

ENERGY TRANSFER IN PHYCOBILIPROTEINS AS  
STUDIED BY PHOTOCHEMICAL HOLE BURNING

J. Friedrich<sup>a</sup>, H. Scheer<sup>b</sup>, B. Zickendraht-Wendelstadt<sup>b</sup>, D. Haarer<sup>a</sup>

a) Fachbereich Physik, Universität Bayreuth, D-8580 Bayreuth  
b) Botanisches Institut, Universität München, D-8000 München

INTRODUCTION

Chlorophyll, the essential part of the photosynthetic reaction center, absorbs light between 450 and 600 nm only weakly and, hence, is rather inefficient in gathering sunlight. To overcome this drawback, photosynthetic organisms developed so called accessory or light harvesting pigments. These pigments gather sunlight efficiently due to their strong absorption in the regions of minimum chlorophyll absorption, and transfer the absorbed energy to the chlorophylls of the reaction center. The phycobiliproteins, e.g. phycoerythrin (PE), phycocyanin (PC) and allophycocyanin (APC) are such pigments (1).

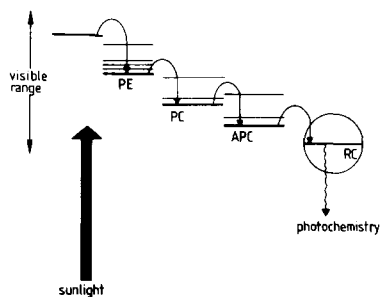


Fig. 1: Schematic representation of absorption and energy transfer by the various antenna pigments in the plant cell.

Fig. 1 shows schematically the sequential energy transfer from PE to PC and APC, from where it is eventually transferred to the chlorophylls. Here, we report photochemical hole burning (PHB) experiments on the isolated pigments, PE and PC. These chromoproteins contain several chromophores which are coupled via energy transfer processes (2,3). The excitation energies of the chromophores within the protein differ slightly because of different microscopic environments and (or) slightly different structures. Hence, the energy transfer is nonresonant in nature.

The spectroscopic investigation of light harvesting pigments is limited by low optical resolution due to inhomogeneous broadening and microscopic disorder. In this study we take advantage of the novel PHB-technique to increase the optical resolution by at least two orders of magnitude, thereby gaining information on the mechanism of the low temperature photochemistry of the chromophores and on the time-scale of the energy transfer process. The high resolution PHB experiments further yield information on the origin of the microscopic disorder and the inhomogeneous line broadening of chromophores attached to protein molecules.

RESULTS AND DISCUSSION

The preparation of the samples was carried out as described in Ref. (4,5). As a matrix for the proteins we used a glycerol/buffer glass.

The results shown in Fig. 2 provide insight into the primary photochemical steps. The numbers label the sequence of hole burning experiments. Obviously, the burning of a lower energy hole does not affect an earlier burnt higher energy hole, but the burning process of a higher energy hole refills a previously burnt, lower energy hole. For example, the burning of hole 3 refills hole 2 but does not affect hole 1. Thus, the PHB process is photo-reversible, and the photoproduct absorbs at energies higher than the reactant.

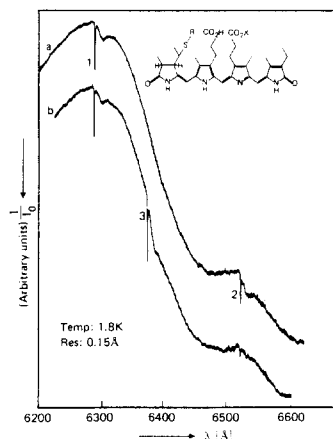
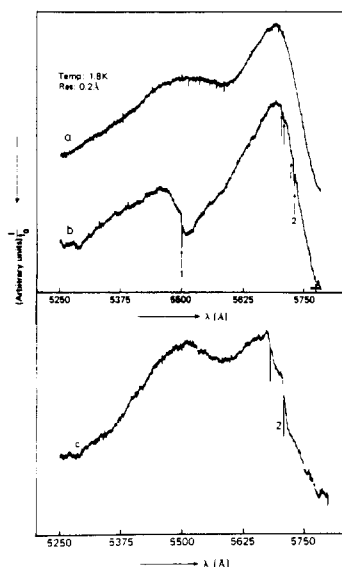


Fig. 2: Sequential PHB in a phycocyanin sample. The numbers give the sequence of PHB experiments. Note that the burning process of hole 3 fills hole 2 but does not affect hole 1.



The reaction is also thermally reversible (not shown). From these arguments one can conclude that the excited product state lies above the excited educt state ( $S_1$ ) from which it is not directly accessible. Therefore, the first step in the PHB reaction has to be an intramolecular relaxation process and, hence, the fluorescence lifetime is an upper limit for the rate of the photoreaction.

The results shown for PC also hold for PE (5). A further feature observed only in PE is shown in Fig. 3. Upon burning in the short wavelength shoulder of the visible band of PE, there appear (in addition to the resonant hole) several nonresonant satellite holes shifted to lower energies by about  $600 \text{ cm}^{-1}$ . These PHB satellites may either be due to vibrational hole burning (6) or to nonresonant energy transfer. The possibility of vibrational hole burning, however, would contradict previous experimental findings. For example, the PHB reaction at  $5500 \text{ Å}$  is as efficient as in the lower transition. If we initiate hole burning photochemistry at  $5500 \text{ Å}$  in a vibrational state of the long wavelength transition at  $5700 \text{ Å}$ , we would simultaneously irradiate into the hole filling process competing with the burning process. We further note that satellite holes only appear on the low energy side of the laser frequency. Fig. 3 (trace c) shows that hole burning in the long wavelength transition does not result in satellite holes in the higher energy shoulder as expected in case of vibrational hole burning. We therefore interpret the PHB satellites in C-PE as being due to nonresonant energy transfer. Three limits can be distinguished with respect to the inherent time scale of the PHB reaction:

Fig. 3: Resonant hole burning and PHB satellites in phycoerythrin. Hole burning in the lowest transition does not create satellites in the higher energy transition (trace c).

- 1) The fast transfer limit, characterized by

$$k_{ET} \gg k_{PR}$$

$k_{ET}$  is the rate of energy transfer and  $k_{PR}$  the rate of the PHB reaction. In this case the excitation energy is transferred to an acceptor before the photoreaction takes place in the resonantly excited chromophore. Only nonresonant holes can be observed.

- 2) The slow transfer limit, characterized by

$$k_{ET} \ll k_{PR}$$

In this case we expect only a resonant hole at the laser frequency.

- 3) The intermediate transfer limit, characterized by

$$k_{ET} \approx k_{PR}$$

Some molecules may undergo a photoreaction while others transfer their excitation energy to an acceptor. A resonant as well as non-resonant holes can be observed.

The intermediate limit would explain our findings. Since the fluorescence lifetime is an upper limit for the rate of reaction it is also an upper limit for the energy transfer rate. Thus, energy transfer in phycoerythrin is, at 2 K, not an ultrashort process but competes with intramolecular decay rates. Another interesting observation along this line of reasoning is the fact that the satellites are rather sharp. In case of nonresonant energy transfer one would generally expect satellite holes with a width on the order of a phonon sideband. From the narrow width ( $10 \text{ cm}^{-1}$ ) of the satellites we conclude that the energy gap between donor and receptor varies only within a narrow range ( $10 \text{ cm}^{-1}$ ). The protein molecule thus seems to keep the chromophores in a highly ordered relative arrangement, and the disorder reflected in the large inhomogeneous width would then be due to the interaction of the protein molecule as a whole with the glass matrix (5,7).

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