Triplet State of Protoporphyrin IX

By JOSEPH LAFFERTY and T. GEORGE TRUSCOTT*
(*Department of Chemistry, Paisley College, Renfrewshire* PA1 2BE, *Scotland*)

Summary Flash photolysis of aqueous solutions of protoporphyrin IX at pH values below 2 results in strong triplet state absorptions while at any higher pH, including physiologically relevant values, no triplet state formation can be detected.

THE metabolic skin photosensitivity disease erythropoietic protoporphyria (epp) is associated with an excess of the pigment protoporphyrin (pp) in the red blood cells and in the skin capillaries. The environment of pp in the skin is not established although the detection of pp in blood plasma may indicate that it is present in the aqueous phase; however the presence of pp in lipid components also cannot be discounted. It has been suggested[1] that the skin damage resulting from 400 nm light absorption by this pigment occurs *via* singlet oxygen produced from the triplet state of pp [equation (1)], where ^{3}pp and ^{1}pp are the triplet

$$^3\text{pp} + \text{O}_2(^3\Sigma_g^-) \rightarrow {}^1\text{pp} + \text{O}_2(^1\Delta_g \text{ or } ^1\Sigma_g^+) \quad (1)$$

and ground singlet states, respectively, of pp.

There are claims[2] that oral treatment with carotenoids, particularly β-carotene, affords protection from epp and that the mechanism[1] of such protection is *via* energy transfer by either or both of the processes (2) and (3).

$$^1\text{O}_2 + \beta\text{-carotene} \rightarrow {}^3\text{O}_2 + {}^3\beta\text{-carotene} \quad (2)$$

$$^3\text{pp} + \beta\text{-carotene} \rightarrow {}^1\text{pp} + {}^3\beta\text{-carotene} \quad (3)$$

There are, however, also claims that β-carotene is of little value in the treatment of epp[3] and since β-carotene can lead to hypervitaminosis A, it is of importance to establish if the treatment of epp with β-carotene is effective.

We report the triplet state formation of pp in methanol, and in aqueous solutions at $\text{pH} < 2$. In particular we note the unexpected effects of higher pH values on the efficiency of triplet state production.

Triplet states were produced and monitored by conventional flash photolysis (time resolution 20 μs) and neodymium laser flash photolysis (time resolution 30 ns) using, for the laser irradiation, the frequency-tripled line of 353·3 nm. Protoporphyrin IX was obtained by acid hydrolysis of the corresponding dimethyl ester, the purity being confirmed by its characteristic absorption spectrum. Aqueous solutions were prepared by adjusting the acid hydrolysate to the appropriate pH by the addition of NaOH, while methanolic solutions were prepared by prior evaporation of the pp acid solution to dryness.

Flash photolysis of the mono- or di-cation (based on the ground state spectra[4] of pp in methanolic solution, or in aqueous solution ($\text{pH} < 2$), resulted in the observation of a strong transient absorption in the wavelength range 400—700 nm with ground-state depletion being observed in the region of the pp Soret band (410 nm). Using both air-saturated and oxygen-saturated solutions the lifetimes of these transient absorptions (typically 150 μs in argon-flushed solutions) were quenched by factors corresponding to second-order rate constants of $1{\cdot}3 \pm 0{\cdot}1 \times 10^9\ \text{l mol}^{-1}\ \text{s}^{-1}$ The lifetime of the transient in methanol was also reduced by the addition of β-carotene and the characteristic triplet absorption of β-carotene (520 nm max.) could then be observed, confirming that the transient is the triplet absorption of pp and, of course, that mechanisms such as (2) and (3) are feasible.

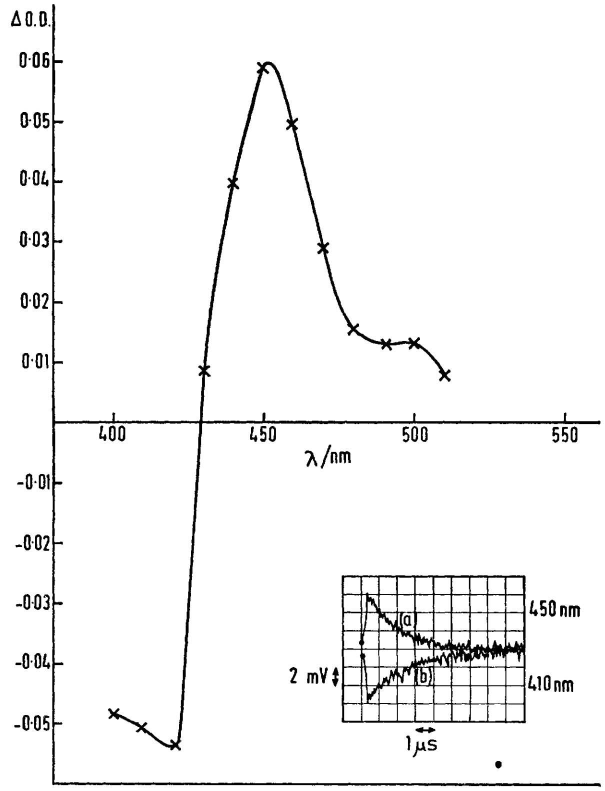

FIGURE. Triplet minus singlet difference spectrum of protoporphyrin IX in acid solution at pH 0. Insert: Typical oscillograms after Nd laser photolysis of protoporphyrin IX in air-saturated solution at pH 0. (a) Transient absorption with max. ΔO.D. = 0·06. (b) Transient depletion with max. ΔOD = −0·05.

The Figure shows the triplet–triplet difference spectrum from 400 to 510 nm for pp in acid solution at pH *ca.* 0, together with typical oscillograms for the decay of the transient absorption (a) and ground state depletion (b).

In order to study the porphyrin molecule under conditions of more biological relevance the pH of a saline solution of pp was adjusted to near 7. At this pH we were unable to detect any triplet state absorption resulting from flashing air-saturated or argon-saturated solutions. Indeed, no triplet can be detected in aqueous solution in the pH range 4—12 over the spectral region 250—750 nm. The disappearance of the triplet at high pH values was reversible in that on adding acid to return the solution pH to <4 the strong pp triplet could again be observed; thus the failure to observe a triplet absorption at $\text{pH} > 4$ is not due to alkaline destruction of pp. Similarly when methanolic solutions of pp are treated with alkali so that pp free base is produced no triplet can be detected. Clearly protonation of the ring nitrogen atoms of pp free base will remove any low-lying $n\pi^*$ energy levels, but, by analogy with other heteroatom-containing molecules this would not account for the lack of triplet-state formation of pp free base.

The most significant factor we note is that no triplet state of free pp appears to be formed in aqueous solutions at physiological pH values. Thus our results under such conditions throw doubt on the involvement of triplet states in the disease epp and on the proposed mechanisms [equations (2) and (3)] by which β-carotene can afford protection from the disease.

We thank Dr. M. Moore for useful discussions and the S.R.C. for grants.

(*Received, 22nd September* 1977; *Com.* 989.)

---

[1] S. J. Chantrell, C. A. McAuliffe, R. W. Munn, A. C. Pratt, and E. J. Land, *J.C.S. Faraday I*, 1977, **73**, 858.

[2] M. M. Mathews-Roth, M. A. Pathak, J. Parrish, T. B. Fitzpatrick, E. H. Kass, K. Toda, and W. Clemens, *J. Invest. Dermatol.*, 1972, **59**, 349; G. Krook and B. Haeger-Aronson, *Acta Dermatovener* (Stockholm), 1974, **54**, 39; C. T. Jansen, *Dermatologica*, 1974, **149**, 363.

[3] M. F. Corbett, A. Herxheimer, I. A. Magnus, C. A. Ramsey, and A. Kobza-Black, *Brit. J. Dermatology*, in the press.

[4] A. Neuberger and J. J. Scott, *Proc. Royal Soc., A*, 1952, **213**, 307.