

# Biophotonics of molecular oxygen: activation efficiencies upon direct and photosensitized excitation

A. A. Krasnovsky, Jr.<sup>1</sup>, N. N. Drozdova<sup>1</sup>, Ya. V. Roumbal<sup>1</sup>, A. V. Ivanov<sup>2</sup>, and R. V. Ambartzumian<sup>3</sup>

<sup>1</sup>A. N. Bach Institute of Biochemistry, Russian Academy of Science, Leninskii pr., 33, Moscow 119071, Russian Federation

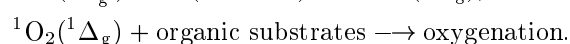
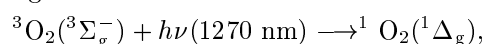
<sup>2</sup>N. N. Blokhin Russian Cancer Center, Russian Academy of Medical Science, Kashirskoye shosse 24, Moscow 115478, Russian Federation

<sup>3</sup>P. N. Lebedev Physics Institute, Russian Academy of Science, Leninskii pr., 53, Moscow 119991, Russian Federation

With a goal of mimicking the mechanisms of the biological effect of low intensity laser radiation, we have measured the rates of oxygenation of chemical traps of singlet oxygen in air-saturated organic solvents and deuterium oxide upon direct (1270 nm) excitation of the  $^1\Delta_g \leftarrow ^3\Sigma_g^-$  transition in oxygen molecules. The data were used for estimation of the molar absorption coefficients of oxygen corresponding to this transition. The obtained values were compared with the radiative lifetimes of singlet oxygen calculated from the quantum yields of singlet oxygen phosphorescence. The quantum efficiencies (the ratios of the photoreaction rates to the irradiation intensities) of oxygenation were compared upon porphyrin-photosensitized and direct oxygen excitation.

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The ground state of molecular oxygen is known to be triplet. Absorption of dark red or infrared (IR) light leads to population of the low-lying singlet states. These singlet states can be efficiently populated by the energy transfer to oxygen from excited triplet molecules of organic photosensitizers. The singlet  $^1\Delta_g$ -state ( $^1O_2$ ) is chemically active and causes oxygenation of biologically active compounds. Therefore, it is responsible for pigment-photosensitized (photodynamic) damage to living cells and tissues<sup>[1]</sup>. However, biological effects are also induced by low-intensity red or IR laser irradiation of the systems, where pigments-photosensitizers are not detected or their concentrations are very low (Refs. [2–4] and references therein). The maxima of the action spectra of certain biological effects resemble the absorption maxima of the dimers,  $(O_2)_2$  and monomer,  $(O_2)$  oxygen molecules, therefore, it was proposed that some of these effects are initiated by reactive  $^1O_2$  molecules produced as a result of laser excitation of molecular oxygen (see Ref. [4] and references therein). A chemistry basis of this concept can be found in pioneering papers<sup>[5,6]</sup>, which were followed by other publications (Refs. [7, 8] and references therein). They showed that red and IR light of the wavelengths corresponding to the absorption bands of oxygen molecules caused oxygenation of chemical traps of singlet oxygen dissolved in Freon 113, carbon tetrachloride and deuterium oxide, saturated with oxygen at very high pressure 130–150 atm. Our goal was to study this phenomenon in natural conditions. As a result, we found that excitation of oxygen by the 1270-nm laser radiation of approximately that power, which was used in biological experiments, led to readily observed oxygenation of the singlet oxygen traps also in solutions saturated with air at the atmospheric pressure. The action spectra of these photooxygenation reactions coincided with the absorption spectrum of the  $^1\Delta_g \leftarrow ^3\Sigma_g^-$  transition in oxygen molecules<sup>[9,10]</sup>. The data are consistent with the following mechanism:



In the present paper, we report additional data supporting this mechanism in air-saturated solutions of the traps in several solvents and compare the quantum efficiencies of the photooxygenation upon direct and photosensitized excitation of molecular oxygen in air-saturated solutions.

Two laser systems were employed in our experiments. One system consisted of the array of continuous diode GaAlAs lasers, radiation of which passed through the fiber light guide and then through an IR cut-off light filter. The spectral band of the eliciting radiation had the maximum at 1267 nm and the half bandwidth about 13 nm. The radiation power at the surface of the irradiated sample was 50 mW. The second system consisted of a wavelength-tunable forsterite laser pumped by the Nd:YAG laser with acousto-optical *Q*-switch operated with 25-kHz repetition rate. It allowed us to obtain monochromatic IR radiation at 1200–1290 nm with 3-nm bandwidth. The average output laser power was 30–120 mW with the peak intensities of individual pulses not exceeding 50 W/cm<sup>2</sup>.

Singlet oxygen traps, tetracene and 1,3-diphenylisobenzofuran (DPIBF) (Aldrich, USA) were used as substrates of oxygenation. The reported molar absorption coefficients corresponding to the main absorption maxima of the traps (474 nm for tetracene and 411–419 nm for DPIBF) are  $1.25 \times 10^4$  and  $2.35 \times 10^4$  (mol/L)<sup>-1</sup>·cm<sup>-1</sup>, respectively<sup>[11,12]</sup>. Reaction with  $^1O_2$  led to bleaching of these absorption bands due to formation of colorless endoperoxides. The rate constants of  $^1O_2$  reactions with tetracene and DPIBF are known to be  $7 \times 10^6$  and  $8 \times 10^8$  (mol/L)<sup>-1</sup>·s<sup>-1</sup>, respectively (Refs. [9, 10] and references therein). The solvents were carbon tetrachloride, benzene, acetone (“chemically pure”, Avogadro, Moscow), hexafluorobenzene (99.9%, Piminvest, Moscow), ethanol (96%, Crystal, Orel), and deuterium oxide (99%, Isotope, St. Petersburg). Solutions of the traps were illuminated in the 10 mm rectangular quartz cell. The volume of the solutions was 1.5 mL. The laser beam was defocused so that the diameter of the illuminated spot on the surface of the cell was 9 mm. The absorption spectra of the solutions were measured

**Table 1. The Rates of Tetracene Photooxygenation upon Irradiation by IR Lasers in Air-Saturated CCl<sub>4</sub>**

Laser	Trap ( $\mu\text{mol/L}$ )	Inhibitor	Photooxygenation Rates (a.u., $\pm 10\%$ )
Diode Array	Tetracene, 50	No	1.0*
Forsterite, 1268 nm, 52 mW	Tetracene, 50	No	1.05
Forsterite, 1268 nm, 120 mW	Tetracene, 50	No	2.4
Diode Array	Tetracene, 70	No	1.1
Diode Array	Tetracene, 30	No	0.9
Diode Array	DPIBF, 40	No	1.1
Diode Array	DPIBF, 10	No	1.1
Diode Array	Tetracene, 50	$\beta$ -Carotene, 140 nmol/L	0.25

\*The photooxygenation rate was equal to  $5.0 \times 10^{-9}$  (mol/L)/s.

using the spectrophotometer Hitachi-3400 (Japan). The experiments were performed as follows. At first, the absorption spectra of the air-saturated solutions of tetracene or DPIBF were measured before IR-irradiation and the absorbances in the main absorption maxima were recorded ( $A_0$ ). Then, the solutions were irradiated during 10–60 min, shaken, and the absorbances ( $A_f$ ) were measured again. The photooxygenation rates ( $V_r$ , (mol/L)/s) were calculated from measuring the illumination time ( $t$ ) needed to decrease the absorbance so that  $\Delta A = A_0 - A_f = 0.05$

$$V_r = 0.05/(\varepsilon_{tr}t),$$

where  $\varepsilon_{tr}$  is the molar absorption coefficient at the absorption maxima of the traps.

Table 1 shows the rates of photooxygenation of the traps in carbon tetrachloride. It is seen that the continuous diode-array and pulsed forsterite lasers caused equal photooxygenation rates if the average power of forsterite laser was equal to the steady-state power of the diode laser. The photooxygenation rates linearly depended on the laser power and did not depend on the concentration of the traps within the indicated concentration range. The photooxygenation rates of tetracene and DPIBF were equal within the error of our experiments. The quencher  $\beta$ -carotene strongly inhibited the photoreactions. The inhibition efficiency of  $\beta$ -carotene coincided with the efficiency of singlet oxygen quenching (see Refs. [9, 10] for more details).

After 15 min purging with oxygen, a fivefold increase in the photoreaction rates was observed. This was directly proportional to the increase of the oxygen concentration in the solutions. In the oxygen-free solutions obtained after 15 min purging with argon, the photodestruction of the traps was not observed.

Figure 1 summarizes results of our measurements of the action spectrum of tetracene photooxygenation in CCl<sub>4</sub>, which were performed using the wavelength-tunable forsterite laser<sup>[9,10]</sup>. The maximum (1273 nm) and the half bandwidth of the action spectrum corresponded (within the range of the experimental error) to those of the absorption and phosphorescence spectra of the electronic  $^1\Delta_g \leftarrow ^3\Sigma_g^-$  transition in oxygen molecules.

Hence, the data indicate that in CCl<sub>4</sub>, the photooxygenation reactions are a result of excitation of oxygen molecules by laser radiation. As shown previously<sup>[10]</sup>, this mechanism leads to the following equations for the

photooxygenation rates:

$$V_r = k_{ox}I_{gen}\tau_{\Delta}[\text{Trap}]/(1 + k_{ox}\tau_{\Delta}[\text{Trap}]), \quad (1)$$

where  $k_{ox}$  is rate constant of the reaction of the traps with singlet oxygen,  $\tau_{\Delta}$  is the  $^1\text{O}_2$  lifetime in the neat solvents without traps and  $I_{gen}$  is the steady-state rate of  $^1\text{O}_2$  generation by laser radiation,

$$I_{gen} = 2.3I_{irr}\varepsilon_{1270}[\text{O}_2]l, \quad (2)$$

where  $I_{irr}$  is the intensity of laser irradiation at 1270 nm in einstein/(L·s),  $[\text{O}_2]$  is the molar concentration of oxygen in air-saturated solutions, and  $l$  is the optical path (1 cm). These equations allowed us to use the data of photochemical experiments for calculation of the molar absorption coefficient ( $\varepsilon_{1270}$ ) corresponding to the spectral maximum of the  $^1\Delta_g \leftarrow ^3\Sigma_g^-$  transition in oxygen molecules. In air-saturated CCl<sub>4</sub>,  $\varepsilon_{1270}$  was found to be  $0.003 \text{ (mol/L)}^{-1}\cdot\text{cm}^{-1}$ <sup>[10]</sup>. This value is 8 orders of magnitude less than the molar absorption coefficient corresponding to the Soret band of porphyrins.

Table 2 summarizes our data on solvent dependence of the rates of DPIBF photooxygenation upon irradiation by the diode GaAlAs laser. Equations (1) and (2) allow for calculation from these data of the relative values of  $\varepsilon_{1270}$  in different solvents. Though the precision of these calculations cannot be very high because many parameters are taken into account, the relative values of

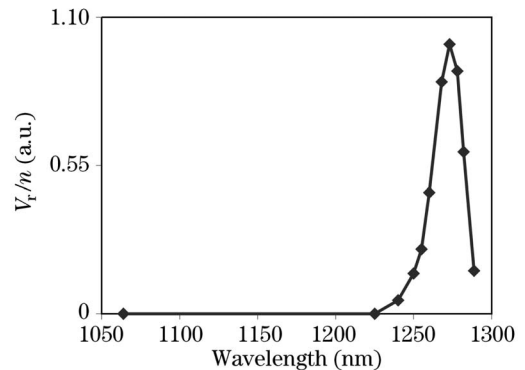


Fig. 1. The action spectrum of tetracene oxygenation in air-saturated carbon tetrachloride upon irradiation by the tunable forsterite laser.  $n$  is the number of photons of laser radiation.

**Table 2. Rates of DPIBF (40  $\mu\text{mol/L}$ ) Photobleaching in the Pigment-Free Air-Saturated Solvents upon Irradiation by the Diode GaAlAs Laser**

Solvents	$\text{CCl}_4$	$\text{C}_6\text{F}_6$	$\text{C}_6\text{H}_6$	Acetone	Ethanol	$\text{D}_2\text{O}^*$
$V_r$ (a.u., $\pm 10\%$ )	1	2.1	0.55	0.4	0.2	0.12
$\tau_{\Delta}^{**}$ ( $\mu\text{s}$ )	27000	15000	31	51	13	45
$[\text{O}_2]$ (mmol/L) <sup>[17]</sup>	2.6	4.4	1.9	2.4	1.65	0.29
$\varepsilon_{1270}$ (a.u.)	1	1.3	1.5	0.7	1.1	1.8
$k_r$ (a.u.) <sup>[13-15]***</sup>	1	1.4	1.6	0.6	0.5	0.2

\*Solutions contained also 0.2% emulgator Cremafore 6E for solubilization of DPIBF.

\*\* $\tau_{\Delta}$  were measured using our phosphorescence spectrometer with pulsed laser excitation<sup>[16]</sup>.

\*\*\* $k_r$  is the probability of the radiative deactivation of singlet oxygen,  $k_r = 1/\tau_r$ .

**Table 3. The Rates of Photooxygenation of Tetracene (40  $\mu\text{mol/L}$ ) upon Porphyrin-Photosensitized and Direct Oxygen Excitation in Air-Saturated  $\text{CCl}_4$** 

Photosensitizer	$\lambda_{\text{ex}}$ and $W^*$	$V_r$ ((mol/L)/s)	$V_r/W$ (a.u.)	$V_r/n_w$ (a.u.)
None	1267 nm, 50 mW	$5 \times 10^{-9}$	1	1
Porphyrin	545 nm, 0.45 mW	$1.2 \times 10^{-7}$	2700	6600

\* $\lambda_{\text{ex}}$  and  $W$  are the excitation wavelength and the irradiation power, respectively;  $n_w$  is the irradiation intensity in number of photons per second. Light absorption by porphyrin was 14% in this experiment.

$\varepsilon_{1270}$  in  $\text{CCl}_4$ ,  $\text{C}_6\text{F}_6$ ,  $\text{C}_6\text{H}_6$  and acetone apparently correlate with the radiative lifetimes ( $\tau_r$ ) for the  $^1\Delta_g \leftarrow ^3\Sigma_g^-$  transition calculated from the quantum yields of photosensitized  $^1\text{O}_2$  phosphorescence<sup>[13-15]</sup>. However, in ethanol and deuterium oxide, the effective values of  $\varepsilon_{1270}$  calculated by the above method are appreciably higher than  $\tau_r$  requires. At the same time, in ethanol and  $\text{D}_2\text{O}$ , a singlet oxygen quencher sodium azide inhibited the photoreactions and purging by pure oxygen increased the photoreaction rates<sup>[9]</sup>. It is noteworthy that the solvents of the first group show negligible absorbance at 1270 nm, while the absorbances of ethanol and  $\text{D}_2\text{O}$  at this wavelength are about 0.1 due to vibrational modes of the OH (OD) groups. In agreement with the mechanism claimed by Singh *et al.*<sup>[8]</sup>, one can propose that the energy transfer to oxygen from vibrationally excited molecules of ethanol and  $\text{D}_2\text{O}$  enhances the singlet oxygen production. Experiments are in progress to test this assumption.

Table 3 presents the oxygenation rates upon porphyrin-photosensitized and direct oxygen excitation. Protoporphyrin IX dimethyl ester was used as a photosensitizer. Monochromatic light from a xenon lamp (1 kW) was employed for porphyrin excitation. The monochromator slit bandwidth was 10 nm. The excitation wavelength (545 nm) corresponded to one of the absorption maxima of the porphyrin. The absorbance of the porphyrin solution at this wavelength was 0.065 in 1-cm cell. Hence, 14% exciting light was absorbed by the porphyrin. The table shows that in this experiment, the quantum efficiency of photosensitized reaction is higher by a factor of 6600 compared to the photooxygenation caused by direct oxygen excitation. Hence, to get the maximal quantum efficiency corresponding to 100% absorption of exciting light, one should multiply this number by 7 that leads to approximately 50000.

Thus, our data demonstrate that photooxygenation re-

actions can be observed due to both photosensitized and direct excitation of oxygen using the light sources of rather moderate power. The photosensitized photooxygenation is much more efficient than photooxygenation caused by direct oxygen excitation, though the difference is not as high as one can expect taking into account the great difference between the molar absorption coefficients of oxygen and porphyrin. The data suggest that in the hydroxyl-group containing solvents (ethanol and deuterium oxide), IR excitation of solvent molecules might appreciably contribute to the laser generation of singlet oxygen.

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