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Interactions of chlorophyll and polypeptide mixture with bacterial reaction centres

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Abstract

In aqueous solutions of chlorophyll (Chl) *a* with synthesized polypeptides, at high ratios of Chl to polypeptides (about 75-150 μM to 500 μM) clusters of polypeptides and pigment molecules were formed. The main absorption maxima of more than one formed cluster were located at about 500 nm (Soret band) and in the region of 720-806 nm (red band). The formation of these clusters was fairly slow (some hours) at room temperature and even slower at 4 °C. The rate of cluster formation increased with the increase in Chl concentration. The addition of the even low amount of reaction centres (RCs), separated from the purple bacteria *Rhodobacter sphaeroides*, to the sample of Chl with polypeptides caused a very strong decrease in the efficiency of cluster formation, and a change in concentration ratios of various pigment-polypeptide aggregates. It was probably a competition between the interaction of Chl with polypeptides and with the RCs. The yield of thermal deactivation of the clusters was high, much higher than that for the RCs alone and it was different for various types of cluster. The clusters absorbing at 725-750 nm were fluorescent with maximum of emission at about 770 nm, whereas clusters absorbing at about 800 nm were nonfluorescent.

Additional key words: absorption spectra; fluorescence spectra; photoacoustic spectra; *Rhodobacter sphaeroides*; thermal deactivation.

Introduction

The interactions of chlorophyll *a* (Chl *a*) with proteins are responsible for the creation of various "forms" of pigment (Shibata *et al.* 1986, Uehara *et al.* 1988) enabling the efficient migration of an excitation from antenna complexes to reaction centres (RCs) in the photosynthetic organisms (Govindjee and Govindjee 1975). The RCs of the investigated purple photosynthetic bacteria are in some extent similar in function to the RC of photosystem 1 (PS1) of green plants (Sauer 1975). The interaction between the RC of bacteria with Chl *a*, which is a main pigment of green plants and algae, can be used as a model of interaction between antenna complexes of PS1 and the RC of plants. In purple bacteria the RC contains bacteriochlorophyll *a* which is an advantage, because absorption and fluorescence maxima of the pigment in the used model of plant RC and maxima belonging to the artificial antenna with Chl *a* are not superimposed. This enables to follow the RC spectra in the pigment-peptide mixture.

The amphiphilic peptide with histidine was used for experiments. This peptide has been investigated (Dudkowiak *et al.* 1998, 1999) in a similar solvent but at lower pigment concentration. In such condition the Chl *a*, introduced by acetone to the peptide aqueous solution, exhibits a spectrum similar to the monomeric form of the pigment but shifted towards long wavelengths because of the pigment interactions with polypeptides (Dudkowiak *et al.* 1998). Now, at higher pigment concentrations, we

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Abbreviations: Chl - chlorophyll; PAS - photoacoustic spectra, photoacoustic signal; PS - photosystem; RC - reaction centre; TD - thermal deactivation.
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are able to obtain large aggregates build from polypeptides and Chl molecules. The absorption, fluorescence, and photoacoustic spectra as well as the influence of the RC presence on the formation of these polypeptide-Chl *a* clusters were investigated.

Materials and methods

Chl *a* purchased from *Sigma* was used without further purification. The pigment dissolved in acetone was added to the peptide solution.

The method of synthesis and characterization of used polypeptide was described in detail by Dudkowiak *et al.* (1998) and Miyake *et al.* (1998). The polypeptide consists of 30 amino acid residues with the following sequence of their amino acid: NH2-EEEQQKKLLEE LKKLHEELKYLLKEEQKKK-COOH. It contains one his tidine residuum located among seven leucines in hydrophobic region of the peptide. As found previously (Dudkowiak *et al.* 1998, 1999), such polypeptide is able to make a ligand to Chl *a* by the histidine residuum. The secondary structure of peptide is characteristic by α-helix formation because the CD spectra (Fig. 1) showed two minima, one at 223 nm related to the α-helix n-π* transition and the second one at 208 nm related to the mixture of two bands: α-helix π–π * transition and random coil π-π * transition (Holtzwarth and Doty 1965, Shoemaker *et al.* 1987, Dudkowiak *et al.* 1998). The spectra repeated after one day showed that α-helix content in a sample is practically not changed during this time. The change from pH = 7.3 to pH = 8.2 did not strongly influence the shape of the CD spectrum.

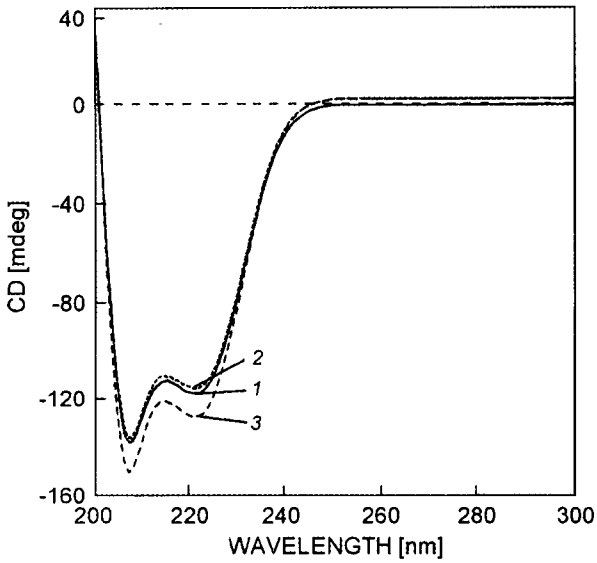

Fig. 1. Circular dichroism spectra of peptides in 30 mM Tris-HCl buffer for the peptide concentration 500 μM. Curves: *1* - just after sample mixing, pH 7.3; *2* - 24 h after sample preparation, pH 7.3; *3* - 24 h after sample preparation, pH 8.2.

RCs were separated from *Rhodobacter sphaeroides* strain R-26 and purified as described by Clayton and Wang (1971) and Hara *et al.* (1999).

The absorption spectra were measured using the *Zeiss Specord M40* spectrophotometer. The fluorescence spectra were obtained by the arrangement constructed in our laboratory in Poznań, and the photoacoustic spectra (PAS) were measured with the one-beam spectrometer built in our laboratory (Frąckowiak 1990) and equipped with the photoacoustic cell (model *300, MTEC Photoacoustic*, USA). All spectra were measured at room temperature (20 °C).

Results

Fig. 2 shows absorption spectra of the Chl *a*-polypeptide mixture suspended in 30 mM Tris-HCl buffer (pH 7.3). In all investigated samples the polypeptide concentration was 500 μM, the same as in previous investigations (Dudkowiak *et al.* 1998, 1999). The Chl *a* concentration of sample *2* was 75 μM. For the same polypeptide at lower pigment concentrations (Dudkowiak *et al.* 1998) absorption maxima are at 436 and 672 nm. Similar maxima (439 and 677 nm) were still observed just after the sample preparation, but already with the shoulders at 475 and 725 nm. One hour after sample preparation new maxima at 505, 750, and 806 nm were developed. The maxima of Chl *a* were then well distinguished at 435 and 671 nm. Red bands showed that at least two different clusters of polypeptides and Chls were formed. After one-day storage the spectrum was practically not changed. An increase in Chl concentration caused an increase in the rate of cluster formation, whereas a decrease of the sample temperature to 4 °C during sample storage diminished this rate (results not shown). The whole absorption strongly decreased when the clusters were formed because some clusters could settle down. After one hour this effect of the absorption decrease was much smaller and the same set of absorp-

tion maxima was still observed. Thus the equilibrium between various aggregated forms was practically reached during 1 h. In aqueous solvents some pigment micelles are formed (Katz *et al.* 1991, Schertz *et al.* 1991). Even in aqueous polyvinyl alcohol films the "wet"

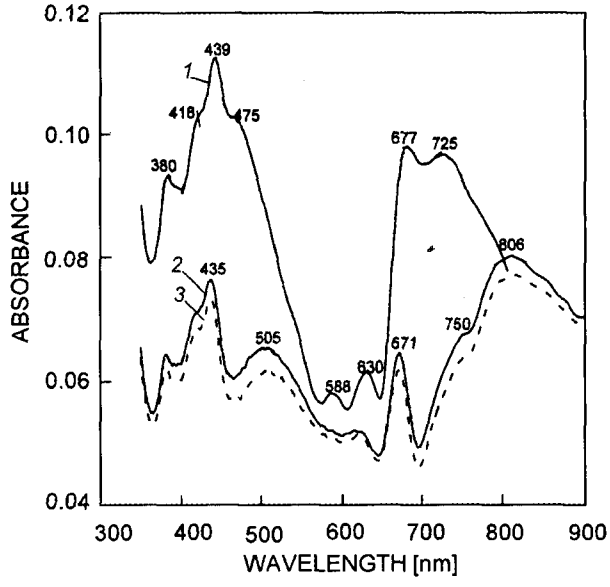

Fig. 2. Absorption spectra of polypeptide-Chl mixture (Chl concentration 75 μM, polypeptide concentration 500 μM). Curves: *1* - just after sample preparation; *2* - 1 h later; *3* - 12 h later.

and "dry" forms of aggregates are observed (Frąckowiak *et al.* 1996). The spectra of Fig. 2 were similar to those observed in other model systems with peptides (Dudkowiak *et al.* 1998) and depended on peptide concentration (not shown). The slow kinetics of formation of such clusters suggests strongly also the peptide-pigment interaction.

Fig. 3 shows the absorption spectra of two samples with RC addition. Even at Chl *a* concentration higher (150 μM) than the previous one and at very low RCs concentration (3 μM), at the same polypeptide concentration the clusters absorbing in long-wavelength region were not formed. The Chl maxima were slightly shifted towards longer wavelengths in comparison to those observed previously (Dudkowiak *et al.* 1998) at low pigment concentrations. The small maxima seen in the long-wavelength region were due to the RC absorption. Similar results were obtained at a lower Chl *a* content and a higher RC concentration (curve *2* in Fig. 3) but with a much lesser shift of Chl maxima to longer wavelengths. The RC maxima were then better pronounced because the concentration ratio of RC to Chl *a* was higher.

Fig. 4 shows the fluorescence spectra of sample without and with RC addition. For both samples the maximum in fluorescence spectra was in the 770-775 nm region, at excitation both in the Soret band and in the red band. Previously (Dudkowiak *et al.* 1998) we found the long wavelength fluorescence band of the Chl-polypeptide samples at 762 nm only when using a polypeptide without histidine. For the polypeptide with histidine, that forms ligand with Chl, the fluorescence maximum was located at about 730 nm. Now, the emission of the monomeric Chl ligated to peptide was observed only in the sample with RC as a low flat maximum near to 705 nm (Fig. 4). Hence the excitation energy might be very efficiently transferred from the pigment monomer and/or the smaller aggregates to one form of the clusters exhibiting strong fluorescence. It was probably the cluster absorbing at about 750 nm because the 25 nm Stokes shift seems to be reasonable. The clusters absorbing at 800 nm are not fluorescent.

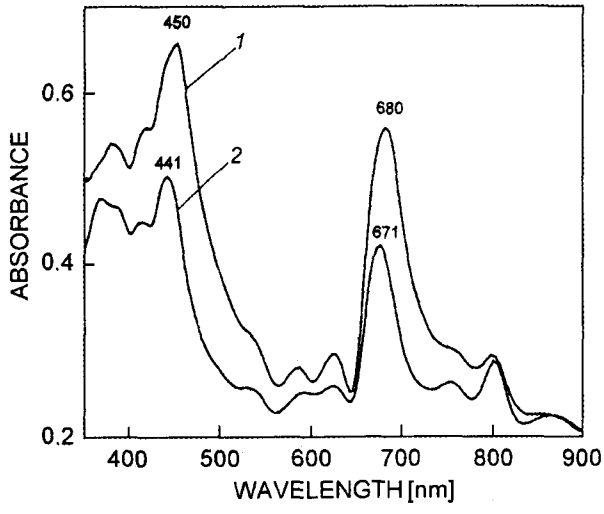

Fig. 3. Absorption of polypeptide-Chl mixture with RC addition. Curves: *1* - Chl concentration c(Chl) = 150 μM, RC concentration c(RC) = 3 μM; *2* - c(Chl) = 75 μM, c(RC) = 7.5 μM. C(polypeptide) in both samples was 500 μM.

Fig. 5 shows absorption spectra and PAS of the RC. The thermal deactivation (TD) yield (Table 1) was calculated in arbitrary, but the same, units for the RC and for the polypeptide-Chl *a* samples without and with RC (PAS in Fig. 6).

The TD was much lower for the RC sample than for the polypeptide-Chl clusters (Table 1), because in the RC the photochemistry and the fluorescence emission successfully competed with the TD, whereas non-fluorescent or slightly fluorescent pigment-polypeptide clusters converted most of their excitation energy into heat. The shapes of the absorption and PAS spectra for RC alone were similar (Fig. 5) whereas the comparison

of absorption spectra (Fig. 6) with PAS of the same samples without and with RC showed strongly different shapes. It was due to various TD yields of different clusters. The absorption and PAS spectra of the Chl-polypeptide samples without RC addition strongly changed in time, therefore for the TD calculation the

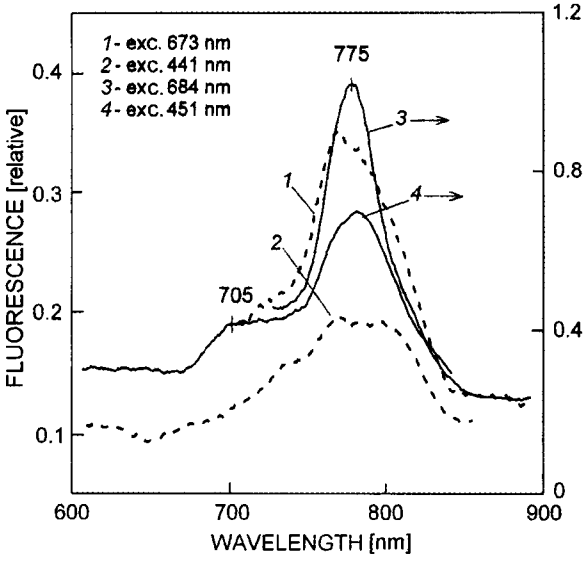

Fig. 4. Fluorescence spectra. Curves *1* and *2*: Chl with polypeptides, c(Chl) 75 μM; *3* and *4*: Chl + polypeptides + RC c(Chl) 150 μM, c(RC) 3 μM, c(polypeptides) in both samples 500 μM. Wavelengths of excitation are given.

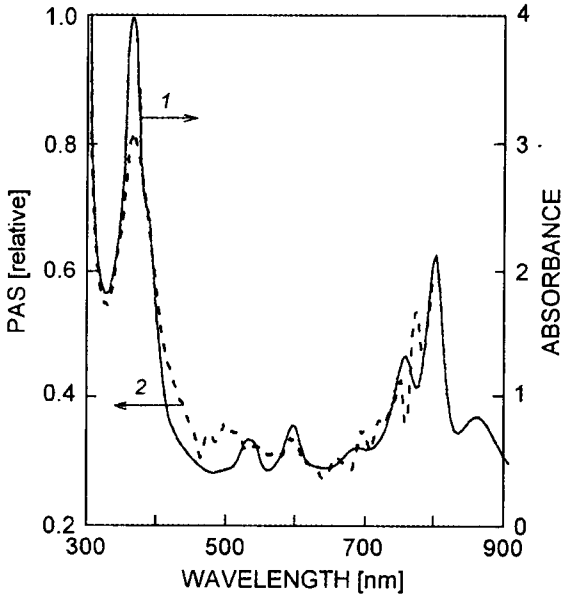

Fig. 5. Absorption and photoacoustic spectra of bacterial RC: curve *1* - absorption; curve *2* – PAS; RC concentration 35 μM.

Table 1. Thermal deactivation yield (in arbitrary units, the same for all samples).

Sample	Wavelengths region [nm]	Thermal deactivation
RC	370	2.0
	800	2.6
Polypeptides with Chl *a*	430	4.6
	589	6.9
	660-680	3.8
Polypeptides with Chl *a* and RC	430	1.7
	585-589	2.5
	670	1.5

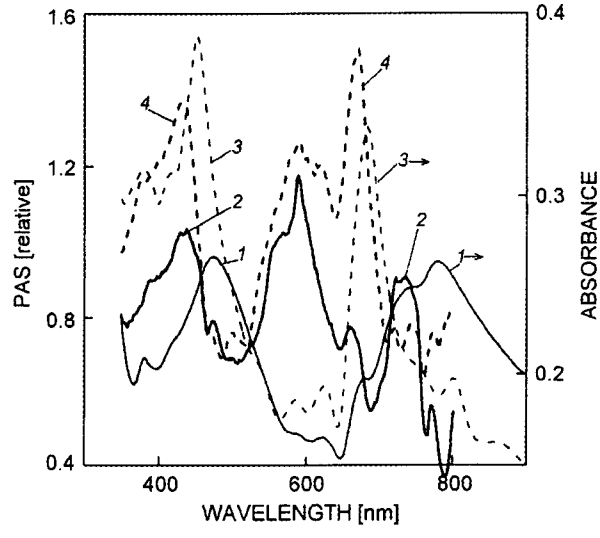

Fig. 6. Photoacoustic (*2*, *4*) and absorption (*1*, *3*) spectra: curves *1* and *2*: Chl + polypeptide; curves *3* and *4*: Chl + polypeptide + RC. Concentration of: polypeptides 500 μM; c(Chl) = 150 μM; c(RC) = 3 μM.

spectra of the samples at the same time after sample mixing were taken (Fig. 6). The RC addition changes the ratios of the PAS maxima. In some cases it is due to stronger absorption, *e.g.*, in the case of maximum at 660-670 nm when the absorption was much higher in the sample with RCs than without them. The maximum 660-680 nm belongs to the monomeric Chl *a* ligated to peptide. The sample with RC showed very low TD, in the sample without RC it was more than double (Table 1). Thus in the last case the contributions from aggregated pigment forms were higher than for the sample with RC. The maximum in 570-590 nm region, due to clusters, was characterized by a very high TD yield (Table 1 and Fig. 6). It had a high TD value, but the TD for sample without RC was still higher. The absorption in Soret band was shifted towards longer

wavelengths in the sample without than with RCs. This effect was due to oligomer formation. The monomeric and oligomeric Soret bands in Fig. 6 were not yet well separated, as it is shown in Fig. 2, because the samples were investigated within shorter time after preparation than the samples presented in Fig. 2. The long-wavelength (700-800 nm) absorption shows that oligomerization of sample in Fig. 6 is also progressing. The shape of absorption in this region gives evidence that more than one type of oligomers was formed. TD for wavelengths longer than 800 nm was not established because of experimental possibilities of our arrangement, but the 780-800 nm region indicates that it was not very high also in samples with absorption of such clusters. Anyway, the TD in this region was lower than the TD in the 730-750 nm range. The PAS amplitude in the 730-750 nm region decrease as a result of RC addition was in agreement with the absorption spectra. It is unexpected that the 800 nm absorption did not give high TD, because this form does not fluoresce. Probably radiant energy caused some conformational changes of clusters than changed excitation energy into heat.

Discussion

The spectral properties of Chls located in organisms depend strongly on the interaction with the surrounding proteins and lipids as well as on mutual pigment aggregation, and these properties can be mimicked in model systems (Katz *et al.* 1991, Schertz *et al.* 1991, Planner *et al.* 1997, Dudkowiak *et al.* 1998, 1999). The contributions from various interactions depend on the structure of photosynthetic apparatus—they are different in various giant antenna complexes such as phycobilisomes of cyanobacteria and chlorosomes of green bacteria as well as in antennas of green plants (Sauer 1975). But it is possible to find close analogies between molecular interactions of pigments with macromolecules in these systems. On molecular level the competition between interactions with polypeptides and other pigment molecules are important. The model system investigated in our work delivers information about this competition.

At high concentrations of Chl and peptide, several types of cluster can be formed. These forms are characterized by different yields of TD and therefore some of them can play role of traps converting the excess of photons into heat. The presence of RC complexes in a sample strongly changes the peptide-pigment interactions. The strong competition between spontaneous formation of the polypeptide-pigment clusters and the interaction of pigment and polypeptide molecules with RC is thus found. On this stage of investigations we cannot decide if these interactions occur with the polypeptides ligated to Chl, such as formed at low pigment concentrations, or with the separated pigment and polypeptide molecules. The first possibility seems more plausible, because in the sample with RC absorption maxima are observed that are characteristic for ligands.

We found a strong affinity between the mixture of Chl molecules with peptides and RC complexes. This affinity is well seen even between RC of purple bacteria and Chl *a*, the antenna pigment of green plants. It could be a model of interactions between RCs and antenna complexes occurring in all photosynthetic organisms.

Hence it is possible to conclude:

(*1*) The interactions between Chl *a* and polypeptides at high concentrations of both components differ strongly from the interactions between the same components in samples of lower pigment concentrations. More than one type of the polypeptide-Chl clusters are formed. These types are characterized by strongly shifted absorption bands and different yields of thermal deactivation of excitation.

(*2*) The presence of RCs in the samples perturbs strongly the interaction between the polypeptides and pigment molecules. It suggests that also *in vivo* the interactions between antenna complexes and RC can have some influence on the structure of the pigment-protein complexes located near to RC.

(*3*) Just after introducing a large amount of pigment to the polypeptide sample, maxima characteristic for the monomeric Chl-polypeptide ligands are observed. The oligomers are formed in a rate dependent on the pigment concentration and sample temperature. Thus oligomers may be formed at the expense of Chls ligated to polypeptides.

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