THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

pubs.acs.org/JPCB

Antioxidative Reaction of Carotenes against Peroxidation of Fatty Acids Initiated by Nitrogen Dioxide: A Theoretical Study

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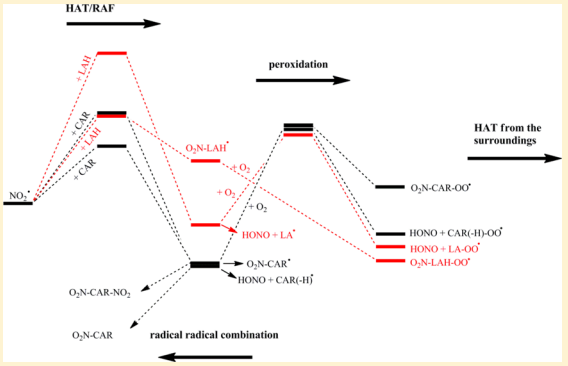
Supporting Information

ABSTRACT: In this study, we investigated the antioxidative functions of carotenes (CARs) against the peroxidation of lipids initiated by nitrogen dioxide using density functional theory. The hydrogen-atom transfer (HAT), radical adduct formation (RAF), and electron transfer (ET) mechanisms were investigated. We chose β-carotene (β-CAR) and lycopene (LYC) and compared their $NO_2^\bullet$ initiations and peroxidations with those of linoleic acid (LAH), the model of the lipid. We found that for CARs ET is more likely to occur in the most polar (water) environment than are HAT and RAF. In less polar environments, CARs react more readily with $NO_2^\bullet$ via HAT and RAF than does the lipid model, LAH. Comparatively, reaction barriers for the RAF between CARs and $NO_2^\bullet$ are smaller than those for the HAT. The additions of O_2 to the radical intermediates $O_2N{-}CAR^\bullet$ and $CAR(-H)^\bullet$ involve sizable barriers and are endergonic. Other than HAT of LAH, we revealed that lipid peroxidation is likely to be initiated by $-NO_2$ addition and the subsequent barrierless addition of O_2. Finally, LYC is a more effective antioxidative agent against $NO_2^\bullet$-initiated lipid peroxidation than is β-CAR.



1. INTRODUCTION

Nitrogen dioxide ($NO_2^\bullet$) is a major component of cigarette smoke and also an environmental pollutant. It has been demonstrated that $NO_2^\bullet$ induces lipid peroxidation,[1,2] which involves several steps. The first step is initiation (eq 1), in which a radical species ($NO_2^\bullet$ in this case) abstracts hydrogen from the substrate RH (of the lipid), generating the radical $R^\bullet$. The second step is propagation, in which the radical forms the peroxide radical (eq 2); the peroxide then abstracts another hydrogen from the substrates (eq 3). With the supply of O_2, lipids are continuously oxidized. Without an antioxidant, the chain reaction can only be terminated by radical recombination; an example is the formation of alkyl peroxide (eq 4).

$$\text{Initiation: } RH \rightarrow R^\bullet \quad (1)$$

$$\text{Propagation: } R^\bullet + O_2 \rightarrow R - OO^\bullet \quad (2)$$

$$ROO^\bullet + RH \rightarrow ROOH + R^\bullet \quad (3)$$

$$\text{Termination: } ROO^\bullet + R^\bullet \rightarrow ROOR \quad (4)$$

Carotenes (CARs) are phytochemicals which play important roles in protecting biological systems from deleterious biological reactions. In addition, CARs are antennae compounds in the light-harvesting units in chlorophyll and bacteriochlorophyll. Nevertheless, CARs also protect biological tissues from the harmful effects caused by singlet oxygen and triplet bacteriochlorophyll.[3,4] It has been well demonstrated that dietary CARs offer antioxidation benefits in many ways.[5,6] For example, Di Mascio et al. have shown that lycopene (LYC, Scheme 1) is very effective in quenching singlet oxygen.[7] Rock demonstrated that β-carotene (β-CAR) (Scheme 1) protects the skin and membranes and exhibits anticancer activity.[8]

In the propagation step of lipid peroxidation, a chain-breaking compound, i.e., an antioxidant, would react with the radical species to form less reactive products. The peroxidation of lipids is thus terminated by these compounds. CARs have long been recognized as chain-breaking species.[5,9−12] Among the common carotenoids, β-CAR and LYC were demonstrated to be the most efficient.[5,6,9,10,13−20] In particular, β-CAR has been shown to scavenge peroxyl radicals effectively under a low pressure of oxygen.[9,21] The beneficial function of CARs to human health has been attributed to their highly conjugated polyene structure (see Scheme 1).

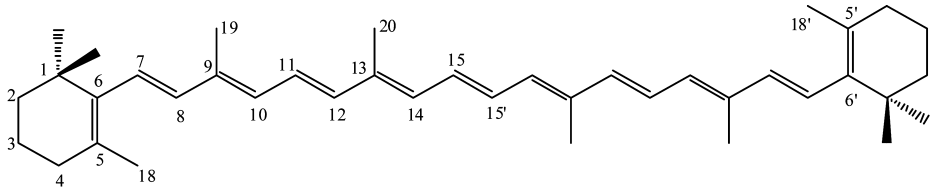
However, adverse effects of supplemental β-CAR have been reported in the case of smokers and workers exposed to asbestos.[22,23] Furthermore, it was reported by Liebler et al. that β-CAR is not very efficient when it comes to preventing lipid peroxidation.[24] Nevertheless, at high concentrations β-CAR was been demonstrated by Truscott et al. to be pro-oxidative.[25,26] Knowledge of the mechanism involved in the chain-terminating reaction of CARs against lipid peroxidation is important for the application of these phytochemicals.

Several possibilities have been proposed for the scavenging of $NO_2^\bullet$ by CARs.[9,26−32] The reaction could occur via electron

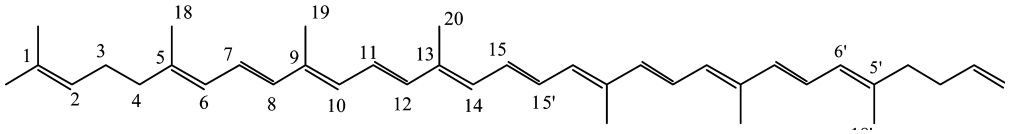
Received: April 30, 2015
Revised: June 22, 2015
Published: June 24, 2015

DOI: 10.1021/acs.jpcb.5b04142
J. Phys. Chem. B 2015, 119, 9640−9650

Scheme 1. Structures and Numbering for β-Carotene (β-CAR) and Lycopene (LYC)



β-CAR



transfer (ET), hydrogen-atom transfer (HAT), or radical adduct formation (RAF)

$$\text{Electron transfer: CAR} + \text{NO}_2^{\bullet} \rightarrow \text{CAR}^{\bullet+} + \text{NO}_2^{-} \quad (5)$$

$$\text{Hydrogen-atom transfer: CAR} + \text{NO}_2^{\bullet} \rightarrow \text{CAR}(-\text{H})^{\bullet} + \text{HONO} \quad (6a)$$

$$\text{CAR} + \text{NO}_2^{\bullet} \rightarrow \text{CAR}(-\text{H})^{\bullet} + \text{HNO}_2 \quad (6b)$$

$$\text{Radical adduct formation: CAR} + \text{NO}_2^{\bullet} \rightarrow \text{ONO} - \text{CAR}^{\bullet} \quad (7a)$$

$$\text{CAR} + \text{NO}_2^{\bullet} \rightarrow \text{O}_2\text{N} - \text{CAR}^{\bullet} \quad (7b)$$

In general, ET is favored in polar environments, while HAT and RAF are favored in nonpolar environments such as in cell membranes or micelles.[26,29]

With regard to $NO_2^{\bullet}$, several experimental studies have been reported. Böhm et al. found that β-CAR and LYC are capable of protecting membranes in aqueous solutions. They found spectroscopic evidence for the existence of ET and RAF products[33] and suggested replacing β-CAR with LYC as an anticancer agent, owing to the enhanced antioxidative capacity of the latter. Everett et al. found conclusive evidence that $NO_2^{\bullet}$ reacts with β-CAR through ET in *tert*-butanol/water.[34−36] Using cigarette smoke, Liebler el al. observed 4-nitro-β-CAR in a liposome model.[37] They also examined the oxidation of cellular lipids by cigarette smoke.[38] In addition to 4-nitro-β-CAR, epoxy-β-CARs and β-apocarotenals were identified as well.[38] Mukherjee et al. were able to detect the NO_2−β-CAR$^{\bullet}$ addition adduct in chloroform.[39] More recently, Lowe et al. reported a wide range of products from the reaction of β-CAR and cigarette smoke in hexane.[40] The reaction products suggest that the HAT at C4 of β-CAR plays an essential role in initiating free radical attacks.[40]

A theoretical study of the reaction between $NO_2^{\bullet}$ and β-CAR in different solvents was performed by Zúñiga et al.[41] They discovered that the ET reaction is thermodynamically favorable in polar solvents. Hydrogen abstraction was exergonic, while the addition reaction was endergonic for all investigated solvents. Porter et al. have shown that the computed C−H and C−OO bond dissociation energies (BDEs) of lipid models are correlated to the HAT of substrates and the O_2 addition of the resulting radicals.[42] The antioxidative capacity of a CAR has been evaluated in terms of its electron-donating tendency[43−46] and C−H BDE.[47] It was revealed by Galano et al. that, among the various peroxyl radicals, ET reactions occur only in CARs with electron-withdrawing groups.[44] In a study by Galano's group, where the transition states for HAT and RAF between CARs and peroxide were located within a continuum model that simulated benzene and water,[46] RAF was found to be kinetically and thermodynamically more favorable.

In less polar environments, ET was found to be less favorable.[26] On the basis of the results of in vitro experiments, Burton and Ingold proposed that β-CAR scavenges peroxyl radicals through RAF, as shown in eqs 7a and 7b.[9] They also proposed a mechanism in order to explain the pro-oxidative behavior of β-CAR at high oxygen pressures.[9] In this mechanism, an auto-oxidative reaction involving O_2 addition to the RAF radical intermediate occurs (eq 8); the peroxide thus would undergo HAT (eq 9)

$$\text{ROO} - \text{CAR}^{\bullet} + \text{O}_2 \rightarrow \text{ROO} - \text{CAR} - \text{O}_2^{\bullet} \quad (8)$$

$$\text{ROO} - \text{CAR} - \text{O}_2^{\bullet} + \text{RH} \rightarrow \text{ROO} - \text{CAR} - \text{O}_2\text{H} + \text{R}^{\bullet} \quad (9)$$

The O_2 affinities of several radicals have been measured in different solvents.[29,48] Using laser flash photolysis on the reactions of CARs with acylperoxyl radicals in polar and nonpolar solvents, El-Agamey and McGarvey demonstrated that the reactivity of carotenoid-addition radicals toward oxygen is low.[29] It was proved that in nonpolar solvent, RAF radical does not react with oxygen.[29] In a later study, however, the authors discovered that RAF radical of CARs with shorter π-conjugation systems undergo reversible addition with oxygen.[48] The rate constant for the addition of O_2 to a RAF radical is inversely proportional to the length of its conjugated system.[49]

In less polar environments, CARs react with $NO_2^{\bullet}$ via HAT or RAF. Either way, the resulting radical intermediate should be relatively nonreactive toward O_2 addition (eq 2). Once the peroxide of the radical intermediate is formed (eq 8), it may rearrange into epoxide or cyclic ether.[26,29,50] In our previous theoretical study, we have shown that these reactions involve barriers that are higher than those of HAT or RAF;[51] thus, we will not consider the epoxide or cyclic ether reaction routes in this study.

Inspired by the above-mentioned theoretical studies, we investigated the reactions involved in the chain-breaking activities of β-CAR and LYC.[51,52] We found that the chain-terminating function of CAR is attributed to the trapping of the radical and the suppression of the addition of O_2. Furthermore, a hydrogen abstraction mechanism also contributes to the protective function of CARs, although to a lesser extent.

DOI: 10.1021/acs.jpcb.5b04142
J. Phys. Chem. B 2015, 119, 9640−9650

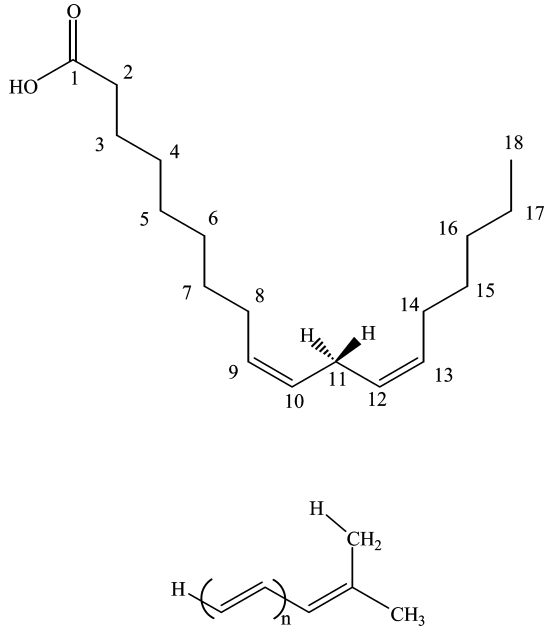
2. COMPUTATIONAL APPROACH

In this study, we used the three-parameter hybrid, generalized gradient approximation (GGA) exchange, and correlation functional B3LYP[53,54] along with the 6-31G(d) basis for geometry optimizations. The transition states (TSs) and minima were located using analytic gradients and verified using the harmonic vibrational frequencies. With these geometries, we obtained single-point energies using the kinetic energy density-dependent meta-GGA functional MPWB1K.[55] The 6-31++G(d,p) basis set was used in the single-point computations. The choice is based on a previous theoretical study, in which we compared the reaction energies of HAT and the RAF of model compounds, obtained using various density functional theories (DFTs) and basis sets, with those obtained using G3 theory.[56] It was observed that the MPWB1K/6-31++G(d,p)//B3LYP/6-31G(d) approach provides most accurate predictions and that the results are not affected by replacing the DFT-optimized geometries with those obtained using B3LYP/6-31G(d).[52] On the basis of these comparisons, we chose MPWB1K/6-31++G(d,p)//B3LYP/6-31G(d) in this study. Unless mentioned otherwise, the MPWB1K/6-31++G(d,p) energies should be assumed for all discussions.

The solvent effects of benzene and water were accounted for using MPWB1K/6-31++G(d,p) and the polarizable continuum model (PCM) developed by Tomasi et al. and Barone et al.[57,58] We encountered convergence difficulties using the United Atom approach of PCM, particularly in cases of the TSs of the HATs. For this reason, we used individual spheres for the hydrogens (RADII=UFF option in Gaussian), in order to overcome this problem.

We used linoleic acid (LAH) as our model lipid. The polyenes M_n (Scheme 2, n = 1−4) were used in the HATs of LAH by peroxides. The computations were performed using the Gaussian03 program series.[59]

Scheme 2. Structures of Linoleic Acid (LAH) and the Model Polyenes (M_n, n = 1−4)



3. RESULTS AND DISCUSSION

N versus O Hydrogen Abstraction and Addition of Nitrogen Dioxide. Table 1 summarizes reaction enthalpies and Gibbs energies (in kcal/mol, at 298 K) for the HAT and RAF reactions of CARs and LAH with $NO_2^\bullet$. In addition, R_{TS} (C−H bond distance for the TSs of HAT and O−C or N−C bond distances for the TSs of RAF) and ν_{imag} (magnitude of the imaginary frequency of the TSs) are also summarized. $\Delta G^{\ddagger}_{sol}$ and ΔG_{sol} are the Gibbs energies including solvation effects of benzene. We investigated the HAT and RAF of $NO_2^\bullet$ at the most favorable locations which have been revealed by previous theoretical studies.[41,52,60] For the HAT, these are C4−H on β-CAR and LY, and C11−H on LAH; for RAF the locations are C5 on β-CAR and LYC and C10 on LAH. The data used correspond to benzene, unless specified otherwise.

The HAT of $NO_2^\bullet$ occurs via either oxygen (−ONO HAT) or nitrogen ($-NO_2$ HAT) termini, resulting in HONO (nitrous acid) and HNO_2, respectively. For −ONO HAT, the TSs involving the cis conformation have a lower energy than those involving the trans conformation, while *trans*-HONO has a lower energy (by 0.6 kcal/mol using G3 theory) than does *cis*-HONO. The energies of *cis*-HONO were used for the data summarized in Table 1. The structures for the TSs involved in the HAT and RAF by $NO_2^\bullet$ are illustrated in Figures 1 and 2, respectively.

For the three substrates, the −ONO HAT reactions are more exergonic in the gas phase and in benzene. The $\Delta G^{\ddagger}$ and $\Delta G^{\ddagger}_{sol}$ values for the −ONO HAT of CARs are lower than their counterparts for the $-NO_2$ HAT. We observed that the C−H distances for −ONO HAT were shorter than their counterparts for the $-NO_2$ HAT reactions, and the magnitudes of the imaginary vibrational frequencies (ν_{imag}) for the TSs of −ONO HAT were smaller than those of $-NO_2$ HAT. The reaction barriers and reaction energies were lower in benzene than in the gas phase. Among the various substrates, β-CAR and LYC are more susceptible to HAT than is LAH, with the $\Delta G^{\ddagger}_{sol}$ and ΔG_{sol} values of LYC being slightly lower than those of β-CAR.

For the RAF, the $-NO_2$ additions are kinetically and thermodynamically favored more than are their −ONO addition counterparts. Furthermore, the $-NO_2$ addition to LYC involves a smaller reaction barrier and is more exergonic than is the addition to β-CAR. The $-NO_2^\bullet$ addition to LAH involves a higher barrier ($\Delta G^{\ddagger}_{sol}$ of 16.27 kcal/mol, on C10) than those for the CARs. The formation of O_2N−LAH$^\bullet$ is endergonic; in contrast, the formation of LA$^\bullet$ via the HAT is exergonic. It is later shown that the O_2 affinity of O_2N−LAH$^\bullet$ is overwhelmingly greater than that of LA$^\bullet$ (vide infra). Thus, this is the most important peroxidation process.

Of the two mechanisms, RAF is kinetically more favorable and has noticeably lower reaction barriers (~6 kcal/mol) than does HAT. The reactions of CARs are similarly exergonic for HAT and RAF reactions.

Mechanisms: Hydrogen-Atom Transfer, Radical Adduct Formation, and Electron Transfer in Different Solvents. Previous discussions on the chain-terminating functions of CARs have taken into account the HAT and RAF mechanisms. These previous studies consider only low-polarity environments (i.e., gas phase and benzene). We performed computations for a wider range of solvents. The reaction energetics in benzene, acetonitrile, and water solvents are listed in Table 2. The B3LYP and MPWB1K data are shown in the table for comparison.

The trend that can be observed in Table 2 is that $\Delta G^{\ddagger}_{sol}$ and ΔG_{sol} are lower for the more polar solvents. Furthermore, it is seen that for β-CAR and LYC, ET is strongly exergonic in acetonitrile and water, while for LAH, ET is highly endergonic.

A comparison of the two functionals shows that the energetics obtained using B3LYP and MPWB1K differ in three directions: (1) the $\Delta G^{\ddagger}_{sol}$ values of HAT obtained using B3LYP are lower,

DOI: 10.1021/acs.jpcb.5b04142
J. Phys. Chem. B 2015, 119, 9640−9650

Table 1. Reaction Enthalpies and Gibbs Energies (in kcal/mol, at 298 K) for the HAT and RAF of CARs and LAH with $NO_2^\bullet$,[a] R_{TS}[b] (in Angstroms), and Magnitude of the Imaginary Frequency of TS (ν_{imag}, in i cm^{-1})

	$\Delta H^\ddagger$	ΔH	$\Delta G^\ddagger$	ΔG	$\Delta G^\ddagger_{sol}$	ΔG_{sol}	R_{TS}[b]	ν_{imag}
			$NO_2^\bullet$ + CAR → HONO + CAR(−H,4)$^\bullet$					
β-CAR	10.30	−8.60	20.17	−8.46	16.41	−10.64	1.309	1357
LYC	8.46	−9.65	18.88	−10.64	15.75	−12.05	1.312	1349
LAH(11)	15.17	−0.75	23.28	−2.71	22.34	−4.63	1.308	1495
			$NO_2^\bullet$ + CAR → HNO_2 + CAR(−H,4)$^\bullet$					
β-CAR	12.02	−1.71	21.52	−1.11	18.40	−5.15	1.325	1525
LYC	9.77	−2.52	19.68	−3.31	17.55	−6.63	1.315	1462
LAH(11)	14.96	6.38	23.40	4.63	22.35	0.82	1.339	1633
			$NO_2^\bullet$ + CAR → ONO−CAR(5)$^\bullet$					
β-CAR	6.38	−10.94	17.85	0.71	13.89	−3.31	2.136	308
LYC	3.70	−19.05	15.55	−5.17	13.38	−8.03	2.186	298
LAH(10)	12.01	2.67	21.27	12.06	19.89	11.44	1.914	449
			$NO_2^\bullet$ + CAR → O_2N−CAR(5)$^\bullet$					
β-CAR	2.20	−19.76	13.99	−6.74	10.90	−9.78	2.262	225
LYC	−2.33	−23.24	11.29	−10.63	9.83	−12.03	2.204	271
LAH(10)	7.85	−1.27	17.70	8.88	16.27	7.29	1.983	381

[a]The locations of HAT and RAF reactions are specified in parentheses. Solvation effects of benzene were included using the PCM model [b]R_{TS} represent C−H bond distances for the TSs of HAT, and O−C and N−C bond distances for the TSs of RAF reactions.

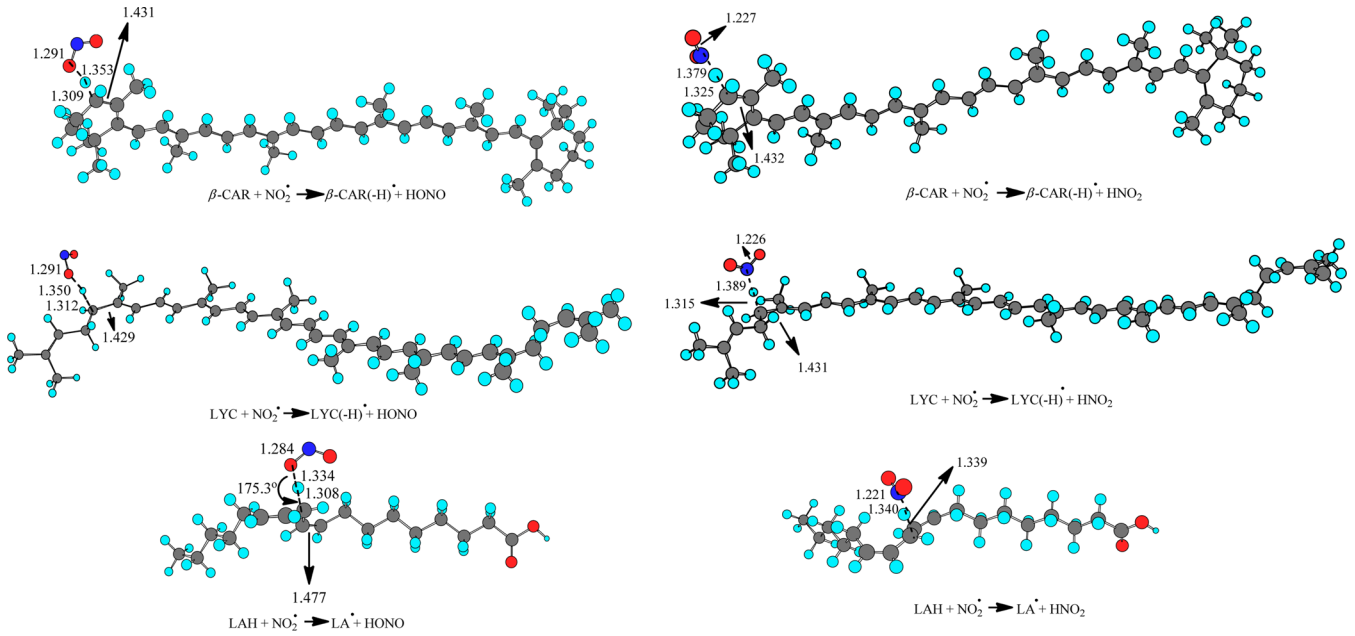


Figure 1. Optimized structures and selected geometrical parameters (bond lengths in Angstroms and bond angles in degrees) for the TSs involved in the HAT of β-CAR, LYC, and LAH. Corresponding reactions are shown under the illustrations.

(2) the ΔG_{sol} values of RAF obtained using B3LYP are several kcal/mol higher than those obtained using MPWB1K, and (3) the ΔG_{sol} values of the ET products obtained using B3LYP are lower by approximately 7−8 kcal/mol than those obtained using MPWB1K. Thus, using B3LYP, ET is the most energetically favorable reaction in acetonitrile and water, while using MPWB1K, the exergonicities of HAT and RAF are comparable to those of ET. Furthermore, we see that in benzene, HAT and RAF are similarly exergonic when MPWB1K is used, while using B3LYP, RAF is less exergonic by 6−7 kcal/mol. Kinetically, the $\Delta G^\ddagger_{sol}$ values of HAT are higher than those of RAF for both β-CAR and LYC for all solvents.

In the three solvents, LAH undergoes RAF at a faster rate than it does HAT; however, the HAT products are lower in energy than those of RAF. As was observed for the CARs, the $\Delta G^\ddagger_{sol}$ and ΔG_{sol} values of the LAH reactions are lower for the more polar solvents. Although the energetics obtained using these two functionals may differ by up to several kcal/mol in some cases, the conclusions are similar. We will restrict ourselves to MPWB1K and benzene as a solvent in the discussion that follows.

Peroxidation of Radical Intermediates. In the propagation step, radical intermediates, including CAR(−H)$^\bullet$, ONO−CAR$^\bullet$, and O_2N−CAR$^\bullet$, undergo O_2 addition and form peroxyl radicals. The values of the energetics for the addition of O_2 to these radicals are summarized in Table 3.

For CAR(−H)$^\bullet$, there are two energetically favorable possibilities for the addition of O_2. The first is to add O_2 on the proximal carbon (C4, see Scheme 1), where the hydrogen atom has been abstracted by $NO_2^\bullet$. Alternatively, the addition can occur

DOI: 10.1021/acs.jpcb.5b04142
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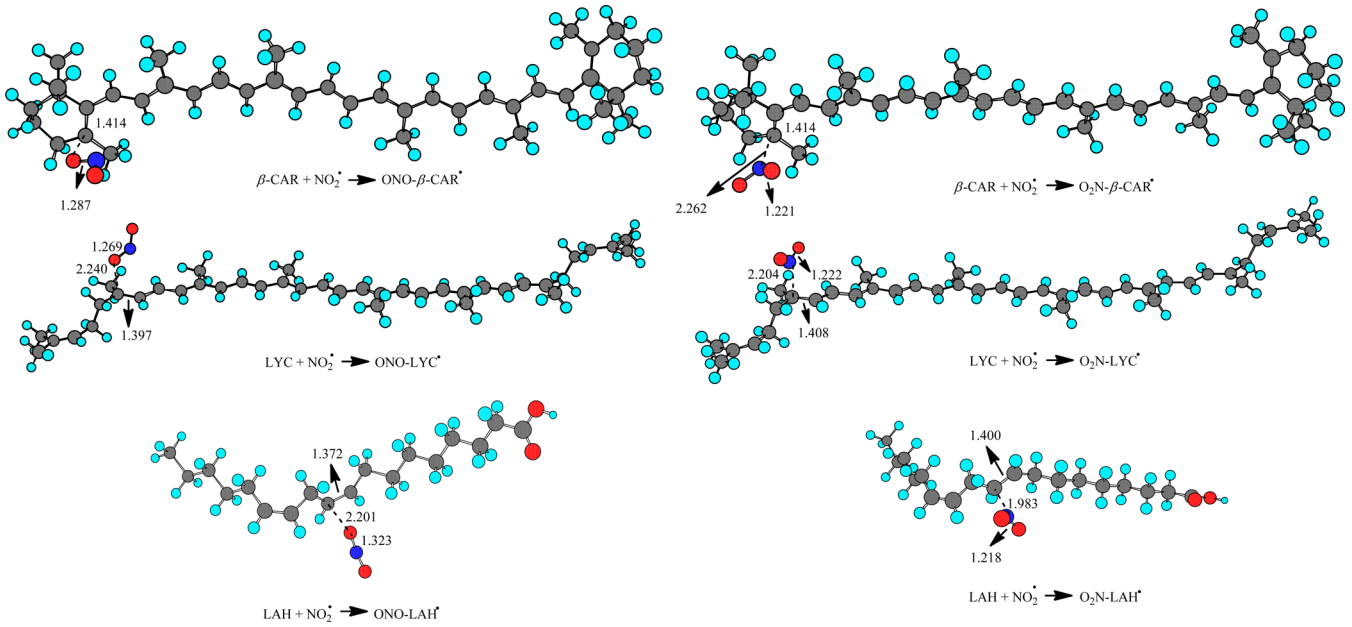

Figure 2. Optimized structures and selected geometrical parameters for the TSs involved in the RAF of β-CAR, LYC, and LAH.

Table 2. Reaction Gibbs energies (in kcal/mol, at 298 K) for the HAT and RAF of CARs and LAH with $NO_2^{\bullet}$ [a]

	$\Delta G^{\ddagger}_{sol}$			ΔG_{sol}		
	benzene	acetonitrile	water	benzene	acetonitrile	water
			$NO_2^{\bullet}$ + CAR → HONO + CAR(−H,4)$^{\bullet}$			
β-CAR	16.41(11.50)	12.33(7.53)	12.32(7.50)	−10.64(−10.44)	−12.40(−12.10)	−12.46(−12.35)
LYC	15.75(11.64)	11.75(7.69)	11.75(7.68)	−12.05(−10.77)	−13.32(−11.96)	−13.43(−12.10)
LAH(11)	22.34(19.17)	20.29(17.27)	20.62(17.60)	−4.63(−5.01)	−6.09(−6.54)	−6.29(−6.76)
			$NO_2^{\bullet}$ + CAR → HNO_2 + CAR(−H,4)$^{\bullet}$			
β-CAR	18.40(13.16)	13.99(9.08)	14.06(9.13)	−5.15(−5.17)	−9.19(−8.85)	−9.35(−9.19)
LYC	17.55(13.59)	13.48(9.72)	13.51(9.77)	−6.63(−5.59)	−10.17(−8.80)	−10.41(−9.05)
LAH(11)	22.35(19.49)	19.77(17.13)	20.01(17.39)	0.82(0.20)	−2.92(−3.36)	−3.23(−3.66)
			$NO_2^{\bullet}$ + CAR → ONO−CAR(5)$^{\bullet}$			
β-CAR	13.89(13.10)	9.67(8.91)	9.41(8.68)	−3.31(3.33)	−4.47(2.02)	−4.47(1.99)
LYC	13.38(11.42)	8.81(7.31)	8.68(7.25)	−8.03(−0.17)	−8.33(−1.20)	−8.43(−0.86)
LAH(10)	19.89(20.01)	18.01(18.23)	18.16(18.39)	11.44(16.41)	10.86(15.61)	11.12(15.85)
			$NO_2^{\bullet}$ + CAR → O_2N−CAR(5)$^{\bullet}$			
β-CAR	10.90(9.73)	6.85(5.93)	6.70(5.79)	−9.78(−1.27)	−12.26(−3.84)	−12.30(−3.90)
LYC	9.83(8.87)	4.44(4.28)	6.24(5.37)	−12.03(−3.60)	−13.97(−5.29)	−13.78(−5.14)
LAH(10)	16.27(16.92)	14.07(14.87)	14.21(15.01)	7.29(13.13)	5.25(11.05)	5.37(11.4)
			$NO_2^{\bullet}$ + CAR → NO_2^{-} + CAR$^{\bullet+}$			
β-CAR				26.72(19.41)	−10.98(−18.13)	−12.50(−19.69)
LYC				23.03(15.82)	−13.66(−21.12)	−15.10(−22.58)
LAH				69.69(65.65)	22.72(18.80)	20.75(16.83)

[a]The locations of HAT and RAF reactions are specified in parentheses. Solvation effects of benzene, acetonitrile, and water were included using the PCM model. Data were computed using MPWB1K/6-31++G(d,p) and B3LYP/6-31++G(d,p) (in parentheses).

at the distal, π-conjugated carbon (C5′). The structures of the TSs for the addition of O_2 to the radicals resulting from the HAT and the RAF reactions are illustrated in Figures 3 and 4, respectively. As can be seen from Table 3, the addition of O_2 is more likely to occur on the proximal carbon kinetically and thermodynamically; this results in CAR(−H,4−4)−OO$^{\bullet}$. Furthermore, the addition of O_2 to LA$^{\bullet}$ involves a much smaller barrier, both in the gas phase and in benzene, than that for CAR(−H)$^{\bullet}$.

In contrast to the addition of O_2 to CAR(−H)$^{\bullet}$, ONO−CAR$^{\bullet}$ and O_2N−CAR$^{\bullet}$ react with O_2 through comparable barriers. The reactions of these carotene systems with O_2 are endergonic. As can be seen from Table 3, it is remarkable that the O_2 additions of ONO−LAH$^{\bullet}$ and O_2N−LAH$^{\bullet}$ are highly exergonic and that these reactions are barrierless.

On combining the results summarized in Tables 1 and 3, we can see that the $-NO_2^{\bullet}$ addition to LAH ($\Delta G^{\ddagger}_{sol}$ of 16.27 kcal/mol) and the subsequent addition of O_2 (ΔG_{sol} of −19.36 kcal/mol) is a kinetically more feasible route than the other ones involved in the peroxidation of LAH with $NO_2^{\bullet}$. In the presence of O_2, this reaction is considered the most likely (most deleterious) route for the peroxidation of LAH.

HAT of Peroxide Radicals. The peroxides could abstract another hydrogen from the surroundings. In contrast, the HAT reactions of the carbon-centered radicals involve significantly

DOI: 10.1021/acs.jpcb.5b04142
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Table 3. Reaction Enthalpies and Gibbs Energies (in kcal/mol, at 298 K) for the O_2 Addition to Carbon-Centered Radicals Resulting from HAT and RAF of CARs and LAH[a]

	$\Delta H^{\ddagger}$	ΔH	$\Delta G^{\ddagger}$	ΔG	$\Delta G^{\ddagger}_{sol}$	ΔG_{sol}	R_{C-O}(TS)	ν_{imag}
			O_2 + CAR(−H)$^{\bullet}$ → CAR(−H)−OO(4,4)$^{\bullet}$					
β-CAR(−H)$^{\bullet}$	19.20	−4.70	28.98	5.89	24.64	4.30	2.107	351
LYC(−H)$^{\bullet}$	13.71	−2.14	24.52	11.53	22.89	9.91	2.047	397
LA$^{\bullet}$(11,9)	11.79	−16.18	21.03	−5.42	18.54	−7.37	2.124	310
			O_2 + CAR(−H)$^{\bullet}$ → CAR(−H)−OO(4,5′)$^{\bullet}$					
β-CAR(−H)$^{\bullet}$	17.61	−0.06	27.78	10.53	25.42	7.29	2.101	327
LYC(−H)$^{\bullet}$	16.15	−2.03	27.66	11.95	26.35	10.38	2.058	381
LA$^{\bullet}$(11,13)	15.71	−11.63	25.58	−1.23	20.43	−3.65	2.114	323
			O_2 + ONO−CAR$^{\bullet}$ → ONO−CAR−OO(5,5′)$^{\bullet}$					
ONO−β-CAR$^{\bullet}$	15.58	3.99	27.10	16.66	26.26	13.27	2.030	401
ONO−LYC$^{\bullet}$	12.27	−2.53	23.27	8.73	26.17	7.17	2.051	397
ONO−LAH$^{\bullet}$(10,9)		−31.06		−18.41		−20.12		
			O_2 + O_2N−CAR$^{\bullet}$ → O_2N−CAR−OO(5,5′)$^{\bullet}$					
O_2N−β-CAR$^{\bullet}$	19.90	4.16	31.54	16.35	24.12	12.95	2.028	404
O_2N−LYC$^{\bullet}$	12.25	−2.90	23.97	9.76	23.08	7.89	2.048	397
O_2N−LAH$^{\bullet}$(10,9)		−28.27		−18.15		−19.36		

[a]The locations of HAT/RAF and O_2 addition reactions are specified in parentheses.

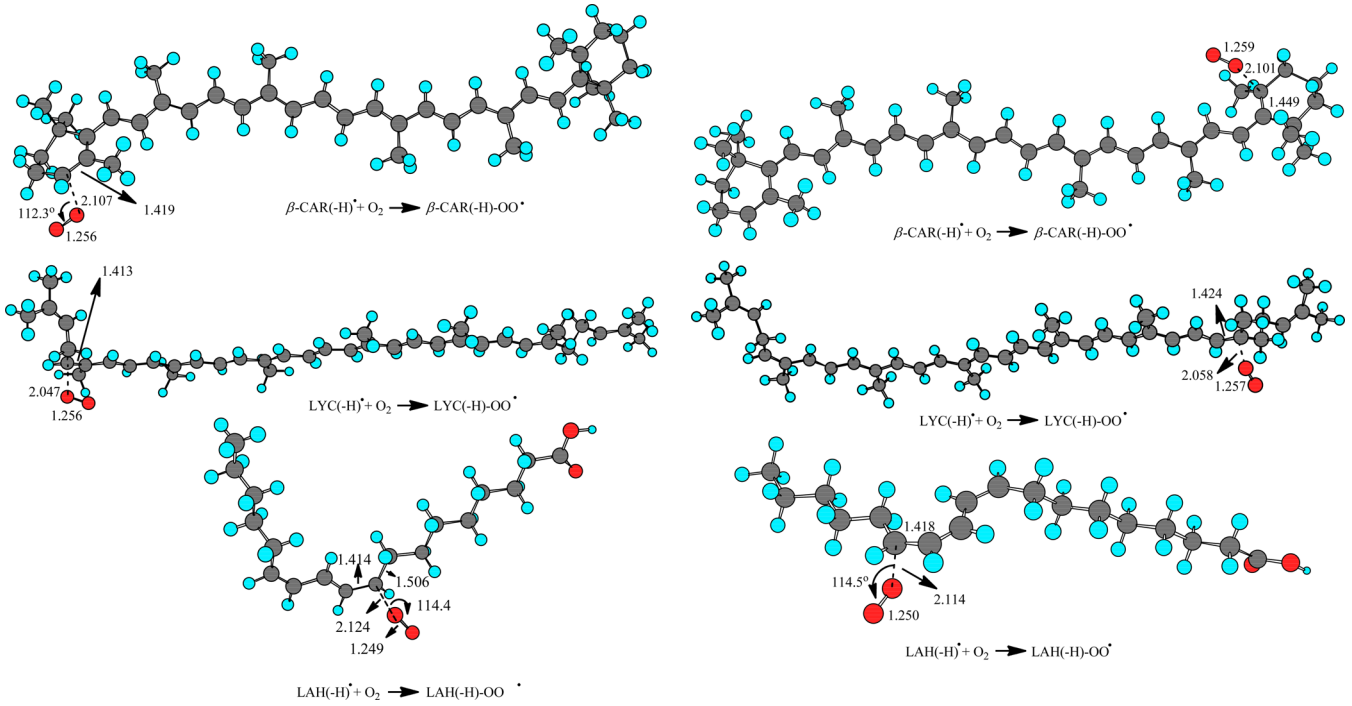

Figure 3. Optimized structures and selected geometrical parameters for the TSs involved in the O_2 additions to β-CAR(−H)$^{\bullet}$, LYC(−H)$^{\bullet}$, and LA$^{\bullet}$. Reactions on the proximal (left) and distal (right) carbons are shown.

higher barriers.[51] We used LAH as the substrate and reacted it with model peroxides, which included M_n(−H)−OO$^{\bullet}$, ONO−M_n(−H)−OO$^{\bullet}$, and O_2N−M_n(−H)−OO$^{\bullet}$ (n = 1−4, see Scheme 2).

As the size of the model peroxides increased through an extension of the conjugated systems, it can be seen (Table 4) that the reaction barriers and reaction energies remained similar. Furthermore, the R_{C-H} and ν_{imag} values of the TSs were very similar for the models of different sizes. On the basis of this observation, we estimated $\Delta G^{\ddagger}_{sol}$ to be ∼27 kcal/mol and ΔG_{sol} to be ca. −7 kcal/mol.

Termination of Radical Intermediates. The intermediate radicals O_2N−CAR$^{\bullet}$ and CAR(−H)$^{\bullet}$ play a central role with respect to the ability of CARs to offer protection against peroxidation. The most likely fate of these radicals is to react with another radical. We added another $NO_2^{\bullet}$ to them and investigated their reactions via the HAT and radical recombination.

The HAT of CAR(−H)$^{\bullet}$ by $NO_2^{\bullet}$ results in CAR(−H_2); the corresponding results are summarized in Table 5 (complete results, including those for −ONO HAT, are summarized in the Supporting Information). The reactions extend the conjugated system of the substrates and thus are exergonic, with $\Delta G^{\ddagger}_{sol}$ being smaller than 20 kcal/mol. Potential energy surfaces for the HAT of O_2N−CAR$^{\bullet}$ by $NO_2^{\bullet}$ are similar, that is, the reactions involve small barriers, and are exergonic.

The radical recombination of CAR(−H)$^{\bullet}$ and O_2N−CAR$^{\bullet}$ with $NO_2^{\bullet}$ is barrierless. Table 5 summarizes energetics for the most exergonic reactions, that is, for the O_2N additions.

DOI: 10.1021/acs.jpcb.5b04142
J. Phys. Chem. B 2015, 119, 9640−9650

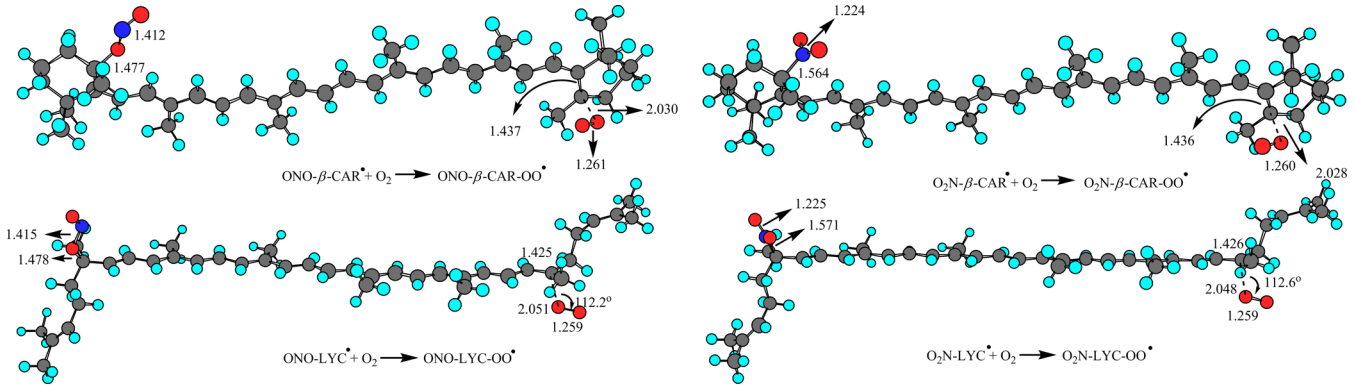

Figure 4. Optimized structures and selected geometrical parameters for the TSs involved in the O_2 additions to the RAF radicals of β-CAR and LYC. Reactions of the ONO-addition (left) and O_2N-addition (right) radicals are shown.

Table 4. Reaction Enthalpies and Gibbs Energies (in kcal/mol, at 298 K) for the HAT of LAH by the Model Peroxides of CAR(−H)−OO•, ONO−CAR−OO•, and O_2N−CAR−OO•

	$\Delta H^{\ddagger}$	ΔH	$\Delta G^{\ddagger}$	ΔG	$\Delta G^{\ddagger}_{sol}$	ΔG_{sol}	R_{C-H}	ν_{imag}
			CAR(−H)−OO• + LAH → CAR(−H)−OOH + LA•					
M_1(−H)−OO•	13.49	−4.04	22.80	−5.51	24.94	−6.96	1.291	1665
M_2(−H)−OO•	13.44	−4.04	23.47	−5.47	25.47	−6.94	1.291	1666
M_3(−H)−OO•	13.68	−4.01	24.16	−5.49	26.34	−6.96	1.291	1666
M_4(−H)−OO•	13.59	−4.00	24.41	−5.50	26.59	−6.95	1.292	1670
			ONO−CAR−OO• + LAH → ONO−CAR−OOH + LA•					
ONO−M_1(−H)−OO•	14.24	−3.64	23.99	−5.07	26.35	−6.44	1.292	1684
ONO−M_2(−H)−OO•	13.85	−3.45	26.02	−4.92	28.53	−6.31	1.292	1698
ONO−M_3(−H)−OO•	14.10	−3.36	26.64	−4.80	29.15	−6.17	1.294	1703
ONO−M_4(−H)−OO•	14.76	−3.37	25.03	−4.90	27.74	−6.29	1.293	1702
			O_2N−CAR−OO• + LAH → O_2N−CAR−OOH + LA•					
O_2N−M_1(−H)−OO•	14.02	−3.78	23.60	−5.34	26.06	−6.71	1.290	1672
O_2N−M_2(−H)−OO•	14.30	−3.55	24.10	−5.02	26.96	−6.37	1.292	1695
O_2N−M_3(−H)−OO•	14.52	−3.47	24.85	−4.99	27.26	−6.35	1.292	1695
O_2N−M_4(−H)−OO•	14.62	−3.40	24.35	−5.04	26.74	−6.76	1.294	1702

Table 5. Reaction Enthalpies and Gibbs Energies (in kcal/mol, at 298 K) for the HAT and Radical Recombination of the Radical Intermediates[a]

	$\Delta H^{\ddagger}$	ΔH	$\Delta G^{\ddagger}$	ΔG	$\Delta G^{\ddagger}_{sol}$	ΔG_{sol}	R_{C-H}	ν_{imag}
			NO_2• + CAR(−H,4)• → HONO + CAR(−H_2,4,4′)					
β-CAR(−H)•	7.39	−14.95	18.29	−15.23	13.98	−16.42	1.284	1156
LYC(−H) •	7.15	−18.74	20.78	−17.44	16.96	−19.64	1.281	1086
LA•(11,8)	1.60	−25.40	13.95	−25.32	10.36	−27.07	1.284	1119
			NO_2• + O_2N−CAR(5)• → HONO + O_2N−CAR(−H,5,4′)					
O_2N-β-CAR•	10.02	−14.22	22.62	−13.54	16.76	−16.05	1.257	956
O_2N-LYC•	7.55	−15.18	22.46	−12.19	17.80	−14.25	1.252	930
O_2N-LAH•(10,9)	−9.99	−39.43	2.27	−40.96	1.74	−42.46	1.332	1304
			NO_2• + CAR(−H,4)• → O_2N−CAR(−H,4,4)[b]					
β-CAR(−H)•		−32.08		−19.87		−21.36		
LYC(−H)•		−30.49		−17.11		−18.84		
LA•(11,9)		−42.87		−30.77		−32.96		
			NO_2• + O_2N−CAR(5)• → O_2N−CAR−NO_2(5,5′)[b]					
O_2N−β-CAR•		−25.88		−11.88		−14.95		
O_2N−LYC•		−29.70		−15.19		−17.04		
O_2N−LAH• (10,9)		−57.55		−46.59		−46.77		

[a]The locations of the first and second HAT/RAF and HAT/radical recombination are specified in parentheses. [b]The reactions are barrierless.

The detailed results are given in the Supporting Information. It is interesting to note that the radical recombinations of LA• and O_2N−LAH• are much more exergonic than those of their carotene counterparts. This implies that, at low oxygen concentrations, LA• and O_2N−LAH• can be neutralized by NO_2•.

Protective Role of Carotenes. The Gibbs energy profiles (ΔG_{sol}) for the reactions of NO_2• with β-CAR and LAH are

DOI: 10.1021/acs.jpcb.5b04142
J. Phys. Chem. B 2015, 119, 9640−9650

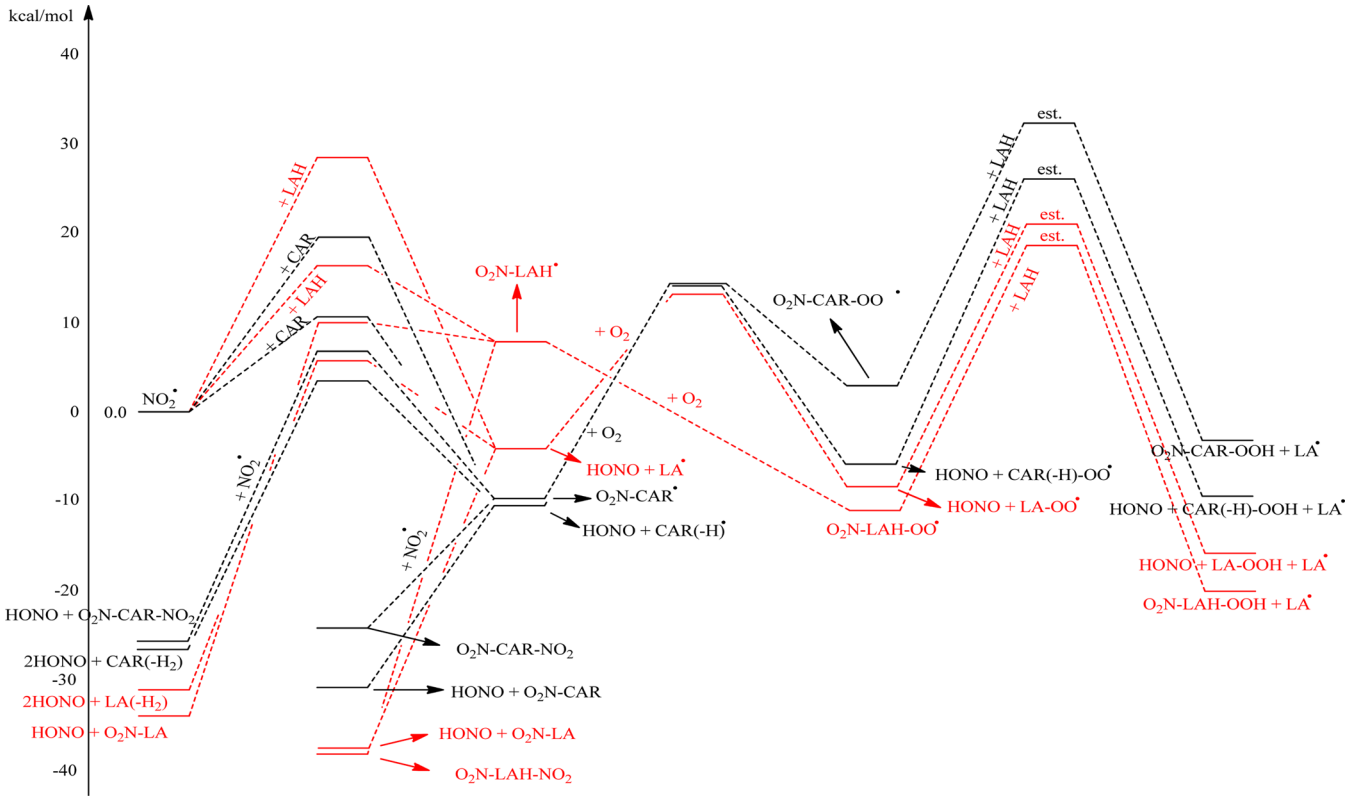

Figure 5. Energy profile (ΔG_{sol}) for the reactions involved in the $NO_2^•$-initiated peroxidation of lipids in the presence of β-CAR.

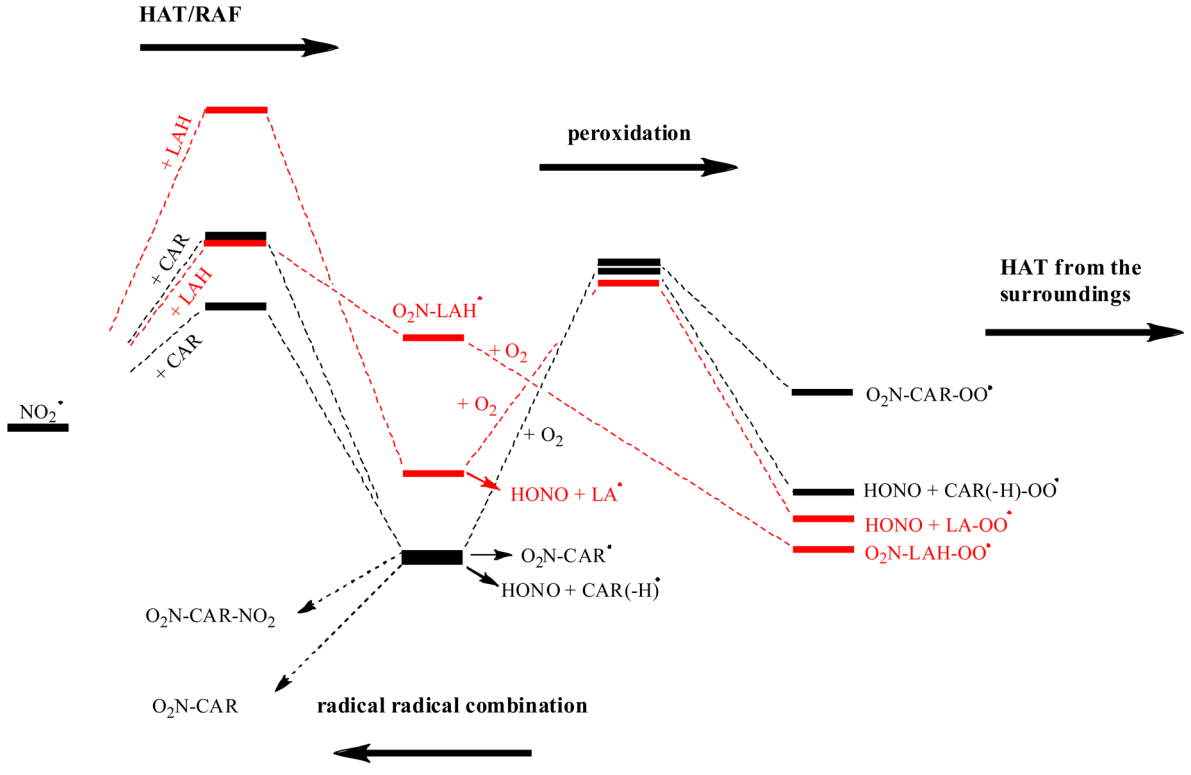

Figure 6. Simplified energy profile for the antioxidative function of CARs.

illustrated in Figure 5 (the energy profile of LYC is similar to that of β-CAR, see Supporting Information). In the figure, we show only the results for the RAF by $-NO_2$ and the HAT by $-ONO$.

In order to inhibit the $NO_2^•$-initiated peroxidation of LAH, the CAR undergoes the HAT or the RAF to form thermodynamically favorable radical intermediates, CAR($-H$)$^•$ and $O_2N-CAR^•$. The formation of these radicals is more exergonic than are those

DOI: 10.1021/acs.jpcb.5b04142
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for the formation of LA$^{\bullet}$ and O_2N−LAH$^{\bullet}$. O_2N−LAH$^{\bullet}$ undergoes O_2 addition without a barrier. Thus, this is the deleterious reaction path of concern. The resulting radical intermediates of CAR do not bind with O_2 readily. The additions of O_2 to CAR(−H)$^{\bullet}$ and O_2N−CAR$^{\bullet}$ are endergonic, while in contrast, the additions of O_2 to LA$^{\bullet}$ and O_2N−LAH$^{\bullet}$ are exergonic. In the absence of CAR, O_2N−LAH−OO$^{\bullet}$ or LA−OO$^{\bullet}$ are formed, which would undergo further HAT from the surroundings.

A simplified energy profile is illustrated in Figure 6. The protective function of a CAR is demonstrated on the basis of its ability to compete with LAH for $NO_2^{\bullet}$ and through the low O_2 affinity of the resulting radical intermediates. The radical intermediates of CAR species thus serve as $NO_2^{\bullet}$ reservoirs and are terminated via radical recombination.

On comparing the protective functions of β-CAR and LYC, it can be seen that the $\Delta G^{\ddagger}_{sol}$ values for the HAT and RAF of LYC are slightly smaller than those for the HAT and RAF of β-CAR and the reactions for LYC are more exergonic (Table 1). In contrast, for O_2 addition, it can be see that (see Table 2) the $\Delta G^{\ddagger}_{sol}$ values for the β-CAR and LYC radicals are comparable. The tendency of LYC to form radical intermediates CAR(−H)$^{\bullet}$ or O_2N−CAR$^{\bullet}$ is stronger. The result supports the hypothesis of Böhm et al. that LYC is more protective than β-CAR against the peroxidation of lipids initiated by $NO_2^{\bullet}$.[33]

4. CONCLUSION

In this study, the antioxidative functions of carotenes against the peroxidation of fatty acids (lipids) initiated by nitrogen dioxide were investigated using density functional theory. It was demonstrated that β-CAR and LYC are capable of intercepting the $NO_2^{\bullet}$ radical from fatty acids through the formation of stable radical intermediates. Our observations can be summarized as follows.

(1) For CARs, ET is more likely to occur in the most polar (water) environment than are HAT and RAF. The ET reactions of β-CAR and LYC are exergonic in polar solvents, while the ET reaction of LAH is endergonic.

(2) For the HAT of CARs, −ONO HAT is kinetically and thermodynamically more favored than is $-NO_2$ HAT. For the RAF of CARs, $-NO_2$ addition is kinetically and thermodynamically more favorable than is −ONO addition. RAF ($-NO_2$) involves TSs that are obviously lower in energy than those of HAT (−ONO). In contrast, the ΔG_{sol} values of the HAT and RAF reactions of CARs are comparable when MPWB1K is used, while HAT is strongly thermodynamically favored when B3LYP is used.

(3) For LAH, RAF is thermodynamically more favorable while HAT is favored kinetically.

(4) The reaction barriers for the RAF of CARs are lower than that of the RAF of LAH. The reaction barriers for the HAT of CARs are comparable to those for the RAF of LAH. The RAF and HAT mechanisms both contribute to the antioxidative functions of CARs. LYC is a more effective antioxidative agent than is β-CAR.

(5) The formation of O_2N−LAH−OO$^{\bullet}$ is proposed to be the most deleterious peroxidation reaction of concern.

(6) CAR radicals [CAR(−H)$^{\bullet}$ and O_2N−CAR$^{\bullet}$] are less reactive toward O_2 and thus are reservoirs of $NO_2^{\bullet}$ radicals. Indeed, the CARs investigated in this study are capable of competing for $NO_2^{\bullet}$ with LAH. Both the HAT and the RAF mechanisms contribute to the antioxidative function. Reaction barriers for the RAF between CARs and $NO_2^{\bullet}$ are smaller than those for the HAT.

(7) The termination of the radical intermediates [O_2N−CAR$^{\bullet}$ and CAR(−H)$^{\bullet}$] by another radical is barrierless via radical−radical recombination. In contrast, radical termination via HAT involves a noticeable barrier. The experimental observation of 4-O_2N−β-CAR was confirmed by our study. We expect that NO_2−CAR−NO_2 will be observed in future experiments as well.

■ ASSOCIATED CONTENT

Supporting Information

Detailed computational results and reaction energy profiles. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcb.5b04142.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge the support provided by the National Science Council of Taiwan, Republic of China. We also thank the National Center for High-Performance Computing for computer time and facilities.

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