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60-965 Poznań, pl. M. Skłodowskiej-Curie 2, telefon 313-216

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61-821 Poznań, ul. Ogrodowa 11, telefon 525-425

CONTENT

A. LECTURES

ENERGY TRANSFER IN C-PHYCOCYANIN IN DIFFERENT STATES OF AGGREGATION STUDIED BY PICOSECOND TIME-RESOLVED FLUORESCENCE

P. Geiselhart and S. Schneider
Institut für Physikalische Chemie der Technischen Universität München, Lichtenbergstraße 4, D - 8046 Garching

R. Fischer, S. Siebzehnrübl and H. Scheer
Botanisches Institut der Ludwig-Maximilians-Universität Menzingerstraße 67, D - 8000 München 19

Introduction

The phycobilisomes are light-harvesting complexes of blue-green and red algae. They are highly organized assemblies of the biliproteins allophycocyanin and phycocyanin (PC), often containing also phycoerythrin or phycoerythrocyanin [1,2]. The pigments of these biliproteins are tetrapyrrol chromophores bound covalently to the polypeptide chains.
In case of PC, which is discussed in this contribution, the monomeric unit consists of the α-polypeptide chain with one phycocyanobilin chromophore (α84) and the β-chain with to chromophores (β84 and β155) [3,4]. From X-ray analysis of crystallized PC the structure of the chromoprotein, the conformations, distances and approximate relative orientations of the chromophores have become known [5,6].

Materials and Meth.

Preparation of the PC-samples in different states of aggregation are discribed elsewhere [7,8]. The samples were excited at low intensity ($\approx 10^{13}$ photons/pulse) by the output of a mode-locked picosecond dye-laser at a repetition rate of 82 MHz. The fluorescence decay was recorded with a Hamamatsu synchroscan streak camera.

Results and Discussion

A) α-subunit

In fig. 1 the fluorescence decay curves of the α-subunit containing only one chromophore (λ_{max} = 616 nm) are shown for different detection wavelengths. They are clearly dominated by a component with a decay time of 1.1-1.2 ns, which must be assigned to the native chromophore-protein arrangement. A second component with lifetime of only 30 ps and a small relative amplitude (< 10%) was found. It must be assigned to non-native chromophore conformations. These could be inherent to native α-subunits or due to slight deteriorations by the sample preparation procedures.

B) β-subunit

The β-subunit contains two chromophores, β84 (λ_{max} ≈ 624 nm) and β155 (λ_{max} ≈ 595 nm) [9]. Excitation wavelength was chosen

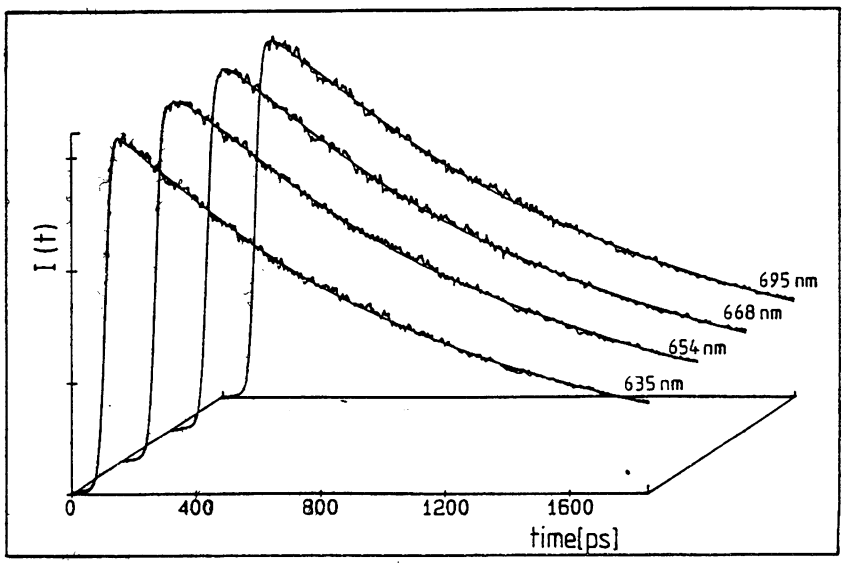

Fig.1 : Time resolved fluorescence of PC α-subunits at different detection wavelengths (λ_{exc} = 620 nm)

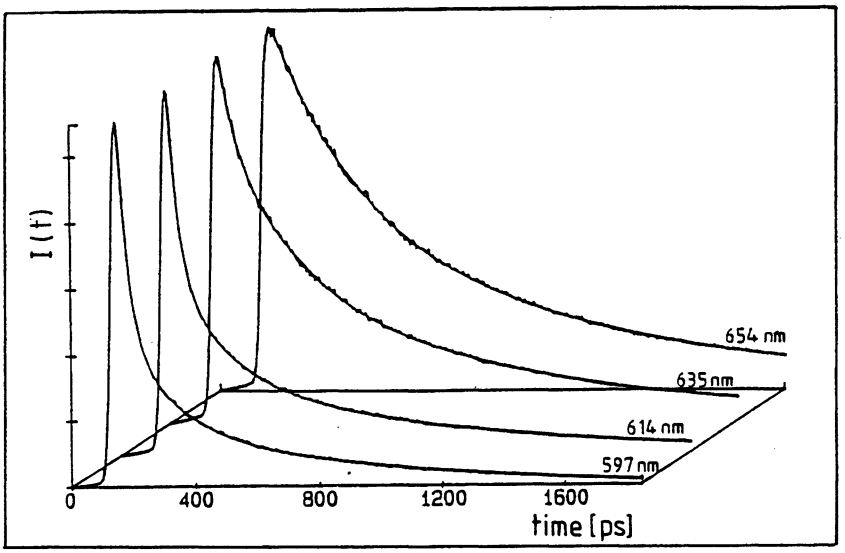

Fig.2 : Time resolved fluorescence of PC β-subunits at different detection wavelengths (λ_{exc} = 580 nm)

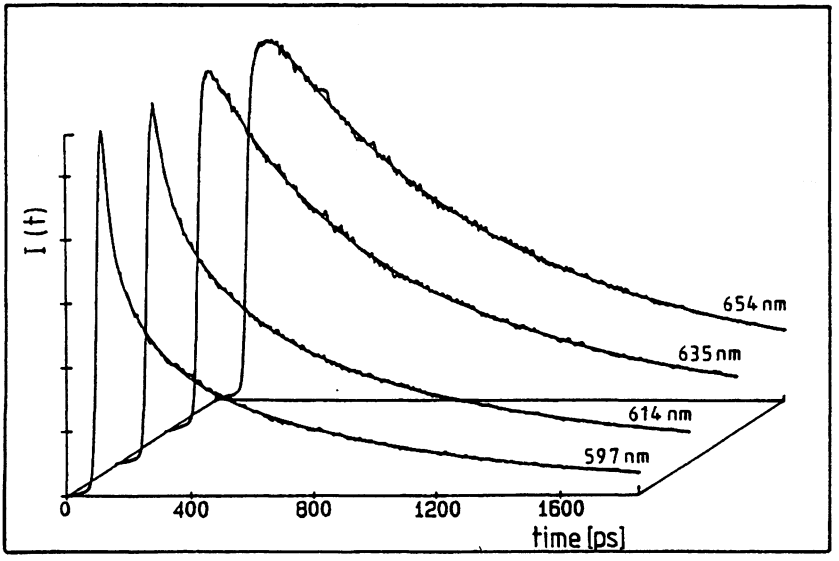

Fig.3 : Time resolved fluorescence of PC monomers at different detection wavelengths (λ_{exc} = 580 nm)

to be 580 nm in order to excite predominantly the "sensitizing" chromophore β155. To fit the decay curves in fig. 2, three components with lifetimes of 25-30 ps, ≈200 ps and ≈800 ps, resp., were necessary. The short lifetime of 25-30 ps with large amplitudes under short wavelength detection is assigned to the energy transfer time from the β155 to the β84 chromophore. The longest lifetime of ≈800 ps, which is significantly shorter than the lifetime of the α-subunit, must be the charcteristic lifetime of the terminal β84 chromophore-protein arrangement. The intermediate component (≈200 ps) is predominantly observed under long-wavelength detection conditions and must therefore be connected with another β84-conformation. This chromophore could be less stabilized by the surrounding polypeptide in the isolated β-subunit than it is in the complete monomeric unit and therefore adopt a different conformation with a characteristic lifetime of about 200 ps.

C) Monomer (αβ)

Since the monomer is a system of three coupled chromophores, three exponentials should be necessary and sufficient to describe the time course of the observed fluorescence. The fits based on a 3-exponential decay-law (fig. 3), are in very good agreement with the experimental traces. The deduced lifetimes are 20-30 ps, ≈ 200 ps and 800-900 ps, resp.. There are no faster energy transfer processes than in the β-subunit, that could be correlated with the addition of the third chromophore (α84); the long lifetime is also comparable to that of the β-subunit. But at long wavelength detection a delayed rise of the fluorescence (negative amplitude of the exponential in the fit) with time-constant of about 30 ps is observed. Since this delayed rise is not observed in the β-subunit (directly evident in comparison of fig. 2 and fig. 3) it proves that the α84 chromophore in the monomer acts as an additional acceptor for the energy transfer from the "sensitizing" chromophor β155.

Conclusions

We believe that the observed heterogeneities in chromophore-protein arrangement are typical for biliproteins, independent of the state of agregation. Therefore in trimeric and hexameric units a distribution of energy transfer rates and lifetimes of terminal acceptors must be expected. This distribution explains why in the larger aggregates systematic variations with excitation and detection wavelength are observed for the lifetimes deduced from 3-exponential fits [10].

Acknowlegement

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