet$ leads a thermodynamically stable adduct $[\mathrm{CAR{-}OH}]^\bullet$, the O–O dissociation of $[\mathrm{CAR{-}OOH}]^\bullet$ is not only exergonic but also has larger exergonicity than the corresponding reactions for $[\mathrm{CAR{-}OOCH_3}]^\bullet$ and $[\mathrm{CAR{-}OOCH_2CH{=}CH_2}]^\bullet$. Therefore, even though the O–O dissociation of $[\mathrm{CAR{-}OOH}]^\bullet$ is not expected to occur spontaneously, it is predicted to occur in the vicinity of another molecule readily to react with the leaving $^\bullet$OH radical fragment. Since this is a highly reactive radical, it can be hypothesize that many other species could act as the assisting molecule when the process takes place in the physiological environments.

So far, we had only considered the addition of one free radical to CAR, and the possible decomposition of the formed adduct for reactions involving peroxyl radicals. But is it possible, or even likely, that the radical adducts $[\mathrm{CAR{-}R_1}]^\bullet$ formed in the first RAF process react with a second free radical? To answer that question second addition reactions have also been considered in the present work:

$$[CAR-R_1]^\bullet + R_2^\bullet \rightarrow R_2-CAR-R_1$$



The Gibbs free energies for such processes was calculated according to

$$\Delta G^0_{\mathrm{add2}} = G_{\mathrm{R_2-CAR-R_1}} - G_{[\mathrm{CAR-R_1}]^\bullet} - G_{\mathrm{R_2^\bullet}} \quad (4)$$

where $R_2^\bullet$ represents the second free radical that is added to the previously formed radical adduct. Since the number of possible R_1R_2 combinations is huge, in the present work we have studied only two variations: $R_1^\bullet = R_2^\bullet$, and $R_1^\bullet = {}^\bullet\mathrm{OH}$ with $R_2^\bullet$ = any of the other studied free radicals. We have chosen the second addition to the radical adduct $[\mathrm{CAR{-}OH}]^\bullet$ since $^\bullet$OH is the most reactive among the studied free radicals and in order to see if there is any

Table 3 Gibbs free energies for the dissociation–addition reactions ($\Delta G^0_{\mathrm{dis1-add1}}$) at 298 K (in kcal/mol) $[\mathrm{CAR{-}OOR}]^\bullet$ + CAR → CAR>O + $[\mathrm{CAR{-}OR}]^\bullet$

	BC	ZEA	CANTA	ASTA
Water				
R = H	−35.21	−44.48	−34.46	−29.57
R = CH_3	−31.45	−37.67	−26.08	−22.79
R = $CH_2CH{=}CH_2$	−27.12	−36.55	−24.10	−21.92
Benzene				
R = H	−33.97	−41.31	−33.04	−31.79
R = CH_3	−29.43	−37.78	−26.04	−20.86
R = $CH_2CH{=}CH_2$	−27.98	−35.24	−22.23	−19.49

Table 4 Adiabatic Gibbs free energy at 298 K (in kcal/mol) for the second radical addition to CAR ($\Delta G^0_{\mathrm{add2}}$)

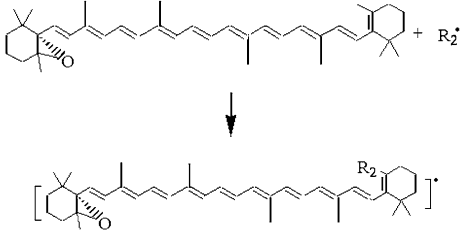
	BC	ZEA	CANTA	ASTA
$R_2^\bullet = R_1^\bullet$				
Water				
$^\bullet$OH	−44.74	−42.12	−37.61	−32.69
$^\bullet$OOH	−16.46	−14.87	−9.74	−4.32
$^\bullet OCH_3$	−24.47	−25.31	−12.40	−14.52
$^\bullet OOCH_3$	−12.21	−11.66	2.13	−1.41
$^\bullet CH_3$	−29.00	−31.85	−25.12	−23.85
$^\bullet$SH	−14.23	−14.37	−10.03	−10.41
$^\bullet OCH_2{-}CH{=}CH_2$	−26.17	−25.69	−9.95	−10.00
$^\bullet OOCH_2{-}CH{-}CH_2$	−7.16	−5.82	3.17	5.54
Benzene				
$^\bullet$OH	−46.68	−44.22	−42.15	−28.62
$^\bullet$OOH	−17.19	−11.94	−9.20	−7.04
$^\bullet OCH_3$	−25.35	−25.39	−12.12	−16.83
$^\bullet OOCH_3$	−13.54	−11.70	−2.48	−1.51
$^\bullet CH_3$	−31.24	−31.49	−26.12	−23.76
$^\bullet$SH	−13.08	−14.40	−12.05	−9.82
$^\bullet OCH_2{-}CH{=}CH_2$	−26.30	−29.37	−9.20	−10.58
$^\bullet OOCH_2{-}CH{-}CH_2$	−9.05	−10.12	2.54	−5.61
$R_1^\bullet$ = OH				
Water				
$^\bullet$OH	−44.74	−42.12	−37.61	−32.69
$^\bullet$OOH	−15.33	−12.33	−8.99	−4.58
$^\bullet OCH_3$	−25.10	−21.69	−17.32	−14.48
$^\bullet OOCH_3$	−14.64	−10.29	1.76	2.76
$^\bullet CH_3$	−32.58	−27.15	−25.07	−22.59
$^\bullet$SH	−19.89	−12.64	−8.94	−6.15
$^\bullet OCH_2{-}CH{=}CH_2$	−24.24	−19.64	−11.64	−8.49
$^\bullet OOCH_2{-}CH{-}CH_2$	−10.79	−9.05	−0.88	0.41
Benzene				
$^\bullet$OH	−46.68	−44.22	−42.15	−28.62
$^\bullet$OOH	−16.14	−15.47	−11.00	−3.24
$^\bullet OCH_3$	−25.81	−23.15	−15.45	−12.97
$^\bullet OOCH_3$	−16.41	−10.84	−3.81	1.05
$^\bullet CH_3$	−33.40	−28.40	−25.31	−20.66
$^\bullet$SH	−20.24	−13.89	−10.95	−2.42
$^\bullet OCH_2{-}CH{=}CH_2$	−23.67	−21.36	−13.88	−10.54
$^\bullet OOCH_2{-}CH{-}CH_2$	−13.55	−9.78	−3.79	1.86

difference when the second free radical is different than the first one. The results for the second addition are shown in Table 4. All the reactions are exergonic, except those of CANTA and ASTA with $^\bullet OO{-}CH_3$ and $^\bullet OO{-}CH_2{-}CH{=}CH_2$. The O–O bond is elongated in the adducts with respect to the isolated radicals as it was previously analyzed for the first additions. Therefore, the same explanation applies in this case for the finding that the second additions of peroxyl radicals are the least energetically favored, compared to the other studied free radicals.

Comparing the results for the second addition, when the second free radical is different from the first one, it is possible to see that the tendency is similar. The second addition is likely to occur in both systems. Apparently the difference or similarity between the two radicals trapped by CAR is not relevant to the viability of the second addition reactions. Their exergonicity seems to be influenced by both the nature of the reacting free radical and the characteristics of the CAR, as it was the case for the first additions. The most exergonic reactions are with BC and ZEA in both cases while the least energetically feasible are with CANTA and ASTA. Therefore, it seems that the presence of a carbonyl group on the terminal rings of CAR lower their reactivity toward a second free radical. According to this tendency, BC and ZEA are better free radical scavengers through RAF mechanism than CANTA and ASTA, in view of the fact that the formers can trap two radicals more efficiently. This seems to be relevant since when two radicals are scavenged, the radical chain reaction terminates and, as a result, it can have consequences for the effectiveness of the global antioxidant capacity of CAR.

As discussed above, when peroxyl radicals react with CAR, one of the possible products of reaction are epoxidated species (CAR>O). The question now is if they are as good free radical scavenger molecule as the parent CAR or at least good enough to trap another free radical. Therefore, the reactions of CAR>O with a second free radical through RAF reactions have also been computed according with the following scheme:

$$CAR>O+R_2^\bullet \rightarrow [R_2-CAR>O]^\bullet$$



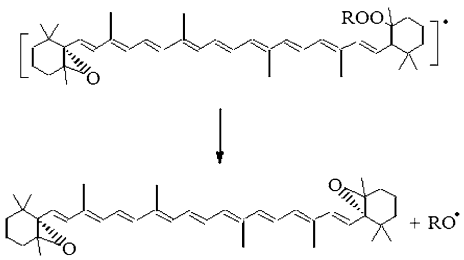
The corresponding Gibbs free energies are calculated according to:

$$\Delta G^0_{dis1\_add2} = G_{[R_2-CAR>O]^\bullet} - G_{CAR>O} - G_{R_2^\bullet} \qquad (5)$$

The energy releases associated with free radical additions to CAR>O (Table 5) are similar to those involving the non-epoxidated CAR. This is relevant since it means that both CAR and CAR>O are equally efficient as free radical scavengers. Also in this case, second additions are expected to take place in both environments. The exergonicity of the reactions is predicted to be controlled mainly by the nature of the free radical, with •OH radical leading to the most exergonic process and peroxyl radicals to the least exergonic ones.

According to the product analysis from experimental works, when the CAR>O species react with another peroxyl radical, a second dissociation process yielding diepoxy CAR (O<CAR>O) is also possible:

$$[ROO-CAR>O]^\bullet \rightarrow O<CAR>O+RO^\bullet$$



The corresponding Gibbs free energies are reported in Table 6 and calculated according to:

$$\Delta G^0_{dis2} = G_{O<CAR>O} + G_{RO^\bullet} - G_{[ROO-CAR>O]^\bullet} \qquad (6)$$

with R = H, CH_3 or CH_2CHCH_2. This process is energetically feasible when R = CH_3 or CH_2CHCH_2, in both polar and non-polar environments. However, when R = H, the adiabatic Gibbs free dissociation energy is small, and we can conclude that the reaction will be reversible. This behavior is exactly the same as the previously described for the O–O dissociation processes of [CAR–OOR]•, and therefore, the same explanation applies.

In both cases, [CAR–OOR]• and [ROO–CAR>O]•, it can be expected an energy barrier for the O–O bond dissociation in the epoxy molecule. To definitely describe the free radical scavenger capacity of the radical adducts, kinetic calculations are also necessary. However, such calculations exceed the scope of the present work.

Table 5 Adiabatic Gibbs free energy at 298 K (in kcal/mol) for the radical addition to epoxidated CAR ($\Delta G^0_{dis1-add2}$)

$R_2^\bullet$	BC	ZEA	CANTA	ASTA
Water				
•OH	−31.28	−39.75	−31.09	−32.48
•OOH	−7.51	−13.04	−0.84	−3.38
•OCH_3	−12.65	−16.05	−9.43	−10.49
•$OOCH_3$	−2.92	−6.10	4.38	5.21
•CH_3	−23.50	−23.45	−18.59	−21.43
•SH	−8.65	−10.46	−2.60	−7.23
•OCH_2–CH=CH_2	−15.76	−15.20	−10.73	−14.96
•$OOCH_2$–CH–CH_2	−3.84	−5.09	4.87	5.84
Benzene				
•OH	−33.04	−37.94	−33.26	−37.82
•OOH	−8.01	−12.78	−4.34	−9.40
•OCH_3	−13.82	−19.69	−13.29	−14.14
•$OOCH_3$	−3.92	−6.92	2.59	0.47
•CH_3	−23.57	−25.47	−20.54	−25.36
•SH	−8.98	−11.27	−4.96	−13.09
•OCH_2–CH=CH_2	−17.80	−17.39	−12.56	−19.89
•$OOCH_2$–CH–CH_2	−4.26	−6.63	1.38	0.41

As discussed above for the first dissociation–addition reaction, in this case, it can also be possible that another CAR molecule promotes the dissociation throughout a concerted addition reaction. The products of this second dissociation–addition reaction are the diepoxy CAR and another CAR radical adduct, as shown in the following scheme.

$$[ROO-CAR>O]^\bullet + CAR \rightarrow O<CAR>O+[CAR-OR]^\bullet$$

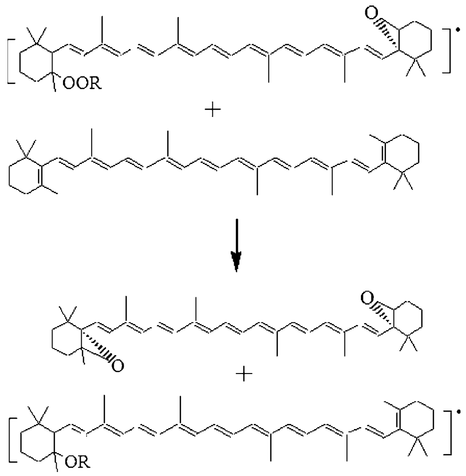


Table 7 reports the Gibbs free energies for such processes, calculated according to:

$$\Delta G^0_{dis2-add2} = G_{O<CAR>O} + G_{[CAR-OR]^\bullet} - G_{[ROO-CAR>O]^\bullet} - G_{CAR} \qquad (7)$$

Table 6 Gibbs free energies for the second dissociation processes leading to O<CAR>O ($\Delta G^{0}_{\mathrm{dis2}}$) at 298 K (in kcal/mol) [ROO–CAR>O]$^{\bullet}$ → O<CAR>O + RO$^{\bullet}$

	BC	ZEA	CANTA	ASTA
Water				
R = H	−5.31	1.78	−2.60	2.52
R = CH_3	−17.76	−13.00	−15.67	−13.91
R = $CH_2CH{=}CH_2$	−17.32	−14.51	−16.64	−15.04
Benzene				
R = H	−4.77	2.06	−0.40	6.62
R = CH_3	−16.36	−11.30	−14.83	−10.74
R = $CH_2CH{=}CH_2$	−16.35	−11.93	−13.96	−11.02

Table 7 Gibbs free energies for the second dissociation–addition reactions ($\Delta G^{0}_{\mathrm{dis2-add2}}$) at 298 K (in kcal/mol) [ROO–CAR>O]$^{\bullet}$ + CAR → O<CAR>O + [CAR–OR]$^{\bullet}$

	BC	ZEA	CANTA	ASTA
Water				
R = H	−39.31	−37.04	−36.46	−29.91
R = CH_3	−34.91	−33.50	−29.36	−26.12
R = $CH_2CH{=}CH_2$	−29.98	−32.92	−28.90	−26.66
Benzene				
R = H	−38.87	−38.37	−35.98	−33.19
R = CH_3	−33.58	−35.05	−30.94	−26.43
R = $CH_2CH{=}CH_2$	−30.96	−29.21	−27.66	−25.06

All dissociation–addition reactions were found to be exergonic both in polar and non-polar environments. Again, $^{\bullet}$OH is not one of the products of the reaction in this case, and then the O–O dissociation reaction of [HOO–CAR>O]$^{\bullet}$ becomes significantly exergonic. Actually, the exergonicity of the reaction is slightly larger in this case due to the higher reactivity of $^{\bullet}$OH, compared to the alcoxyl radicals $^{\bullet}OCH_3$ and $^{\bullet}OCH_2CH{=}CH_2$.

One important aspect that arises from the above discussion is that some of the products formed through RAF mechanism are not free radicals, and therefore, they do not propagate radical damage to any further extent while there are other products that are free radical adducts. Fortunately the latter are not as reactive as the starting free radicals, but nevertheless, they can be involved in the radical chain propagation. After the second addition to [CAR–R_1]$^{\bullet}$, the product of the reaction, R_2–CAR–R_1, is a closed shell systems, and the radical chain propagation is terminated. This is also the case for the epoxy and diepoxy species. The epoxy CARs were found to be as efficient as its parent CAR for scavenging free radicals through the RAF mechanism. When there is an adequate amount of CAR, the termination products that are expected to be formed are R_2–CAR–R_1, CAR>O, [R_2–CAR>O]$^{\bullet}$, and O<CAR>O. The OR$^{\bullet}$ species that can be produced through dissociation, during the reactions of peroxyl radicals with CAR, can be captured by either CAR, radical adducts [CAR–R_1]$^{\bullet}$ or CAR>O. There is only one product [R_2–CAR–O]$^{\bullet}$ that is a free radical, and therefore, may be involved in further radical chain propagation, but as mentioned above, with a lower reactivity than the starting free radicals: $^{\bullet}$OH, $^{\bullet}$OOH, $^{\bullet}O{-}CH_3$, $^{\bullet}OO{-}CH_3$, etc.

4 Conclusions

Some concluding remarks can be drawn from the present work, which might be relevant to the further fate of the species formed through the free radical-scavenging activity of CAR in physiological environments:

- Free radical addition reactions to CAR are controlled to a larger extent by the nature of the free radical than by the nature of the radical scavenger (CAR).
- Each CAR molecule can scavenge at least two free radicals and terminate the propagation chain.
- Thermodynamic calculations support the experimental product identification for CAR reacting with peroxyl radicals.
- The epoxy species formed after the peroxyl radical additions to CAR has free radical-scavenging capabilities similar to their parent CAR, through RAF processes.
- ASTA and CANTA are predicted to be less reactive, when reacting through RAF mechanism, than BC and ZEA.

Acknowledgments This study was made possible due to funding from the Consejo Nacional de Ciencia y Tecnología (CONACyT), as well as resources provided by the Instituto de Investigaciones en Materiales IIM, UNAM. The work was carried out, using a KanBalam supercomputer, provided by DGSCA, UNAM and the facilities at Laboratorio de Supercómputo y Visualización en Paralelo of UAM Iztapalapa. The authors would like to acknowledge both Oralia L. Jiménez A and María Teresa Vázquez for their technical support. A.M. is grateful for financial support from DGAPA-UNAM-México, and R.V. for the financial support from CONACYT, through project 49057-F.

References

1. Boyd NF, McGuire V (1991) Free Rad Biol Med 10:185
2. Nelson RL (1992) Free Rad Biol Med 12:161
3. Knekt P, Reunanen A, Takkunen H, Aromaa A, Heliovaara M, Hakuunen T (1994) Int J Cancer 56:379
4. Omenn GS, Goodman GE, Thornquist MD (1996) N Engl J Med 334:1150
5. Riemmersma RA, Wood DA, Macityre CCA, Elton RA, Gey KF, Oliver MF (1991) Lancet 337:1

6. Salonen JT, Nyyssoner K, Korpela H, Tuomilehto J, Seppanen R, Salonen R (1992) Circulation 86:803
7. Street DA, Comstock G, Salkeldy R, Klag M (1994) Circulation 90:1154
8. Kushi LH, Folsom AR, Prineas RJ, Mink PJ, Wu Y, Bostick R (1996) N Engl J Med 334:1156
9. Stephens NG, Parsons A, Schofield PM, Kelly F, Cheesman K, Mitchisnon MJ, Brown MJ (1996) Lancet 347:781
10. Panasenko OM, Nova TV, Azizova OA, Vladimirov YA (1991) Free Rad Biol Med 10:137
11. Steinberg D (1991) Circulation 84:1421
12. Janero DR (1991) Free Rad Biol Med 11:129
13. Hodis HN, Mack WJ, LaBree L, Cashin-Hemphill L, Sevanian A, Johnson R, Azen S (1995) J Am Med Assoc 273:1849
14. Butterfield DA, Hensley K, Harris M, Mattson M, Carney J (1994) Biochem Biophys Res Commun 200:710
15. Hensley K, Carney JM, Mattson MP, Aksenova M, Harris M, Wu JF, Floyd RA, Butterfield DA (1994) Proc Natl Acad Sci USA 91:3270
16. Butterfield DA, Martin L, Carney JM, Hensley K (1996) Life Sci 58:217
17. Butterfield DA (1997) Chem Res Toxicol 10:495
18. Fay DS, Fluet A, Johnson CJ, Link CD (1998) J Neurochem 71:1616
19. Krinsky NI (1993) Annu Rev Nutr 13:561
20. Edge R, McGarvey DJ, Truscott TG (1997) J Photochem Photobiol 41:189
21. Burton GW, Ingold KU (1984) Science 224:569
22. Everett SA, Dennis MF, Patel KB, Maddix S, Kundu SC, Willson RL (1996) J Biol Chem 271:3988
23. Woodall AA, Britton G, Jackson M (1997) J Biochim Biophys Acta 1336:575
24. Martínez A, Vargas R, Galano A (2009) J Phys Chem B 113:12113
25. Martínez A, Rodríguez-Gironés MA, Barbosa A, Costas M (2008) J Phys Chem A 112:9037
26. Martínez A (2009) J Phys Chem B 113:3212
27. Martínez A (2009) J Phys Chem B 113:4915
28. Galano A, Alvarez-Diduk R, Ramirez-Silva MT, Alarcon-Angeles G, Rojas-Hernández A (2009) Chem Phys 363:13
29. Galano A, Vargas R, Martínez A (2010) Phys Chem Chem Phys 12:193
30. Galano A (2007) J Phys Chem B 111:12898
31. Galano A, Francisco-Márquez M (2009) J Phys Chem B 113:11338
32. Martínez A, Barbosa A (2008) J Phys Chem B 112:16945
33. Liebler DC, McClure TD (1996) Chem Res Toxicol 9:8
34. Mortensen A (2002) Free Rad Res 36:211
35. El-Tinay AH, Chichester CO (1970) J Org Chem 35:2290
36. Mordi RC, Walton JC, Burton GW, Hughes L, Ingold KU, Lindsay DA (1991) Tetrahedron Lett 32:4203
37. Hoshino M, Konishi R, Seto H, Seki H, Sonoki H, Yokoyama T, Shimamori H (2001) Res Chem Intermed 27:189
38. Sawaki Y, Ogata Y (1984) J Org Chem 49:3344
39. Gaussian 03, Revision D.01, Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Montgomery JA Jr, Vreven T, Kudin KN, Burant JC, Millam JM, Iyengar SS, Tomasi J, Barone V, Mennucci B, Cossi M, Scalmani G, Rega N, Petersson GA, NakatsujiH,HadaM,Ehara M,ToyotaK, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Klene M, Li X, Knox JE, Hratchian HP, Cross JB, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Ayala PY, Morokuma K, Voth GA, Salvador P, Dannenberg JJ, Zakrzewski VG, Dapprich S, Daniels AD, Strain MC, Farkas O, Malick DK, Rabuck AD, Raghavachari K, Foresman JB, Ortiz JV, Cui Q, Baboul AG, Clifford S, Cioslowski J, Stefanov BB, Liu G, Liashenko A, Piskorz P, Komaromi I, Martin RL, Fox DJ, Keith T, Al-Laham MA, Peng CY, Nanayakkara A, Challacombe M, Gill PMW, Johnson B, Chen W, Wong MW, Gonzalez C, Pople JA (2004) Gaussian Inc., Wallingford
40. Dunning TH, Hay PJ (1976) In: Schaefer HF III (ed) Modern theoretical chemistry, vol 3. Plenum, New York, pp 1–28
41. Cances MT, Mennucci B, Tomasi J (1997) J Chem Phys 107:3032
42. Mennucci B, Tomasi J (1997) J Chem Phys 106:5151
43. Mennucci B, Cances E, Tomasi J (1997) J Phys Chem B 101:10506
44. Tomasi J, Mennucci B, Cances E (1999) J Mol Struct 464:211