SINGLET OXYGEN REACTIONS IN AQUEOUS SOLUTION. PHYSICAL AND CHEMICAL QUENCHING RATE CONSTANTS OF CROCIN AND RELATED CAROTENOIDS

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ABSTRACT : Thermal decomposition of 3-(1,4-epidioxy-4-methyl-1,4-dihydro-1-naphthyl)propionic acid (2) has been used to measure both the physical and chemical quenching rate constants of ${}^{1}O_{2}$ (k_{Q} and k_{R} , respectively) by water-soluble 8,8'-diapocarotenoids such as crocin (1a) and its derivatives (1b,c). The value of k_{Q} was found to be slightly lower than the diffusion controlled rate constant in all the solvents examined, whereas k_{R} showed strong dependence on the medium, going from ca. 10⁸ in H₂O to 10⁶ M⁻¹s⁻¹ in DMF and DMSO. These findings suggest that both energy- and electron-transfer mechanisms are involved in the quenching of O_{2} (${}^{1}\Delta_{g}$) by crocinoids.

The protective role played by carotenoids¹ in photodynamic oxidation² continues to be a matter of considerable chemical, biological and industrial interest Although crocin (la), the water-soluble pigment of saffron³, is a well recognized quencher of singlet oxygen⁴, no determination of quenching rate constants has been reported so far. On the other hand, estimation of such constants is crucial to a better use of crocin as an ${}^{1}O_{2}$ -monitor as well as to understanding the physical and chemical mechanisms by which carotenoids interact with singlet oxygen in vitro⁵ and in vivo^{2,4}.

We report here a measurement of rate constants for both reaction and physical quenching of O_2 $({}^{1}\!\Delta_{g})$ by crocin $(\underline{l}a)$ and its derivatives $(\underline{l}b,\underline{c})$ in water and other solvents.



To avoid undesired side reactions accompanying a sensitized photooxygenation, e.g. <u>cis-trans</u> isomerization of the polyene chain^{1b,3} and $0\frac{1}{2}$ production⁶, the water-soluble 1,4-endoperoxide of 3-(4-methyl-l-naphthyl)propionic acid (2) was chosen as a thermal source of singlet oxygen⁷. In a typical experiment two separated solutions (10 ml) of crocin (1a, 4.1×10^{-5} M) and 2 (94% by KI-starch test; 5.3×10^{-3} M) in 0.1 M phosphate buffer (pH 7.4) were mixed at 0°C. After deoxygenation by nitrogen bubbling, the resulting solution was rapidly heated to $35\pm1^{\circ}$ C and allowed to stand at $35\pm0.1^{\circ}$ C (under N₂). Electronic absorption spectra of samples taken at 3-min. intervals were recorded in the range 350-520 nm after rapid cooling to 0°C and appropriate dilution with the buffer. The disappearance of crocin was monitored by the decrease in 443 nm absorption.

For all the experiments performed, linear relationships of the type : $\ln \begin{bmatrix} C \end{bmatrix}_t = -k_C t + \text{cost.}$ (where $\begin{bmatrix} C \end{bmatrix}_t$ is the concentration of the carotenoid in the reaction mixture at time t) were obtained with satisfactory correlation coefficients in the interval 1-25 min. This appeared coherent with a steady-state approximation and allowed the rate of bleaching, V_t , to be calculated at a given $\begin{bmatrix} C \end{bmatrix}_t$.

According to the following sequence of reactions for the 10_2 -mediated interactions between 2 (AO₂) and the carotenoid (C) :

1)
$$AO_2 \xrightarrow{k_D} A + {}^{1}O_2$$

2) ${}^{1}O_2 \xrightarrow{k_S} {}^{3}O_2$
4) ${}^{1}O_2 + C \xrightarrow{k_R} Products$
(bleaching)⁸

quantitative kinetic analysis gives :

$$V = - \frac{d[c]}{dt} = k_{D} \left[AO_{2} \right] \cdot \frac{k_{R} [c]}{k_{S} + (k_{Q} + k_{R}) [c]}$$

where k_D is the decomposition rate constant of the endoperoxide⁹ corrected for the yield in ${}^{1}O_2$ (82%)⁷, k_S the rate constant for the natural decay of ${}^{1}O_2^{-10}$, k_Q and k_R the bimolecular rate constants for physical and chemical quenching of ${}^{1}O_2$, respectively. At a given time t (typically 3 min. after reaching the reaction temperature) :

$$\frac{Z(t)}{v_t} = a \cdot \left(\frac{1}{[c]_t}\right) + b \qquad \text{where} \quad a = \frac{k_s}{k_R} ; \quad b = \frac{k_R + k_Q}{k_R}$$

and $Z(t) = k_D \left[AO_2 \right]_t = k_D \left[AO_2 \right]_0 \exp(-k_D t) \left(\left[AO_2 \right]_0 \text{ being the initial concentration} of the endoperoxide). Thus the plot of <math>(Z(t) / V_t)$ vs. $(l / \begin{bmatrix} C \\ t \end{bmatrix})$ (for the same instant t) gives a straight line, if one carries out a set of experiments using the same parametric variable, $\left[AO_2 \right]_0$, but varying the initial concentration of the carotenoid. The quenching rate constants, k_R and k_Q , can then be calculated from the slope a and the intercept b.

The data of Table 1 indicate that in water 8,8'-diapocarotenoids (seven non-carboxy double bonds) have a quenching capacity for O_2 ($\frac{1}{\Delta}_g$) comparable to that shown in organic solvents by other polyene compounds having a more extended conjugation^{5b-f}. Unlike those, however, crocinoids exhibit an appreciable rate of chemical reaction (bleaching).

Table 1 - ${}^{1}O_{2}$ -quenching rate constants ($M^{-1}s^{-1}$) and oxidation potentials of crocin and its derivatives at 35+0.1°C

	SOLVENT (pH)	$10^{-8} \kappa_{R}^{(a)}$	$10^{-9} k_Q^{(a)}$	Eox (V vs. SCE) ^(b)
CROCIN (la) ^(c)	H ₂ O (7.4)	0.56	1.8	0.58
EMI-CROCIN (1b) (c)	H ₂ O (7.4)	1.3	5.1	0.57
CROCETIN $(lc)^{(d)}$	H ₂ O (7.8)	2.5 ^(e)	5.5 ^(f)	0.55
CROCETIN (lc)	DMF	< 0.05	$\simeq 7$	0.82
CROCETIN (lc)	DMSO	< 0.05	≃ 2	0.87

(a) mean values of 4 independent measurements each based on 5 bleaching expts. (r > 0.996); (b) peak potentials measured by cyclic voltametry (glassy carbon electrode and SCE reference electrode; 0.1 M KCl and 0.1 M TEAP as supporting electrolytes in H₂O and organic solvents, respectively); (c) isolated from alcoholic extract of saffron according to *Ref.* 3 (R_t 10.2 and 16.1 min. for la and lb, respectively, in analytical HPLC); (d) obtained by acidic hydrolysis of la^{1b}; (e) 4 in D₂O (*Ref.* 5a); (f) 2.5 in D₂O (*Ref.* 5a).

Taking into account the fact that triplet crocetin (1c) results from guenching of singlet molecular oxygen in D₂O, Matheson and Rodgers proposed^{5a} a slightly endothermic¹¹ energy transfer as the major ${}^{1}O_2$ -scavenging mechanism. In agreement with this suggestion we found that 13-<u>cis</u>-crocin (3)¹² undergoes ${}^{1}O_2$ -induced isomerization to the all-<u>trans</u>-polyene (1a) with high efficiency¹³. In fact, using the decrease of the <u>cis</u>-peak³ at 328 nm as a measure of double bond inversion (in short-term experiments characterized by no significant bleaching of absorbance at 443 nm) we obtained the following second order rate constants in water : k_I (isomerization) = $1.2 \times 10^9 \text{ M}^{-1} \text{s}^{-1}$ and $k_O = 6.1 \times 10^8 \text{ M}^{-1} \text{s}^{-1}$.

On the other hand, an electron-transfer mechanism cannot be ruled out¹³. Inspection of the data of Table 1 reveals that a marked decrease of the k_R (about two orders of magnitude) is found on going from water to solvents characterized by lower dielectric constants, more positive E_{ox} for crocetin, and more negative redox potentials for O_2/O_2^{-1} (E° = -0.71 V vs. SCE in DMF^{14a} and -0.58 V vs. SCE in DMSO^{14a}, adjusted for unit conc. of O_2^{-14b}). This strongly suggests that electron transfer is involved at least in the bleaching of 8,8'-diapocarotenoids in aqueous solution¹⁵.

Thus our results show that an electron-transfer process could be a major factor in the decomposition of carotenoids in fruit juices lb and in vivo 4 in the presence of light and oxygen.

Acknowledgements

We thank Dr. V. Malatesta (Farmitalia, Milan) for measuring oxidation potential and Ministero Pubblica Istruzione (M.P.I., Rome, Italy) for financial support.

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- ll-The diffusion-controlled rate constant for crocetin and molecular oxygen in water at 35°C can be calculated $\simeq 2 \times 10^{10}$ M⁻¹s⁻¹ by means of Smoluchowski equation

 $k_{diff} = 4 \pi N (D_{c} + D_{o}) R_{co} / 1000$

where the diffusion coefficients of the carotenoid (D_C) and of oxygen (D_O) are assumed to be $0.3-0.5 \times 10^{-5}$ and 3.07×10^{-5} cm² s⁻¹, respectively, and the encounter radius R_{CO} = 7 Å (values estimated according to : R.C. Reid, J.M. Prausnitz and T.W. Sherwood, "The Properties of Gases and Liquids", 3rd ed., McGraw-Hill, New York, 1977, pp. 57-60 and 566-582; Landolt-Börnstein, II Bd., 5 Teil, BdTeil a, Springer Verlag, Berlin, 1969, p. 611.

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(Received in UK 19 June 1987)