CARBON NANOTUBES QUENCH SINGLET OXYGEN GENERATED BY PHOTOSYNTHETIC REACTION CENTER PROTEINS

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1. Introduction

The primary events of photosynthesis take place in the reaction center protein (RC), where the energy of light is converted into chemical potential. Under conditions when the RC photochemistry is oversaturated (excess light and/or the photochemical processes are blocked) reactive oxygen species (ROS, including, e.g., singlet oxygen ($^1O_2$), superoxide anion (O_2^-), and hydroxyl radicals ($OH^\cdot$)) are formed with large probability[1,2].
There is a large interest to reduce the formation of the ROS components because they decrease the efficiency of the photochemical energy conversion, in addition, they react with the intracellular components resulting in their degradation (the RC itself as well).
Different mechanisms are developed in nature in order to decrease the ROS concentration, including specific enzyme reactions (e.g. peroxidases, superoxide dismutases) and/or decaying the concentration of long lived excited species (e.g. energy transfer from chlorophyll triplets to carotenoids, **Fig. 1**). Carbon nanotubes (CNT), in artificial systems, are also known to react with singlet oxygen[3].

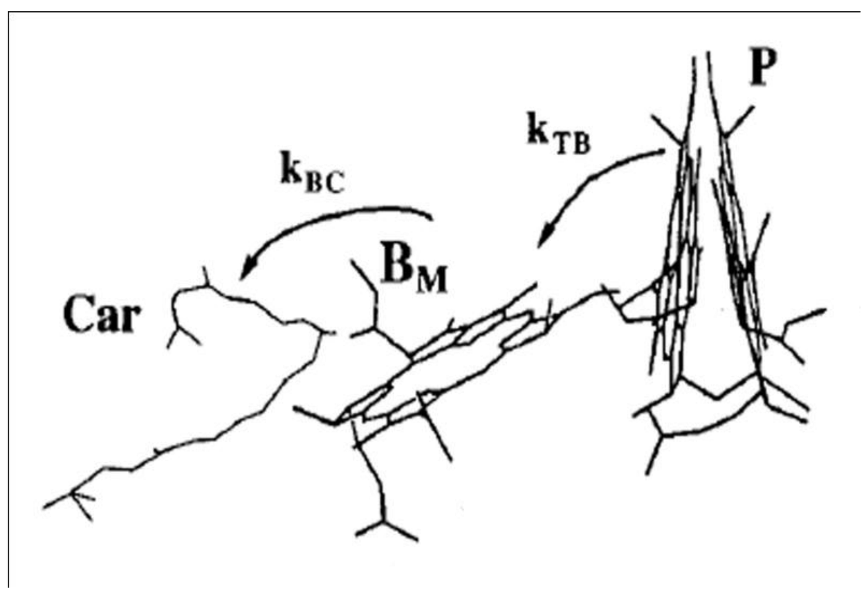

Fig. 1. Schematic representation of the triplet energy transfer from the excited primary electron donor (bacteriochlorophyl dimer, P) to the the carotenoid, Car) in wild type *Rhodobacter (Rb.) sphaeroides* purple bacterium. B_M: bacteriochlorophyll monomer; k_{TB} and k_{BC} are the rate constants for the triplet energy transfer.

In our work we used 1,3-diphenylisobenzofuran (DPBF), a dye that has a specific color reaction, to measure the singlet oxygen concentration after excitation a sensitizer (methylene blue) as a model system and photosynthetic reaction center in the absence or presence of carbon nanotubes. Our results indicate that the absorption change of the DPBF at 420 nm decreased in the presence of carbon nanotubes, indicating that carbon nanotubes are good quenchers of this ROS species.

2. Aims

According to recent publications[1,2] carotenoids has essential role in singlet oxygen quenching. Our aim is to show
- the possibility of using DPBF to indicate the change in the amount of $^1O_2$ generated by RC in our model systems;
- the difference of $^1O_2$ induction between the carotenoid containing wild type *Rb. sphaeroides* 2.4.1. and the carotenoidless mutant R-26 strains;
- the possibilities of quenching $^1O_2$ by CNT.

3. Experiments

Sample preparation
RCs of *Rb. sphaeroides* purble bacteria were prepared by detergent solubilization and purified by ammonium sulfate precipitation, followed by anion-exchange chromatography.

Measurements
The reaction solution containing DBPF, methylene blue and/or RC were illuminated with red light (λ>720 nm) in a 0.5 cm cuvette and whole absorption spectra were measured in every 15th seconds by a UNICAM UV-4 double-beam spectrophotometer. The reaction mixtures contained DPBF as reactant and methylene blue, RC or CNT in TRIS buffered (pH:8.00) detergent suspension according to the aim as indicated in the corresponding figures.

3. Results and discussions

3.1. Measuring $^1O_2$ quantity in model system

After the illumination and measuring the absorption spectra the logarithm of the absorption at 420 nm as compared to the untreated sample was plot as a function of the illumination time (dose-response curve, **Figs. 2,3**). Figures indicate that after illumination there is no change in the absorption of methylene blue (665 nm) while that of the DPBF decreases gradually.

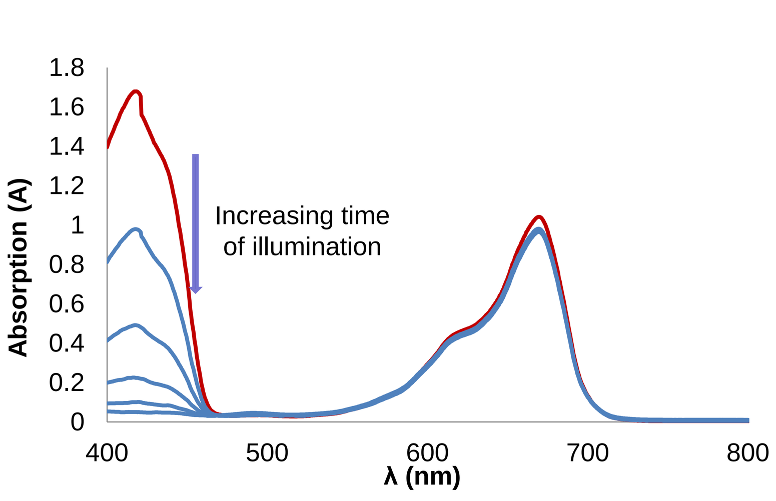

Fig. 2. The absorption spectra of the DBF and methylene blue solution after series of illumination. Arrow indicates increasing illumination times.

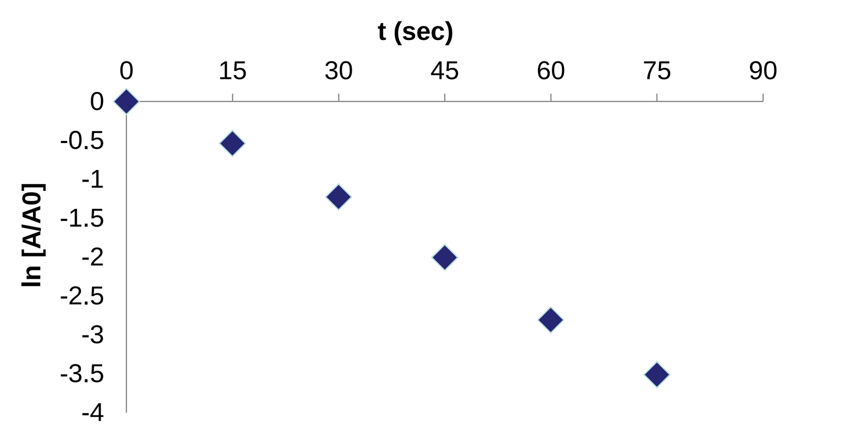

Fig. 3. The change in the DPF absorption at 420 nm as a function of time in a semilogarithmic representation. Data points are taken from spectra represented in Fig. 2.

3.2. Carbon nanotubes quench $^1O_2$ generated by methylene blue

Figure 4 indicates that addition of carbon nanotubes with increasing concentration quenches $^1O_2$ generated by methylene blue.

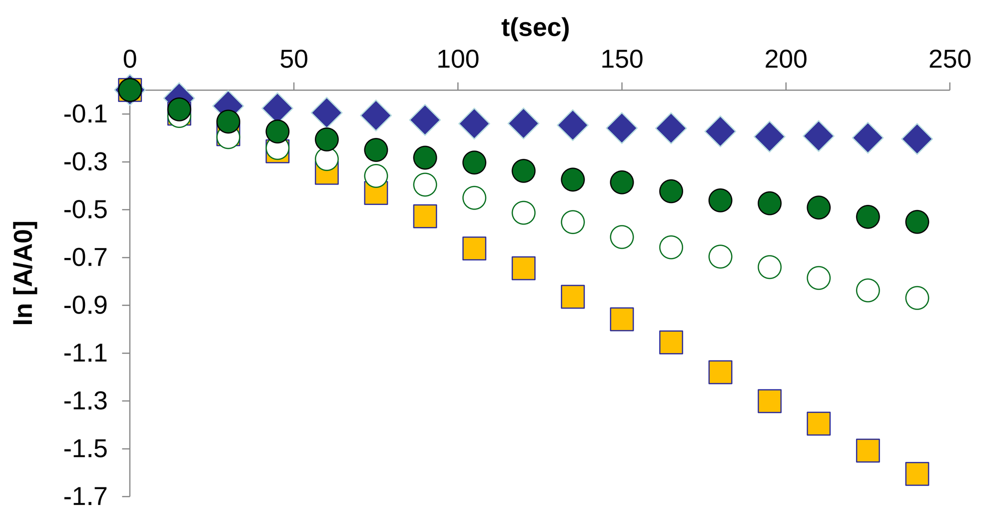

Figure 4.
♦ – Spontaneuos degradation of DPBF,
● – DPBF, methylene blue and CNT (7 μg/ml),
o – DPBF, methylene blue and CNT (14 μg/ml),
■ – DPBF and methylene blue.

3.3. Quenching $^1O_2$ by carbon nanotubes generated by photochemistry of reaction centers

DPBF does not affect on the RC photochemistry
In the first experiment we were curious weather the DPBF has an effect on the kinetics of electron transfer within the reaction center protein. The flash induced absorption change was measured after single saturating flash excitation in the presence and absence of this chemical. Figure 5 shows that there is no change in the RC kinetics, at least at the concentration we used.

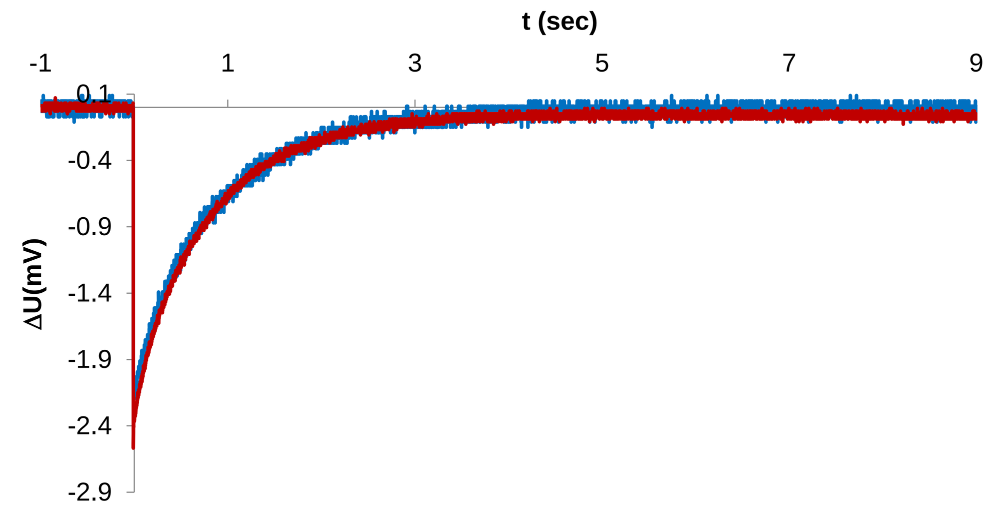

Figure 5. The flash induced absorption change of RC solution at 860 nm after single saturating flash excitation in the absence (blue line) and presence of DPBF (red line). Measurement was taken in detergent suspension.

Comparing the amount of singlet oxigen generated by RC R26 and 2.4.1.
Carotenoid containing 2.4.1 RCs produce less amount of $^1\mathbf{O_2}$ then R-26, as they have an efficient quenching mechanism (cf. Fig. 1). The dose-response curves show the difference between the two type RCs.

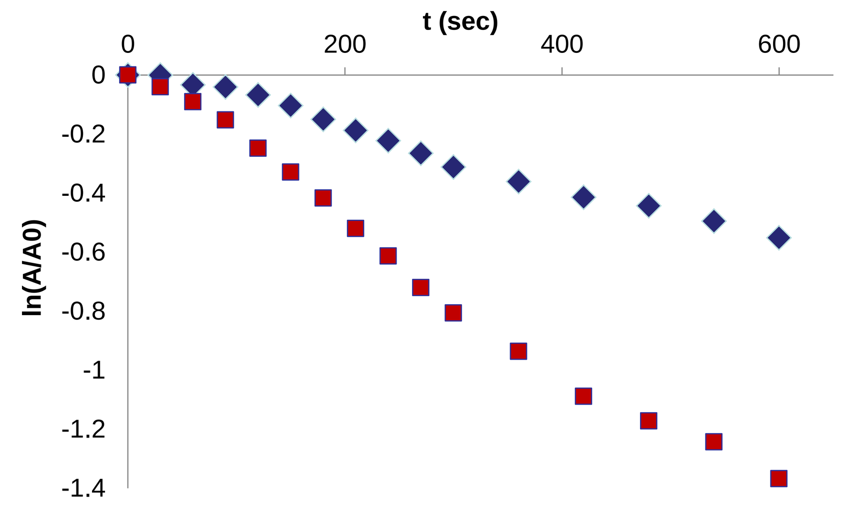

Figure 6. Dose-response curve of absorption change induced by DPBF- $^1O_2$ reaction in carotenoid containing (2.4.1.) and carotenoid less (R-26) RCs.
♦ – RC-241
■ – RC-R26

$^1O_2$ quenching effect of carbon nanotubes in RC solutions
Figure 7 shows that CNT quenches the amount of $^1O_2$ generated by the photochemistry of RCs purified from the carotenoid less R-26 strain.

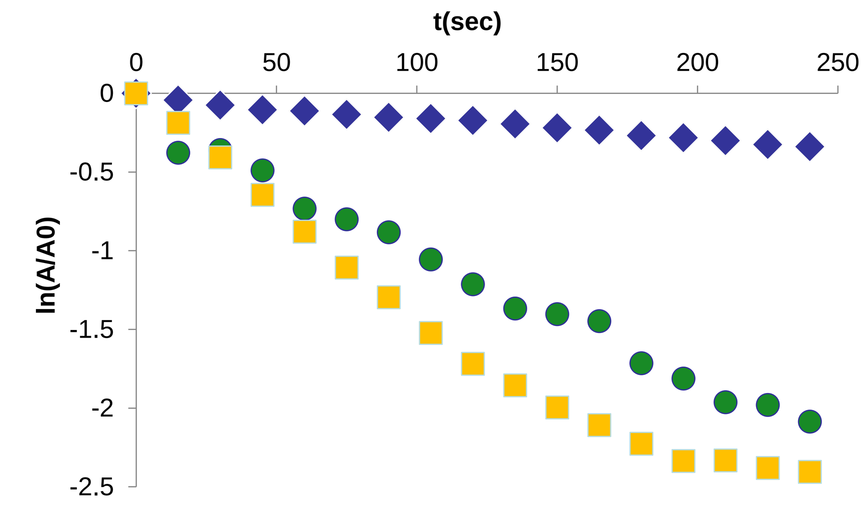

Figure 7. Dose-response curve of absorption change induced by DPBF- $^1O_2$ reaction in carotenoid less (R-26) RCs in the presence and absence of CNT.
♦ – Spontaneus degradation of DPBF
● – RC-R26 + CNT
■ – DPBF and RC-R26

4. Summary

By using the specific dye (DPBF) we proved the difference of $^1O_2$ producing potential between carotenoid containing RC-241 and carotenoidless RC-R26.
We showed that carbon nanotubes are able to quench $^1O_2$ either in model system containing methylene blue or in the photochemistry of RCs.

Acknowledgement
This work was supported by grants from Switzerland through the Swiss National Science Foundation (IZ73Z0_128037/1) and Swiss Contribution (SH/7/2/20) and by the Hungarian OTKA (K81180 and K84133) .

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