LIGHT INDUCED SINGLET OXYGEN PRODUCTION AND DETECTION GENERATED BY BACTERIAL REACTION CENTRE-CARBON NANOTUBES COMPOSITES

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Abstract

Nowadays the photoelectric energy transformation is one of the most promising alternative energy sources. Plants and bacteria have their unique apparatus,the photosynthetic reaction centreprotein (RC)to convert the light into chemical potential. Generation of singlet oxygen accompanying the photochemistry of isolated reaction centre protein of non-sulfur purple bacteria(*Rhodobacter(Rb.) sphaeroides*) have been studied by measuring oxygen uptake by conventional oxygen electrode. In case of oversaturated illuminating conditionsbesides the RC photochemistry the excess energy is captured by triplet states of chlorophyll molecules. The triplet energyisthen dissipated by heat or by sensitizingthe formation of various reactive oxygen species (ROS), especially by singlet oxygen ($^1O_2$). Under these conditions the protein subunits are damaged and the efficiency of photochemistry decreases. The aims of our work are to find conditions in which the concentration of these harmful compounds can be reduced. The possible role of carbon nanotubes (CNT), which are known to quench the singlet oxygen is investigated in the CNT/RC hybrid bio-nanocomposites.

Introduction

During photosynthesis, the light energy is converted into chemical potential in the reaction centre protein (RC) with extremely high efficacy. Under conditions of excess light and/or when 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 inside the complex with large probability.[1,2]ROS components can decrease the efficiency of the photochemical energy conversion, e.g. by reacting with the intracellular components resulting in their degradation (the RC itself as well).

Different mechanisms are developed in nature in order to reduce 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). Carbon nanotubes (CNT), in artificial systems, are also known to react with singlet oxygen.[3]

Experimental

Carotenoid-less*Rb. sphaeroides* R-26 purple bacterial cells were grown photoheterotrophically. RCs were prepared bydetergent (LDAO:N,Ndimethyldodecylamine-N-oxide, Fluka)solubilization and purified by fractionalized ammonium sulfate precipitation, followed by DEAE Sephacel (Sigma)anion-exchange chromatography.

The photochemical reactions were carried out in a solution that contained 1,3-diphenylisobenzofuran (DPBP), and RC or CNT/RC composite in a 0.5 cm spectroscopic cuvette that was illuminated with 808 nm continuous wave diode laser (DL) light and whole

absorption spectra were measured in every fifteenth seconds by an AVANTES CCD fibre optic spectrophotometer. The reaction mixtures contained DPBF and RC or CNT/RC composite in TRIS buffered (10 mM TRIS, 100 mMNaCl, 0, 03% LDAO, pH:8.0) detergent suspension.

Singlet oxygen concentration was also determined by measuring oxygen uptake with a Clark type electrode (1µM RC; 250 mM histidine; phosphate buffer pH 7; 30 °C; light intensity 500 µE).[4] The effect of carbon nanotubes was detected in blend samples and also after immobilizing the RC on the CNT surface with physical sorption – and chemical binding as well.

Results and discussion

Effect of carbon nanotubes on the equilibrium concentration of $^1O_2$ in RC solutions was determined in two different ways. One method was the use of ROS sensitive DPBF die that has a characteristic spectral change after reacting with singlet oxygen. The other method was the measure of oxygen uptake directly by a Clark type oxygen electrode in the presence of histidine.

Singlet oxygen produced by RC could be detected with the use of DPBF. Figure 1 shows the bleaching of the absorption peak of DPBF at 420 nm in the function of the illumination time. Carotenoid-less R-26 reaction centre wasbound to functionalized and non-functionalized single-walled (SWCNT) and multi-walled (MWCNT) carbon nanotubes with different binding methods indicated on the graph.

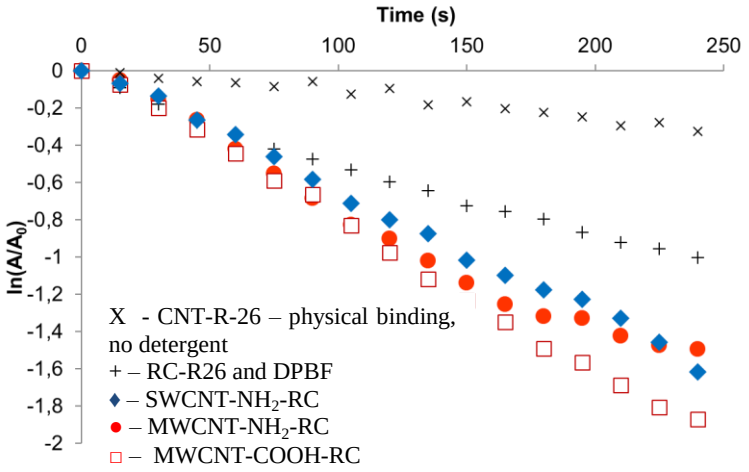

Figure 1. Dose-response curve of absorption change induced by DPBF- $^1O_2$ reaction in carotenoid less (R-26) RCs and CNT/RC suspensionsafter different binding procedure on the surface of amine – and carboxyl functionalized single – and multiwalled carbon nanotubes (SWCNT-NH_2, MWCNT-NH_2, MWCNT-COOH)

On Figure 2 oxygen uptakemeasured with Clark type electrode is shown in the presence of histidine. RCs purified from the carotenoid-less mutant R-26 bacterial strain was used with constant, 1 µM concentration. Carboxyl-functionalised multiwalled carbon nanotubes were applied. Measurements were done using different reaction centre/multi-walled carbon nanotube volume ratios.Three different MWCNT/RC suspensions were prepared.First, RC and MWCNT blend samples, second, RC was bound on the MWCNT with physical sorption and third, RC was bound chemically through the carboxyl-groups of the tubes with the use of crosslinker molecules.Blend samples were measured with four different CNT concentrations and composites were made with two different MWCNT ratios with physical sorption and chemical binding method as well.

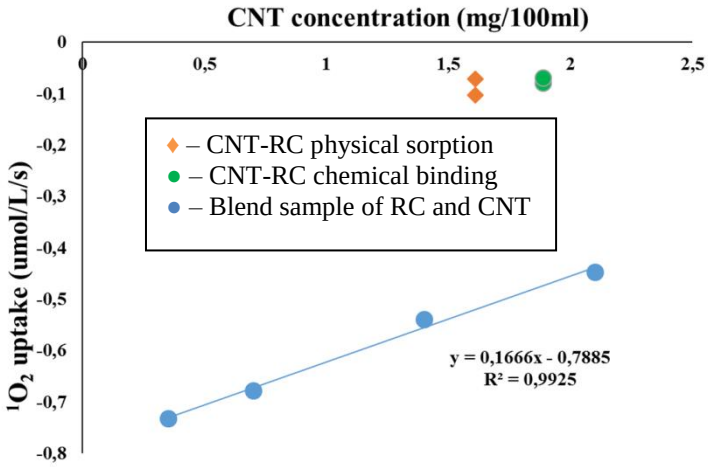

Figure 2. Oxygen uptake in Clark type electrode in the function of MWCNT concentration with three different MWCNT/RC suspensions(Blend samples; MWCNT/RC after physical sorption and MWCNT/RC after chemisorption).[RC]=1 μM.

Conclusion

According to our results with both measuring techniques, MWCNT is able to quench singlet oxygen with bigger efficiency after physical sorption of the RC on the nanotube's surface, than after chemical binding or in the case of the blend samples. This can probably be explained with the smaller diffusion distance between the peptide complexes and the CNTs.
Under our present experimental conditions, we do not see difference in the equilibrium concentration of $^1O_2$ in SWCNT/RC and MWCNT/RC complexes, however, we do see the effect of functionalization. -COOH functionalization shows slightly larger $^1O_2$ production (in line with slightly lower photoactivity of this sample, cf. Fig. 1).

Acknowledgements

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