

Note

Theoretical Elucidation on Different Lipid-Oxidation Potentials of Aminoxy Antioxidants

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To elucidate the different lipid-oxidation potentials of aminoxy antioxidants, a kind of combined density functional theory (DFT) method was employed to calculate C—H bond dissociation enthalpies (BDEs) of a model linoleic acid (LH) and O—H BDEs of hydrogenated aminoxy. The higher the O—H BDE is, the more potent the aminoxy to abstract the H-atom from LH and the stronger the LH-oxidation potential. Accordingly, the prooxidant activity differences of aminoxy were elucidated by the different O—H BDEs of hydrogenated aminoxy, which were further clarified in terms of distinct electronic effects of the substituents.

Keywords AM1, aminoxy antioxidant, density functional theory, H-abstraction reaction, lipid oxidation, O—H bond dissociation enthalpy

Introduction

Recently, more and more attention was given to antioxidants, owing to their wide application in pharmaceutical industry, food industry and chemical industry.¹⁻³ Apart from the conventionally used phenolic antioxidants, aminoxy, such as 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), quinolinic and indolinonic aminoxy (Fig. 1, 1—6), also showed good antioxidant activities.⁴⁻⁷ They can effectively scavenge carbon-, nitrogen-, and oxygen-centered radicals ($R\cdot$, $R_2N\cdot$, $RO\cdot$, $ROO\cdot$, $O_2\cdot^-$) by directly coupling with them or by imitating superoxide dismutase (SOD).⁸⁻¹³

The character of these antioxidants is that themselves are radicals. It may be argued that although these radicals are rather stable, they are still possible to abstract the H-atom from surrounding fatty acids to give rise to lipid oxidation. Through experimental studies, it was found that some aminoxy, such as TEMPO, 2, 3, and 4 indeed stimulated the autoxidation of linolenic acid at high or low concentration, and TEMPO was the strongest stimulator.⁴ However, some aminoxy, such as 5 and 6, did not react with fatty acid, *i. e.*, methyl linoleate, at appreciable rate.^{4,7} To get deeper insight into the lipid-oxidation mech-

anisms of aminoxy and to design better aminoxy antioxidants, the different lipid-oxidation potentials of aminoxy were explained by means of quantum chemical calculations.

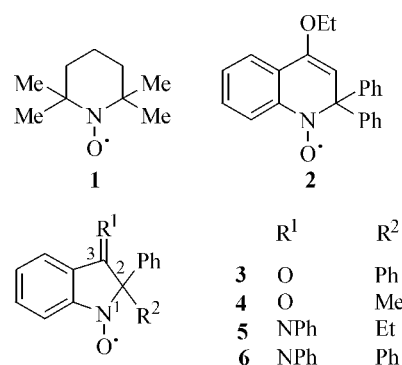


Fig. 1 Molecular structures of aminoxy.

Methods

Density functional theory (DFT) has been used extensively to investigate the structure-activity relationships for antioxidants, with the advantages of accuracy and convenience.¹⁴⁻¹⁹ Furthermore, Wright and co-workers²⁰⁻²⁵ proposed the combined DFT methods to calculate physicochemical parameters for large molecules, being more applicable and economical than the pure DFT methods.

The combined DFT method calculation procedures employed in this paper are as the following. The molecular geometries were optimized, firstly, by molecular mechanic method, and then, by semiempirical quantum chemical method AM1.²⁶ Finally, (RO)B3LYP function on the basis set of 6-311 + G(2d,2p) was used to calculate single point energy (SPE). The molecular enthalpy in gas-phase at 298 K consists of (RO)B3LYP/6-311 + G(2d,2p) calculated SPE, AM1 calculated zero-point vibrational energy (ZPVE) and vibrational contribution to energy (scaled by a factor of 0.973),²⁴ translational, rotational and PV-work

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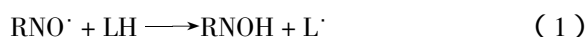
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terms. All of the calculations were accomplished by Gaussian 98 program.

Results and discussion

Selection of theoretical parameter to characterize the lipid-oxidation potential of aminoxyl

From the physico-chemical point of view, the lipid (LH)-oxidation potential of an aminoxyl radical (RNO^\cdot) is governed by the variation of free energy (ΔG) of the H-abstraction reaction between LH and RNO^\cdot [Eq. (1)].



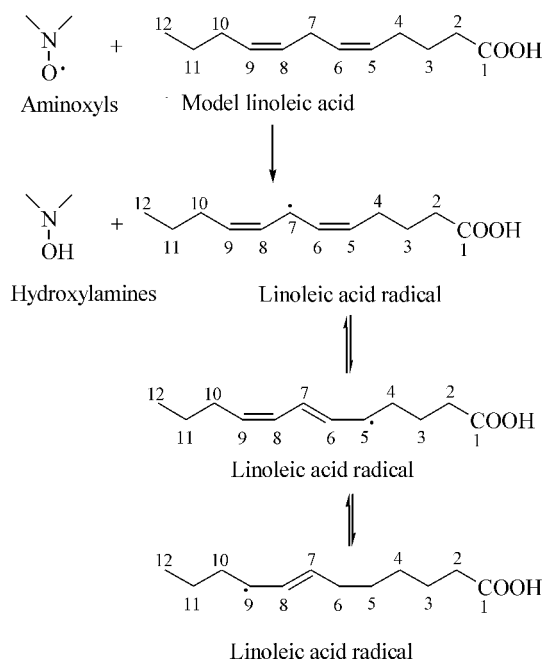
A negative ΔG implies that the reaction is facilitated, while a positive ΔG means that the reaction is prohibited. Thus, to investigate the LH-oxidation reactions theoretically, ΔG of the reactions need to be calculated. As known to all, ΔG is determined by variation of enthalpy (ΔH), variation of entropy (ΔS) and relative ratio of LH to RNO^\cdot . Taking into account that ΔS pertaining to a H-abstraction reaction is near to zero in many cases,^{27,28} ΔH can be used as a theoretical parameter to measure the intrinsic H-abstraction activity. Furthermore, as shown in Eq. (1), the H-abstraction reaction consists of the C—H bond dissociation process for LH and the O—H bond formation process for RNO^\cdot . The ΔH for the H-abstraction reaction equals the difference between C—H bond dissociation enthalpy (BDE) and O—H bond formation enthalpy. And the latter is actually the minus of O—H BDE. Thus, if the C—H BDE is smaller than the O—H BDE, the H-abstraction reaction will take place, otherwise, the reaction will be difficult to occur. Accordingly, to elucidate the different LH-oxidation potentials of RNO^\cdot , C—H BDEs of fatty acids and O—H BDEs of hydroxylamines should be calculated at first.

C—H BDEs of model fatty acid

To simplify the calculation, a model linoleic acid was constructed (Scheme 1). As known to all, the allylic hydrogens in fatty acids are subject to be abstracted. Hence, only hydrogens at positions 4, 7, and 10 of model linoleic acid were considered in the calculations. As shown in Table 1, the C—H BDEs for positions 4 and 10 are comparable to those for 1-butene (349.4 kJ/mol), 1-pentene (348.8 kJ/mol) and *cis*-2-pentene (345.4 kJ/mol),²⁹ in agreement with the previous conclusion that the BDE calculated with the present method is acceptable with an expecting error of ± 8 kJ/mol.²¹ The C—H BDE for position 7 gets *ca.* 54 kJ/mol lower, compared with those for other positions, resulting from the fact that the carbon-centered radical pertinent to position 7 can be better stabilized than radicals at other positions through stronger resonance effect (Scheme 1). In fact, as shown in Table 2, the unpaired electron on C(7) distributes well on C(5) and C(9), in

contrast to the unpaired electron on C(4) or C(10) which only extends to C(6) or C(8). So the C—H BDE corresponding to C(7) is the lowest.

Scheme 1 H-abstraction reaction of aminoxyls and model linoleic acid and the resonance effect in the fatty acid radical



According to the above theoretical analysis, if the O—H BDE of an antioxidant is near to 305 kJ/mol, the antioxidant-derived radical will likely abstract the allylic H-atom from position 7 of the fatty acid. And the lower the O—H BDE is, the safer the antioxidant-derived radical.

O—H BDEs of hydroxylamines

As to the O—H BDEs for hydroxylamines, only the experimental value for hydrogenated TEMPO (TEMPOH) is available, *i. e.*, 297.69 kJ/mol³⁰ or (291.21 ± 12.55) kJ/mol.³¹ Considering the fact that the concentration of LH is much higher than that of RNO^\cdot in experimental systems, it is understandable that TEMPO is a lipid-oxidation trigger. O—H BDEs for TEMPOH and hydrogenated **2–6** were also calculated with the combined DFT method (Table 1). However, the calculated O—H BDE for TEMPOH is only 277.22 kJ/mol, about 20 kJ/mol lower than the experimental value. Thus, it seems that the combined method is not very accurate to give the absolute O—H BDEs for hydroxylamines, which may result from the deficiency of DFT method, because the O—H BDE of TEMPOH calculated by pure DFT method (RO)B3LYP/6-31G(d,p) was even lower (271.71 kJ/mol). However, the calculated results are significant to predict the relative order of the O—H BDEs, namely, TEMPO > **2** > **3** ~ **4** > **5** ~ **6**, which is just in accordance with the experimental finding that TEMPO was the strongest prooxidant, **5** and **6** had no prooxidative activity, and **2**, **3**, **4** were in the middle of

Table 1 Theoretical thermodynamic parameters and C—H or O—H bond dissociation enthalpies (BDEs) for model linoleic acid (LH) and hydrogenated aminoxylys

	E_T (hartree) ^a	E_{TC} (hartree) ^b	H (hartree) ^c	BDE (kJ/mol) ^d
LH	-619.950824	0.319873	-619.638648	
L-(H-4) ^e	-619.304659	0.306084	-619.005899	354.70
L-(H-7) ^f	-619.324705	0.305563	-619.026452	300.74
L-(H-10) ^g	-619.305170	0.306021	-619.006472	353.20
1	-483.855132	0.277687	-483.584003	
1 -H	-484.469981	0.289621	-484.187240	277.22
2	-1093.909578	0.402242	-1093.517257	
2 -H	-1094.525202	0.416527	-1094.118981	273.25
3	-975.957970	0.312897	-975.652581	
3 -H	-976.571128	0.326248	-976.252749	269.16
4	-784.177580	0.254909	-783.928614	
4 -H	-784.789680	0.267974	-784.528001	267.11
5	-1034.720067	0.384489	-1034.345019	
5 -H	-1035.329092	0.398321	-1034.940586	257.08
6	-1187.176828	0.406097	-1186.780756	
6 -H	-1187.791253	0.426317	-1187.375507	254.94

^a Total electronic energy. ^b Thermal correction to energy. ^c Enthalpy. ^d BDE = $(H_R + H_H - H_P) \times 627.5095 \times 4.184$, in which H_P is the enthalpy of parent molecule, H_R the enthalpy of radical, and H_H the enthalpy of hydrogen atom, -0.49765 hartree. ^e Lipid radical derived from H(4)-abstraction. ^f Lipid radical derived from H(7)-abstraction. ^g Lipid radical derived from H(10)-abstraction.

Table 2 Spin density on the carbon atom of model linoleic acid radical

No.	H(4) removed	H(7) removed	H(10) removed
1	0.003	-0.000	0.000
2	0.003	0.001	-0.000
3	0.019	-0.002	-0.001
4	0.425	0.017	-0.003
5	0.037	0.268	-0.004
6	0.420	0.026	-0.001
7	0.032	0.325	0.028
8	-0.002	0.023	0.416
9	-0.005	0.276	0.037
10	-0.003	0.009	0.434
11	-0.001	0.002	0.015
12	0.000	0.001	0.002

the range.

As known to all, electron-donating groups are beneficial to stabilize the oxygen-centered radical and to reduce the O—H BDE, whereas electron-withdrawing groups have an opposite effect.^{13,14,19,32} Therefore, the O—H BDE differences associated with hydrogenated **3**, **4** and **5**, **6** essentially arise from the different substituent effects of =O and =NR at position 3. Since =O is a stronger electron-withdrawing group than =NR, it destabilizes the aminoxy radicals more than =NR and raises the O—H BDEs of hydrogenated **3** and **4**, in comparison with those of **5** and **6**. This is supported by the highest occupied molecular orbital (HOMO) energy levels (E_{HOMO}) of **3**—**6**. The order of E_{HOMO} is: **5** (-0.22238 a.u.) \sim **6** (-0.22393 a.u.) $>$ **3** (-0.23129 a.u.) \sim **4** (-0.23277 a.u.), manifesting the stronger electron-withdrawing effect of =O than that of

=NR.

In summary, the prooxidant activity differences of aminoxylys have been elucidated by the different O—H BDEs of hydrogenated aminoxylys. The higher the O—H BDE is, the more potent the aminoxylys to abstract the H-atom from LH and the stronger the prooxidant activity. The difference in O—H BDEs can be further clarified in terms of electronic effects of the substituents in aminoxylys.

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