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PHOTOTHERMAL SPECTROSCOPY OF BACTERIOCHLOROPHYLL - LIPOPROTEIN COMPLEXES

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SUMMARY: Photoacoustic spectra at room and 85 K temperatures as well as photothermal beam deflection spectra of bacteriochlorophyll - lipoprotein complexes from purple bacterium *Chromatium minutissimum* were measured. Spectra were compared and obtained differences were tentatively explained by various inertion of these two methods. Photothermal beam deflection method measure the heat which is generated in close surroundings of absorbing pigment molecule, whereas usage of more inert photoacoustic signal is averaged over contributions from various pigments located in a larger sample volume and therefore is similar to absorption spectra.

Photoacoustic spectroscopy (PAS) is used to study the spectrum of that part of absorbed light energy which is transformed into heat through nonradiative deexcitation processes (1). The light energy absorbed by the pigments of photosynthetic organisms can be transferred to other molecules, exchanged into heat, emitted as fluorescence or delayed emission, or used for photochemical reactions. There is a competition among these all possibilities, but in the case of antenna pigments, very efficient is the process of excitation energy transfer to the next

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participant in the donor-acceptor chain tunneling the excitation to reaction centers (2). In various types of antenna chromophores the division of excitation energy between various paths of deexcitation is different, what is closly related to their physiological role in photosynthesis. A part of excitation energy converted into heat can be measured by photothermal methods (3,4). The steady state thermal deactivation of photosynthetic systems up to now is predominantly measured applying photoacoustic spectroscopy (2-4). From technical reason this method deliver data averaged over contributions from pigments located in rather large sample volume, therefore it is not possible to distinguish between contributions from closly located chromophores having various photothermal properties. Recently (5) has been shown that photothermal deflection spectroscopy, in which signal is detected by the periodic laser beam deflection caused by the gradient of the index of refraction produced in the fluid (gas or liquid) near the illuminated sample, is very sensitive tool for the investigations of photosynthetic pigments in organisms and in model systems (mono and multilayers of chlorophylls). The photothermal beam deflection method is less inert than photoacoustic spectroscopy therefore it could give information about heat generated in close surroundings of absorbing pigment molecules. In this paper we compare the photothermal spectra obtained for the same photosynthetic antenna complexes by means photothermal beam deflections and photoacoustic spectroscopy methods. The suspensions of the bacteriochlorophyll - lipoprotein complexes from purple bacterium *Chromatium minutissimum* were taken as the samples.

The differences between the spectra were tentatively explained by the different delay of measured thermal deactivation signal in respect to absorption of light.

MATERIALS AND METHODS

Bacterium *Chromatium minutissimum* was cultured at 303 K under 1000 lux in Parsen medium (7). Pigment-lipoprotein complexes

were separated according to Moskalenko (8) from 4-5 days culture. Complexes in 0.04 M Tris buffer with 0.022 M glycin (pH 9.2) were introduced to PVA film as previously (9). The polarized fluorescence and absorption spectra of investigated sample where reported previously (10). In *Chromatium minutissimum* purple bacteria three types of antenna complexes (6), denoted according their near - IR - absorption maxima as B870, B800-820 and B800-850, occurr but under special culturing conditions a large amount of B800-820 is synthesized.

The photoacoustic spectra at room (298 K) and low temperatures (85 K) were measured with a single-beam photoacoustic spectrometer constructed in Trois Rivieres (11,12). The frequency of light beam modulation was 35 Hz (at room temperature) and 11 Hz (at 85K). The light beam intensity at 680 nm was 2.68 W/m^2.

The photothermal beam deflection (PD) spectrometer allow rapid measurements of thermal dissipation of absorbed light on the basis of "mirage effect" was described in details previously (5). In this method the modulated heat emission from illuminated with intensity-modulated light was measured *via* the detection of the periodic deflection of a laser beam parallel to the sample surface.

RESULTS AND DISCUSSION

Fig.1 shows photothermal spectra obtained by means of the PD and PAS methods. As it follows from the comparison of PD and PAS spectra there are some similarities, but also big differences between the results obtained using PAS and PD methods. Analogy between positions of low temperature PAS maxima of a sample in PVA film with room temperature PD spectrum in fluid PVA can be found but the maxima are shifted and the relations between intensities are dramatically changed. There is a fundamental difference between these two methods in the time constant of measured effects: in PD method the measured signal is less delayed in time in respect to the process of absorption than in PAS. At 37 Hz used for PAS the energy stored, luminescence emission etc occurring in about 4 ms is competing with thermal deactivation occurring at the same time, whereas in case of PD only energy storage in intermediate closer to the photoexcitation, with a shorter time of around 0.25 ms are influencing the measured spectrum. There is also the second serious difference between the conditions of measuring photosynthetic samples with these two methods: PAS is measured in the hermetically closed cell, which causes very

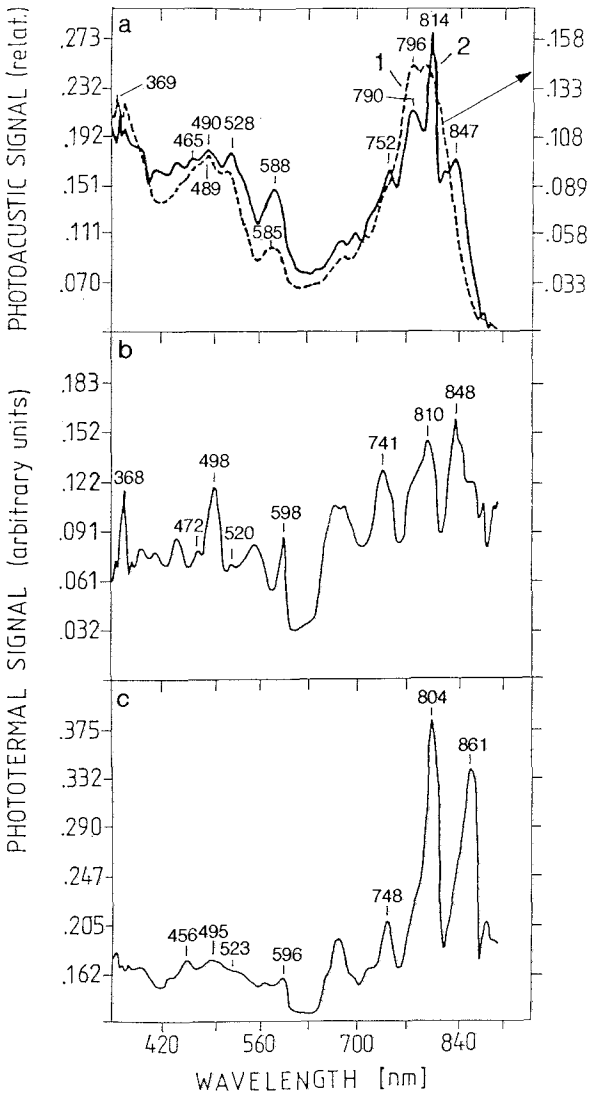

Fig. 1. Photothermal spectra of bacteriochlorophyll - lipoprotein complexes a/ PAS of complexes immobilized in PVA film at room (1) and low (2) temperature.
b/ Photothermal deflection (PD) spectra of sample in buffer.
c/ PD spectra of sample in fluid PVA.

artificial gaseous conditions. As it was shown previously (13,14) the photosynthetic complexes embedded in PVA film can still be photochemically active, therefore photochemical reactions occurr in reaction centers can not be excluded. The next difference

between both methods is an illuminating sample light intensity: because of lower PAS than PD method sensitivity in a first case usually higher light intensity has to be applied.

As it follows from Fig. 1 at room temperature in films values of PAS signal are in carotenoids (Car) region about 0.06 whereas in buffer measured by PD about 0.08. In both cases signal is normalized on the intensity unit of incident light by the division by "carbon black" spectrum. Taking into account usually higher efficiency of thermal deactivation in solid film than in fluid solvent the sensitivities of both methods (under used light conditions) are comparable. Comparing PAS at 85K with PD in buffer and PVA one can see that the contribution to thermal deactivation done by various Car are different. But generally in this region low temperature PAS of PVA film and PD spectra of PVA solution have similar amplitude and character. Much bigger differences between PAS and PD spectra occur in red and near - IR region. Measurements done by PD for a sample in buffer gives the highest maximum at 848 nm. This maximum can be seen only as a shoulder in low temperature PAS. In PVA sample measured by PD two very well resolved maxima at 804 nm and 861 nm are observed. Therefore in the absorption region of B800-820 complexes only one maximum (at 804 nm) appears which can be also related to B800-850 complex because the second large maximum is at 861 nm. Maximum at about 850 nm is observed also in PAS (85 K) for a film sample and in a buffer using PD. The thermal deactivation in Pheophytin is in PD spectra less efficient than in PAS. From the observed spectra it seems that the distribution of this part of absorbed energy which is converted into heat is rather a slow process and strongly depends on a medium in which measurements are carried out and on the interaction between a given type of complexes and this medium. PD spectra are strongly exposed contributing from complexes located close to reaction center. A more inert PAS method is averaging effects giving signal predominantly on the amount of the energy absorbed; it means similar to an absorption spectrum. It seems that some complexes are specially efficient "traps" for

excitation and that in such complexes energy is converted into heat. Using a slow PAS method we are measuring a system after reaching some equilibrium state. In this stage of investigation is not possible to decide which factor, of the three mentioned above, is predominantly responsible for the differences between PD and PAS results, but most interesting is a possibility, that PD is showing the thermal deactivation in a close surrounding of an absorbing pigment molecule. If it is true, the increase of signal in Q_y region of band can be related with some strongly dissipating acceptor of energy being located in the vicinity of absorbing bacteriochlorophyll (BChl) molecules. From Soret (S_2) excitation is converted to S_1 level but probably in Soret band are excited various BChl complexes. Therefore the effect is in some extent averaged. It is more specific in the red region in which contributions of various complexes are better resolved and the effect of the deactivation of these complexes by PD method is more specific. Lowering in temperature slacks the reactions, therefore the low temperature PAS is in some extent similar to PD spectra. This problem will be the subject of further studies using nonmodulated background illumination of various intensity in order to distinguish a part of energy used for photochemistry in traps from other parts emitted as luminescence or converted into heat. Presented results strongly suggest that using beam deflection method is possible to distinguish between contributions to thermal deactivation spectra from various closly located in complexes chromophores exhibiting different yield of thermal deactivation and as a result also different yield of excitation energy transfer in donor-acceptor chain.

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