P–E3–17

SUBPICOSECOND SPECTROSCOPY OF THE PHOTOACTIVE YELLOW PROTEIN

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Purpose: Elucidation of the primary steps of the photocycle of the photoactive yellow protein from *Ectothiorhodospira halophila*, containing a new chromophore: thiol ester-linked p-coumaric acid.

Methods: Using a laser system of <200 fs pulse duration, based on a Ti:S pumped optical parametric generator and a white light continuum generator, transient absorption was measured at 20 wavelengths (from 430-550 nm) with excitation at 400 and 460 nm. The data were globally analysed.

Results: A satisfactory fit required three components. Next to a non-decaying component, the lifetimes were 0.61 and 2.5 ps (400 nm excitation) and 0.78 and 3.8 ps (460 nm excitation). All Decay Associated Spectra (DAS) showed bleaching and absorption. In addition, the first and second DAS exhibited stimulated emission.

Conclusions: The first intermediate state of the PYP photocycle is formed in ~0.6 ps and has a lifetime of ~3 ps. Despite the different chromophores, obtained lifetimes strikingly resemble those of early intermediates of bacteriorhodopsin, appearing due to chromophore photoisomerization.

P–E3–18

EARLY CHARGE SEPARATION IN MODIFIED BACTERIORHODOPSINS

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The primary events of the bacteriorhodopsin (bR) photocycle, studied in previous ultrafast absorption kinetic experiments, can be summarised as follows:

$$bR_{570} + h\upsilon \longrightarrow bR^{*}_{trans} \xrightarrow{100\text{–}200\,fs} bR^{*}_{cis} \xrightarrow{300\text{–}500\,fs} J_{625} \xrightarrow{3\text{–}5\,ps} K_{590} \cdots ,.$$

The time resolution (~3 ps) presently available for protein electric response signal (PERS) of bR is not enough to assign its early components to this scheme.

Here we report ultrafast PERS data on
(i) bR mutants with considerably prolonged lifetime of the excited state and
(ii) bR samples of artificial retinal pigment with blocked trans-cis isomerization.

P–E3–19

STRUCTURE AND REACTIONS OF THE Cu_A CENTER OF SUBUNIT II FROM CYTOCHROME ba_3 OF *Thermus thermophilus*

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Purpose: To investigate the relation between the geometric and electronic structures of the binuclear center of Cu_A in cytochrome oxidase and to study the effect of Hg(II) on the metal center.

Methods: S-, X- and Q-band EPR was used combined with simulations and CNDO/S calculations on a 109-atom system.

Results and conclusions: The two Cu ions were nearly identical with $g_x = 1.996$, $g_y = 2.011$, $g_z = 2.187$, $|A_x|$~15 G, $|A_y| = 29$ G and $|A_z| = 28.5$ G. The linewidth was M_I-dependent in a manner suggesting a large anisotrpy in the A-tensor. Theoretical CNDO/S calculations, based on the X-ray structure of the *Paracoccus denitrificans* enzyme, yielded a singly occupied antibonding orbital. Each Cu is π^*-bonded to one S and σ^*-bonded to the other. Hg(II) was found to extrude one of the Cu ions, leaving the other in the reduced state.

P–E3–20

INTERNAL ELECTRON TRANSFER IN CYTOCHROME *C* OXIDASE DURING REDUCTION

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Purpose: Full description of the spectral and redox behavior of heme a_A (Fe_A) and heme a_B (Fe_B) that deserves mechanistic analyses.
Methods: Cytochrome *c* oxidase purified from bovine heart muscle was reduced with sodium dithionite under anaerobic conditions in a stopped-flow rapid-scan apparatus. The spectral changes recorded for 30 min were subjected to data analyses by global fitting to derive kinetic parameters.
Results: Based on a reaction model consisting of 11 species with specific spectral features the observed spectral changes were reproduced satisfactorily, thus rigorous analyses of the reaction mechanism being enabled.
Conclusions: The redox potential of Fe_A is higher than that of Fe_B by 80 mV. This relationship and the intrinsic absorption spectra of Fe_A and Fe_B in both oxidized and reduced states are unchanged throughout the reduction process. The redox potentials of both Fe_A and Fe_B are independent of the redox states of Cu_B. Thus any assumption like a 'neoclassical model' is needed to explain rather complicated spectral changes obtained here.