111. Singlet-Oxygen Quenching by Carotenoids: Steady-State Luminescence Experiments

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The near-infrared luminescence of singlet oxygen ($^1O_2$) has been measured in order to determine the efficiency of $^1O_2$ quenching by two carotenoid compounds, β-carotene and canthaxanthin. 1*H*-Phenalen-1-one and rose bengal have been used as photosensitizers in those steady-state luminescence experiments. *Stern-Volmer* analysis of the $^1O_2$ luminescence in solutions of CCl_4 and CD_3OD, containing different concentrations of the carotenoids, has shown a very efficient quenching by canthaxanthin. The rate constants are about a factor of 2 below the diffusion limited values for the given solvents, confirming earlier results in benzene. In comparison, the efficiency of $^1O_2$ quenching by β-carotene is slightly lower than that by canthaxanthin in non-polar solvents and is reduced by an order of magnitude in CD_3OD, due to the aggregation of this quencher.

Introduction. – Singlet oxygen ($^1O_2$; $^1\Delta_g$) is most often the reactive species in photosensitized oxidations; it is produced by electronic energy transfer from the triplet state of a sensitizer (generally a dye) to oxygen *(Reactions 1–3)*.

$$\text{Sens} \xrightarrow{h\nu} {}^1\text{Sens}^* \tag{1}$$

$$^1\text{Sens}^* \xrightarrow{k_{\text{isc}}} {}^3\text{Sens}^* \tag{2}$$

$$^3\text{Sens}^* + {}^3O_2 \xrightarrow{k_{\text{et}}} \text{Sens} + {}^1O_2 \tag{3}$$

Because of the efficiency with which they protect biological systems from photodynamic damage [1–5], carotenoids are of considerable interest in the pharmaceutical and cosmetic industries [6–8]. They are known to deactivate singlet oxygen by physical quenching [1] [9] [10],

$$^1O_2 + Q \xrightarrow{k_q} {}^3O_2 + {}^3Q^* \tag{4}$$

and, thus, to prevent its chemical reaction with other substrates. This quenching has been investigated for different carotenoids (*e.g.* β-carotene), and benzene has been chosen as a suitable solvent [9–13]. The efficiency of the physical quenching of $^1O_2$ seems to be related

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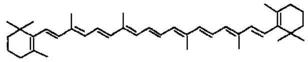
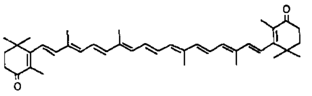
to the number of conjugated double bonds exhibited by the quencher [1] [2] [11]: polyenes with fewer than nine conjugated double bonds have triplet energies above that of $^1O_2$ ($^1\Delta_g$) and are, hence, much less efficient physical quenchers.

We have focussed our work on canthaxanthin, a compound used by some cosmetics manufacturers as an orally taken tanning agent. Measuring the luminescence of $^1O_2$ at 1270 nm [14–17] and using *Stern-Volmer* plots, we have determined the bimolecular rate constant of the physical quenching of $^1O_2$ by canthaxanthin, in comparison with that of all-*trans*-*β*-carotene, more widely investigated. Experiments have been run in a non-polar and in a polar solvent in order to evaluate the effect of the medium on the efficiency of the quenching of $^1O_2$ by these two carotenoids.



all-*trans*-*β*-carotene



canthaxanthin

Method of Analysis. – $^1O_2$ is produced by photosensitization *(Reactions 1–3)*, using rose bengal or 1*H*-phenalen-1-one as sensitizers. Rose bengal is known to be a very efficient $^1O_2$ sensitizer [18] [19], and we have shown that this is also the case for phenalenone [20] which presents the additional advantage of being soluble in polar as well as in non-polar solvents.

The signal of the $^1O_2$ emission at 1270 nm, measured upon continuous excitation of the sensitizer, is directly proportional to the quantum yield of $^1O_2$ luminescence (Φ_e), the proportionality factor depending on the equipment used and on the number of absorbed photons.

In the absence of a $^1O_2$ quencher, the luminescence quantum yield (Φ_e°) can be written as:

$$\Phi_e^\circ = \Phi^\circ(^1O_2)\cdot k_e/(k_e + k_d) \qquad (5)$$

where $\Phi^\circ(^1O_2)$ is the quantum yield of $^1O_2$ production by the chosen sensitizer; k_e and k_d are the radiative and the non-radiative rate constants, respectively, of $^1O_2$ deactivation in the solvent used.

If a $^1O_2$ quencher is added to the solution, Φ_e is given by:

$$\Phi_e = \Phi(^1O_2)\cdot k_e/(k_e + k_d + (k_r + k_q)[Q]) \qquad (6)$$

where k_q and k_r are the bimolecular rate constants of the physical quenching of $^1O_2$ by Q and of the chemical reaction between $^1O_2$ and Q, respectively.

If the rate of the quenching of the triplet state of the sensitizer by Q (k'_q [Q]) is negligible in comparison with the rate of energy transfer from this same triplet state to oxygen (k_{et} [O_2]), *i.e.* if

$$k_{et}[^3O_2] \gg k'_q[Q], \qquad (7)$$

then

$$\Phi(^1O_2) = \Phi^\circ(^1O_2) \qquad (8)$$

In this case, a *Stern-Volmer* analysis of the quenching leads to a linear relationship between the ratio of the luminescence signals in the absence and in the presence of quencher (I_0/I) and the concentration of the quencher:

$$I_0/I = \Phi_e^0/\Phi_e = 1 + (k_r + k_q)\cdot\tau(^1O_2)\cdot[Q] \qquad (9)$$

where $\tau(^1O_2) = 1/(k_e + k_d)$ is the $^1O_2$ lifetime in the absence of quencher.

The value of $(k_r + k_q)$ can be calculated from the slope of $I_0/I = f([Q])$, if $\tau(^1O_2)$ is known. If $k_r \ll k_q$, this method allows the direct determination of k_q.

Results. – Solutions of 1*H*-phenalen-1-one (absorbance of about 0.4 at the excitation wavelength) containing up to 3×10^{-7} mol·l^{-1} of the carotenoid in CCl_4 and up to 10^{-5} mol·l^{-1} in CD_3OD have been irradiated at 366 nm. In the absence of quencher, a stable signal is obtained for the $^1O_2$ luminescence upon irradiation of the sensitizer (stationary concentration of $^1O_2$). However, in the presence of β-carotene or canthaxanthin, the initial signal increases during the irradiation, more strongly in the case of β-carotene in CD_3OD. This increase may be explained by a reaction between the sensitizer (1*H*-phenalen-1-one) and the quencher (*e.g.* H abstraction), leading to a partial consumption of the quencher and, thus, to an increase of the $^1O_2$ concentration during the irradiation time. For the *Stern-Volmer* analysis, the intensities of the signals have been measured at the beginning of the irradiation and two different samples have been prepared for each concentration of quencher.

The corresponding *Stern-Volmer* plots for canthaxanthin and β-carotene are linear (*Figs. 1* and *2*), as expected by assuming diffusion-controlled energy transfer for the quenching of the triplet state of the sensitizer by oxygen, as well as by the quencher. In fact, the former reaction is at least 200 times faster than the latter, since the concentrations of oxygen in air saturated CCl_4 and CD_3OD are 2.6×10^{-3} mol·l^{-1} and 2.1×10^{-3} mol·l^{-1}, respectively [21], and condition (*7*) is met.

The lifetime of $^1O_2$ in CCl_4 is about 28.5 ms [22]: this value is an average of the results obtained by different authors using steady-state or time-resolved emission spectroscopy

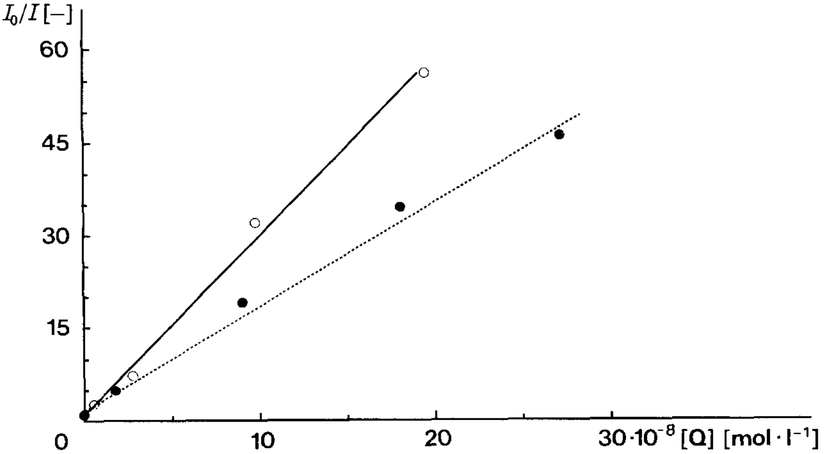


Fig. 1. Stern-Volmer *plots of the quenching of* $^1O_2$ *by* β*-carotene* (·····) *and canthaxanthin* (——) *in* CCl_4 (sensitizer: 1*H*-phenalen-1-one, λ_{ex} = 366 nm)

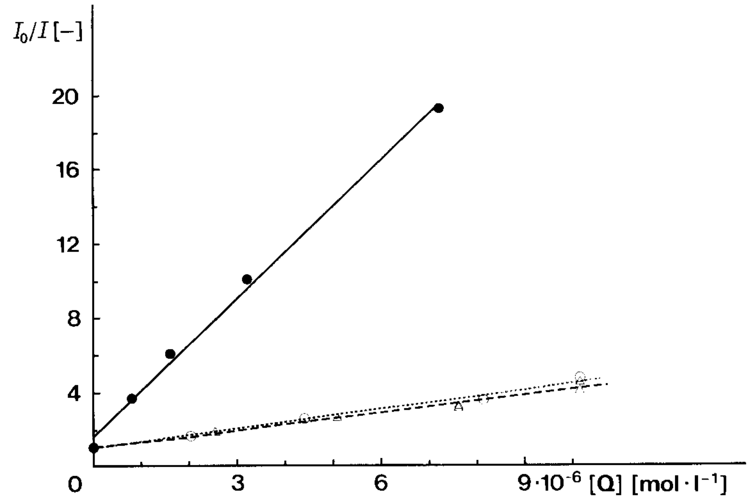


Fig. 2. Stern-Volmer *plots of the quenching of* $^1O_2$ *by β-carotene* (· · · · · : sensitizer 1*H*-phenalen-1-one (λ_{ex} = 366 nm); – – – – –: sensitizer rose bengal (λ_{ex} = 546 nm)) *and by canthaxanthin* (——: sensitizer 1*H*-phenalen-1-one)

at 1270 nm [23–25]. Using this average lifetime, rate constants of physical quenching of $^1O_2$ by β-carotene and canthaxanthin of 5.9×10^9 and 1.0×10^{10} l · mol^{-1} · s^{-1}, respectively, are obtained *(Table)*. The value for β-carotene is close to the one reported in the literature, in the same solvent [26].

In CD_3OD, the lifetime of $^1O_2$ (227 μs [27]) is more than 100 times shorter than in CCl_4 and, consequently, higher concentrations of quencher must be used for the *Stern-Volmer* analysis. However, condition (*7*) still holds, and the *Stern-Volmer* plots are linear *(Fig. 2)*. The value of k_q obtained for β-carotene in CD_3OD is lower by a factor of 8 when compared with k_q of canthaxanthin *(Table)*. To check, if this low value obtained for k_q might be a consequence of a possibly lower precision of the analysis (luminescence signals increasing rapidly during irradiation due to the reaction between 1*H*-phenalen-1-one and β-carotene), the experiments with β-carotene have been repeated using rose bengal as a sensitizer. In this latter case, the luminescence signals are stable, in the presence as well as in the absence of β-carotene. The *Stern-Volmer* analysis *(Fig. 2)* leads to a value of k_q of

Table. *Rate Constants of Quenching of* $^1O_2$ *by β-Carotene and Canthaxanthin in* CCl_4*, and in* CD_3OD (k_q in l · mol^{-1} · s^{-1}, sensitizer: 1*H*-phenalen-1-one, unless otherwise specified)

Quencher	Solvent	k_q [l · mol^{-1} · s^{-1}]	k_q [lit.] [l · mol^{-1} · s^{-1}]
β-Carotene	CCl_4	$5.9 (\pm 0.6) \times 10^9$	$7 (\pm 2) \times 10^9$ [26]
	C_6H_6		$1.25–1.38 \times 10^{10}$ [9] [10] [12][a])
	CD_3OD	$1.5 (\pm 0.1) \times 10^9$	
		$1.4 (\pm 0.1) \times 10^9$ [b])	
Canthaxanthin	CCl_4	$1.0 (\pm 0.1) \times 10^{10}$	
	C_6H_6		$1.45 (\pm 0.2) \times 10^{10}$ [10][a])
			$1.78 (\pm 0.2) \times 10^{10}$ [28][a])
	CD_3OD	$1.2 (\pm 0.1) \times 10^{10}$	

[a]) Literature values in C_6H_6 are given for comparison. [b]) Sensitizer: rose bengal.

1.4×10^9 l·mol^{-1}·s, very close to the one found when 1*H*-phenalen-1-one is used as the sensitizer *(Table)*.

Discussion. – The rate constants of quenching of $^1O_2$ by *β*-carotene and canthaxanthin in benzene have been determined by monitoring the triplet state of the carotenoids by flash photolysis or pulse radiolysis [9] [10] [12] [28] and are close to the rate constant of diffusion (k_{diff}) which is about 3.0×10^{10} l·mol^{-1}·s^{-1}, when oxygen is one of the reaction partners [29]. The results reported in the *Table* show that these two carotenoids are also very efficient $^1O_2$ quenchers in CCl_4, where the diffusion-controlled rate constant should be slightly lower than in benzene because of the higher viscosity of the former solvent [29] [30]. In non-polar solvents, canthaxanthin appears to be more efficient than *β*-carotene *(Table)*. As already suggested by other authors [10], this could be due to its lower triplet energy, canthaxanthin containing 13 conjugated double bonds (11 C=C and 2 C=O) instead of 11 for *β*-carotene.

In the case of canthaxanthin, k_q seems, independently of the solvent, to be about a factor of 2 lower than the diffusion-limited rate constant. For *β*-carotene, however, k_q is 20 times smaller than k_{diff} in CD_3OD: this lower quenching efficiency may be linked to the low solubility and the aggregation of *β*-carotene in this solvent. In fact, within the concentration range used for the *Stern-Volmer* analysis, a net deviation from the *Beer-Lambert* law is found. Moreover, the molar absorption coefficient (ε_{max} (450 nm) $= 30800 \pm 500$ l·mol^{-1}·cm^{-1} for a concentration of *β*-carotene of 4×10^{-6} mol·l^{-1}) is much lower than in benzene (ε_{max} (466 nm) = 122000 l·mol^{-1}·cm^{-1} [31]) or in carbon tetrachloride (ε_{max} (463 nm) $= 103700 \pm 1000$ l·mol^{-1}·cm^{-1}). No aggregational effect of canthaxanthin has been detected in any of the solvents used. This compound, having a higher polarity due to its two C=O groups, is apparently better solvated in CD_3OD than *β*-carotene and, hence, a better reference for kinetic measurements in this domain, as aggregational effects may be avoided. Consequences of these findings in applying carotenoids as $^1O_2$ quenchers in biological systems are under investigation.

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Experimental. – 1*H*-Phenalen-1-one *(EGA Chemie)* and rose bengal *(Fluka)* have been used as $^1O_2$ sensitizers. The purified carotenoid pigments, *β*-carotene and canthaxanthin, were provided by *F. Hoffmann-La Roche & Co AG* (Basel). CCl_4 *(Fluka, puriss.)*, CH_3OH *(Fluka, puriss.)*, and CD_3OD (> 99.9% d_4, *Dr. Glaser*) have been used as solvents. Absorption spectra were recorded on a *UV-260 Shimadzu* spectrophotometer. The $^1O_2$ luminescence measurements have been made with solns. of identical absorbance (A = 0.4) at the excitation wavelength (366 nm for 1*H*-phenalen-1-one and 546 nm for rose bengal). Solns. containing different concentrations of the carotenoids were prepared, from standard solns. of the sensitizers. A fluorescence cell (1 cm × 1 cm) containing the sample was placed on an optical bench and irradiated through a H_2O filter (10 cm) and a monochromator (*Jobin Yvon*, 12-nm band width) with a Xe/Hg lamp (*Osram*, 1 kW). The luminescence of $^1O_2$ was collected at 90° by a mirror, chopped at a frequency of 19 Hz, and measured by means of a Ge detector *(Judson)* connected to a preamplifier and to a lock-in amplifier (model *5101, EG&G, Princeton Applied Research*). Signals were recorded on a graphic plotter connected to the lock-in amplifier.

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