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A theoretical study on photobleaching mechanisms of hypocrellins

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Abstract

In this paper, quantum chemical method RHF/6-31G(d,p)//AM1 was employed to investigate the photobleaching behaviors of hypocrellins. It is found that frontier charge rather than Mulliken charge is an applicable parameter to predict the $^1O_2$- and $O_2^{\cdot-}$-addition positions of the pigments; accordingly some experimental observations on the photobleaching mechanisms of hypocrellins were elucidated. In addition, some theoretical predictions that were not observed in the previous experiments will be helpful to direct the further study.

Keywords: Ab initio; Addition reaction; AM1 method; Hypocrellins; Photobleaching

1. Introduction

As naturally occurring perylenequinonoid pigments (PQPs), hypocrellins A and B (HA and HB, Scheme 1) have attracted much attention in recent years [1–9]. They hold many excellent properties as potent photodynamic medicine, such as: (i) high yields of reactive oxygen species (ROS), i.e., singlet oxygen ($^1O_2$) and superoxide anion radical ($O_2^{\cdot-}$); (ii) low toxicity, for instance, natural products containing HA and HB have been used as folk medicine in China over hundreds of years; (iii) high metabolic rate in human body; (iv) high thermal and photostability. In addition, in a recent research [10], we revealed that hypocrellins and other PQPs show promise in their use as novel fungicides to inhibit the growth of various fungi. Nevertheless, to develop the pigments further, it is necessary to know their photobleaching behaviors.

The photobleaching mechanisms of HA, HB and its butylamino-derivative (BAHB, Scheme 1) have been investigated in various systems [11–15]. It was found that in nonpolar solvents singlet oxygen ($^1O_2$) mechanism predominates in the photooxidation of HA and HB, while in polar solvents both $^1O_2$ and superoxide anion radical ($O_2^{\cdot-}$) mechanisms are involved [14,15]. For BAHB, both $^1O_2$ and $O_2^{\cdot-}$ play important roles in its photobleaching in various organic solvents [14]. In addition, endoperoxide (**1**) and dioxetane (**2**) (Scheme 2) were recognized as preliminary oxidation products of hypocrellins [12–15]. Interestingly, **1** is unstable and can regenerate parent hypocrellin by releasing $^1O_2$, while both **1** and **2** can give the final oxidation product (Scheme 2) [14,15]. Since theoretical methods have made great contributions to understanding the physicochemical properties of hypocrellins [16–22], we attempt to investigate the photobleaching behaviors of hypocrellins

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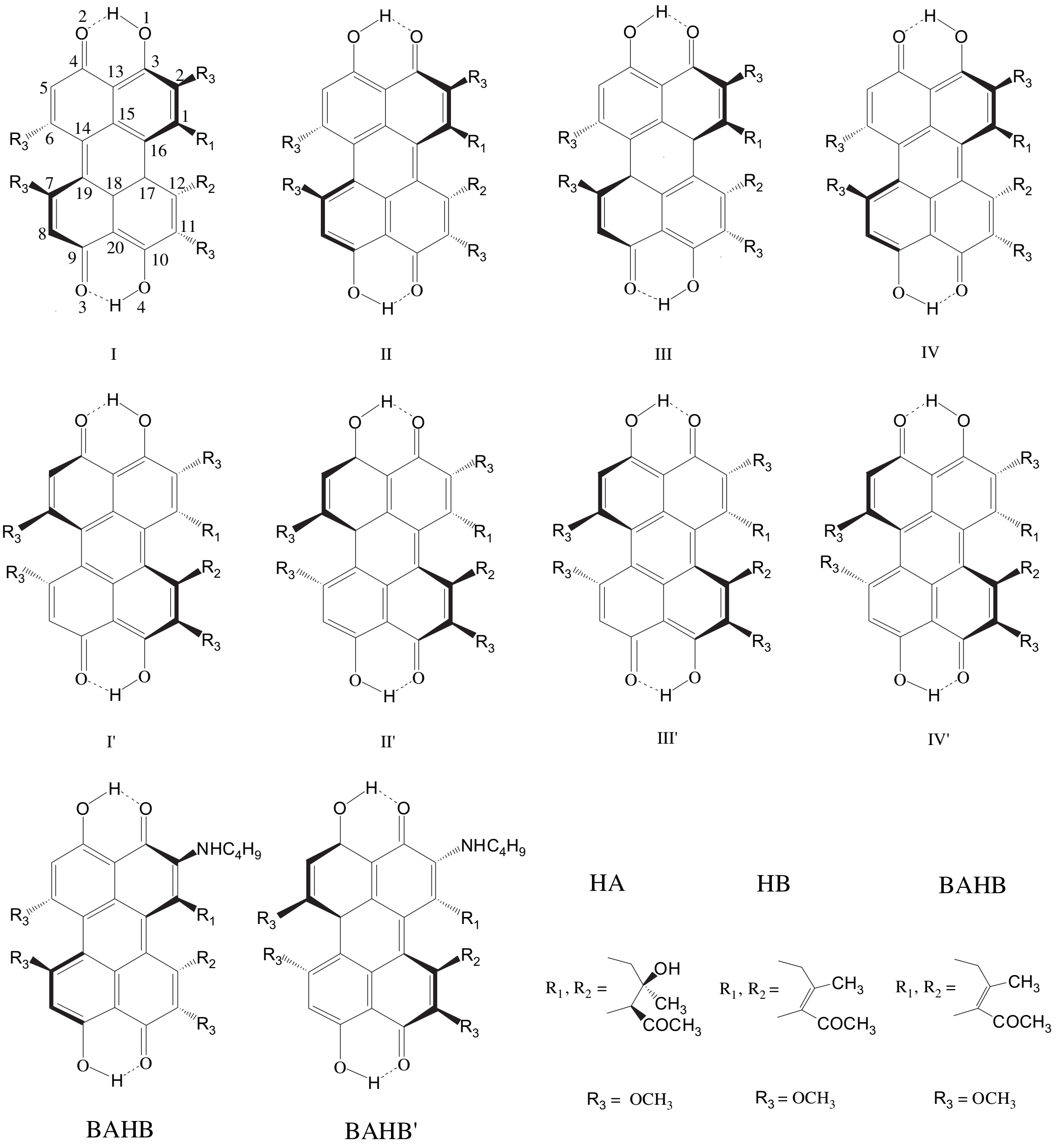


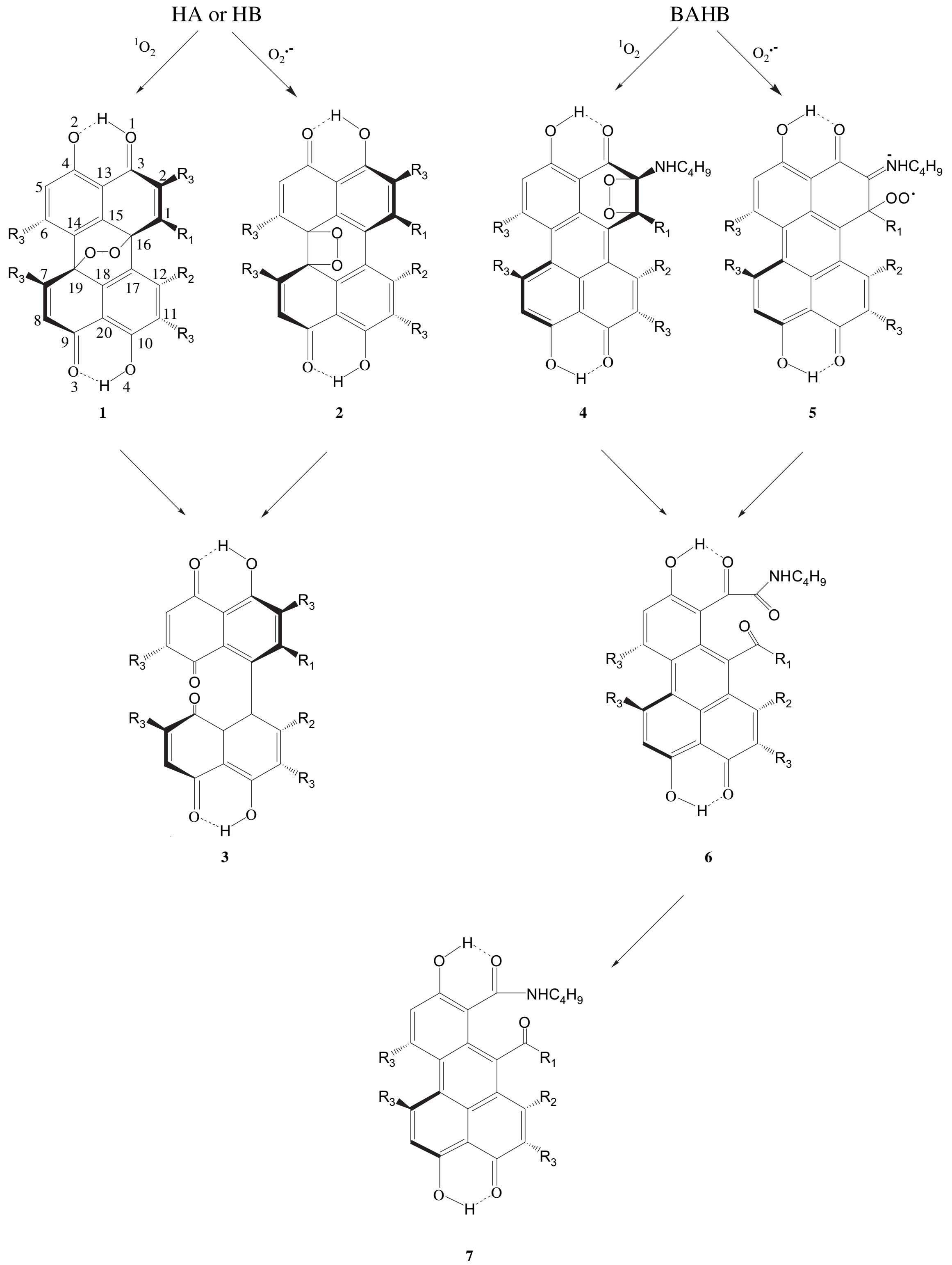
Scheme 1. The structures of isomers of HA, HB and BAHB.

by quantum chemical calculation, which will provide a deeper insight into the process.

2. Methods

The molecular geometries were optimized, firstly, by molecular mechanic method, and then, by semiempirical quantum chemical method AM1 [23]. Finally, restricted Hartree-Fock (RHF) Hamiltonian on basis set of 6-31G(d,p) was used to calculate the single point energy (SPE) in vacuum at 298.15 K. The molecular energy consists of RHF/6-31G(d,p)-calculated SPE and AM1-calculated zero point vibrational energy (ZPVE) and vibrational correction to the energy (scaled by a factor of 0.973). This method takes advantages of economy and accuracy and has been justified by former studies [22,24].

To investigate the $^1O_2$ and $O_2^{\cdot -}$ mechanisms, we have to select proper physicochemical parameters to characterize both mechanisms theoretically. Since $^1O_2$-addition reaction is electrophilic, whereas $O_2^{\cdot -}$-adduction is nucleophilic, according to Fukui et al.'s frontier orbital theory [25], the proper theoretical parameters for measuring $^1O_2$- and $O_2^{\cdot -}$-addition activity should be frontier charge density (Q_f) for highest occupied molecular orbital (HOMO) and for lowest unoccupied molecular orbital (LUMO), respectively. Q_f can be



Scheme 2. Photobleaching mechanisms of HA, HB and BAHB proposed by previous experiments.

calculated conveniently according to the following equation:

$$Q_{\mathrm{f}}^{j} = 2\sum_{i=1}^{n} c_{ji}^{2} \qquad (1)$$

in which, c_{ji} is the coefficient for orbital i of atom j in the frontier orbital (HOMO or LUMO). The quantum chemical calculations were accomplished by Gaussian 98 program [26].

Table 1
Thermodynamic parameters for isomers of HB

	SPE (hartree)[a]	TCE (hartree)[b]	Enthalpy (hartree)
HB I	−1823.744002	0.535984	−1823.222490
HB II	−1823.738665	0.536283	−1823.216862
HB III	−1823.733016	0.536048	−1823.211441
HB IV	−1823.732602	0.536089	−1823.210987
HB I′	−1823.743996	0.535985	−1823.222483
HB II′	−1823.738676	0.536282	−1823.216874
HB III′	−1823.733011	0.536049	−1823.211435
HB IV′	−1823.732619	0.536086	−1823.211007

[a] Single point energy.
[b] Thermal correction to energy which includes zero point vibrational energy.

3. Results and discussion

3.1. The most stable isomers of hypocrellins

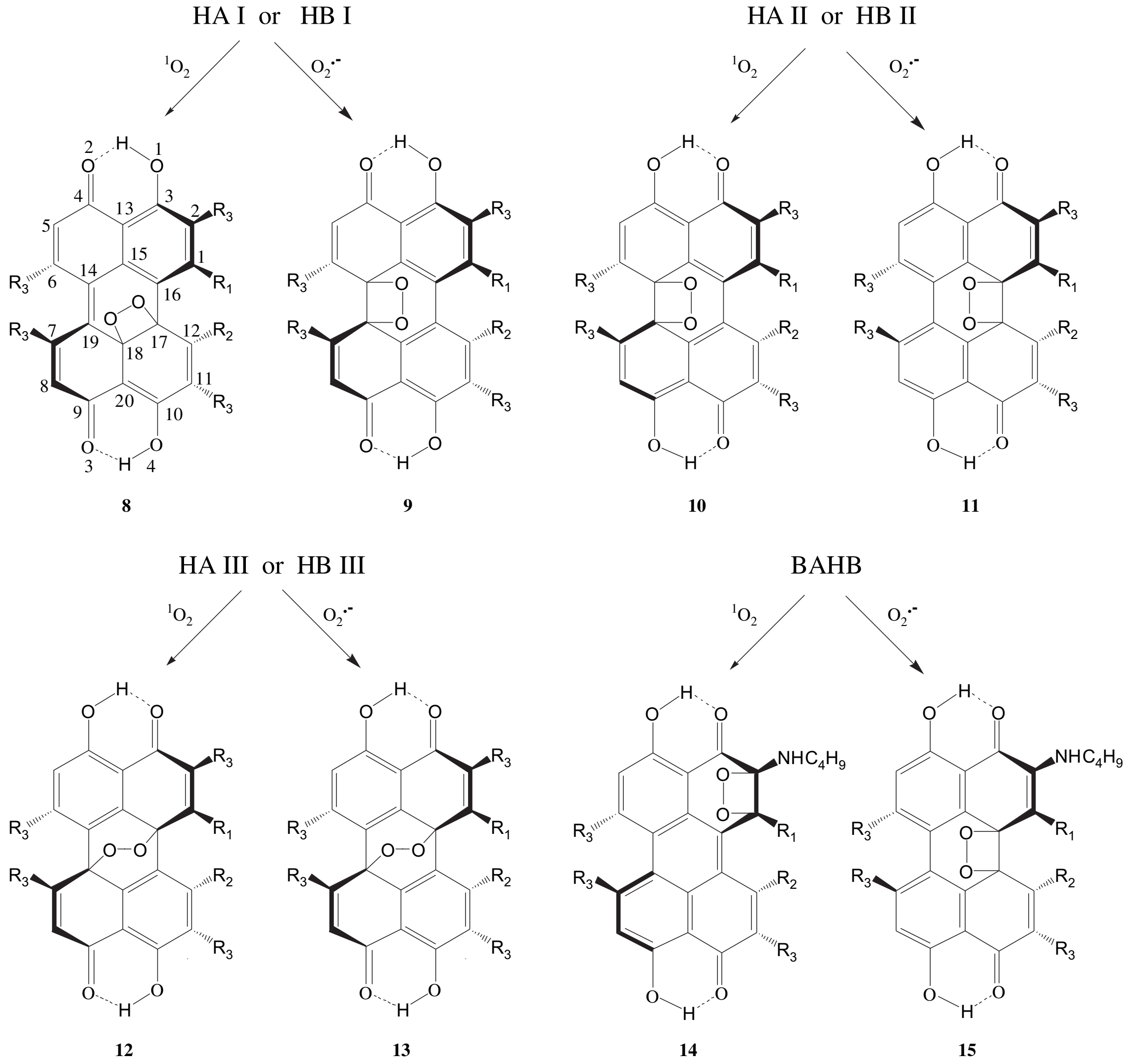
Theoretically, hypocrellins exist in eight conformations (Scheme 1). To investigate the photobleaching mechanisms of hypocrellins, we attempt to use the most stable isomers as starting points. For HA, isomers I, II and III are the most stable ones, which were supported by experimental determination and theoretical calculation [22,27,28]. While for HB, it can be found from Table 1 that the 4,9- or 3,10-quinones are more stable than the 3,9- or 4,10-quinones, which is identical with HA and can be elucidated in terms of resonance theory [22]. However, the left-handed-helical isomers of HB are comparably stable to the right-handed counterparts which is different from HA and possibly results from the difference in their seven-member side rings. Nevertheless, considering the fact that only isomer I was observed in the crystal structure of HB [29], we prefer to use the left-handed isomers I, II and III of HB in the present study. As to BAHB, because of the effect of amino group, it exists as 3,10-quinones (Scheme 1) [20]. To compare with HA and HB, only left-handed-BAHB is taken into consideration in this work.

3.2. Singlet oxygen (${}^1O_2$) mechanism

Table 2 lists the Q_f for HOMO of HA, HB and BAHB, from which one can predict the most likely attacking positions of ${}^1O_2$. The higher the Q_f, the more probable the atom to be attacked. It is interesting to note that HA and HB exhibit very similar Q_f, suggesting that both of them hold the same ${}^1O_2$-addition position. According to the highest Q_f (highlighted in bold, Table 2), 1,4-addition will occur at 16 and 19 positions in isomer III, while 1,2-addition at (17,18), (14,19) and (1,2) positions will take place in isomers I, II and BAHB, respectively (Scheme 3). The predicted 1,4-addition in isomer III and 1,2-addition in BAHB are in good agreement with the experimental observation (Scheme 2) [14]. However, the prediction on isomer I is a little different from the experimental conclusion, in which the 1,2-addition occurred at 14 and 19 positions, as in isomer II [14]. As the isomers I and II undergo fast

Table 2
Frontier charge density (Q_f) for HOMO and Mulliken charge density (in parentheses) of HA, HB and BAHB

	HA I	HA II	HA III	HB I	HB II	HB III	BAHB
C1	0.033 (−0.009)	0.001 (−0.013)	0.025 (−0.012)	0.033 (0.008)	0.001 (0.013)	0.043 (−0.007)	**0.192** (−0.023)
C2	0.016 (0.338)	0.006 (0.298)	0.105 (0.296)	0.020 (0.340)	0.003 (0.295)	0.129 (0.318)	**0.142** (0.272)
C3	0.104 (0.416)	0.005 (0.581)	0.016 (0.576)	0.107 (0.419)	0.005 (0.590)	0.021 (0.583)	0.017 (0.580)
C4	0.003 (0.600)	0.087 (0.463)	0.031 (0.470)	0.003 (0.600)	0.090 (0.465)	0.023 (0.474)	0.009 (0.461)
C5	0.017 (−0.310)	0.008 (−0.259)	0.020 (−0.263)	0.016 (−0.308)	0.010 (−0.258)	0.018 (−0.264)	0.014 (−0.259)
C6	0.002 (0.522)	0.040 (0.500)	0.030 (0.513)	0.002 (0.523)	0.039 (0.502)	0.023 (0.516)	0.013 (0.501)
C7	0.000 (0.524)	0.043 (0.501)	0.006 (0.522)	0.000 (0.527)	0.044 (0.504)	0.007 (0.524)	0.007 (0.5038)
C8	0.005 (−0.310)	0.005 (−0.258)	0.074 (−0.314)	0.005 (−0.309)	0.004 (−0.258)	0.066 (−0.313)	0.052 (−0.263)
C9	0.002 (0.603)	0.085 (0.462)	0.007 (0.602)	0.002 (0.606)	0.081 (0.463)	0.008 (0.604)	0.030 (0.465)
C10	0.118 (0.429)	0.006 (0.598)	0.033 (0.4366)	0.120 (0.430)	0.007 (0.594)	0.025 (0.438)	0.003 (0.595)
C11	0.044 (0.345)	0.010 (0.303)	0.013 (0.351)	0.049 (0.346)	0.016 (0.311)	0.027 (0.3508)	0.094 (0.3009)
C12	0.018 (0.008)	0.001 (0.006)	0.050 (0.008)	0.020 (−0.079)	0.002 (−0.091)	0.058 (−0.076)	0.040 (−0.080)
C13	0.100 (−0.194)	0.120 (−0.224)	0.113 (−0.217)	0.096 (−0.195)	0.118 (−0.224)	0.086 (−0.223)	0.038 (−0.216)
C14	0.049 (−0.040)	**0.155** (−0.061)	0.005 (−0.050)	0.046 (−0.039)	**0.168** (−0.064)	0.006 (−0.050)	0.008 (−0.051)
C15	0.106 (0.009)	0.099 (0.029)	0.085 (0.018)	0.105 (0.004)	0.100 (0.022)	0.091 (0.010)	0.045 (0.016)
C16	0.100 (−0.042)	0.020 (−0.032)	**0.188** (−0.048)	0.102 (−0.000)	0.015 (0.007)	**0.179** (−0.002)	0.119 (0.025)
C17	**0.139** (−0.030)	0.030 (−0.036)	0.006 (−0.020)	**0.138** (−0.027)	0.038 (−0.029)	0.009 (−0.020)	0.116 (−0.055)
C18	**0.130** (0.008)	0.096 (0.028)	0.068 (0.007)	**0.129** (0.606)	0.097 (0.023)	0.053 (0.006)	0.023 (0.036)
C19	0.019 (−0.038)	**0.141** (−0.054)	**0.187** (−0.049)	0.018 (−0.047)	**0.129** (−0.059)	**0.184** (−0.058)	0.138 (−0.076)
C20	0.077 (−0.192)	0.130 (−0.224)	0.090 (−0.193)	0.072 (−0.195)	**0.130** (−0.223)	0.094 (−0.196)	0.003 (−0.230)
O1	0.074 (−0.680)	0.045 (−0.678)	0.105 (−0.690)	0.076 (−0.682)	0.041 (−0.682)	0.113 (−0.701)	0.004 (−0.664)
O2	0.047 (−0.675)	0.064 (−0.678)	0.016 (−0.673)	0.044 (−0.674)	0.067 (−0.679)	0.011 (−0.673)	0.077 (−0.676)
O3	0.028 (−0.674)	0.062 (−0.676)	0.079 (−0.686)	0.026 (−0.676)	0.058 (−0.676)	0.077 (−0.689)	0.025 (−0.679)
O4	0.087 (−0.676)	0.050 (−0.664)	0.019 (−0.672)	0.088 (−0.681)	0.055 (−0.667)	0.013 (−0.676)	0.017 (−0.682)



Scheme 3. Photobleaching mechanisms of HA, HB and BAHB proposed by theoretical calculations.

tautomerization through an intramolecular hydrogen atom transfer reaction [17,22], it is difficult to determine the structure of the transient intermediate product for the addition reaction, such as **2**. As a result, dioxetane **10** might be missed in the previous experiments. Therefore, the theoretical prediction provides new clues to directing the further study.

Table 2 also lists the Mulliken charges for each atom. It can be seen that this parameter cannot account for the $^1O_2$-addition position at all, which offers further justification for the effectiveness of frontier charges in characterizing $^1O_2$-addition reaction.

3.3. Superoxide anion radical ($O_2^{\cdot -}$) mechanism

Table 3 lists the Q_f for LUMO of HA, HB and BAHB, from which one can predict the most likely attacking positions of $O_2^{\cdot -}$. The higher the Q_f, the more probable the atom to be attacked. The similar Q_f between HA and HB suggests that both pigments have the same $O_2^{\cdot -}$-addition position. According to the highest Q_f (highlighted in bold, Table 3), 1,4-addition will occur at 16 and 19 positions in isomer III, while 1,2-addition at (14,19), (16,17) and (16,17) positions will take place in isomers I, II and BAHB, respectively (Scheme 3). The predicted 1,2-addition in isomer I is just in line with the experimental observation (Scheme 2) [14]. However, the predicted addition products for isomers II and III were not observed in experiments, which also probably result from the fast tautomerization between various isomers. Although 1-position of BAHB was proposed to be the attacking site of $O_2^{\cdot -}$, the calculated corresponding Q_f is rather low. In contrast, 2-position holds rather high Q_f, which

Table 3
Frontier charge density (Q_f) for LUMO of HA, HB and BAHB

	HA I	HA II	HA III	HB I	HB II	HB III	BAHB
C1	0.049	0.065	0.047	0.046	0.065	0.044	0.032
C2	0.071	0.118	0.098	0.073	0.120	0.101	**0.145**
C3	0.042	0.079	0.054	0.037	0.075	0.057	0.092
C4	0.060	0.042	0.084	0.058	0.041	0.089	0.044
C5	0.073	0.040	0.045	0.071	0.037	0.042	0.040
C6	0.079	0.067	0.101	0.074	0.062	0.101	0.072
C7	0.081	0.068	0.060	0.081	0.070	0.058	0.064
C8	0.075	0.043	0.061	0.070	0.040	0.057	0.032
C9	0.061	0.040	0.042	0.064	0.043	0.043	0.040
C10	0.044	0.072	0.088	0.045	0.074	0.084	0.073
C11	0.074	0.121	0.084	0.082	0.135	0.091	0.122
C12	0.049	0.056	0.077	0.054	0.062	0.081	0.070
C13	0.044	0.018	0.012	0.045	0.016	0.010	0.021
C14	**0.211**	0.070	0.093	**0.213**	0.073	0.095	0.071
C15	0.021	0.024	0.014	0.020	0.019	0.010	0.029
C16	0.060	**0.174**	**0.166**	0.057	**0.168**	**0.157**	**0.187**
C17	0.062	**0.192**	0.078	0.051	**0.181**	0.068	**0.143**
C18	0.020	0.026	0.014	0.023	0.028	0.013	0.026
C19	**0.208**	0.072	**0.188**	**0.201**	0.070	**0.182**	0.056
C20	0.045	0.023	0.033	0.054	0.021	0.038	0.017
O1	0.013	0.096	0.066	0.011	0.093	0.071	0.015
O2	0.086	0.014	0.029	0.084	0.014	0.031	0.114
O3	0.088	0.014	0.064	0.092	0.014	0.065	0.013
O4	0.013	0.090	0.028	0.012	0.091	0.026	0.086

suggests that this position is apt to be attacked by $O_2^{\cdot -}$.

Alike in $^1O_2$-addition reaction, Mulliken charges fail in elucidating the $O_2^{\cdot -}$-addition position either, indicating that frontier charges are indeed proper parameters to investigate the photobleaching behaviors of hypocrellins.

In summary, frontier charge rather than Mulliken charge can be used to predict the $^1O_2$- and $O_2^{\cdot -}$-addition positions, by which some experimental observations on the photobleaching mechanisms of hypocrellins were elucidated. In addition, some theoretical predictions that were not observed in the previous experiments will be helpful to direct the further study. Therefore, theoretical method can provide a deeper insight into the photobleaching mechanisms for hypocrellins and may be used to investigate the photobleaching behaviors of other pigments.

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