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C. SYBESMA

Biophysics Laboratory
Vrije Universiteit Brussel
B-1050 Brussels
Belgium

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4. Photosynthetic Electron Transport I: Plants and Algae

POLARIZED TIME-RESOLVED FLUORESCENCE OF C-PHYCOCYANIN FROM MASTIGOCLADUS LAMINOSUS.

W. John[o], H. Scheer[o], P. Hefferle[+], S. Schneider[+]
[o]Botanisches Institut der Universität München, FRG
[+]Institut für Physikalische und Theoretische Chemie,
Technische Universität München, Garching, FRG.

Introduction: Phycobiliproteins are photosynthetic light-harvesting pigments in blue-green and red algae, where they are organized - together with uncolored peptides - into complex structures, the phycobilisomes, attached to the outer thylakoid surface. Within the phycobilisome the energy is probably transferred by the Förster mechanism, but the details are yet insufficiently understood. In an approach complementary to the investigation of phycobilisomes and large fractions thereof, we are currently studying isolated phycobiliproteins and their aggregates by time-resolved fluorescence spectroscopy. The expected understanding of their chromophore-protein and chromophore-chromophore interactions should give useful information on the energy transfer in the more complex systems. Since fluorescence is one of the most sensitive indicators for any conformational changes of the chromophore, it can also be used to study the (partial) denaturation of biliproteins. Here we wish to report on the fluorescence of C-Phycocyanin and its subunits from Mastigocladus laminosus.

Biliproteins: M. laminosus was grown on Castenholz medium and harvested after 3 days. C-Phycocyanin was isolated with minor modifications as described earlier (1). The subunits were separated by preparative electrofocusing on Sephadex G 75 in 8M urea on a Servalyt gradient, pH 4-9. The α und ß subunits were eluted and renatured on a Biogel P6 column without delay. Phycocyanin aggregates (trimers = $(\alpha,ß)_3$) were dissociated with NaSCN(1M) to monomers (α,ß). The sedimentation coefficients of the samples (extrapolated to t = 0), are shown in table 1. The molecular weights and sedimentation coefficients of the dissociated pigments correspond to the (α,ß) monomer, those of the aggregates are between di- and trimers (shape, partial dissociation?) and have been interpreted as trimers $(\alpha,ß)_3$ from comparison with earlier results of other laboratories. All measurements were done in potassium phosphate buffer (80 mM, pH 6.0).

Measurement and Data Analysis: The fluorescence decay kinetics were measured with a synchronously pumped mode-locked dye laser (rhodamine 6G, 80 MHz repetition rate, pulse width $\lesssim$ 2psec) and a repetitively working streak camera (Hadland Photonics). The time resolution of this system is appx. 25 psec without deconvolution procedure. The apparatus allows measurements with small excitation intensities (10^{13} photons per pulse and cm^2) with good signal to noise ratio due to the high repetition rate. Polarizers were placed in the excitation and emission path for polarized measurements.

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From the separately recorded polarized fluorescence decay curves $I_{\perp}(t)$ and $I_{//}(t)$, the functions $I_0(t) = I_{//}(t)+2\cdot I_{\perp}(t)$ and $D(t) = I_{//}(t)-I_{\perp}(t)$ are calculated (dotted curves in the figures). The best fits for both functions ($I_0(t)$ and $D(t)$) are determined under the assumption of a biexponential response function by means of a Marquardt-Algorithm. The fit curves as well as their decay times and relative amplitudes are shown in the figures.

Results and Discussion:

Rather complex decay laws can be expected to describe the fluorescence decay curves in view of the composite nature of the systems under study. The presently available S/N ratio and dynamic range does not allow a distinction between the predictions of the various models. Although it is found that all decay curves can be fitted well as convolutions of a biexponential, the rate constants presented in this paper must still be considered as purely descriptive. (table 1)
The calculated isotropic fluorescence decay ($I_0(t)$) agrees well with the experimentally observed decay when the polarizer is set at the magic angle. The anisotropic decay components ($D(t)$) are principally shorter lived, and their fast decaying contribution is more pronounced than in the isotropic decay. Depolarization by rotation of the entire protein can be excluded with the molecular weight being in the range between 18 (α-subunit) and 120 kDalton (trimer $(\alpha,\beta)_3$). Local motions of the chromophore region are also unlikely, because the chromophores are believed to be held rigidly by the protein, and large angular motions would be required for the observed depolarization. The alternative depolarization mechanism is then energy transfer among neighbouring, nonparallel chromophores, which has already been studied by several groups by static polarized fluorescence measurements of biliproteins[2]. We relate therefore the fast decaying component of the fluorescence to energy transfer between sensitizing (="s") and fluorescing (="f") chromophores as well as to transfer among the "f"-chromophores, which would both lead to depolarization. It is, however, not the absolute value of the short components, but rather their difference (isotropic vs. anisotropic) from which this time constant can ultimately be derived. In the descriptive two-exponential formalism, energy transfer time constants between 20 and 100 psec are estimated.

A monoexponential decay would be excepted on this basis for the α-subunit, which carries only a single chromophore. A biexponential was recorded instead, similar to the results found for the α-subunit of phycocyanin in Spirulina platensis (these authors, in the press). Aggregation and energy transfer among the aggregates is unlikely in view of the sedimentation data. We rather suggest that two different subsets of the pigment exist in this subunit, either already in the native pigments, or as a result of the subunit separation. The latter requires denaturation with urea over long times (about 16 hours) and subsequent renaturation, which may lead to incomplete restoration of the original status of the chromophore. To our expierence is the separation by electrofocusing most gentle and complete, if judged from SDS-PAGE and absorption spectroscopy, but possibly

Pigment	T(°C)	Isotropic decay		Anisotropic decay	
α-Subunit	18	1061(74%)	2681(26%)	634(84%)	2147(16%)
	36	975(71%)	2134(29%)	567(61%)	1297(39%)
	52	689(80%)	2564(20%)	313 -	- -
β-Subunit	18	363(51%)	1644(49%)	191(47%)	1041(53%)
	36	259(49%)	1075(51%)	101(53%)	769(47%)
	52	277(68%)	1191(32%)	95(37%)	469(63%)
Monomer	18	428(52%)	1520(48%)	272(59%)	2200(41%)
$(\alpha\beta)_1$	36	- -	- -	228(52%)	1600(48%)
	52	304(65%)	1650(35%)	202(58%)	1200(42%)
Trimer	18	462(55%)	2100(45%)	206(23%)	1375(77%)
$(\alpha\beta)_3$	36	307(53%)	1600(47%)	132(70%)	1800(30%)
	52	280 -	2060 --	95(73%)	1100(27%)

Table 1: Fluorescence decay characteristics of C-Phycocyanin from Mastigocladus laminosus in different aggretation states, and of its subunits. The decay curves were fit with a biexponential, for which the time constants τ(in psec) and the relative amplitudes (%, in brackets) are given.

not yet gentle anough. The interpretation of two subsets of the α-subunit with each individual polypeptide being disaggregated and bearing only one isolated chromophore is supported by the unusually long time constant of the "fast" component (as compared to all other pigments in which energy transfer is possible) and by the comparably slow depolarization.
The fluorescence decay of all samples becomes faster at higher temperatures. This is in contrast to earlier results with PC from Spirulina platensis (these authors, in the press), which showed a decreased decay rate for the excited state population at higher temperature. This may reflect an adaption of the thermophilic Mastigocladus laminosus.
The results are summarized in the figs. 2 and 3. The data for intact phycobilisomes and for entire cells are shown for comparison in fig. 4. The fluorescence is rapidly depolarized in both cases due to the large number of possible energy acceptors in these units. The long-lived component in phycobilisomes corresponds to the radiative lifetime of the terminal acceptor, allophycocyanin ($\lambda_{em} \gg 650$ nm); the comparable short lifetime observed in whole cells reflects the quenching by the chlorophylls in the photosynthetic membrane. (figs. not shown in this summary).

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