

**POZNAŃ TECHNICAL UNIVERSITY
POLISH BIOPHYSICAL SOCIETY**

**PHOTOBIOLOGY
AND
BIOTECHNOLOGY**

**PROCEEDINGS
OF INTERNATIONAL SYMPOSIUM**

**JUNE 27-30 · 1989
POZNAŃ · POLAND**



UER028023126158

THE INTERNATIONAL SYMPOSIUM ON
"PHOTOBIOLOGY AND BIOTECHNOLOGY"

Poznań, Poland, June 27–30, 1989

Is organized by:

Institute of Physics, Poznań Technical University,
Institute of Environmental Engineering, Poznań Technical
University,
Institute of Commodity Science, Academy of Economics in
Poznań.

Under the auspices of the Polish Biophysical Society, Polish
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Wydanie I. Nakład 520+30 egz. Arkuszy wyd. 10,7. Arkuszy druku 11,75. Papier offsetowy kl. III 71 g. Przyjęto do druku 13.02.1989 r. Podpisano do druku 22.03.1989 r. Druk ukończono w kwietniu 1989 r. Zamówienie nr S/74/89.K-8/114

Wykonano w Zakładzie Graficznym Politechniki Poznańskiej
61-821 Poznań, ul. Ogrodowa 11, telefon 525-425

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

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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 ($\alpha 84$) and the β -chain with two chromophores ($\beta 84$ and $\beta 155$) [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 Methods

Preparation of the PC-samples in different states of aggregation are described 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 ($\lambda_{\max} = 616 \text{ nm}$) 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, $\beta 84$ ($\lambda_{\max} \approx 624 \text{ nm}$) and $\beta 155$ ($\lambda_{\max} \approx 595 \text{ nm}$) [9]. Excitation wavelength was chosen

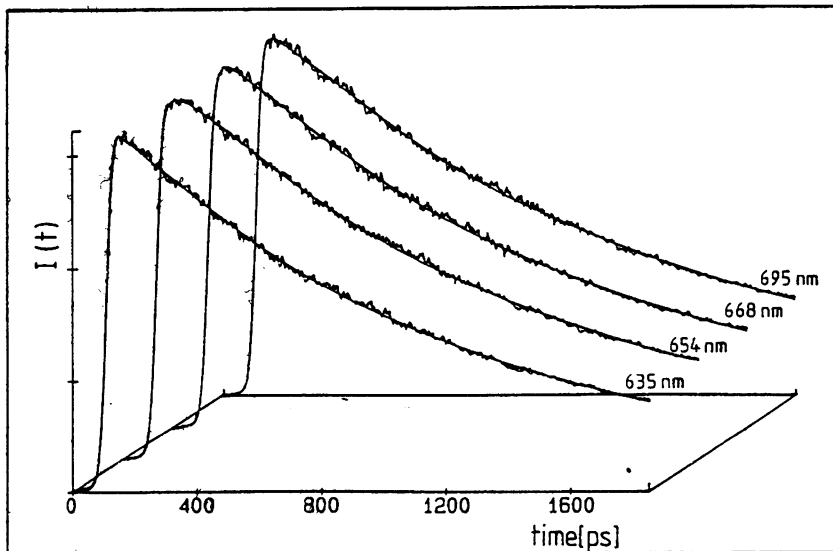


Fig.1 : Time resolved fluorescence of PC α -subunits at different detection wavelengths ($\lambda_{\text{exc}} = 620 \text{ nm}$)

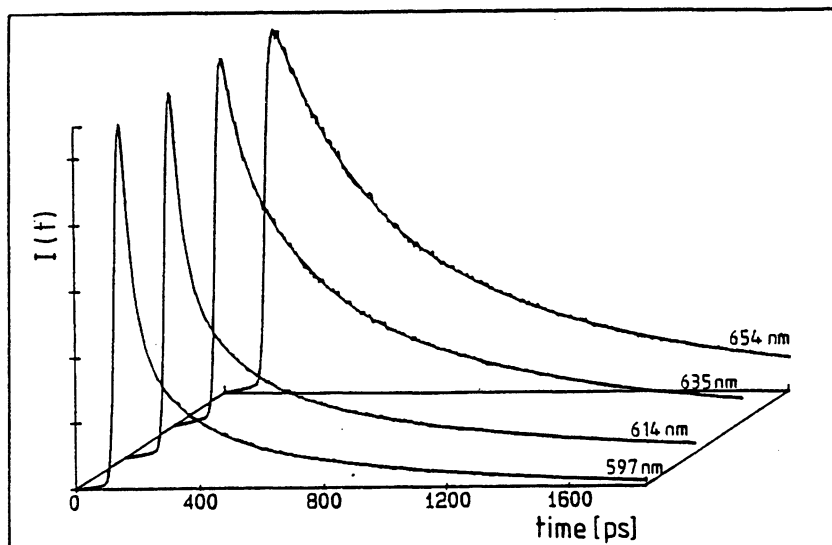


Fig.2 : Time resolved fluorescence of PC β -subunits at different detection wavelengths ($\lambda_{\text{exc}} = 580 \text{ nm}$)

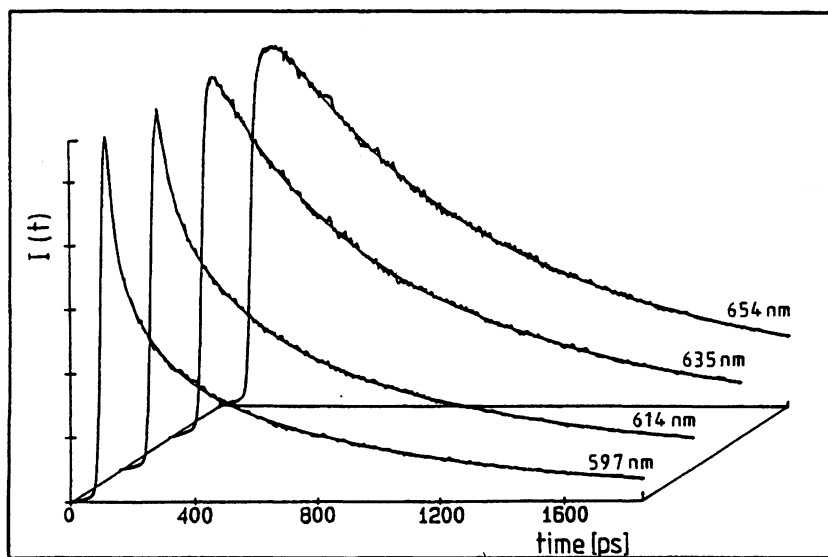


Fig.3 : Time resolved fluorescence of PC monomers at different detection wavelengths ($\lambda_{\text{exc}} = 580 \text{ nm}$)

to be 580 nm in order to excite predominantly the "sensitizing" chromophore $\beta 155$. 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 $\beta 155$ to the $\beta 84$ chromophore. The longest lifetime of ≈ 800 ps, which is significantly shorter than the lifetime of the α -subunit, must be the characteristic lifetime of the terminal $\beta 84$ chromophore-protein arrangement. The intermediate component (≈ 200 ps) is predominantly observed under long-wavelength detection conditions and must therefore be connected with another $\beta 84$ -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 ($\alpha\beta$)

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 ($\alpha 84$); 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 $\alpha 84$ chromophore in the monomer acts as an additional acceptor for the energy transfer from the "sensitizing" chromophore $\beta 155$.



Conclusions

We believe that the observed heterogeneities in chromophore-protein arrangement are typical for biliproteins, independent of the state of aggregation. 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].

Acknowledgement

Financial support by Deutsche Forschungsgemeinschaft (SFB 143) and Fonds der Chemie is gratefully acknowledged. Thanks are also due to Hamamatsu Photonics Europe for the loan of the streak camera.

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