Quenching of Singlet Oxygen by Haematoporphyrin Derivative (and Haematoporphyrin) and its Consequences on the Efficiency of Photodynamic Cancer Therapy

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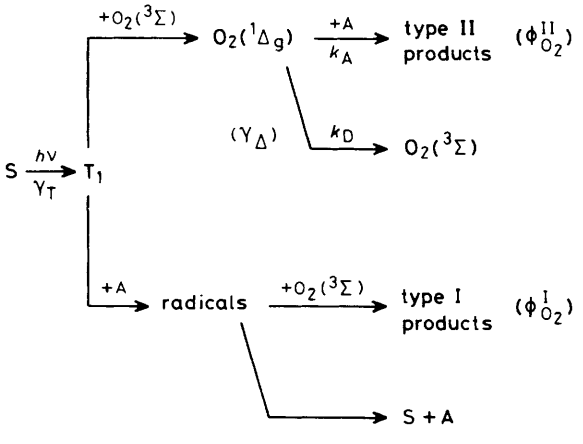
Haematoporphyrin derivative used in photodynamic therapy and haematoporphyrin quench singlet oxygen that they produce (rate constants 5×10^8 and 8×10^8 dm^3 mol^{-1} s^{-1}, respectively) with the consequence that in photodynamic therapy an increase of the sensitizer concentration may induce a very noticeable reduction of the efficiency of the photo-oxidative damage to tumour cells.

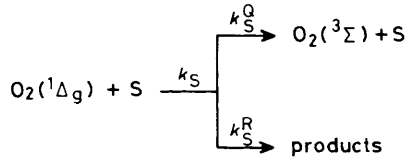
In recent years there has been a rising interest in the ability of haematoporphyrin (Hp) and of some related compounds, namely haematoporphyrin derivative (Hpd) or a fraction of its components to photosensitize cell death in tumours.[1,2] The use of these compounds (S) relies largely on their significantly increased localization in tumour cells (A) over that in normal cells.[3] Upon illumination these porphyrins sensitize the photo-oxidative damage of cells. The mechanism of attack is generally thought to involve the production of singlet oxygen $O_2(^1\Delta_g)$ *via* the Hp or Hpd triplet state T_1 (type II photo-oxidation), although alternative pathways involving radicals such as porphyrin radicals or superoxide O_2^- (type I photo-oxidation) cannot be entirely excluded[4,5] (see Scheme 1).

The *in vivo* local photosensitizer concentration is believed to be an important factor in photodynamic therapy (PDT) because tumour response depends on it. However, very little is known about the effect of the concentration [S] during the irradiation time if one excepts its incidence on the absorbed light intensity and the formation of aggregates in concentrated aqueous solutions. These aggregates may favour the radical mechanism.[4] From *in vitro* measurements, we now report that an increase of [S] induces a reduction of the efficiency of the type II photo-oxidative damage of cells characterized by quantum yield $\phi^{II}_{O_2}$ and then enlarges the relative importance of the type I mechanism (quantum yield $\phi^{I}_{O_2}$). This conclusion is the consequence of the discovery of a new process involving Hp and Hpd, namely the physical quenching of singlet oxygen by the photosensitizer (k_S^Q). This process (Scheme 2) is the main result of the interaction between $O_2(^1\Delta_g)$ and S since $k_S^R < k_S^Q$ for Hp and Hpd[6] as for other dyes.[7–11]



Scheme 1

The rate constants k_S^Q were obtained by determining the quantum yield $\phi^{II}_{O_2}$ of oxygen consumption during the Hp and Hpd sensitized photo-oxygenation of furfuryl alcohol (F) for various [F] and [S] as previously described.[12,13] Table 1 summarizes the main results. It appears that Hp deactivates $O_2(^1\Delta_g)$ very efficiently since the quenching rate constant in methanol is close to the highest values obtained for k_S^Q at this time (for comparison 7.3×10^8 dm^3 mol^{-1} s^{-1} for chlorophyll a in benzene and only 4.4×10^7 dm^3 mol^{-1} s^{-1} for tetraphenylporphyrin in the same solvent[14]). This value is only weakly affected by addition of 10% water to the methanolic solution, showing that H_2O does not induce any specific effect on k_S^Q. In connection with the similarity of k_S^Q values for MeOH and MeOH/H_2O solutions it is interesting to note that in this last mixture, Hp is monomeric.[15,16] We recently demonstrated that, in benzene solution, chlorophylls and related compounds show a linear relation between log k_S^Q and their half-wave oxidation potentials and concluded that (i) the interaction between dyes and $O_2(^1\Delta_g)$ may involve an exciplex and (ii) the physical quenching may be envisaged as a



Scheme 2

Table 1. Rate constants for the physical quenching of singlet oxygen by the sensitizer.

Sensitizer[a]	Solvent	$\tau_\Delta \times 10^6$[b] /s	$k_S^Q \times 10^{-8}$[c] /dm^3 mol^{-1} s^{-1}
Hp	MeOH	10.4	7.7 ± 0.8
Hp	MeOH/H_2O (9:1)	8.0	8.0 ± 0.8
Hpd	MeOH	10.4	5.1 ± 0.6

[a] Hp, from Aldrich, was used as purchased; Hpd was prepared from Hp by the method described by D. Kessel, P. Thomson, B. Musselman, and C. K. Chang, *Photochem. Photobiol.*, 1987, **46**, 563; absorption and fluorescence spectra of Hp and Hpd were verified to be closely related to those given in the literature. [b] The values for water and MeOH are given by M. A. J. Rodgers, *J. Am. Chem. Soc.*, 1983, **105**, 6201; for MeOH/H_2O solvents a linear variation of k_D *versus* the mole fraction of MeOH was assumed (N. Miyoshi, M. Ueda, K. Fuke, Y. Tanimoto, M. Itoh, and G. Tomita, *Z. Naturforsch., Teil B*, 1982, **87**, 649). [c] The molecular weight of Hpd being uncontrolled, all the results refer to $M = 598.7$ of Hp for sake of comparison.

spin–orbit-induced intersystem crossing within the exciplex.[14] It seems likely that the same process may also be responsible for the quenching by Hp or Hpd.

The rate constant k_S^Q for Hpd is relatively close to that found for Hp. As Hpd consists of several porphyrins including Hp, protoporphyrin, and hydroxyethylvinyldeuteroporphyrin (a total of about 50%) and of ether-linked or ester-linked porphyrin units ranging from two to six,[1] it is likely that the quenching process is essentially related to the interaction of $O_2(^1\Delta_g)$ and a porphyrin ring, both for Hpd and Hp. This result is very important in PDT. If singlet oxygen does not interact with S, the quantum yield of tumour necrosis by type II mechanism is given by $\phi_{O_2}^{II} = \gamma_\Delta.\phi_A$ with $\phi_A = k_A.[A]/(k_A.[A] + k_D)$. In fact, by taking the quenching of singlet oxygen by Hpd into account the probability ϕ_A of $O_2(^1\Delta_g)$ addition is reduced to equation (1).

$$\phi_{A,S} = \frac{k_A[A]}{k_A[A] + k_S^Q[S] + k_D} = \phi_A/\left(1 + \frac{k_S^Q[S]}{k_A[A] + k_D}\right) \quad (1)$$

It follows that $\phi_{O_2}^{II}$ is reduced by the same factor. From the value obtained for k_S^Q and with k_A, [S], and [A] respectively in the reasonable ranges of 10^4—10^6 $dm^3\ mol^{-1}\ s^{-1}$ (ref. 17), 20—60 μM,[18] and 10^{-3}—10^{-1} M, a crude estimation of $\phi_{A,S}/\phi_A$ can be made. The photo-oxidation efficiency may be reduced by 3 to 11% in aqueous media ($k_D = 2.4 \times 10^5\ s^{-1}$) and by 7 to 48% in nonaqueous media ($k_D = 3.3 \times 10^4\ s^{-1}$). Thus, this photosensitizer concentration effect cannot be neglected.

On the other hand, if a type I mechanism is also involved in the photo-oxygenation process, its relative importance will rise with [Hpd] because its efficiency is not affected by the sensitizer concentration. In PDT this concentration effect may be partially compensated by the photobleaching effect recently demonstrated.[19] This photobleaching, which results in a loss of photodynamically active porphyrin, leads to a decrease of the absorbed light intensity but, in turn, an increase of $\phi_{A,S}$. From a practical point of view, the physical quenching of singlet oxygen, as the photobleaching (and in conjunction with it?), may contribute to the improved therapeutic ratio.[19]

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