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HIGH-RESOLVED OPTICAL SPECTROSCOPY OF MODEL PHOTOSYNTHETIC SYSTEMS

(Presented by K. K. Rebane)

Introduction

Light energy conversion in photosynthesis has to be generally considered as a cooperative event in which many chlorophyll molecules together with the auxiliary pigments, proteins, lipids and with electron-transport agents participate as a photosynthetic unit. The different chlorophyll forms *in vivo* which are involved in the primary photosynthetic processes (light energy absorption, transfer and accumulation) cannot be attributed to different chemical compounds; the essential difference between them must be sought mainly in the molecular architecture of the antenna and reaction-center pigment-protein complexes.

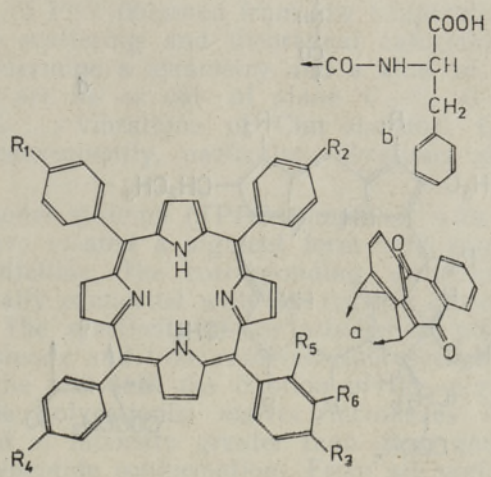
The central problem to be solved, using the model systems, is therefore the problem of the pigment-protein interaction in the broad sense. From this point of view, the protein interacting with a porphyrine molecule, can be modeled at differently complex levels: i) as a set of individual aminoacids; ii) as the aminoacids in the peptide chain; iii) as a peptide chain in a given (ordered) conformation and iv) as some higher multipeptide structure. Beside some general conclusions, there are very few theoretical and experimental data available which could contribute to the answer of the question at what above-mentioned level and to what extent the protein can influence the physical properties of the interacting pigment.

The tool necessary for the study of such a complex problem as the pigment-protein interactions really are, is low temperature and high resolution spectroscopy. The optical spectroscopy of complex organic molecules has been intensively developed in two last decades. Especially, advantages of lasers together with an optical cryotechnique produced a remarkable increase of the spectral resolution as well as qualitative improvements of our understanding of intramolecular energy flow. New spectroscopical techniques, such as Shpolskii and site selection spectroscopy, were established. With improved lasers (e. g. tunable single-mode frequency lasers) and with the decreasing sample temperature, hole-burning experiments have been achieved even on chlorophyll-like molecules [1].

Materials and methods

In vivo photosynthetic systems are very large and complex objects containing chlorophylls, proteins, quinones, membranes, etc. in some not, till now, completely known functional and structural organization. Therefore the spectroscopy of well-defined model photosynthetic systems prepared to the special request provide an attractive basis for interpreting results obtained on photosynthetic systems isolated *in vivo* from plants and bacteria.

The basic-building blocks of the photosynthetic apparatus are pigment-proteins. The protein molecular matrix has the S_1, S_2 electronic states separated by a large energy gap ($\approx$3 eV) from those of porphyrins, so its function is predominantly structural: it ensures the basic conformational property of the complex, i. e. the fixation of distance and geometry of bonded porphyrine molecules both as regards each other and also in regard to protein. The protein influence on bonded pigments can be described conveniently in terms of the perturbational molecular field V which must be able to reflect also the regularity of protein polypeptide conformation and the chirality of the protein structure. We have studied this influence on the set of model systems consisting of the tetra-anionic water-soluble porphyrins (TPPC, TPPS) (see Fig. 1) with sequential polycationic helical polypeptides. The complexes are formed spontaneously by the ionic interactions between the oppositely charged groups [2], which leads to: i) the red shift of the bonded porphyrine long-wavelength absorption band (from 640 nm for free pigment to 650—660 nm for different polypeptide matrices); ii) the observation of two new absorption bands in Soret region

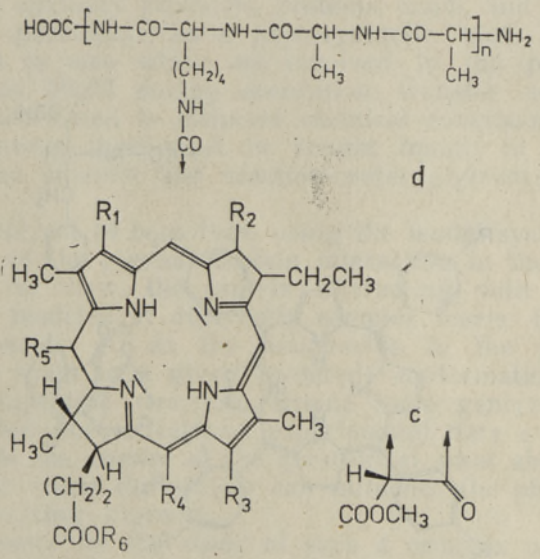


	R_1	R_2	R_3	R_4	R_5	R_6
TPP	H	H	H	H	H	H
TPPC	—COOH	—COOH	—COOH	—COOH	H	H
TPPS	$—SO_3H$	$—SO_3H$	$—SO_3H$	$—SO_3H$	H	H
PARA—TPP—L—Phe	$—CH_3$	$—CH_3$	b	$—CH_3$	H	H
ORTHO—TPP—L—Phe	$—CH_3$	$—CH_3$	$—CH_3$	$—CH_3$	b	H
ME—TPP	$—COOCH_3$	$—COOCH_3$	$—COOCH_3$	$—COOCH_3$	H	H
MC—TPP	$—CH_3$	$—CH_3$	—COOH	$—CH_3$	H	H
TPPA Q	—COOH	—COOH	a	—COOH	H	a

Fig. 1. The structures and abbreviations of tetraphenylporphyrine-based model systems.

(359—450 nm); iii) the strong optical activity of originally achiral porphyrine molecule as manifested by the intense CD bands in Soret region. To summarize: the specific chlorophyll-protein structural features, i.e. the fixation of a pigment on a regular molecular structure and the chirality of this matrix, were found to serve as a natural selective factor for the directed tuning of physical properties of the system.

We, especially, studied a set of isolated porphyring in different matrices as a reference for complex model photosynthetic systems (see Figs 1, 2): free base phthalocyanine (H_2-PHTH) in *n*-alkanes and polyethylene, pheophorbide-a (PHEO) in *n*-alkanes and dimethylformamide (DMF), a set of five derivatives of chlorin (Chn-t, 7-C-Chn-t, MChn-d, 13-A-MChn-d, 20-Cl-MChn-d) in *n*-alkanes and dichlormethan + methanol, chlorophyll-a (Chl-a) in *n*-alkanes and polystyrene foils, and a set of tetraphenylporphyrins (TPP, M-TPP, TE-TPP, ...) in *n*-alkanes and in toluene. The porphyrin-aminoacid interaction was investigated on *ortho*- and *para*-TPP phenylalanine systems [3] (see Fig. 1); however, porphyrin-polypeptide interaction was studied on PHEO bonded to a synthetic Lys-Ala-Ala polypeptide [4] (B-PHEO) (see Fig. 2). Furthermore, the aggregation effects of M-TPP, *para*-TPP and B-PHEO systems were examined including the fast energy transfer within the polypeptide chain in B-PHEO. Very recently extraordinary attention was paid to porphyrin-quinone interactions on TPP-AQ systems [5] (see Fig. 1).



	R_1	R_2	R_3	R_4	R_5	R_6
Chn-t	$-CH=CH_2$	$-CH_3$	$-CO_2CH_3$	$-CH_2CO_2CH_3$	H	$-CH_3$
7—C Chn-t	$-CH=CH_2$	$-C\equiv N$	$-CO_2CH_3$	$-CH_2CO_2CH_3$	H	$-CH_3$
MChn-d	$-CH_2-CH_3$	$-CH_3$	H	$-CH_2CO_2CH_3$	H	$-CH_3$
13—A—Mchn-d	$-CH_2-CH_3$	$-CH_3$	$-CO-CH_3$	$-CH_2CO_2CH_3$	H	$-CH_3$
20—Cl—Mchn-d	$-CH_2-CH_3$	$-CH_3$	H	$-CH_2CO_2CH_3$	Cl	$-CH_3$
Chl a	$-CH=CH_2$	$-CH_3$	c	c	H	fytol
Pheo a	$-CH=CH_2$	$-CH_3$	c	c	H	H
B—Pheo a	$-CH_2=CH_2$	$-CH_3$	c	c	H	d

Fig. 2. The structures and abbreviations of chlorine-based model systems.

Results and discussion

The obtained results can be summarized as follows:

— The frequencies of normal vibrations of phthalocyanines, chlorines, pheophorbides, chlorophylls, and tetraphenylporphyrins are connected with vibrations of particular molecular skeletons. The same frequencies can be found in model TPP and PHEO model photosynthetic systems as well [3–6].

— The site distribution functions of isolated TPP and PHEO molecules embedded in low temperature matrices are Gaussian curves with fwhm 100 — 280 cm^{-1}, depending on solvents.

— The porphyrin TPP — aminoacid (phenylalanine) interaction does not produce any significant changes in the frequency of normal vibrations (FNV) and site distribution functions. Much more intense is the effect of aggregation of M-TPP and *para*-TPP molecules in frozen *n*-octane. This aggregation produces significant broadening of site distribution functions (fwhm 100 — 240, 250 cm^{-1}) and the red shift 110 cm^{-1} [7]. Similar effect was observed on B-PHEO long-chain polypeptide model system, where fwhm of particular site distribution functions was changed from 280 to 460 cm^{-1}, and 150 cm^{-1} red-shifted [3–5].

— The decrease of symmetry and an increasing number of different substituents leads to an increase of active vibrations. This trend was observed in the literature and in our work, where on Zn-Chn only 24 FNV were found, on Chn it was 24, 30, 34, 40 FNV, on bacterio-Chn it was 21 — 22 FNV, on isobacterio-Chn 29 FNV, on *mesotetrapropyl*-Chn 42 FNV. We have found 26 FNV on MChn-d, whereas on 20-Cl-MChn-d 39 FNV, and on 13-A-MChn-d 58 FNV. Analogically on Chn-t it was 39 FNV, and on 7-C-Chn-t 45 FNV.

— A comparison of FNV obtained from low temperature fluorescence spectra, IR, Raman scattering and theoretical calculations enables in a special case to determine a symmetry and a kind of vibrations [8]. In most cases they are in- or out- of plane C—C, C—C—H, C—N, C—C—N, C—N—H, ... vibrations of Chn skeleton. Chn is a large macrosystem and, consequently, particular vibrations are usually not characteristic.

— In the two model systems (TPPC complexes with model polytripeptides) [2] we have created a pigment form with significantly shortened fluorescence lifetime. The corresponding quenching mechanism seems to be specifically connected with the regular conformation of the polypeptide matrix. The specificity of the alfa-helical polypeptide influence on the TPPC steady and time resolved fluorescence properties can be rationalized by the fact that the fixation of the pigment onto the helical polytripeptide polycationic matrix introduces the porphyrine into the charge field of intensity greater than that generated by any other nonhelical polypeptide conformation. From all spatial curves, the helix needs the maximal length of polypeptide chain to fill a given distance between *C* and *N* termini, so the alfa-helical polytripeptide ensures the highest possible spatial density of the sources of the discussed field.

— The TPP-quinone interaction in model photosynthetic systems does not produce any significant changes in frequencis of normal vibrations. The life-time τ determined from hole burning experiments $\tau \sim 2$ ps (see Fig. 3) is much higher than those observed on *in vivo* bacterial photosynthetic reaction centres [9]. These results support the idea of abovementioned papers that very fast electron transfer in femtosecond scale is

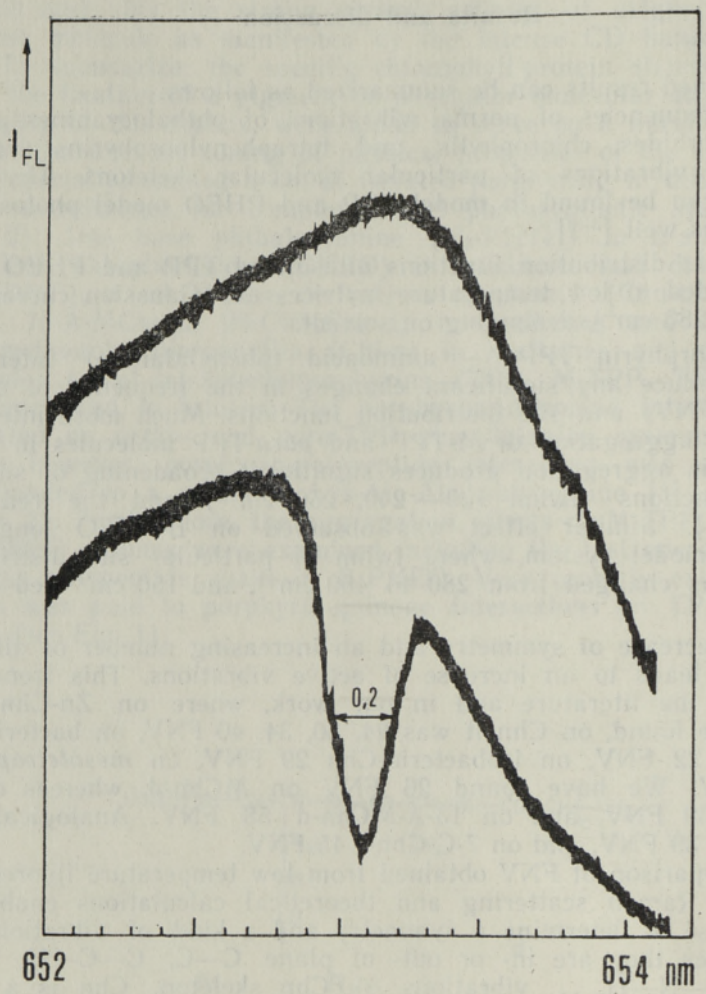


Fig. 3. Hole burning spectrum of tetraphenylporphyrin antraquinone system in toluene at 4K. The spectrum in the bottom represents the hole burned by pulsed dye laser into the excitation spectrum in the top.

strongly coupled with the protein environment. The study of systems consisting of TPP, especially Zn-TPP, quinones and model proteins is in progress.

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FOTOSÜNTEESI MUDELSÜSTEEMIDE OPTILINE KÕRGLAHUTUSSPEKTROSKOOPIA

Porfüriinide molekulide tetraanioonide ja polüpeptiidmaatriksi vastastikmõju on uuritud madalatemperatuurilise laserspektroskoopia meetodil. On määratud pigmendimolekulide normaalvõnkumiste sagedused ning arutletud tetrafenüülporfiini ja polüpeptiidi kompleksi fluorestsentsi eluea lühenemise üle.

И. ХАЛА, К. ВАЦЕК, М. АМБРОЖ, К. ГРОФ, П. ПАНЧОШКА, И. ПЕЛАНТ

ОПТИЧЕСКАЯ СПЕКТРОСКОПИЯ ВЫСОКОГО РАЗРЕШЕНИЯ МОДЕЛЬНЫХ СИСТЕМ ФОТОСИНТЕЗА

Методом низкотемпературной лазерной спектроскопии изучены пигмент-белковые взаимодействия тетра-анионов порфиринов в матрицах полипептидов. Определены частоты нормальных колебаний молекул пигментов. Обсуждается возможность сокращения времени затухания флуоресценции тетрафенилпорфина в комплексе с полипептидом.