Available online at www.sciencedirect.com

SCIENCE DIRECT®

Dyes and Pigments 66 (2005) 15–17

DYES and PIGMENTS

www.elsevier.com/locate/dyepig

Singlet-oxygen-generating activity of deuterated perylenequinonoid pigments

Qian Zhao, Hong-Yu Zhang*

Laboratory for Computational Biology, School of Life Sciences, Shandong University of Technology, Zibo 255049, PR China

Received 20 April 2004; received in revised form 21 May 2004; accepted 11 August 2004
Available online 18 October 2004

Abstract

To investigate whether intramolecular H-atom transfer (IHT) is critical to the high yields of $^1O_2$ of perylenequinonoid pigments (PQPs), the $^1O_2$-generating activities of parent and deuterated PQPs were determined by means of chemical trapping. It was found that deuterium substitution has little influence on the $^1O_2$-yields of PQPs and thus IHT is unlikely a prerequisite of high $^1O_2$-yields of PQPs.

Keywords: Intramolecular H-atom transfer; Perylenequinonoid pigment; Singlet oxygen

1. Introduction

Naturally occurring perylenequinonoid pigments (PQPs, Scheme 1), including hypocrellin A (HA), hypocrellin B (HB), cercosporin (CP) and hypericin (HYP), etc., have long been known as excellent photosensitizers [1–10]. They hold great potential as photodynamic medicine or fungicide, because many attractive properties are shared by PQPs, 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; (iii) high metabolic rate in human body; (iv) high thermo- and photo-stability [1–10].

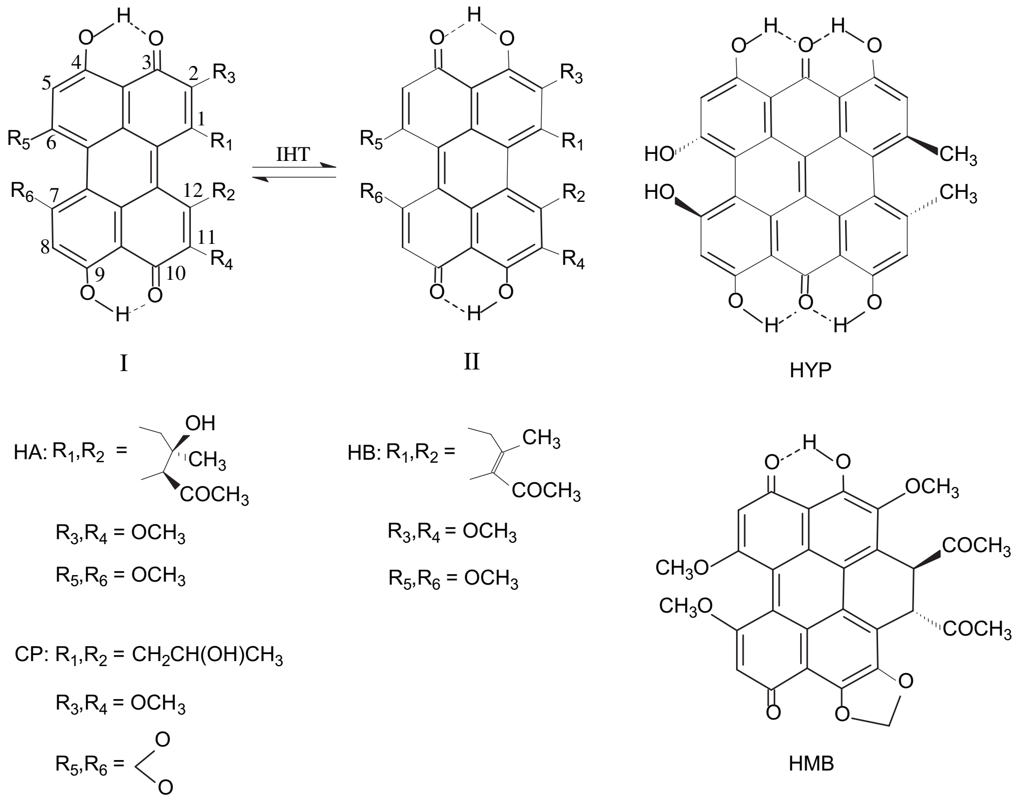
The structure–activity relationship (SAR) of PQPs has indicated that 4,9-dihydroxy-3,10-perylenequinone is the active center of the pigments [11], and the phenolic hydroxyl groups at positions 4 and 9 seem playing a critical role in the photo-generation of singlet oxygen ($^1O_2$), because when the hydroxyls of HA, HB and CP were methylated or acetylated, the quantum yields of $^1O_2$ dropped drastically from ~0.8 to ~0.2 [11]. Since PQPs undergo intramolecular H-atom transfer (IHT) in ground and excited states [12], it was conjectured that the IHT is crucial for PQPs to retain the high yields of $^1O_2$ [13], because the thermal vibration pertinent to the IHT could couple to the radiationless transition and facilitates the intersystem crossing from the singlet-excited state to the triplet-excited state. However, this notion was challenged by two recent findings: (i) the $^1O_2$-yield of HYP was efficiently enhanced by methylating the hydroxyls [14]; (ii) the photosensitizing activity of hypomycin B (HMB, Scheme 1), which contains only one hydroxyl group, was comparable to that of HA [13], but no expected IHT was observed in the excited state of HMB [15]. Thus, it is very interesting to reconcile the contradictory photo-physicochemical behaviors of HA, HB, CP, HMB and HYP, which will provide deeper insight into the photosensitizing mechanisms of PQPs.

As HA exhibits a deuterium isotope effect in the IHT, but HYP not [12], it is reasonable to infer that if IHT plays a role in the photo-generation of $^1O_2$, the deuterium isotope effects on the $^1O_2$-yields of HA and

---

* Corresponding author. Tel./fax: +86 533 2780271.
E-mail address: zhanghy@sdut.edu.cn (H.-Y. Zhang).

0143-7208/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.dyepig.2004.09.002



Scheme 1. Structures of some typical PQPs.

HYP will be different. In this paper, employing chemical trapping methodology, we determined the $^1O_2$-yields of parent and deuterated HA and HYP. The results will be helpful to understand the intriguing photo-physico-chemical behaviors of PQPs.

2. Materials and methods

2.1. Chemicals

HA was extracted from *Hypocrella bambuase*, a parasitic fungus growing in the southwestern mountains of Yunnan Province of the People's Republic of China, and was purified by column chromatography and recrystallizing according to the routine procedures [16,17]. HYP was purchased from Planta Naturstoffe Chemical Company. The purity of HA and HYP are higher than 98%. 9,10-Diphenylanthracene (DPA) was purchased from Aldrich Chemical Company. Methanol-d_4 was obtained from Cambridge Isotope Laboratories, Inc.

2.2. Preparation of deuterated PQPs

As the lifetime of $^1O_2$ in deuterated solvent is about 10 times longer than that in general solvent [18], to compare the $^1O_2$-yields of parent and deuterated PQPs, we had to prepare deuterated PQPs in a general solvent. The procedure is as follows. HA (1.0 mg) or HYP (1.0 mg) was dissolved in 1.0 ml of methanol-d_4 and placed at room temperature for 48 h in the dark to ensure the substitution of deuterium. Then, the methanol-d_4 was evaporated to give deuterated HA or HYP. Finally, methanol was used to dissolve the deuterated HA or HYP again to give the expected samples, which were immediately used in the $^1O_2$-detection.

2.3. Determination of relative $^1O_2$-yield

The DPA-bleaching method was used to determine the relative $^1O_2$-yields of HA, HYP and deuterated pigments [19]. During the experiments, the sample containing PQP and DPA was illuminated by a projector (Panasonic PT-L1501) and the absorbance of DPA at 374 nm (where PQPs have the weakest absorbance) was recorded by a Unico UV-2102 spectrophotometer as a function of irradiation time to measure the $^1O_2$-generating ability. The irradiation intensity was 24000 lux, measured by an illuminometer ST-80C. The concentration of DPA was maintained at 1.88×10^{-4} M, under which it could trap $^1O_2$ efficiently but not quench the excited state of PQP [19]. All of the experiments were carried out in triplicate.

3. Results and discussion

As shown in Fig. 1, upon illumination the absorbance of DPA at 374 nm declined drastically, indicating that both parent and deuterated PQPs can efficiently generate $^1O_2$. It is interesting to note that although the $^1O_2$-yields of deuterated PQPs are a little lower than those of parent ones, there is no evident difference between the deuterium isotope effects on HA and HYP, which means that deuterium substitution has little influence on the $^1O_2$-yields of PQPs and thus IHT is unlikely a prerequisite of high $^1O_2$-yields of PQPs.

Recently, the IHT in PQPs was considered governed by the skeleton vibration of the pigments, because the zero-point energy of H-atom lies above the IHT barrier, and therefore the H-atom is freely delocalized between the two oxygen atoms until being trapped by the tautomerized conformations of PQPs [12]. Accordingly, it is reasonable to speculate that it is carbon-skeleton vibration rather than IHT that is responsible for the high $^1O_2$-yields of PQPs and the big difference in photosensitizing activity of methylated HA, HB, CP and HYP can be interpreted in terms of distinct vibrational modes of their carbon-skeletons. Apparently, HYP contains three more conjugated rings than HA, HB and CP, so the vibrational mode of the former is definitely different from those of the latter. As a result, if being methylated, the tautomerization in HA, HB and CP is completely disrupted and thus the vibrational modes get rather simple, whereas methylated HYP still reserves complex vibrational modes of carbon-skeleton, which may be potent to accelerate the intersystem crossing and thus enhance the $^1O_2$-yield. This notion can also explain why HMB has no IHT but exhibits high photosensitizing activity. That is, although the conformational isomer of HMB is not stable and thus is incapable of trapping the H-atom [20], its skeleton vibration is sufficient to lead to the high-efficiency intersystem crossing. Hence, it seems if the carbon-skeleton vibration rather than IHT is taken into consideration, the contradictory observations on $^1O_2$-generating mechanism of PQPs can be unified.

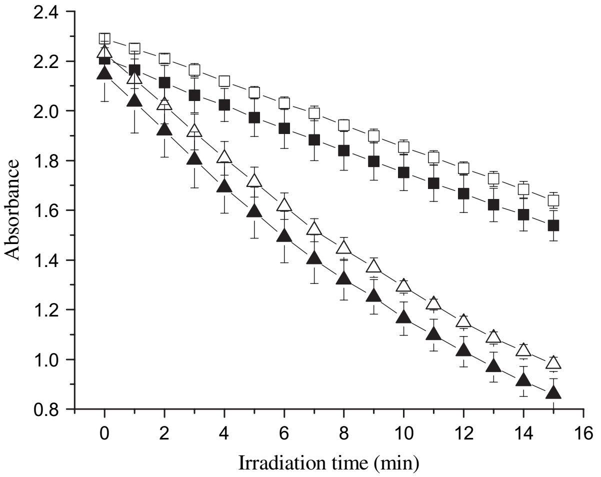


Fig. 1. Change of absorption of 9,10-diphenylanthracene (1.88×10^{-4} M) at 374 nm during the irradiation of perylenequinonoid pigment solution. (■) Hypericin, 3.2×10^{-5} M; (□) deuterated hypericin, 3.2×10^{-5} M; (▲) hypocrellin A, 2.4×10^{-5} M; (△) deuterated hypocrellin A, 2.4×10^{-5} M.

In brief, the $^1O_2$-yields of PQPs are little influenced by deuterium substitution. Consequently, not IHT but probably carbon-skeleton vibration is responsible for the high yield of $^1O_2$, which is of significance in directing the photosensitization study on related pigments, such as anthraquinonoid derivatives, and in designing novel photosensitizers as well.

Acknowledgement

This work was partially supported by the National Key Project for Basic Research (2003CB114400). We are grateful to Prof. J.W. Petrich, Iowa State University, for his helpful suggestion in preparing deuterated PQPs.

References

[1] Weiss U, Merlini L, Nasini G. Prog Chem Org Nat Prod 1987;52:1.
[2] Duran N, Song PS. Photochem Photobiol 1986;43:677.
[3] Jiang LJ. Chin Sci Bull 1990;35:1608.
[4] Jiang LJ. Chin Sci Bull 1990;35:1681.
[5] Diwu Z, Lown JW. Photochem Photobiol 1990;52:609.
[6] Diwu Z. Photochem Photobiol 1995;61:529.
[7] Zhang HY, Zhang ZY. Adv Free Radic Life Sci 1999;7:41.
[8] Falk H. Angew Chem Int Ed 1999;38:3116.
[9] Jiang LJ, He YY. Chin Sci Bull 2000;45:2019.
[10] Xing MZ, Zhang XZ, Sun ZL, Zhang HY. J Agric Food Chem 2003;51:7722.
[11] Diwu ZJ, Lown JW. J Photochem Photobiol B: Biol 1993;18:131.
[12] Petrich JW. Int Rev Phys Chem 2000;19:479 and references therein
[13] Zhang HY, Liu W, Liu WZ, Xie JL. Photochem Photobiol 2001;74:191.
[14] Roslaniec M, Weitman H, Freeman D, Mazur Y, Ehrenberg B. J Photochem Photobiol B: Biol 2000;57:149.
[15] Chowdhury PK, Das K, Datta A, Liu WZ, Zhang HY, Petrich JW. J Photochem Photobiol A: Chem 2002;154:107.
[16] Li C, Wang HQ, Chen YT, Xie JL. J Mol Catalys 2000;14:300.
[17] Liu WZ, Li WL, Shen YX, Chen YT, Xie JL. Chin Tradit Herb Drug 2001;32:771.
[18] Rodgers MAJ. Photochem Photobiol 1983;37:99.
[19] Diwu ZJ, Lown JW. J Photochem Photobiol A: Chem 1992;64:273.
[20] Chen DZ, Kong DX, Zhang HY. Acta Chim Sin 2002;60:234.