# Quenching of singlet oxygen luminescence by fatty acids and lipids

## Contribution of physical and chemical mechanisms

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By the use of photosensitized luminescence of singlet oxygen ($^1O_2$) in $CCl_4$, the rate constants for quenching $^1O_2$ by saturated and unsaturated fatty acids were determined. The experimental data suggest that saturated fatty acids quench $^1O_2$ via a physical mechanism, presumably as a result of energy transfer to the vibrational sublevels of CH- and COOH-groups. Unsaturated fatty acids are predominantly chemical quenchers. The relative contribution of the physical quenching depends on the number of double bonds in fatty acid molecules. It was found that the quenching activity of egg phosphatidylcholine is approximately equal to the sum of quenching activities of the lipid fatty acids. The data obtained may be used for prediction of the efficiency of singlet oxygen quenching by any lipids whose fatty acid composition is known.

*Fatty acid* *Lipid* *Egg yolk phosphatidylcholine* *Singlet oxygen* *Singlet oxygen luminescence* *Quenching of singlet oxygen*

## 1. INTRODUCTION

It is common knowledge that biological membrane lipids undergo photosensitized peroxidation in living organisms and model systems [1–6]. An essential role in this process is generally ascribed to singlet molecular oxygen in $^1\Delta_g$ state, $^1O_2$, which is formed as a result of energy transfer from triplet molecules of sensitizers to $O_2$ [3–6]. Therefore, an investigation of mechanisms underlying the lipid–singlet oxygen interactions is of substantial interest. One of the approaches to this problem is the measurement of rate constants of $^1O_2$ quenching ($K_q$) by structurally different lipids and fatty acids. Earlier such measurements were performed by analyzing the destruction of the substances in the course of photosensitized oxygenation. Hence, the rate constants of chemical quenching ($K_{ox}$) were estimated [5,7]. Meanwhile, it is well known that the hydroperoxides formed by a reaction between $^1O_2$ and lipids are unstable. Their spontaneous decomposition leads to a formation of free radicals which induce further peroxidation ([7] and references therein). Thus, the $K_{ox}$ values may reflect the sum of the two indicated types of peroxidation and not only the reactions of $^1O_2$. On the other hand, organic substances are known to be physical quenchers of $^1O_2$. As proposed in [8], the quenching activity of simple chemically inert organic molecules is a result of energy transfer from $^1O_2$ to high-frequency vibrations of hydrogen atoms.

Here, we aimed to determine the $K_q$ values for saturated and unsaturated fatty acids under conditions in which the free radical peroxidation does not affect the results of the measurements. We tried to evaluate the contribution of the described physical quenching to the total activity of fatty acids and lipids by quenching the photosensitized luminescence of singlet oxygen in solutions.

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This method has been employed for determination of the $K_q$ values for lipids and other substances [9–12].

## 2. MATERIALS AND METHODS

The luminescence of $^1O_2$ was measured on a device with a phosphoroscope and a photomultiplier S-1 cooled with dry ice [10]. Tetraphenylporphin ($10^{-6}$ M) was used as a photosensitizer, $CCl_4$ (analytical grade) served as a solvent. The luminescence was excited with monochromatic light, 652 nm, and measured at 1270 nm. The chromatographic standards of fatty acids (Sigma) were used without further purification; egg yolk phosphatidylcholine was prepared following the usual procedure and purified chromatographically [13]. Before use, the unsaturated fatty acids were stored at −50°C in soldered ampules with argone. The fatty acid composition of phosphatidylcholine was analyzed after methylation on a Perkin-Elmer F-22 gas chromatograph.

## 3. RESULTS AND DISCUSSION

The experimental data demonstrate that an addition of fatty acids and lipids to porphyrin solutions results in quenching of the singlet oxygen luminescence. To minimize the peroxidation of the quenchers low intensities of exciting light were used. The efficiency of peroxidation was controlled by measuring the changes in intensity of oxygen luminescence upon illumination. When the quenchers were destroyed, the luminescence intensity gradually increased during the illumination period.

The experimental conditions were selected so that no changes in the luminescence intensity were observed within the first 5 min of illumination of any solutions used in our experiments.

Luminescence quenching (fig.1) can be described in terms of the Stern–Folmer equation, i.e.:

$$\frac{L_o}{L_q} = 1 + K_q \tau_o C_q$$

where:

$L_o$ and $L_q$ = the luminescence intensities in the absence and presence of quenchers;

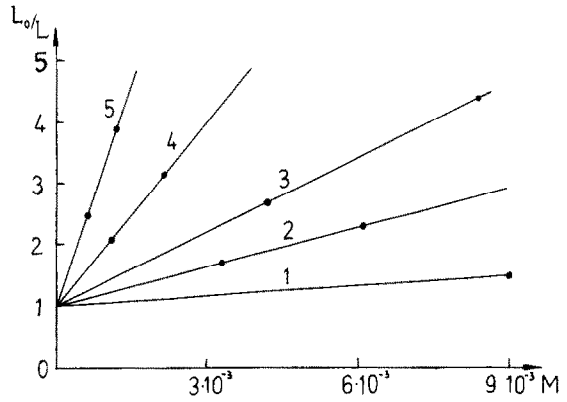


Fig.1. Quenching of singlet oxygen luminescence by acetic (1), stearic (2), oleic (3), linoleic (4) and arachidonic (5) acids. $L_o$ and $L$, intensities of luminescence in the absence and presence of the quenchers.

$\tau_o$ = the luminescence lifetime in the absence of quenchers;

$C_q$ = the concentration of quenchers.

The $\tau_o$ value equal to 24 ms was determined using a phosphoroscope [9]. The values of $K_q$ obtained are given in tables 1 and 2.

### 3.1. *Quenching by saturated fatty acids*

The $K_q$-values for saturated fatty acids vary within the range of $(2.3–11) \times 10^3$ $M^{-1}.s^{-1}$ (table 1) and are close to the $K_q$-values obtained for simple organic substances, such as alkanes, alcohols and acetone [12]. These constants apparently characterize the physical quenching of $^1O_2$ since no destruction of the quenchers was observed either in our studies or in [4,5]. An increase in the number of hydrogen atoms ($n_H$) in the molecules of saturated fatty acids is accompanied by an increase of $K_q$. However, the $K_q/n_H$ ratio is not constant and reaches its maximal value for the smallest molecules (table 1). This allowed us to assume that $K_q$ is a sum of the rate constants for $^1O_2$ quenching by hydrogen atoms of the CH- and COOH-groups, i.e.:

$$K_q = n_H \times K_{CH} + K_{COOH}$$

The data from table 1 correspond to this formula at $K_{CH} = 2.1 \times 10^2$ $M^{-1}.s^{-1}$ and $K_{COOH} = 1.7 \times 10^3$ $M^{-1}.s^{-1}$. Hence, the quenching activity of a single COOH-group is much higher than that

Table 1

Rate constants of $^1O_2$ quenching by saturated fatty acids

| Acid | Formula | $K_q \times 10^{-3}$ ($M^{-1}.s^{-1}$) | $K_q/n_H$ | $\Delta K_q/\Delta n_H$ |
|---|---|---|---|---|
| Acetic | $CH_3COOH$ | 2.3 | 580 | 0 |
| Caproic | $C_5H_{11}COOH$ | 3.9 | 320 | 200 |
| Lauric | $C_{11}H_{23}COOH$ | 6.1 | 250 | 190 |
| Palmitic | $C_{15}H_{31}COOH$ | 8.0 | 250 | 210 |
| Stearic | $C_{17}H_{35}COOH$ | 9.0 | 250 | 210 |
| Arachidic | $C_{19}H_{39}COOH$ | 10 | 250 | 210 |
| Behenic | $C_{21}H_{43}COOH$ | 11 | 250 | 210 |

$n_H$ is the number of hydrogen atoms in an acid molecule; $\Delta K_q$ and $\Delta n_H$ are the differences between $K_q$ and $n_H$ for the given acid minus those for acetic acid; errors of determination of the $K_q$ values are ± 5%

of a single CH-group. This conclusion correlates qualitatively with the quenching theory [8], since according to our measurements the IR absorption of a single OH-group in the spectral region of the singlet oxygen emission is higher than that of a single CH-group [12].

### 3.2. *Quenching by unsaturated fatty acids*

One double bond in oleic acid increases $K_q$ by the factor of 2 as compared to the saturated fatty acid having the same $n_H$ (table 2). An increase of the number of double bonds enhances the $K_q$ values. This effect is not due to the physical quenching described above, since $n_H$ is reduced with an increase in the number of double bonds in fatty acid molecules.

It is probable that an increase of $K_q$ is a result of a chemical reaction between $^1O_2$ and unsaturated fatty acids [3–6]. Indeed, at sufficiently high intensities of excitation we observed a significant decrease of the quenching activity of fatty acids upon illumination.

It is known that oxygenation of unsaturated fatty acids is caused by the reactivity of allylic and doubly allylic hydrogen atoms [3–7]. For this reason we made an attempt to present $K_q$ as a sum:

$$K_q = K_{ph} + K_1 + K_2$$

where:

$K_{ph}$ = the rate constant of physical quenching similar to that for saturated fatty acids;

$K_1$ and $K_2$ = the rate constants of quenching of $^1O_2$ by allylic and doubly allylic hydrogen atoms, respectively.

The data of table 2 can be described by this formula with the following values of the constants: $K_{ph} = 7 \times 10^3$, $K_1 = 1.1 \times 10^4$, $K_2 = 2.7 \times 10^4$ $M^{-1}.s^{-1}$. Hence, the allylic and doubly allylic hydrogen atoms possess the highest quenching activity. The contribution of $K_{ph}$ to $K_q$ varies from 40% for oleic acid to ~5% for docosahexaenoic acid. Our data do not allow us to conclude if some additional physical mechanism contributes to the overall quenching activity of the unsaturated fatty acids. A comparison of $K_q$ with $K_{ox}$ obtained in [4] does not clarify the problem, since $K_q < K_{ox}$ (table 2). An analysis of the reasons for this disagreement requires further experiments. It is possible that the reported $K_{ox}$ values are too high due to the con-

Table 2

Rate constants of $^1O_2$ quenching by unsaturated fatty acids

| Acid | Formula | $n_{db}:n_a:n_{da}$ [a] | $K_q \times 10^{-4}$ ($M^{-1}.s^{-1}$) | $K_{ox} \times 10^{-4}$ [b] ($M^{-1}.s^{-1}$) |
|---|---|---|---|---|
| Oleic | $C_{17}H_{33}COOH$ | 1:2:0 | 1.7 | 7.4 |
| Linoleic | $C_{17}H_{31}COOH$ | 2:3:1 | 4.2 | 13 |
| Linolenic | $C_{17}H_{29}COOH$ | 3:4:2 | 8 | 19 |
| Arachidonic | $C_{19}H_{31}COOH$ | 4:5:3 | 10 | 24 |
| Docosahexaenoic | $C_{21}H_{31}COOH$ | 6:7:5 | 15 | – |

[a] $n_{db}$, $n_a$ and $n_{da}$ are the numbers of double bonds, allylic and doubly allylic hydrogen atoms in acid molecules

[b] The values of $K_{ox}$ were obtained for methyl esters of the fatty acids in pyridine [4]

Table 3

$K_q$ value and fatty acid composition for egg yolk phosphatidylcholine

| Fatty acid[a] | $\alpha_a$ | $(2.\alpha_a.K_{qa}) \times 10^{-3}$ $(M^{-1}.s^{-1})$ |
|---|---|---|
| 10:0 | 0.0015 | 0.02 |
| 16:0 | 0.28 | 4.5 |
| 18:0 | 0.16 | 2.9 |
| 16:1 | 0.01 | 0.33 |
| 18:1 | 0.345 | 12 |
| 18:2 | 0.165 | 14 |
| 20:2 | 0.035 | 7 |
| Lipid | 0.996 | 41 (60[b]) |

[a] The ratio of the number of carbon atoms related to the number of double bonds

[b] The experimentally determined value of $K_q$ for the lipid

tribution of free radical peroxidation or else $K_{ox}$ might be strongly dependent on the solvent or esterification of the fatty acids.

### 3.3. *Quenching by lipids*

To estimate the activity of fatty acids in lipid molecules we examined the fatty acid composition of egg yolk phosphatidylcholine and compared the $K_q$ value obtained by direct measurement with the value calculated by the formula:

$$K_q = \Sigma\ (2.\alpha_a K_{qa})$$

where:
$\alpha_a$ = the relative content of a fatty acid;
$K_{qa}$ = its quenching rate constant.

The values presented in table 3 demonstrate that the calculated and experimental values of $K_q$ are in a reasonable agreement. Thus, it might be concluded that the quenching activity of fatty acids is similar in acid solutions and lipids.

On the basis of this observation one can predict the $K_q$ values for various lipids. It might be expected, for instance, that the minimal activity of phospholipids should correspond to the activity of two molecules of saturated lauric acid; i.e., $K_q = 1.2 \times 10^4\ M^{-1}.s^{-1}$. The maximal value of $K_q$ equal to $3 \times 10^5\ M^{-1}.s^{-1}$ should be characteristic of phospholipids with two molecules of docosahexaenoic acid. Experimental values of $K_q$ for some natural phospholipids in [11] lie within the indicated interval.

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