Bio-Nanocomposites - Optoelectronics, Energy Conversion, Biosensors

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Abstract
Biological materials are developed by Nature for extremely efficient, specific and sensitive functions. There are more and more demonstrations recently that it is possible to attach them to nanomaterials combining the advantageous properties of both components. These new types of materials, called *bio-nanocomposites,* open possible directions for new generations of practical applications, e.g. energy conversion and storage, integrated optoelectronics in memory and micro imaging, or sensitive components of analytical (biosensor) devices, etc. In our laboratory recently we have successfully bound representatives of redox proteins (photosynthetic reaction centers and horse radish peroxidase) to inorganic nanostructures and the optoelectronic properties and possible directions of applications are investigated. Examples for applications in environmental field are presented here.

Introduction
There are many examples that biological materials are used in technical applications (e.g., environmentally friendly biodegradable items and biosensor devices) and vice versa, technical developments (e.g., actuators, artificial tissues, and drug delivery systems) are used in biological systems [1-3]. Because biological materials are developed by Nature for extremely efficient, specific and sensitive functions the use of their exceptional capacity would be beneficial in hybrid systems combing them with inorganic materials. The bio-nanocomposites are of special interest because of their combined advantageous properties and the possibility of the appearance of some new characteristics. Combining different materials (organic or inorganic as well) such substances can be created that pose not only the advantageous properties of the used materials but also new, attractive features can appear. This way there is a good chance to design and tune precisely the characteristics of substrates we need for certain application.
Different biological materials like drugs [4-5], peptides [6], proteins [7], and nucleic acids [8] can be used in combination in nano-hybrid systems as targeted accumulations in vitro and in vivo, e.g. for cancer treatments. Specific biological recognitions are suitable for antibacterial and antiviral targeting by nanomaterials decorated by e.g. antibiotics or antibodies. The unique optical properties of carbon nanotubes can be used for biological imaging [9].
There are many potential applications of bionanocomposite materials in new generations of devices such as biosensors, integrated (opto)electronic devices (switches or converters), photoelectric energy conversion, and (single-molecule) imaging [2,10].
In our laboratory we bound two representatives of redox active proteins (photosynthetic reaction center (RC) and horse radish peroxidase enzyme (HRP)) successfully to various inorganic carrier matrices. Functionalized and non-functionalized single (SWCNT) and multi walled (MWCNT) carbon nanotubes [11], carbon nanotube bundles [12], indium tin oxide

(ITO) [13], porous silicon (PSi) [14] and conducting polymers [15] were used and the functional activities of these samples were demonstrated.

Aims

The aim of our work is to create functional bio-nanocomposite materials from nano-structured carriers and RC or HRP enzyme proteins. This work requires a continuous design of a measuring system to investigate the basic characteristic (optical characteristics, electric conductivity, redox properties, etc.) of our nanosystems. We would like to design a model or a prototype of a device for possible future application (integrated optical, electric conductance, photocurrent, imaging, biosensors, etc.). There are auxiliary processes providing connecting points for other projects, e.g., using other relevant redox proteins and matrices, or phenomena, which can be investigated.

Materials and methods

Biological samples RCs were prepared routinely from *Rhodobacter* (*Rb.) sphaeroides purple* bacterial strains by standard protein purification procedures. HRP was obtained from Sigma. These proteins were bound to different carrier matrices by different methods (physical and chemical binding), which is also routine in our laboratory.

Inorganic arrier matrices Functionalized and non-functionalized single (SWCNT) and multi walled (MWCNT) carbon nanotubes, carbon nanotube bundles were obtained from our collaboration partners (Prof. Klára Hernádi, SZTE AKKT, and Prof. László Forró (EPFL, Switzerland, Lausanne). ITO was purchased from Precision Glass (Germany), porous silicon (PSi) was obtained from Vivechana Agarwal and Gabriela Palestino (Mexico). Conducting polymers were prepared in collaboration with Prof. Csaba Visy (SZTE DPCMC).

Experimental methods Steady state and kinetic sbsorption spetra were measured by commercial bench top (Unicam) and home built spectrophotometers [16], respectively. Composite electrode samples were prepared and photocurrents were measured by a Metrohm PGSTAT204 potentiostat/galvanostate. Light induced conductivity changes were measured by a programmable Keithley 2400 multimeter.

Results and discussions

RC-nanostructures

We already have shown that the integral membrane protein, the photosynthetic reaction center, could be attached to single-walled carbon nanotubes with the apparent binding to specific site(s). Evidence was given that one of the effects of carbon tubes binding included the stabilization of light-induced charge(s) in photosynthetic energy conversion. The attachment of nanotubes increased the lifetime of the $P^+Q_B^-$ state within the protein , probably due to the slow charge recombination between the oxidized primary electron donor, P^+, and negative semiquinone forms, Q_A- and/or Q_B^-, the reduced primary and secondary electron acceptor quinones within the protein, respectively. After the excitation, the intraprotein charge movements are followed by slower reorganization of the protein structure (cf. Figure 1).

Specific interactions between the CNTs and RCs can be investigated if specialized chemical (covalent) binding are achieved by several strategies [17]. Common or engineered functional groups (like amine, carboxyl or sulfhydryl) or specialized cross linkers can be used. Several homo- (e.g., glutaraldehyde) and heterobifunctional (e.g., sulfo-SMCC, *N*-(1-Pyrene)iodoacetamide) cross-linkers can be used to bind specific and nonspecific sites of the RCs to different carrier matrices.

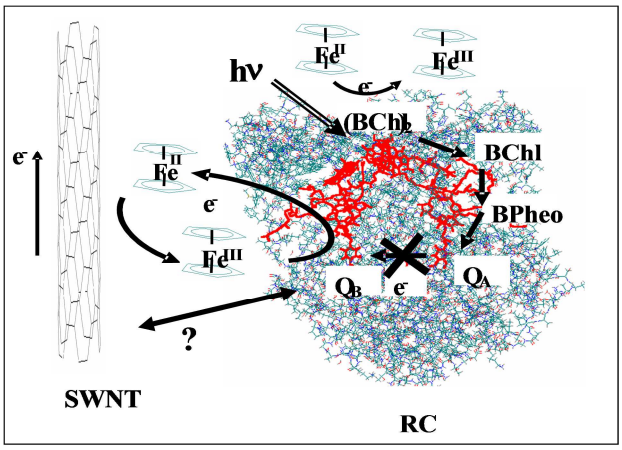

Figure 1. Schematic presentation of the positions of cofactors, and the path of the electron flow in the RC protein and of the possible interaction between the carbon nanotube and the RC. $(BChl)_2$: primary electron donor bacteriochlorophyll dimer; BChl: bacteriochlorophyll monomer; BPheo: bacteriopheophytine; Q_A: primary quinone; Q_B: secondary quinone. Black arrows: the electron transport route. X indicates theelectron transport step, which is blocked by the inhibitor, terbutryn. Fe^{II} and Fe^{III} are reduced and oxidized ferrocene, respectively [11].

A promising approach is to bind the RCs to ITO (a transparent conducting electrode) in a photo-electrochemical cell through conducting polymer (Poly(3,4-ethylenedioxythiophene) (PEDOT), Poly[bis(4-phenyl)(2,4,6-trimethylphenyl) amine] (PTAA)). The conducting polymer connects larger amount of RCs and wires electrons to the working electrode. The RC/PTAA/CNT complex showed photochemical activity as indicated by flash photolysis experiments and a photocurrent was measured which was sensitive to the electron transport mediator applied (externally added electron donor/acceptor or specific inhibitor (like terbutryn).

Photosynthetic RC proteins offer unique applications, for example, their use in the nanostructures or in the optoelectronic systems. In these systems the electron – arising from charge separation – is trapped in the redox components of the RC or its molecular environment and, among other things, can participate in electric circuits. The fabrication of systems for efficient light-energy conversion (e.g., photovoltaics), integrated optoelectronic systems or biosensors (e.g., for specific detection of pesticides) can be visualized for the near future.

Measuring light-induced change in the current (called photocurrent) in an electrochemical cell is an elegant demonstration of the suitability of the photosynthetic systems for photovoltaics, or other practical applications in optoelectronics (e.g. for sensing elements for specific compounds, like pesticides). Two of our RC based composites were successfully tested and found to be active in electrochemical cells. It has been demonstrated that continuous redox turnover of nanocomposite prepared from PTAA/MWCNT and RCs bound to ITO can be driven by light if quinone is added to the solution for mediating the electron transport between the working and the counter electrode.

Real time sensing of H_2O_2 by HRP biosensor

The accurate and sensitive determination of H_2O_2 is very important in laboratory experiences because it is a product of reactions catalysed by several oxidase enzymes in living cells and it is essential in environmental and pharmaceutical analyses. The fabrication of a device with the sensing component of enzyme protein based (bio-)sensors is a promising way for this purpose because the function of biological molecules is very specific, sensitive and selective. HRP is the most commonly used enzyme in H_2O_2 detection because it can oxidize xenobiotics in the presence of H_2O_2. In order to define the limit of detection (LOD) of H_2O_2 we made calibrations with guaiacol and amplex red (AR), which chemicals are substrates of HRP. The accumulation of the reaction products, tetraguaiacol and resorufin, then can easily be detected by absorption or emission spectroscopy. In our experiments an enzyme electrode was fabricated from ITO, MWCNT and HRP. LOD was calculated in both cases. The H_2O_2

decomposition was about 0.15 nM H_2O_2/sec in the case of guaiacol and 0.075 nM H_2O_2/sec in the case of AR. Our measurements indicated that the enzyme activity yields 122.8 M [H_2O_2]/(M [HRP] · sec) with guaiacol and 49.5 M [H_2O_2]/(M [HRP] · sec) with AR [18].

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