

analysis of complex biological structures as compared to isotropic CD.

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Conformation changes after primary charge separation in photosynthetic reaction centers

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Light induced conformation changes of quinone depleted photosynthetic reaction centers (RCs) purified from the carotenoid-less *Rhodobacter sphaeroides* R-26 were investigated by transient absorption (TA) and grating (TG) methods. Surprisingly, the decay of the TA signal measured at 860 nm was divided into a 15 ns and a 40 μ s components. The latter coincides with the life time of the TG signal, which was assigned earlier [Nagy et al. (2008) FEBS Lett. 582, 3657-3662] to spectrally silent conformational changes. The nature of the 40 μ s phase was investigated further. Although, the probability of chlorophyll triplet formation under our measuring conditions was small, possible contribution of the triplet states was also studied. The presence of carotenoid in the wild type RCs eliminated the 40 μ s component indicating the role of carotenoid in the energy transfer within the RCs. There was no significant effect of the molecular oxygen on the TA. This fact may be explained if the chlorophyll triplets inside the protein have reduced accessibility to molecular oxygen. A differential effect of osmotic potential and viscosity on conformation changes accompanying the primary charge separation was measured by the effect of ficoll, glucose and glycerol as compared the TA to the TG signals.

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Variable chlorophyll fluorescence: in part a yield change due to light-induced conformational change

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On a dark-to-light transition the chlorophyll fluorescence rises from a minimum intensity (F_0) to a maximum intensity (F_M). Conventionally, this rise is interpreted to arise from the reduction of the primary quinone acceptor, Q_A , of photosystem II - although this cannot explain all presently available observations. In untreated leaves, at room temperature, the fluorescence rise follows the reduction of the electron transport chain (ETC). Once induced, ~30-40 % of the variable fluorescence intensity relaxes within 100 ms in darkness and can be re-induced within 3 ms as long as the ETC remains reduced. Analyzing the fluorescence relaxation kinetics, +/- DCMU, ~30% of the amplitude cannot be explained by Q_A

re-oxidation. Special properties of this phase determined on DCMU-inhibited samples: at cryogenic temperatures (below -40°C), where the Q_A/S_2 recombination is blocked, it still relaxes and it exhibits a strong temperature dependence with an apparent $E_a \approx 12$ kJ/mol, whereas the reduction of Q_A is nearly temperature insensitive. A fluorescence yield change, driven by light-induced conformational change in the reaction center complex, can explain all these observations.

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Tuning function in bacterial light-harvesting complexes

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Purple photosynthetic bacteria are able to synthesize a variety of different light-harvesting complexes, sometimes referred to as LH2, LH3 and LH4. Here we have investigated the structural origins of these different forms and the manner in which the sequence tunes the absorption spectrum of the light-harvesting system. We then consider the functional consequences of this tuning for the organization of the light harvesting system and on the ecology of the organisms. Specifically by spectroscopic techniques, in particular circular dichroism and resonance Raman spectroscopy, we have been able to obtain information on the organization of the bacteriochlorophyll binding sites in the unusual LH4 of *Roseobacter denitrificans*. This provides a picture of how different peripheral light-harvesting complexes are able to modulate the absorption spectrum. The structure and organization of this complex is the put in the context of the the recently published variability of the light-harvesting complexes. In particular the observation of their ability to form mixed complexes containing different polypeptides. We examine quantitatively the possible reasons for maintaining such variability by considering the transport properties of membranes containing either pure or mixed complexes and show that mixed complexes can permit light-harvesting to continue during adaptation. We then consider the different constraints that may be behind this type of adaptation in different bacteria and the conditions under which different types of antenna system might be optimal. Finally we integrate this into the evolutionary context of adaptation to variable light intensity and the ecological niches where such organisms are found.

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Interaction between photosynthetic reaction centers and ITO

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Photosynthetic reaction center proteins (RC) purified from *Rhodobacter sphaeroides* purple bacterium were deposited on the surface of indium-tin-oxide (ITO), a transparent conductive

oxide, and the photochemical/physical properties of the composite was investigated. The kinetics of the light induced absorption change indicated that the RC was still active in the composite and there was an interaction between the protein cofactors and the ITO. The electrochromic response of the bacteriopheophytin absorption at 771 nm showed an increased electric field perturbation around this chromophore on the surface of ITO compared to the one measured in solution. This absorption change is associated with the charge-compensating relaxation events inside the protein. Similar life time, but smaller magnitude of this absorption change was measured on the surface of borosilicate glass. The light induced change in the conductivity of the composite as a function of the concentration showed the typical sigmoid saturation characteristics unlike if the chlorophyll was layered on the ITO which compound is photochemically inactive. In this later case the light induced change in the conductivity was oppositely proportional to the chlorophyll concentration due to the thermal dissipation of the excitation energy.

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The supramolecular organization of photosystem II in vivo studied by circular dichroism spectroscopy

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The light reactions of photosynthesis in higher plants take place in granal chloroplast thylakoid membranes, which contain chirally organized macrodomains composed of photosystem II (PSII) supercomplexes associated with light harvesting antenna complexes (LHCII). The physiological relevance of this hierarchic organization, which often manifest itself in semicrystalline assemblies, has not been elucidated but the diversity of the supramolecular structures and their reorganizations under different conditions indicates its regulatory role. The present work focuses on the structural and functional roles of different components of LHCII-PSII supercomplexes. We used various growth conditions, influencing the protein composition, and different Arabidopsis mutants (KoCP24, KoCP26, KoPsbW, KoPsbX, Dgd1), with altered organization of the membranes, and measured their circular dichroism (CD) spectra as well as their chlorophyll fluorescence kinetics to characterize the chiral macro-organization of the chromophores and the functional parameters of the membranes, respectively. We show that the formation of chiral macrodomains require the presence of supercomplexes. Our data also reveal specific functions of some of the protein or lipid compounds in the light adaptation processes of plants.

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Excitation energy transfer and non-photochemical quenching in photosynthesis

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The success of photosynthesis relies on two ultrafast processes: excitation energy transfer in the light-harvesting antenna followed by charge separation in the reaction center. LHCII, the peripheral light-harvesting complex of Photosystem II, plays a major role. At the same time, the same light-harvesting system can be 'switched' into a quenching state, which effectively protects the reaction center of Photosystem II from over-excitation and photo-damage. In this talk I will demonstrate how LHCII collects and transfers excitation energy. Using single molecule spectroscopy we have discovered how LHCII can switch between this light-harvesting state, a quenched state and a red-shifted state. We show that the switching properties between the light-harvesting state and the quenched state depend strongly on the environmental conditions, where the quenched state is favoured under 'NPQ-like' conditions. It is argued that this is the mechanism of non-photochemical quenching in plants.

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Photobiology in the soil: arrested chlorophyll biosynthesis in pea epicotyl sections

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The key regulatory step of chlorophyll (Chl) biosynthesis is the NADPH:protochlorophyllide oxidoreductase (POR) catalyzed reduction of protochlorophyllide (Pchl_{ide}) which is light activated in angiosperms. This process is usually described on artificially dark-grown plants.

In this work, we studied epicotyl segments developed under the soil surface, which were dissected from pea plants grown under natural light conditions. Using 77 K fluorescence spectroscopy, pigment analyses, electron microscopy and fluorescence microscopy, we found that upper segments showed transitional developmental stages, i.e. Chl appeared besides Pchl_{ide} and etio-chloroplasts were typical. In regions under 5 cm depth, however, the characteristics of the segments were similar to those of plants germinated artificially in complete darkness, i.e. only Pchl_{ide} and etioplasts were present.

The results of this work prove that these latter symptoms may occur in shaded tissues of fully developed, photosynthetically active plants grown under natural conditions.