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Lipoic Acid and Dihydrolipoic Acid. A Comprehensive Theoretical Study of Their Antioxidant Activity Supported by Available Experimental Kinetic Data

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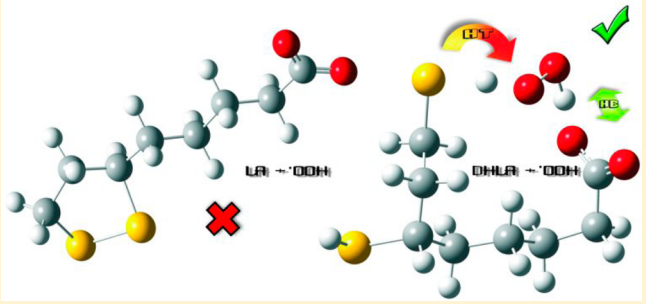
Supporting Information

ABSTRACT: The free radical scavenging activity of lipoic acid (LA) and dihydrolipoic acid (DHLA) has been studied in nonpolar and aqueous solutions, using the density functional theory and several oxygen centered radicals. It was found that lipoic acid is capable of scavenging only very reactive radicals, while the dehydrogenated form is an excellent scavenger via a hydrogen transfer mechanism. The environment plays an important role in the free radical scavenging activity of DHLA because in water it is deprotonated, and this enhances its activity. In particular, the reaction rate constant of DHLA in water with an $HOO^{\bullet}$ radical is close to the diffusion limit. This has been explained on the basis of the strong H-bonding interactions found in the transition state, which involve the carboxylate moiety, and it might have implications for other biological systems in which this group is present.



INTRODUCTION

Lipoic acid (LA) is an endogen organosulfur compound that plays an essential role in the metabolism as a cofactor for several mitochondrial enzymes.[1,2]It can be synthesized enzymatically from octanoic acid and ingested with food mainly from meat, although it can be found in lesser amounts in fruits and vegetables.[3,4]LA is better known than its reduced form, dihydrolipoic acid (DHLA). In vivo they are interconverted via enzymatic redox reactions.[5] Both free LA and DHLA have been detected in cells after administration of LA.[5] Therefore, it is not evident which one is the biologically active form, particularly for functions that have not been explicitly studied. Most endogenously produced LA is not free, because its precursor, octanoic acid, is bound to enzyme complexes prior to the enzymatic insertion of sulfur atoms. Accordingly, for functions other than cofactor, LA or DHLA are obtained from the diet.

It has been reported that both forms (LA and DHLA) have antioxidant properties.[6−9] In chemical experiments in which reduction of LA to DHLA is not expected, LA scavenges both hydroxyl radicals and singlet oxygen, but it has been shown to be incapable of protecting linoleic acid from AAPH-induced oxidation.[10] DHLA, on the other hand, is capable of protecting linoleic acid under the same conditions. This and other tests allowed Zhao et al. to conclude that DHLA is a better antioxidant than LA. However, the ability to scavenge $^{\bullet}OH$ and other very reactive oxidants like $^1O_2$ does not necessarily mean that a certain compound can be classified as an antioxidant, because almost any biological molecule can react with $^{\bullet}OH$ radicals at diffusion controlled rates. In fact $^{\bullet}OH$ reactions having rate constants smaller than 1×10^9 M^{-1} s^{-1} are exceptions.[11] Unfortunately, this is not common knowledge and should be studied more in detail.

Suzuki et al.[12] found that both LA and DHLA are capable of scavenging $^{\bullet}OH$ radicals, but only DHLA is capable of scavenging superoxide radical anions with a rate constant of 3.3×10^5 M^{-1} s^{-1}. This result implies that DHLA is an excellent antioxidant, and that is very important because $O_2^{\bullet-}$ is a relatively mild oxidant that is therefore much more selective and can be useful to discriminate between the antioxidant capacities of different substrates.

LA and DHLA are considered amphiphilic molecules, and they are soluble in both polar and nonpolar environments, a desired property of antioxidants because it means they can act in cytosol as well as in the lipid membrane.

In the last years we have been involved in the development of a theoretical methodology that uses quantum chemistry and statistical thermodynamics methods to obtain rate constants of radical-molecule reactions, known as QM-ORSA.[13]In most cases the rate constants calculated with this methodology are in excellent agreement with experimental values for a wide range of reactivities.[13] In a recent investigation, using this methodology, we have found that glutathione reacts almost exclusively via hydrogen abstraction mechanisms, both with ROS[14] and with carbon radicals produced during guanosine damage by $^{\bullet}OH$ radicals.[15] In both studies it was found, as expected, that

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the thiol group is responsible for the antioxidant capacity of glutathione. Additionally, it was established that the closest carboxylate, which is absent in most thiols, plays an essential role in the enhancement of this activity when the radical contains a hydrogen atom that is capable of forming an H bond with carboxylate. In this contribution, we intend to use this methodology to explore the antioxidant capacity of LA and DHLA with several radicals in order to determine, at the molecular level, the mechanism of their antioxidant activity.

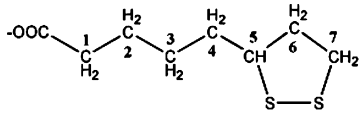
Based on the chemical structures of LA and DHLA, the tested mechanisms are hydrogen transfer (HT), and single electron transfer (SET). Detailed kinetic data are also provided for the first time, and the influence of the environment polarity on the scavenging activity has been assessed.

We have chosen a set of radicals with very different reactivities: the $^{\bullet}OH$ radical has been included because it is commonly used in experiments and it is the main source of biological damage in living organisms; $^{\bullet}OCH_3$ is used as an example of an alkoxyl radical; $HOO^{\bullet}$, $^{\bullet}OOCH_3$, and $^{\bullet}OOCHCH_2$ are examples of alkyl peroxyl radicals, and $^{\bullet}OOCCl_3$ is a model for very electronegative peroxyl radicals. In this way, we can easily test radicals of very different reactivities, which would be a tremendous task for experimental investigation. In addition, radicals presenting intermediate to low reactivity have been recommended for the studying of the relative scavenging activity of different compounds.[16,17]

To the best of our knowledge, the in vivo "equilibrium", as well as the interconversion rates in biological media, has not been quantitatively studied to date. The elucidation of whether LA or DHLA is the most active antioxidant could encourage this kind of work.

The chemical structure, or structures, responsible for the antioxidant capabilities of these compounds, as well as the mechanisms of action and the role of the environment, seem to be important for many researchers in different fields.

The structures of LA and DHLA and the labels used for the studied reaction sites are the following:



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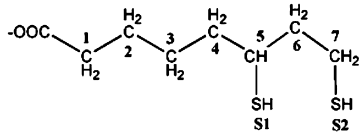

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COMPUTATIONAL DETAILS

All electronic calculations were performed with the Gaussian 09 package of programs.[18] Geometry optimizations and frequency calculations were carried out using the M06-2X[19] functional and the 6-31++G(d,p) basis set, using an ultrafine grid, in conjunction with the SMD continuum model[20] using pentylethanoate and water as solvents to mimic lipid and aqueous environments, respectively. The M05-2X and M06-2X functionals have been recommended and tested for kinetic calculations by their developers.[19] M05-2X has been successfully used by independent authors[21−26] and M06-2X was developed as an improvement over its predecessor M05-2X. It is also among the best performing functionals for calculating reaction energies involving free radicals.[27] SMD is considered to be a universal solvation model, due to its applicability to any charged or uncharged solute in any solvent or liquid medium for which a few key descriptors are known.[20]

Unrestricted calculations were used for open shell systems. Local minima and transition states were identified by the number of imaginary frequencies; local minima have only real frequencies, while transition states are identified by the presence of a single imaginary frequency that corresponds to the expected motion along the reaction coordinate. Relative energies are calculated with respect to the sum of the isolated reactants. Thermodynamic corrections at 298.15 K were included in the calculation of relative energies, which correspond to 1 M standard state. In addition, the solvent effects on entropy loss in the liquid phase have been taken into account according to the free volume theory.[28]

Counterpoise corrections (CP) to the basis set superposition error have not been included, since it has been recently demonstrated that, for a large variety of systems, CP corrected energies systematically differ more from the CBS-extrapolated values than the uncorrected ones. The main reason for such behavior is that the BSSE and the basis set truncation error systematically cancel.[29,30] Moreover, it has been shown that counterpoise corrections tend to overcorrect the BSSE.[31]

The rate constants (k) were calculated using the conventional transition state theory (TST):[32−34]

$$k = \sigma\kappa\frac{k_B T}{h} e^{-(\Delta G^{\ddagger})/RT} \quad (1)$$

where k_B and h are respectively the Boltzmann and Planck constants; $\Delta G^{\ddagger}$ is the Gibbs free energy of activation; σ represents the reaction path degeneracy, accounting for the number of equivalent reaction paths; and κ accounts for tunneling corrections. The latter are defined as the Boltzmann average of the ratio of the quantum and the classical probabilities, and they were calculated using the zero curvature tunneling corrections (ZCT).[35]

For single electron transfer reactions (SET), the barriers were estimated using Marcus theory.[36,37] It relies on the transition state formalism, defining the SET activation barrier ($\Delta G^{\ddagger}_{SET}$) in terms of two thermodynamic parameters, the free energy of reaction (ΔG^{0}_{SET}) and the nuclear reorganization energy (λ)

$$\Delta G^{\ddagger}_{SET} = \frac{\lambda}{4}\left(1 + \frac{\Delta G^{0}_{SET}}{\lambda}\right)^2 \quad (2)$$

The reorganization energy (λ) has been calculated as

$$\lambda = \Delta E_{SET} - \Delta G^{0}_{SET} \quad (3)$$

where ΔE_{SET} is the nonadiabatic energy difference between reactants and vertical products. This approach is similar to the one previously used by Nelsen and co-workers[38] for a large set of self-exchange reactions.

Some of the calculated rate constant (k) values are close to, or within, the diffusion-limit regime. Accordingly, the apparent rate constant (k_{app}) cannot be directly obtained from TST calculations. In the present work the Collins−Kimball theory[39] is used for that purpose:

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$$k_{app} = \frac{k_D k}{k_D + k} \quad (4)$$

where k is the thermal rate constant, obtained from TST calculations, and k_D is the steady-state Smoluchowski[40] rate constant for an irreversible bimolecular diffusion-controlled reaction:

$$k_D = 4\pi R D_{AB} N_A \quad (5)$$

where R denotes the reaction distance, N_A is the Avogadro number, and D_{AB} is the mutual diffusion coefficient of reactants A (free radical) and B (LA or DHLA). D_{AB} has been calculated from D_A and D_B according to reference 41, and D_A and D_B have been estimated from the Stokes−Einstein approach:[42,43]

$$D = \frac{k_B T}{6\pi\eta a} \quad (6)$$

where k_B is the Boltzmann constant, T is the temperature, η denotes the viscosity of the solvent (in our case water ($\eta = 8.91 \times 10^{-4}$ Pa s) and pentylethanoate ($\eta = 8.62 \times 10^{-4}$ Pa s)), and a is the radius of the solute.

For the kinetic study we have not included the endergonic reaction paths because, even if they took place at a significant rate, they would be reversible, and therefore, the formed products would not be observed. However, it should be noted that they might still represent significant channels if their products rapidly react further. This would be particularly important if these further stages were sufficiently exergonic to provide a driving force and if their barriers of reactions were low. This could be the case for the SET reactions in aqueous solution since they yield reactive species and take place at relatively large reaction distances. In addition, slightly endergonic processes can be important when there are no exergonic competing paths, but this was not the case in the present study.

RESULTS AND DISCUSSION

Multireference Diagnostics. Before starting the study of the system of interest with M06-2X, which is based on a single-determinant reference state, it is important to assess whether such a formalism is suitable. In recent publications, Tishchenko et al.[44,45] proposed that the transition states corresponding to H abstraction from the hydroxyl group in phenol by $^{\bullet}OOCH_3$,[44] and from the hydroxyl group in vinyl alcohol by $^{\bullet}OH$,[45] are affected by multireference effects. These findings may lead to the conclusion that transition states of hydrogen atom transfers, from OH groups that are linked to π electron systems, to oxygenated radicals suffer from multireference character. In a more recent publication by the same group,[46] it was found that the transition state of the $HOO^{\bullet}$ hydrogen abstraction from the hydroxyl group in methanol also presents significant multireference character, according to the T1 diagnostic. This suggests that multireference issues may also affect saturated systems. It has been established that a T1 value of 0.02 or greater indicates a significant multireference character for closed-shell systems. On the other hand, for open shell systems such as the ones in the present study, the benchmark value accepted so far is considerably larger and equal to 0.045. Moreover, since it has been shown that density functionals with a high fraction of Hartree−Fock (HF) exchange are often inaccurate for multireference systems,[47−49] such systems would not be properly described using the M06-2X functional. Because of structural similarities with alcohols, thiols could also be affected. However, it should be mentioned that canolol[50] and esculetin,[51] which are phenolic antioxidants were tested, and they certainly do not present significant multireference character.

Consequently in the present work the reliability of this functional has been tested. We have performed the T1 test for the main HT transition states in the DHLA + $HOO^{\bullet}$ reaction and compared the results with those for the previously described systems at CCSD(T)/6-311+G(d)//M05-2X/6-311+G(d,p) level of theory for the transition states corresponding to H abstractions, when the abstraction takes place from the SH moiety. The values obtained from the T1 diagnostic are presented in Table 1. The value for the methanol + $HOO^{\bullet}$ system was taken from ref 46 and was obtained at the CCSD(T)/aug-cc-pVDZ//M06-2X/MG3S level of theory. According to values in Table 1, we can safely conclude that, based on the T1 diagnostic, the thiols + peroxyl radical systems have no important multireference character; moreover, the T1 values are the lowest among the studied systems.

Table 1. Values of T1 Diagnostic for Transition States Involved in H Abstractions by Oxygenated Radicals, for the O−H or S−H Moiety in Different Systems

system	T1 diagnostic
ethenol + $^{\bullet}OH$	0.044[a]
phenol + $^{\bullet}OOCH_3$	0.044[a]
phenol + $^{\bullet}OH$	0.041[a]
methanol + $HOO^{\bullet}$	0.048[b]
canolol + $HOO^{\bullet}$	0.023[b]
esculetin + $HOO^{\bullet}$	0.030[c]
DHLA S1 + $HOO^{\bullet}$	0.019
DHLA S2 + $HOO^{\bullet}$	0.021

[a]From ref 50. [b]From ref 46. [c]From ref 51.

Antioxidant Activity. As is the case for many scavengers,[52−56] the free radical scavenging activity of LA and DHLA can take place through a variety of mechanisms. The ones considered in this work are shown in the following schemes: Scheme 1 for hydrogen transfer (HT) from LA; Scheme 2 for HT from DHLA; and Scheme 3 for single electron transfer (SET) mechanisms for both LA and DHLA. Since in water both acids are deprotonated, the carboxylate form was modeled. In nonpolar media we used protonated forms.

The SET mechanism need not be included in nonpolar environments since the latter do not promote the necessary solvation of the intermediate ionic species yielded by this mechanism. However, just to prove this point, the reaction energies for the SET process were calculated and found to be larger than 73 kcal/mol in all cases.

To obtain an initial screening of the reactivity and thermodynamic viability of the studied reactions we have calculated the Gibbs free energies of all possible reaction channels. They are shown in Tables 2 −5 for LA and DHLA, and for both media (lipid or aqueous).

The first conclusion that can be drawn from thermodynamic data is that, as expected, hydrogen abstraction reactions by $^{\bullet}OH$, for both acids (LA and DHLA) and for almost all positions, are very exergonic. The $^{\bullet}OH$ radical is very reactive, because the O−H bond in the water molecule is very strong; therefore, we expect, as usual, a low selectivity for $^{\bullet}OH$ reactions with LA and DHLA. Thus, we anticipate almost

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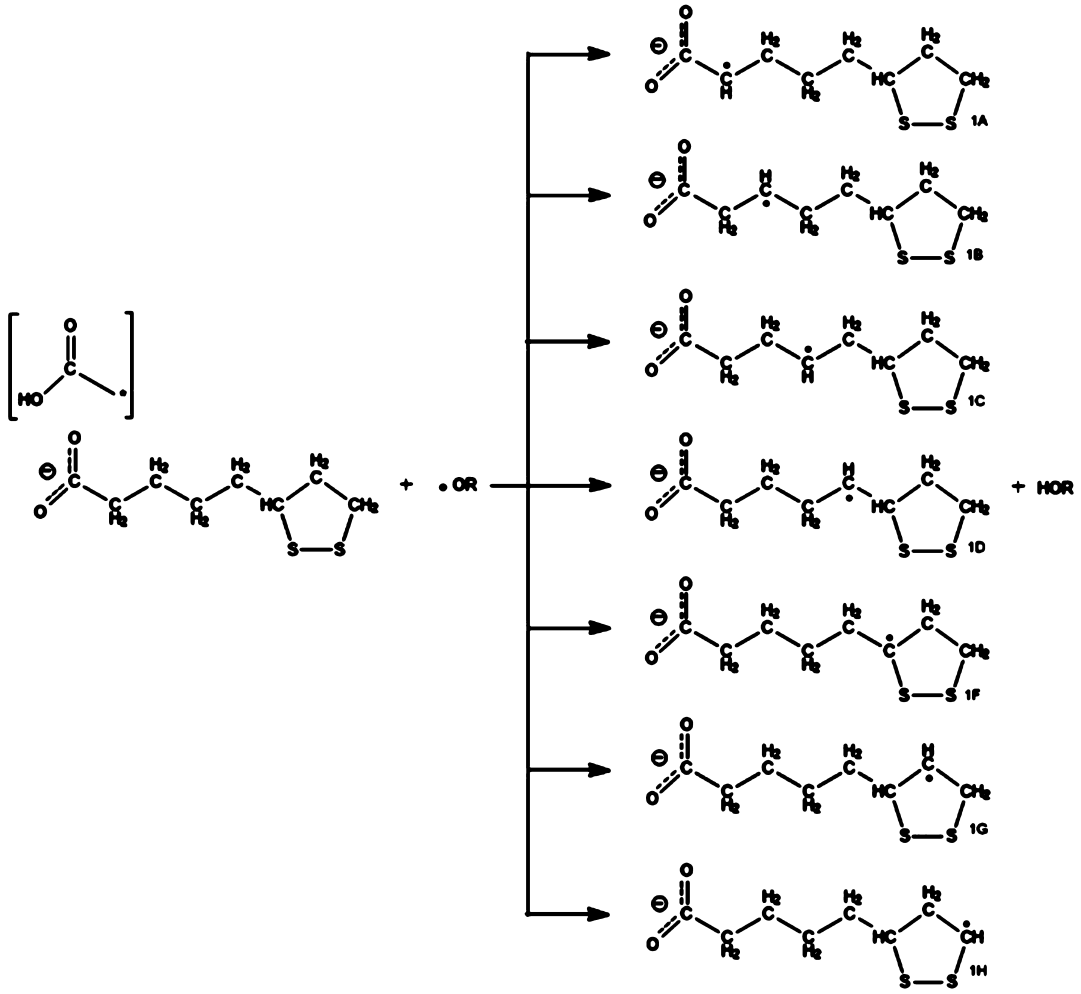
Scheme 1. Studied Reaction Channels for the HT Mechanism from LA



diffusion controlled reactions of •OH with many of the H atoms of the studied molecules, as previously found in the study of the reactions of glutathione with the •OH radical.[14] For this reason we will not include hydrogen transfer reactions with •OH radical in the kinetics section, and assume that they will proceed at diffusion-limited rates. Such reactivity of OH has created a misconception about inevitability of oxidative stress because antioxidants cannot react faster than OH. However, it is important to note that OH is not the enemy to fight; other reactive species, including its precursors, are the actual targets of antioxidants.[57] Qualitatively, the same situation can be observed for the $^{\bullet}OCH_3$ radical; however, these reactions are much less exergonic; therefore, they are probably not as fast as the reactions with •OH. To which extent? This question can be answered only via kinetic calculations.

Regarding H abstraction reaction with the much less reactive alkylperoxyl radicals, the situation changes dramatically. The only bonds that are weak enough to be exergonically broken by these radicals are the S−H ones. The H−OO bond in HOOH is much weaker than the H−O bond in the H_2O molecule, and therefore it is not strong enough to favor the breaking even of a tertiary C−H bond. It is noticeable that there are no exergonic reaction channels for H abstraction from LA by either HOO• or $^{\bullet}OOCH_3$. Accordingly it is not expected that LA can successfully scavenge hydroperoxyl or alkylperoxyl radicals via hydrogen transfer. This great difference between the LA and DHLA reactivities is the first exciting result from this work. LA, contrary to what is commonly assumed, cannot be considered as an antioxidant because, even if it depurates OH and alkoxy radicals, it reacts with them with rate constants that are similar to the ones for reaction with biological targets.

No large differences are observed between results in aqueous and lipid media. However, the reactions in water are systematically more exergonic, by 1−6 kcal/mol, than in lipid media. These differences are larger for the $^{\bullet}OOCCl_3$ reactions.

As expected, in lipid media the SET reactions are largely endergonic for both antioxidants, since the anionic products are not stabilized in a nonpolar solvent. The thermochemistry of the single electron transfer (SET) reaction mechanism in aqueous medium is different than HT. For the reaction of •OH radical with LA the electron loss is exergonic by approximately 6 kcal/mol, whereas the corresponding DHLA reaction is endergonic by 19 kcal/mol. Therefore, it can be concluded that LA is capable of reacting via SET with very electrophilic radicals, whereas DHLA is not reactive via this mechanism. The deprotonated S−H form of DHLA could, in principle, react via SET. However, it is not significantly deprotonated at physiological pH, since the S−H pK_a[58] is close to 10.7. As we will show later, its neutral form is very reactive via HT. Thus, the deprotonated form is not expected to play any role in the antioxidant activity of DHLA as was demonstrated recently for glutathione.[14]

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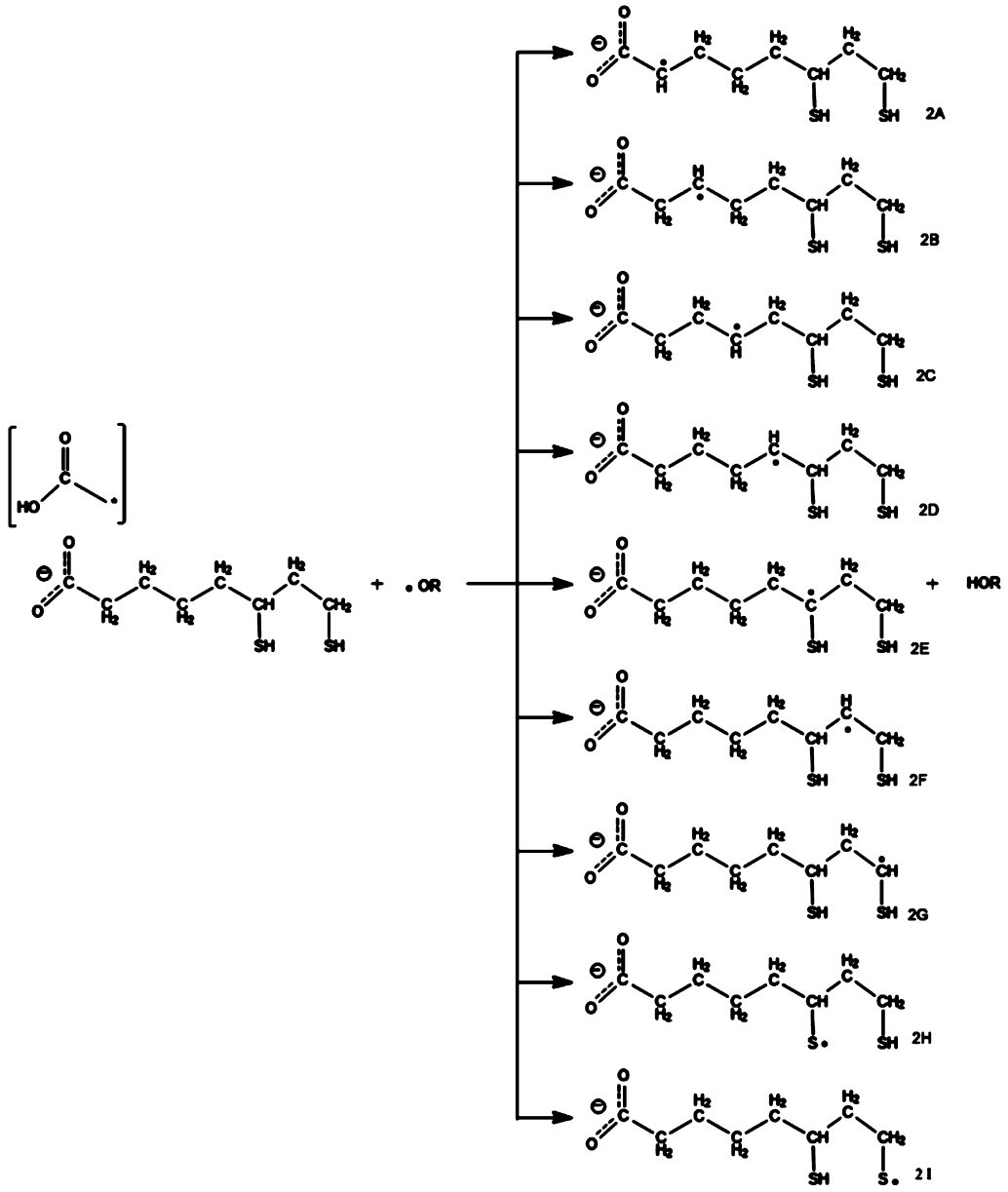
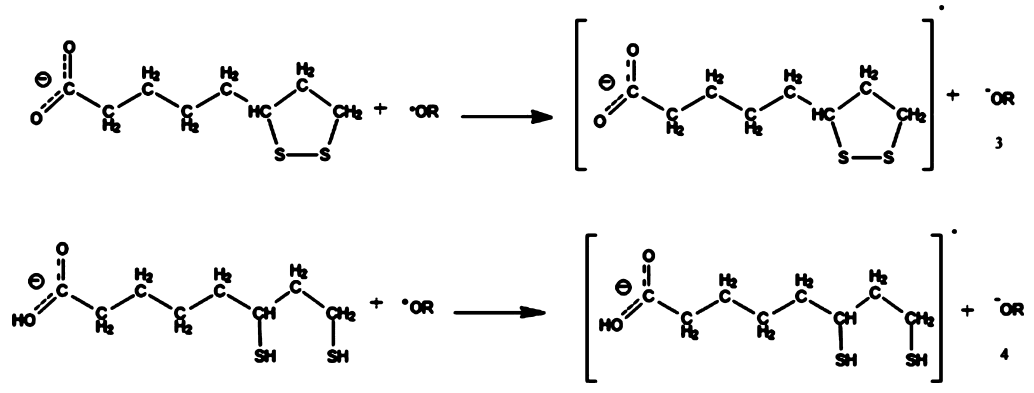
Scheme 2. Studied Reaction Channels for the HT Mechanism from DHLA



Scheme 3. Studied SET Reactions for Both LA and DHLA in Water



From thermodynamic data and comparisons with previous results we can conclude that LA reacts only with very reactive radicals, and that the reaction is diffusion controlled and nonselective. As a consequence, the in vivo antioxidant activity of LA−DHLA can only be attributed to DHLA. In a second stage of this work we will model the kinetics of the exergonic

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Table 2. Reaction-Free Energy, ΔG^0, for Electron Transfer (SET) and Hydrogen Transfer (HT) for LA for Different Radicals (solvent water)

	$^{\bullet}OH$	$^{\bullet}OCH_3$	$HOO^{\bullet}$	$^{\bullet}OOCH_3$	$^{\bullet}OOCHCH_2$	$^{\bullet}OOCCl_3$
SET	−6.1	5.0	17.9	26.0	20.0	3.5
HT-1	−28.1	−12.8	4.1	5.8	4.5	−3.6
HT-2	−23.9	−8.7	8.2	9.9	8.6	0.5
HT-3	−23.6	−8.4	8.5	10.2	8.9	0.8
HT-4	−24.3	−9.0	7.8	9.5	8.2	0.1
HT-5	−29.0	−13.7	3.1	4.9	3.6	−4.6
HT-6	−24.8	−9.6	7.3	9.0	7.7	−0.4
HT-7	−27.4	−12.1	4.8	6.5	5.1	−3.0

Table 3. Reaction Free Energy, ΔG^0, for Electron Transfer (SET) and Hydrogen Transfer (HT) for DHLA for Different Radicals (solvent water)

	$^{\bullet}OH$	$^{\bullet}OCH_3$	$HOO^{\bullet}$	$^{\bullet}OOCH_3$	$^{\bullet}OOCHCH_2$	$^{\bullet}OOCCl_3$
SET	19.1	30.0	43.0	51.2	45.2	28.7
HT-1	−27.9	−12.6	4.3	6.0	4.7	−3.4
HT-2	−24.3	−9.0	7.8	9.5	8.2	0.1
HT-3	−25.4	−10.2	6.7	8.4	7.1	−1.0
HT-4	−23.3	−8.0	8.9	10.6	9.3	1.1
HT-5	−29.1	−13.8	3.1	4.8	3.5	−4.7
HT-6	−26.3	−11.0	5.9	7.6	6.3	−1.8
HT-7	−26.8	−11.5	5.4	7.1	5.8	−2.3
HT-S1	−37.8	−22.6	−5.7	−4.0	−5.3	−13.4
HT-S2	−37.2	−21.9	−5.1	−3.4	−4.7	−12.8

Table 4. Reaction Free Energy, ΔG^0, for Electron Transfer (SET) and Hydrogen Transfer (HT) for LA for Different Radicals (solvent pentylethanoate)

ΔG^0	$^{\bullet}OH$	$^{\bullet}OCH_3$	$HOO^{\bullet}$	$^{\bullet}OOCH_3$	$^{\bullet}OOCHCH_2$	$^{\bullet}OOCCl_3$
SET	49.4	59.8	69.5	70.1	58.7	37.0
HT-1	−24.3	−9.1	8.1	10.2	9.0	1.6
HT-2	−19.9	−4.6	12.6	14.7	13.5	6.0
HT-3	−20.4	−5.1	12.0	14.1	13.0	5.5
HT-4	−22.1	−6.8	10.3	12.4	11.3	3.8
HT-5	−25.6	−10.4	6.8	8.9	7.7	0.3
HT-6	−21.2	−5.9	11.2	13.3	12.2	4.7
HT-7	−24.3	−9.0	8.2	10.3	9.1	1.6
HT-O	6.4	21.7	38.8	40.9	39.7	32.3

Table 5. Reaction Free Energy, ΔG^0, for Electron Transfer (SET) and Hydrogen Transfer (HT) for DHLA for Different Radicals (solvent pentylethanoate)

ΔG^0	$^{\bullet}OH$	$^{\bullet}OCH_3$	$HOO^{\bullet}$	$^{\bullet}OOCH_3$	$^{\bullet}OOCHCH_2$	$^{\bullet}OOCCl_3$
SET	73.5	83.9	93.6	94.2	82.8	61.1
HT-1	−25.4	−10.1	7.0	9.2	8.0	0.5
HT-2	−19.6	−4.3	12.9	15.0	13.8	6.3
HT-3	−21.7	−6.4	10.7	12.8	11.7	4.2
HT-4	−21.8	−6.5	10.6	12.7	11.6	4.1
HT-5	−25.1	−9.9	7.3	9.4	8.2	0.8
HT-6	−21.7	−6.4	10.8	12.9	11.7	4.2
HT-7	−23.4	−8.1	9.0	11.1	10.0	2.5
HT-S1	−35.3	−20.0	−2.8	−0.7	−1.9	−9.4
HT-S2	−33.7	−18.4	−1.3	0.8	−0.4	−7.8
HT-O	−5.9	9.4	26.5	28.6	27.5	20.0

reaction channels with all radicals, except for the most reactive $^{\bullet}OH$ radical, whose reactivity is so large that it abstracts almost any H atom. In this way we shall be able to evaluate the Gibbs free energy barriers and rate constants, and therefore quantitatively assess the primary antioxidant activity.

Kinetic Study. We have performed the kinetic study of all exergonic reaction channels obtained in the thermodynamic study. These reaction channels are the only candidates expected to have large enough rate constants. In the particular case of the $^{\bullet}OH$ radical reactions, their kinetics were excluded because the reactions are expected to be diffusion controlled. The Gibbs free energy of activation ($\Delta G^{\ddagger}$) for the different reaction channels involving LA and DHLA in water, are reported in Table 6 and 8, while those in pentylethanoate are given in Tables 7 and 9.

Table 6. Rate Constants (k) and Gibbs Free Energy of Activation ($\Delta G^{\ddagger}$) for Reactions for Lipoate, in Water

	$^{\bullet}OCH_3$		$^{\bullet}OOCCl_3$	
	$\Delta G^{\ddagger}$ (kcal/mol)	$k(M^{-1}\ s^{-1})$	$\Delta G^{\ddagger}$ (kcal/mol)	$k(M^{-1}\ s^{-1})$
SET	–	5.4×10^6	–	2.2×10^8
HT-1	9.2	8.2×10^6	12.6	4.6×10^4
HT-2	8.3	2.6×10^7	11.1	2.0×10^5
HT-3	10.6	7.4×10^5	14.9	5.7×10^2
HT-4	11.9	1.2×10^5	12.7	2.4×10^4
HT-5	7.9	1.1×10^7	9.1	2.9×10^6
HT-6	12.0	1.2×10^5	13.2	1.7×10^4
HT-7	8.5	6.9×10^6	8.8	4.6×10^6
total	–	5.86×10^7	–	2.26×10^8
total (exp)[12]				1.8×10^8

Table 7. Rate Constants (k) and Gibbs Free Energy of Activation ($\Delta G^{\ddagger}$) for Hydrogen Abstraction from LA, in Pentylethanoate

	$^{\bullet}OCH_3$		$^{\bullet}OOCCl_3$	
	$\Delta G^{\ddagger}$ (kcal/mol)	$k(M^{-1}\ s^{-1})$	$\Delta G^{\ddagger}$ (kcal/mol)	$k(M^{-1}\ s^{-1})$
HT-1	13.7	1.3×10^4	20.3	8.8×10^{-1}
HT-2	13.0	2.4×10^4	–	–
HT-3	13.9	5.4×10^3	–	–
HT-4	14.1	4.8×10^3	–	–
HT-5	10.1	4.8×10^5	11.7	2.9×10^4
HT-6	14.6	3.6×10^3	–	–
HT-7	9.7	1.1×10^6	13.3	2.7×10^3
total	–	1.6×10^6	–	3.2×10^4

The comparison between DHLA and LA again shows that the first is much more reactive than the latter. The trend observed in the Gibbs energy barriers shows that the SH groups are responsible for most of the antioxidant activity, these barriers being the lowest in all cases. The comparison of the LA and DHLA Gibbs free energies of activation shows that, even for the less selective $^{\bullet}OCH_3$ radical, the barrier for the SH site in DHLA is approximately 1.5 kcal/mol lower than the lowest $\Delta G^{\ddagger}$ for LA. For less reactive radicals the reaction is thermodynamically disfavored for LA. From all the studied channels shown in Schemes 1−3 only reaction channels 2H and 2I, see Scheme 2, are responsible for the antioxidant activity of LA−DHLA pair.

It is important to call attention to the exceptionally small $\Delta G^{\ddagger}$ of activation for the reaction of $HOO^{\bullet}$ with DHLA in aqueous solution, which is even lower than the corresponding barrier in the $^{\bullet}OCH_3$ reaction. This situation is not observed in

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Table 8. Rate Constants (k) and Gibbs Free Energy of Activation ($\Delta G^{\ddagger}$) for Hydrogen Abstraction in DHLA, in Water

	$^{\bullet}OCH_3$		HOO$^{\bullet}$		$^{\bullet}OOCH_3$	
	$\Delta G^{\ddagger}$ (kcal/mol)	k (M^{-1} s^{-1})	$\Delta G^{\ddagger}$ (kcal/mol)	k (M^{-1} s^{-1})	$\Delta G^{\ddagger}$ (kcal/mol)	k (M^{-1} s^{-1})
HT-1	9.5	5.6×10^6		–		–
HT-2	10.4	8.8×10^5		–		–
HT-3	11.0	3.9×10^5		–		–
HT-4	11.7	1.7×10^5		–		–
HT-5	8.6	5.4×10^6		–		–
HT-6	10.6	1.2×10^6		–		–
HT-7	9.4	2.0×10^6		–		–
HT-S1	7.3	6.1×10^7	16.2	9.0×10^2	15.7	2.0×10^3
HT-S2	6.9	5.1×10^7	6.4	1.3×10^8	13.4	4.0×10^4
total	–	1.3×10^8	–	1.3×10^8	–	4.2×10^4
total (exp)[a]		1.32×10^8				
		2.92×10^8				

	$^{\bullet}OOCHCH_2$		$^{\bullet}OOCCl_3$	
	$\Delta G^{\ddagger}$ (kcal/mol)	k (M^{-1} s^{-1})	$\Delta G^{\ddagger}$ (kcal/mol)	k (M^{-1} s^{-1})
HT-1	–	–	13.0	2.3×10^4
HT-2	–	–	10.6	6.2×10^5
HT-3	–	–	12.2	4.7×10^4
HT-4	–	–	12.5	3.9×10^4
HT-5	–	–	10.0	8.9×10^5
HT-6	–	–	12.7	3.4×10^4
HT-7	–	–	10.8	2.0×10^5
HT-S1	13.2	6.1×10^4	12.4	2.1×10^5
HT-S2	13.0	3.6×10^4	11.8	2.6×10^5
total	–	9.7×10^4	–	2.3×10^6
total (exp)				2.7×10^7

[a]Assuming that the rate constant of $O_2^{\bullet -}$ is because of its conjugated acid HOO$^{\bullet}$ with 0.0025 molar fraction at pH 7.4.[12]

Table 9. Rate Constants (k) and Gibbs Free Energy of Activation ($\Delta G^{\ddagger}$) for Hydrogen Abstraction from DHLA, in Pentylethanoate

	$^{\bullet}OCH_3$		HOO$^{\bullet}$		$^{\bullet}OOCH_3$	
	$\Delta G^{\ddagger}$ (kcal/mol)	k (M^{-1} s^{-1})	$\Delta G^{\ddagger}$ (kcal/mol)	k (M^{-1} s^{-1})	$\Delta G^{\ddagger}$ (kcal/mol)	k (M^{-1} s^{-1})
HT-1	13.6	1.9×10^4	–	–		
HT-2	14.4	3.1×10^3	–	–		
HT-3	13.6	6.6×10^3	–	–		
HT-4	14.6	3.1×10^3	–	–		
HT-5	8.9	4.9×10^6	–	–		
HT-6	13.8	9.4×10^3	–	–		
HT-7	11.8	5.6×10^4	–	–		
HT-S1	7.1	7.0×10^7	15.19	2.1×10^3	16.2	4.1×10^2
HT-S2	8.7	3.3×10^6	15.46	9.0×10^2	16.6	1.2×10^2
total	–	7.83×10^7	–	2.96×10^3	–	5.3×10^2

	$^{\bullet}OOCHCH_2$		$^{\bullet}OOCCl_3$	
	$\Delta G^{\ddagger}$ (kcal/mol)	k (M^{-1} s^{-1})	$\Delta G^{\ddagger}$ (kcal/mol)	k (M^{-1} s^{-1})
HT-1			20.1	1.11
HT-5			13.6	2.2×10^3
HT-7			14.2	8.7×10^2
HT-S1	15.2	1.5×10^3	13.5	2.2×10^4
HT-S2	16.7	1.1×10^2	13.6	9.3×10^3
total	–	1.6×10^3	–	3.41×10^4

a simulated lipid environment, and the explanation can be found in the structure of the transition state shown in Figure 1. As can be seen in Figure 1a, in the transition state of HOO$^{\bullet}$ in water, the deprotonated carboxyl group and the electron-deficient HOO$^{\bullet}$ hydrogen atom form a very strong H bond between the two moieties. This results in a free energy of activation which is much lower than what would be expected from the bond dissociation energies (BDE) of the breaking S–H bond and the forming H–OOH bond. Although DHLA has two SH groups, only one of them is in a conformation that is appropriate for the formation of this type of hydrogen bonding. The other one can be used to assess the magnitude of the effect; as can be seen from Table 8, the S2 transition state is approximately 10 kcal/mol more stable than for S1. The effect

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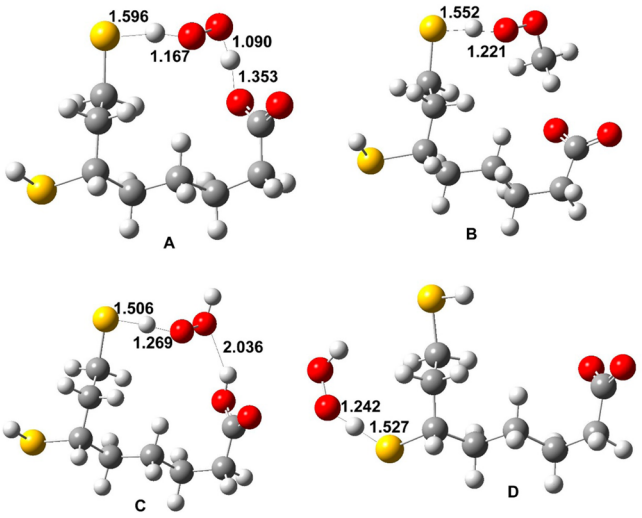

Figure 1. Molecular graphics and main geometrical parameters of selected transition states (A) TS-HT-S2 with OOH in water, (B) TS-HT-S2 with $OOCH_3$ in water, (C) TS-HT-S2 with OOH in pentylethanoate, (D) TS-HT-S1 with OOH in water.

of the carboxylate moiety is so important that, even for the transition state of the rest of the radicals that cannot form a hydrogen bond (all except $HOO^{\bullet}$), the S2 transition state is less energetic than S1, even though the latter is bonded to a tertiary carbon atom while the first is bonded to a secondary carbon atom.

In simulated lipid media the carboxyl group is protonated, and therefore, the H bond in much weaker (Figure 1B) and has no effect on the free energies of activation. Consequently, the S1 position has a slightly lower free energy of activation because S1 is bonded to a tertiary carbon atom. The transition state with $^{\bullet}OCH_3$ (Figure 1-C) has no possibility of forming an especially stable transition state and the situation in both media is similar to the one for $HOO^{\bullet}$ in a lipid medium.

The order of overall reactivities for radicals involved in H abstraction in water is $HOO^{\bullet} \approx {}^{\bullet}OCH_3 > {}^{\bullet}OOCHCH_2 > {}^{\bullet}OOCH_3$, whereas in lipid medium it is $^{\bullet}OCH_3 > {}^{\bullet}OOCHCH_2 > {}^{\bullet}OOCH_3 > HOO^{\bullet}$; i.e. the hydrogen bond in the transition state between H−OO and carboxylate inverts the order of reactivities, transforming the less reactive radical $HOO^{\bullet}$ into one of the most reactive. Taking into account the importance of the $O_2^{\bullet-} + H^+ \rightleftharpoons HOO^{\bullet}$ equilibrium in biological systems, and the great importance of the superoxide radical, this situation could be of great relevance in understanding the role of SH groups close to a carboxylate moiety in living systems.

The Gibbs free energy of activation is an excellent criterion to evaluate the rate of radical scavenging reactions. Moreover, since the units are kcal/mol, they are easily assimilated for those readers that are accustomed to making comparisons using energy units. However, there is an important factor that is missed in classical Gibbs free energies of activation, i.e. the tunneling corrections, which for this kind of reaction have been reported to play an important role.[59−61] Additionally, it is more common to make comparisons in terms of rate constants. For these reasons we have calculated the rate constants for each reaction channel using conventional transition state theory and ZCT tunneling corrections. We are assuming that the variational effects are not important in this case, because in the very few previous studies for HT reactions involving antioxidants it was found to be unimportant.[59,61]

The calculated rate constants are reported in Tables 6 and 7, for the reactions of LA with the most reactive radical, $^{\bullet}OCH_3$, in water and pentylethanoate. In water we have also included the $^{\bullet}OOCCl_3$ radical. Its rate constants are large for almost all positions, independently of the nature of the site, in agreement with the low selectivity of this very reactive radical. It is important to mention that $^{\bullet}OOCCl_3$ and $^{\bullet}OCH_3$ radicals would react with similar rates with structurally equivalent biological molecules. It is well-known that, in order to be considered a good antioxidant, a compound should react faster than the biological targets. This is definitively not the case of LA with most of the studied free radicals. It should be noticed that we have not tabulated the rate constants of $^{\bullet}OOCCl_3$ with LA in lipid media, the reason being that the corresponding reactions are endergonic. In this case, even if the reactions were fast, as expected, the reverse reactions would be even faster because of the low equilibrium constant.

The calculated rate constants for H abstraction from DHLA, in both water and pentylethanoate, are reported in Tables 8 and 9, respectively. The first conclusion is that the reactions are much faster in water than in pentylethanoate. In the case of the very reactive alkoxyl radical the ratio k water/k pentylethanoate is about 2, while for the less reactive $OOCH_3$ the ratio is approximately 78. It is significant that the ratio for OOH radical is more than 4 orders higher (4.3×10^4 M^{-1} s^{-1}), with an overall rate constant, in water, of 1.28×10^8 M^{-1} s^{-1}, i.e. the reaction is almost diffusion controlled. This rate constant positions DHLA among the best OOH scavengers in water, which is the most important medium for biological systems. It is important to notice that this exceptional reactivity is due not only to the presence of the S−H moiety but also to the strong H bond that is formed with COO^- in the transition state. This situation occurs also in the case of glutathione; however, in the present case the lowering in the energy barrier is even more important, probably because the relative positions of SH and COO^- are optimal.

This is one of the most important findings of this contribution. Usually the reactivity of certain positions in a molecule can be evaluated via the stability of the formed products, in agreement with Bell−Evans−Polanyi principle. In the present case, however, a large deviation from this principle occurs because the reaction of $^{\bullet}OCH_3$ at the S2 position is approximately 15 kcal/mol more exothermic than the corresponding reaction with an OOH radical; while the rate constants are very similar, i.e., even though both S−H positions have similar bond dissociation energies (BDE), their reactivities are very different. In the present case the carboxylate anion acts as an intramolecular catalyst in a manner that is similar to the one used by enzymes. The only way to observe such a dynamic interaction is by modeling the transition states, as we have done in this article.

If the rate constant with $HOO^{\bullet}$ radicals in lipid media is used as a comparison criterion, DHLA and 34-DHBA are predicted to react almost as fast as Trolox (3.4×10^3 M^{-1} s^{-1}),[62] which is frequently used as an antioxidant reference. Compared to other antioxidants, the peroxyl radical scavenging activity of this compound was found to be lower than those of carotenes ($\sim 10^5-10^6$ M^{-1} s^{-1}),[63,64] dopamine (8.2×10^5 M^{-1} s^{-1}),[65] canolol (6.8×10^5 M^{-1} s^{-1}),[50] and hydroxytyrosol (6.4×10^5 M^{-1} s^{-1});[66] sesamol (3.3×10^4 M^{-1} s^{-1}),[67] and sinapinic acid (1.7×10^4 M^{-1} s^{-1}).[68] It is similar to those of protocatechuic acid (5.1×10^3),[69] capsaicine (6.5×10^3),[70] and α-mangostin (7.8×10^3 M^{-1} s^{-1});[71] and higher than those of tyrosol ($7.1 \times$

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10^2 M^{-1} s^{-1}),[66] melatonin (3.1×10^2 M^{-1} s^{-1})[72] and caffeine (3.2×10^1 M^{-1} s^{-1}).[56]

In aqueous solution the overall rate constants of the reactions of DHLA with ROO$^\bullet$ are much higher than in nonpolar media. This is mainly caused by the influence of the carboxylate group. Therefore, it can be stated that the anionic forms are much better peroxyl, and particularly HOO$^\bullet$, scavengers than the neutral species. In such a media, DHLA is predicted to react with HOO$^\bullet$ 1.4×10^3 times faster than Trolox (8.96×10^4 M^{-1} s^{-1}).[62] Compared to other antioxidants, DHLA is predicted to have peroxyl radical scavenging activity much higher than those of melatonin (2.0×10^1 M^{-1} s^{-1}),[72] caffeine (3.3×10^{-1} M^{-1} s^{-1}),[56] allicin (7.4×10^3 M^{-1} s^{-1}),[73] thioacrolein (2.9×10^4 M^{-1} s^{-1}),[73] and dopamine (2.2×10^5 M^{-1} s^{-1});[65]and even higher than excellent antioxidants such as canolol (2.50×10^6 M^{-1} s^{-1}),[50] α-mangostin (1.4×10^6 M^{-1} s^{-1}),[71] protocatechuic acid (1.3×10^7 M^{-1} s^{-1}),[69] 2-propenesulfenic acid (2.6×10^7 M^{-1} s^{-1}),[73] glutathione (2.7×10^7 M^{-1} s^{-1}),[14]sesamol (2.4×10^8 M^{-1} s^{-1}),[67] and resveratrol (5.62×10^7).[74] Only piceatannol (1.1×10^9 M^{-1} s^{-1})[74] has a larger rate constant. Therefore, it can be stated that in aqueous solution, at physiological pH, DHLA is among the best peroxyl radical scavengers identified so far.

In order to analyze the relative importance of the different mechanisms and reaction paths on the overall HOO$^\bullet$ scavenging activity of the studied DHLA, the branching ratios have been estimated (Tables S1 and S2, Supporting Information [SI]), according to

$$\Gamma_i^{\mathrm{PE}} = \frac{k_i}{k_{\mathrm{tot}}} \times 100 \tag{7}$$

$$\Gamma_i^{\mathrm{W,pH=7.4}} = \frac{k_i}{k_{\mathrm{overall}}^{\mathrm{W,pH=7.4}}} \times 100 \tag{8}$$

where i represents each particular path

As was pointed out before, the S−H moieties are responsible for the antioxidant activity, and the S−H group located close to the carboxylate group in water solution is responsible for the unexpected scavenging activity in this environment.

In conclusion we can say that, according to our calculated rate constants, in aqueous media DHLA is an excellent antioxidant that, if present in appropriate concentrations, will prevent oxidative damage because it reacts much faster than any biological target with the studied radicals. The latter have been chosen to represent a wide range of reactivities. Particularly, for OOH radicals, the rate constants are so fast that it would prevent oxidative damage even at low concentrations.

It is important to mention that, since the antioxidant action of thiols is due to the homolysis of an S−H bond, in principle the polarity of the solvent would not be expected to have a large influence. However, since a polar solvent increases the acidity of the carboxyl group, thus enabling the formation of carboxylate that in turn can catalyze the reaction, the antioxidant capacity of DHLA increases dramatically in water.

Comparison of the Calculated Rate Constants with Experimental Values. The methodology used in this work has been previously tested against experimentally measured rate constants, and the maximum deviation found between theoretical and experimental rate constants values is a factor of 2, which is about the same as the deviation between any two experimental values.[13] However, as with any modeling, when possible it needs to be tested against experimental data.

Only a few experimental rate constants have been reported for the systems studied in this work. However, these few values allow us to prove again, for these particular systems, that our methodology is correct. We have shown that LA is not a good antioxidant because it reacts only with very reactive radicals such as $^\bullet$OH, $^\bullet$OCH$_3$, and $^\bullet$OOCCl$_3$. This seems to be in contradiction with the fact that the experimental rate constants for the $^\bullet$OH reactions are larger with LA than with DHLA. Our calculations show that, because LA is capable of reacting via SET with very electrophilic radicals, even if it does not react at all with peroxide radicals it reacts faster than DHLA with $^\bullet$OOCCl$_3$. Our calculated rate constant for LA + $^\bullet$OOCCl$_3$ (2.26×10^8 M^{-1} s^{-1}) is in excellent agreement with the experimental value (1.8×10^8 M^{-1} s^{-1}).[12] This value is larger than the one for DHLA + $^\bullet$OOCCl$_3$. In this case the agreement with experiment is not as excellent but is still quite good.

According to Packer et al.[7] LA scavenges neither methylperoxyl radicals nor superoxide radical anions. In this work we arrive exactly at the same conclusions.

Packer et al.[7] also found that the situation is quite different for DHLA. Although it is slightly less reactive with very reactive radicals, it is capable of reacting very fast with HOO$^\bullet$, and therefore scavenges via acid/base equilibrium its conjugated base, the $O_2^{\bullet-}$ radical. It can also scavenge practically any radical, including alkyl peroxide radicals. Our results are in excellent agreement with this qualitative experimental evidence. Regarding the scarce quantitative data available, our calculated rate constant for HOO$^\bullet$ /($O_2^{\bullet-}$) 1.28×10^5 M^{-1} s^{-1} is in perfect agreement with one of the experimental rate constants 1.32×10^5 M^{-1} s^{-1}.[12]

CONCLUSIONS

Lipoic acid itself is not a good primary antioxidant because it does not depurate the less reactive, long-lived, radicals that are of huge importance for oxidative stress. On the other hand almost any molecule in a living organism can react with OH radicals at almost diffusion-controlled rates.

Dihydrolipoic acid is an excellent antioxidant capable of scavenging almost any oxygen-centered radical as well as others with similar reactivity.

While lipoic acid is a mild electron donor that is capable of reacting with very electrophilic radicals, DHLA is an excellent donor of H atoms that reacts essentially via a hydrogen transfer mechanism.

Even though it is important to know which one of LA and DHLA is the one acting as antioxidant within living organisms, it should be recalled that they are in equilibrium via biochemical reactions in vivo. The results of this work illustrate the need for studying in detail the interconverting rate of one into another in order to find out which one is favored in the equilibrium. This of course should be done in vivo. Only if DHLA concentrations are important and the interconversion is fast, it would be relatively unimportant which form is consumed.

Dihydrolipoic acid is one of the few antioxidants that is capable of reacting with HOO$^\bullet$ radicals at rates of the order of 10^8 M^{-1} s^{-1} and it is the only one, to our knowledge, that can react as fast via a hydrogen transfer mechanism. This is related to the unique structure of DHLA that places the −SH and −COO^- moieties at an ideal distance to "trap" the HOO$^\bullet$ radical and transfer an H atom to it, which is exactly the same way the active sites of enzymes work, i.e. the catalyst is near the reactive site in a perfect place.

The methodology used in this study, has proven once again its validity for the study of any kind of reaction rates of ROS with antioxidants, and therefore for antioxidant activity. It seems to be an excellent complement to experimental studies.

These conclusions are in perfect agreement with the few experimental data available, within the expected accuracy of experimental results and theoretical calculations.

■ ASSOCIATED CONTENT

Supporting Information

Calculated branching ratios, details of SET mechanism results, tunneling corrections, Cartesian coordinates of main transition states (those shown in Figure 1), and reactants. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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