

## Is HOMO Energy Level a Good Parameter to Characterize Antioxidant Activity

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**Abstract:** Semiempirical quantum chemical method AM1 was employed to calculate the highest occupied molecular orbital (HOMO) energy levels ( $E_{\text{HOMO}}$ ) for various types of antioxidants. It was verified that the correlation between logarithm of free radical scavenging rate constants ( $\lg k_s$ ) and  $E_{\text{HOMO}}$  substantially arises from the correlation between  $E_{\text{HOMO}}$  and O-H bond dissociation energies (BDE) of antioxidants. Furthermore,  $E_{\text{HOMO}}$  were poorly correlated with the logarithm of relative free radical scavenging rate constants ( $\lg k_s/k_I$ ) for various types of antioxidants that possess complex structures ( $r = 0.5602$ ). So in a broad sense,  $E_{\text{HOMO}}$  was not an appropriate parameter to characterize the free radical scavenging activity of antioxidants.

**Keywords:** AM1, antioxidant, free radical, HOMO energy level, O-H bond dissociation energy, structure-activity relationship.

As free radicals cause degradation of many industrial materials, degeneration of foods, and scores of diseases, such as inflammation, neurodegeneration, and tumors, selecting highly efficient antioxidants with low toxicity is of great importance and in fact, has been paid much attention<sup>1-3</sup>. Moreover, it has been pointed out that theoretical methods will accelerate the selection of new antioxidants<sup>4,5</sup>. Indeed, various theoretical parameters have been found appropriate to characterize the free radical scavenging activity of antioxidants. Roughly speaking, the selected parameters can be separated into two types. One is the indexes that represent the O-H bond dissociation energy (BDE) of antioxidants<sup>6</sup>, including the difference of heat of formation between antioxidant and its free radical generated after H-abstraction reaction ( $\Delta\text{HOF}$ )<sup>4,7</sup> and the relative O-H BDE ( $\Delta\text{H}_{\text{abs}}$ )<sup>8</sup>, *etc.*. The other is that represent the electron-giving ability of the antioxidants, *i.e.*, the highest occupied molecular orbital (HOMO) energy level ( $E_{\text{HOMO}}$ ) and the relative adiabatic ionization potential ( $\Delta\text{H}_{\text{ox}}$ )<sup>4,8,9</sup>.

It has been verified that BDE-like parameters are good theoretical indexes to predict antioxidant activity, because they correlate well with the activation energy of the H-abstraction reaction<sup>6</sup> and consequently correlate well with the logarithm of the rate constant for antioxidant to scavenge free radicals ( $\lg k_s$ )<sup>4,7,10</sup>. Furthermore, these indexes have been used successfully to elucidate the structure-activity relationship of flavonoid antioxidants<sup>11,12</sup>, and has been employed to explain the activity differences of various antioxidants<sup>13-15</sup>. So theoretical methods are fairly useful in this field. The more

interesting discovery is that  $E_{\text{HOMO}}$  is also very good to characterize the activity of some antioxidants, *e.g.*, tocopherols<sup>8,9</sup>. The higher the  $E_{\text{HOMO}}$ , the more active the antioxidants. And a good correlation ( $r = 0.893$ ) between  $E_{\text{HOMO}}$  and  $\lg ks$  was found for tocopherolic antioxidants (TA)<sup>8</sup>. As the calculation of  $E_{\text{HOMO}}$  is much more time-saving than that of  $\Delta\text{HOF}$  or  $\Delta\text{H}_{\text{abs}}$ , the discovery will be of significance in practice. Moreover, the discovery suggests that an electron transfer process is involved in the H-abstraction reaction of TA, which is helpful to clarify the antioxidative mechanism<sup>8,16</sup>. However, the validity of  $E_{\text{HOMO}}$  has not been verified fully.

Obviously, TA all possess identical active center, *i.e.*, *para*-methoxyphenol, and their difference only arises from the substituting groups. As is well known to all, electron-donating groups enhance the  $E_{\text{HOMO}}$ , and at the same time reduce the O-H BDE<sup>4,17</sup>, namely, the groups increase the antioxidant electron-giving ability and H-abstraction reactivity simultaneously. Therefore, to a certain extent the correlation between  $E_{\text{HOMO}}$  and O-H BDE<sup>8</sup> makes it a superficial phenomenon for  $E_{\text{HOMO}}$  to act as an appropriate parameter characterizing the antioxidative activity of TA<sup>18</sup>. Apparently, to verify the effectiveness of  $E_{\text{HOMO}}$ , a deeper investigation is needed, which is the objective of this paper.

## Methods

The  $E_{\text{HOMO}}$  was calculated by semiempirical quantum chemical method AM1<sup>19</sup>, as AM1 was found to be good enough to calculate  $E_{\text{HOMO}}$  for different kinds of molecules<sup>20</sup>, even as good as *ab initio* method HF/3-21G<sup>8</sup>. And AM1 was also very good to calculate  $\Delta\text{HOF}$ , better than other semiempirical quantum chemical methods, such as MNDO and PM3<sup>7,21</sup>. Hence, to verify the effectiveness of  $E_{\text{HOMO}}$  and to compare it with that of  $\Delta\text{HOF}$ , AM1 was selected to calculate the parameter. The procedure is as follows. Molecular mechanic method MMX<sup>22</sup> in program PCMODEL was used to optimize the molecular structures preliminarily. Then AM1 in MOPAC7 was employed to perform a complete geometry optimization.

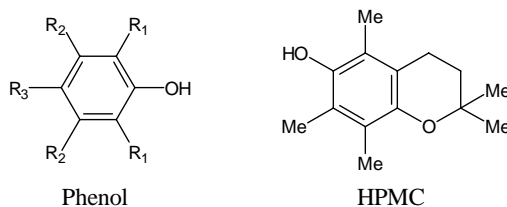
## Results and Discussion

It has been found that there exists a fairly good correlation between experimentally determined O-H BDE and AM1 calculated  $\Delta\text{HOF}$  for 17 phenols (**Table 1**,  $r = 0.9495$ )<sup>23</sup>, which is the origin of the effectiveness of  $\Delta\text{HOF}$  to predict free radical scavenging activity of antioxidants. Noting that the O-H BDE difference results from the different electron-donating ability of the substituting groups, it is reasonable to infer there should exist a correlation between O-H BDE and  $E_{\text{HOMO}}$ . In fact, the linearity between O-H BDE and  $E_{\text{HOMO}}$  is fairly good (**Table 1**,  $r = -0.9336$ ,  $P < 0.0001$ ). Taking into account the correlation between  $\lg ks$  and O-H BDE, it is easy to recognize that the previously observed linearity between  $\lg ks$  and  $E_{\text{HOMO}}$  substantially arises from the correlation between  $E_{\text{HOMO}}$  and O-H BDE. Hence, the effectiveness of  $E_{\text{HOMO}}$  is indeed a superficial phenomenon.

Furthermore, the antioxidant activity is influenced not only by the electron-donating

or electron-withdrawing property of substituting groups, but also by the formation of intramolecular hydrogen bond (IHB)<sup>11-15</sup>. Hydrogen bond will stabilize the free radical generated after H-abstraction, and will enhance the scavenging activity of antioxidant. But  $E_{\text{HOMO}}$  cannot reflect this free radical stabilizing factor. Hence,  $E_{\text{HOMO}}$  cannot be used to predict the scavenging activity for various types of antioxidants possessing IHB. To verify this conjecture,  $E_{\text{HOMO}}$  was calculated for flavonoids, pyrogallols, *etc.* (Table 2). The correlation coefficient between  $E_{\text{HOMO}}$  and logarithm of the relative rate constants ( $\lg k_3/k_1$ ) for 13 antioxidants is very poor (Table 2,  $r = 0.5602$ ), much lower than the value between  $\Delta\text{HOF}$  and  $\lg k_3/k_1$  ( $r = 0.9491$ )<sup>7</sup>. This results from the considerable differences of the antioxidant structures. In addition, previous study showed that  $E_{\text{HOMO}}$  was invalid to elucidate the activity differences of flavonoid antioxidants, but BDE-like parameters were valid<sup>12,14</sup>. Therefore, BDE-like indexes are much better than  $E_{\text{HOMO}}$  to characterize the antioxidant activity. As to whether the H-abstraction reaction involves an electron transfer process, it has to be answered by high level *ab initio* calculation on the transition state of the reaction.

**Table 1** Experimentally determined O-H BDE values, AM1 calculated  $\Delta\text{HOF}$  values and  $E_{\text{HOMO}}$  for 17 phenols



Phenol	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	O-H BDE <sub>exp</sub> <sup>a</sup> (kcal/mol)	$\Delta\text{HOF}$ (kcal/mol) <sup>b</sup>	$E_{\text{HOMO}}$ (eV)
1	H	H	H	88.30	37.66	-9.1142
2	H	H	Me	86.20	36.15	-8.8801
3	H	H	CMe <sub>3</sub>	85.30	36.39	-8.8947
4	H	H	OMe	82.81	33.46	-8.6357
5	Me	H	H	84.50	35.19	-8.8845
6	CMe <sub>3</sub>	H	H	82.80	32.21	-8.8068
7	OMe	H	H	83.16	32.49	-8.6136
8	H	CMe <sub>3</sub>	H	86.62	37.37	-8.9267
9	H	OMe	H	86.70	39.25	-8.9651
10	Me	H	Me	82.73	33.89	-8.7020
11	CMe <sub>3</sub>	H	CMe <sub>3</sub>	81.24	31.08	-8.6277
12	OMe	H	OMe	80.00	30.40	-8.3456
13	CMe <sub>3</sub>	H	Me	81.02	30.90	-8.6075
14	CMe <sub>3</sub>	H	OMe	78.31	28.53	-8.5510
15	Me	H, Me	OMe	79.20	30.88	-8.4170
16	Me	Me	OMe	81.88	33.42	-8.7130
17	HPMC			78.25	30.40	-8.3533

<sup>a</sup> O-H bond dissociation energy determined by experiment<sup>24</sup>.

<sup>b</sup> Difference of heat of formation between phenol and its free radical generated after H-abstraction reaction calculated by AM1 method<sup>23</sup>.

**Table 2.** E<sub>HOMO</sub> (eV) and relative free radical scavenging rate constants for various types of antioxidants

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
E <sub>HOMO</sub>	-8.9021	-9.1520	-8.9457	-8.7098	-9.2727	-8.7449	-8.7502	-9.1121	-8.7742	-9.0108	-9.2903	-8.2910	-8.6083
k <sub>3</sub> /k <sub>1</sub> <sup>a</sup>	110	31	52	105	0.5	50	31	23	250	150	160	290	240

<sup>a</sup>k<sub>3</sub> is the rate constant for antioxidants to scavenge free radicals. k<sub>1</sub> is the rate constant for free radicals to react with methyl linoleate and oxygen. See ref. 25 for the detailed description.

I, quercetin; II, luteolin; III, catechin; IV, fisetin; V, naringenin; VI, kaempferol; VII, rutin; VIII, caffeic acid; IX, nordihydro-guaiaretic acid; X, chlorogenic acid; XI, propyl gallate; XII, vitamin E; XIII, 2,6-di-*tert*-butyl-4-methylphenol (BHT).

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