

REGULARITIES OF INTERMOLECULAR INTERACTION
AND ENERGY TRANSFER IN MIXED ASSOCIATES OF
CHLOROPHYLL AND ITS ANALOGS

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The nature of the interaction of chlorophyll molecules and energy transfer in aggregates is of particular interest in relation to the organization of these molecules in photosynthetic systems. It may be considered that associates and mixed associates of different pigments are the intermediate link between crystals and solutions, so far as there is definite ordering in the structure of these complexes, and the intermolecular interaction energy correspond to the case of localized exciton. Our investigation of model systems is an attempt to study general regularities of the mixed association and estimate the efficiency of energy trapping in mixed aggregates of different pigments under varying the aggregate composition.

The procedures for preparation of mixed aggregates of chlorophyll and its analogs in water-dioxane mixtures (4:1) and methods of spectral-luminescent measurements were essentially the same as in our previous papers [1,2]. Protochlorophyll (PChl) and 4-vinyl-protochlorophyll (4VPChl) of constant concentration C_d ($C_{d\text{initial}} = 1 \cdot 10^{-5} \text{ m/l}$) were used as a donor component (in energy transfer) while the relative acceptor con-

centration (chlorophyll "a" - Chl, Pd-pheophytin "a" - PdPh, Cu-pheophytin "a" - CuPh, bacteriochlorophyll - BChl) was increased from 0,001 C_d up to equimolar one. The sequence in the choice of acceptors Chl, PdPh and CuPh corresponds to the probability deterioration of deactivation nonradiative processes which for CuPh is no less than 10^{13} sec^{-1} . The basic spectral-luminescent characteristics and energetic parameters are listed in Table I. The increasing of relative acceptor concentra-

TABLE I. SPECTRAL-LUMINESCENT PROPERTIES OF AGGREGATED FORMS OF PIGMENTS IN MIXTURE OF DIOXANE-WATER

PIGMENT	ABSOR. λ_{MAX} nm	LUMIN. λ_{MAX} nm	QUANTUM YIELD B	MEAN LIFETIME τ , SEC.	DEGREE OF POLARISATION P, %
PROTOCHLOROPHYLL	637	641	$8,5 \cdot 10^{-4}$	$\sim 2 \cdot 10^{-10}$	34 - 36
4-VINYL-PROTOCHLOROPHYLL	639	642	$1,5 \cdot 10^{-3}$	$\sim 9 \cdot 10^{-11}$	—
CHLOROPHYLL "A"	687	693	$5,3 \cdot 10^{-3}$	$\sim 4 \cdot 10^{-10}$	11 - 12
Pd-PHEOPHYTIN "A"	647	650	$3,0 \cdot 10^{-4}$	$\sim 7 \cdot 10^{-12}$	—
Cu-PHEOPHYTIN "A"	660	—	$< 10^{-5}$	$< 2 \cdot 10^{-13}$	—
BACTERIOCHLOROPHYLL	826	—	$< 10^{-5}$	$< 1 \cdot 10^{-13}$	—
Cu-PROTOPHEOPHYTIN	666	—	$< 10^{-5}$	$< 2 \cdot 10^{-13}$	—

tion in donor matrix resulted in the typical changing of solution absorption spectra (Fig.1) and the transformation of circular dichroism (CD) spectra (Fig.2). It should be noted that in the case of PChl as a donor the optical activity of the donor component is almost

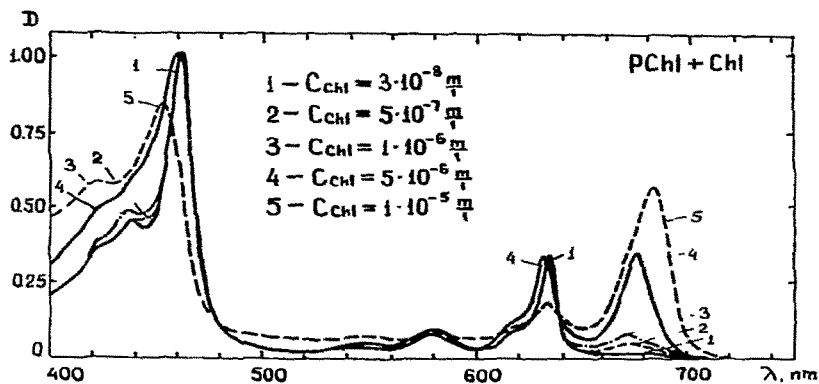


Figure I. Changing of absorption spectra of Pchl and Chl mixed associates in water-dioxane mixture 4:1 under increasing of Chl concentration.

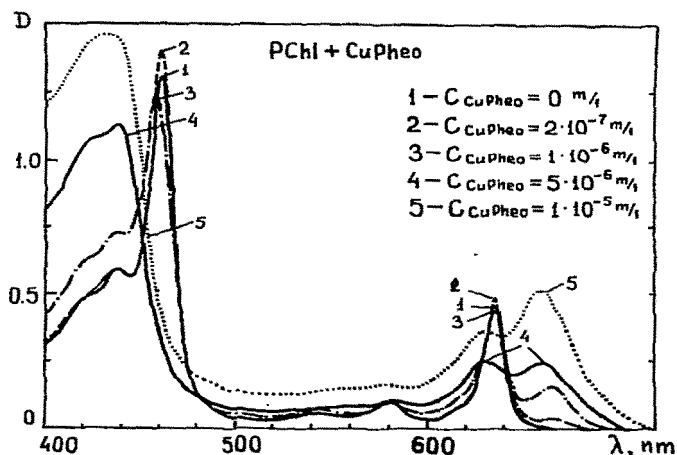


Figure IA. Changing of absorption spectra of Pchl and CuPheo mixed aggregates in water-dioxane mixture 4:1 under increasing of CuPheo concentration.

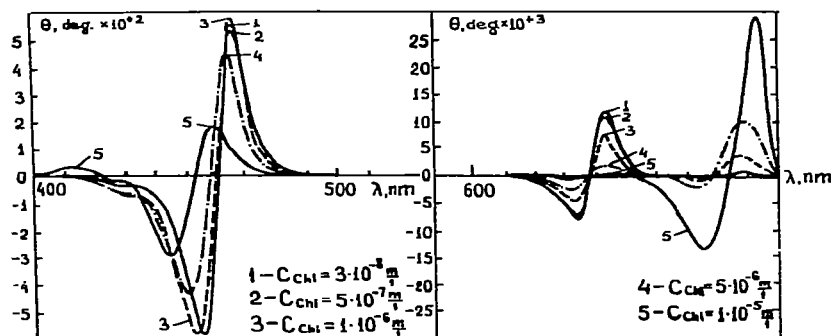


Figure 2. Changing of CD spectra of PChl and Chl mixed associates in water-dioxane mixture 4:1 under increasing of Chl concentration.

reduced up to zero under increasing acceptor concentration. In contrast to this the overturn of the sign dependence of donor bands in CD spectra of 4VPChl has been discovered under similar conditions. Analysis of our results and comparison of the data obtained with spectral characteristics of different pigment aggregated forms in water-dioxane mixtures, investigated earlier [3], permit us to draw the conclusion that under increasing the acceptor (Chl, PdPh, CuPh) concentration in true mixed complexes the pigment-pigment interaction leads to the structure changing of both donor and acceptor parts of the mixed pigment aggregate.

The direct evidence of mixed association in binar solutions is the electronic excitation energy transfer

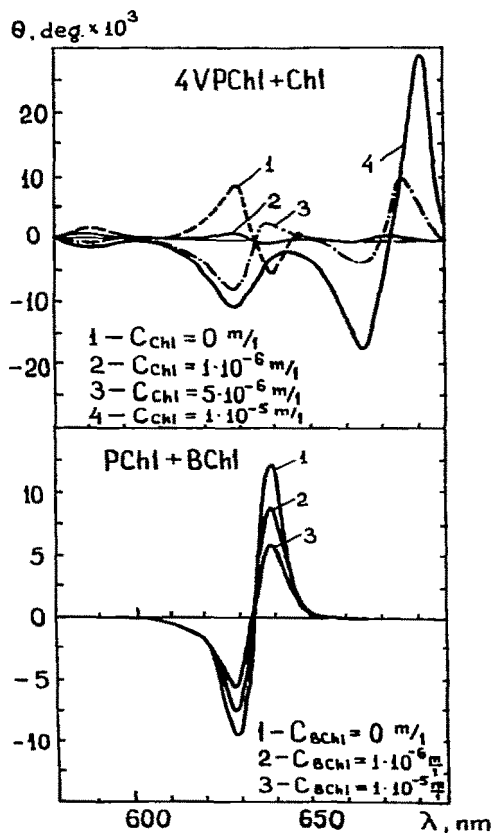


Figure 2A. Changing of CD spectra of 4VPChl and Chl mixed aggregates in water-dioxane mixture 4:1 under increasing of Chl concentration.

which is manifested in the donor luminescence quenching under increasing acceptor concentration and for Chl as an acceptor - in the sensitization of its fluorescence using exciting light of $\lambda=460\text{nm}$ (absorption maximum of donor). The energy migration in this case does take place between molecules belonging to one mixed aggregate so far as under initially used pigment concentrations in solutions ($\approx 10^{-5} \text{ m}/1$) the energy transfer between different molecules of different complexes would be impossible due to their spatial disconnection. The calculation of the experimental values of the transfer efficiency in associate determined by the donor luminescence quenching was made using

$$f_{DA} = 1 - \frac{S_D^*}{S_D^0} \cdot \frac{D_\Sigma - D'_A}{D_D} \cdot \frac{1 - T_\Sigma}{T'_A - T_\Sigma} \quad (1)$$

On the other hand in the case of luminescing acceptor (Chl) assuming that the acceptor is excited only due to the energy transfer process, it is possible to calculate experimental values of the transfer efficiency from sensitization data

$$f_{AD} = S_A^{\text{MEAS}} \cdot \frac{D_\Sigma (1 - T_D^0)}{D_D (1 - T_D)} \cdot \frac{1}{S_D^0} \cdot \frac{B_D^0}{B_A(C)} \quad (2)$$

Here, D and T are the corresponding optical densities and transmittances of solutions; subscript Σ refers to the total absorption (or transmittance); subscripts D and A refer to the donor and acceptor characteristics of complexes; index 0 is connected with optical properties of the standard which is the long-wavelength aggregate of pure donor ($C_{\text{initial}}=10^{-5} \text{ m}/1$) in the water-dioxane mixture (1:4). Values S are corresponding areas

under luminescence spectra; B_D^0 - quantum yield of donor luminescence in the absence of transfer. The factor $B_A(C)$ takes into account the concentration change of acceptor luminescence quantum yield which for Chl had been experimentally measured in the same conditions.

Fig.3 compares experimental investigation results of PChl luminescence quenching by different acceptors in mixed associates. The data obtained for every pair of pigments in the complex are practically independent of excitation wavelength. Furthermore the calculation

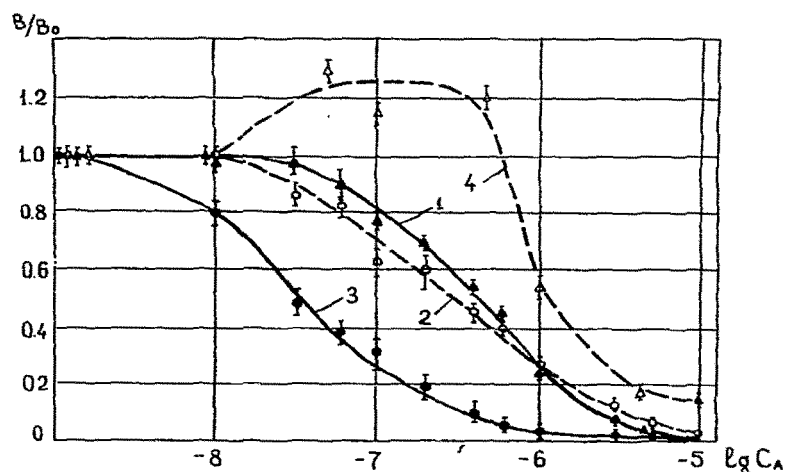


Figure 3. Changing of PChl luminescence relative quantum yield in mixed associates under increasing of relative concentration of different acceptors (1- Chl, 2- PdPh, 3- CuPh, 4- BChl). $C_{PChl} = 10^{-5}$ M/l = const.

using formulas (1) and (2) for PChl \rightarrow Chl system showed that the transfer efficiencies determined by PChl luminescence quenching were practically the same as the migration efficiencies determined by Chl fluorescence sensitization. Therefore, the excitation energy transfer in mixed pigment associates takes place without losses. Fig.3 shows that under similar conditions all acceptors as excitation traps may be ordered as follows: BChl < Chl < PdPh < CuPh. The energy transfer between PChl and CuPh in such systems becomes appreciable when the ratio of donor to acceptor molecules is equal to 1000:1. This fact permits us to conclude that mixed associates of pigments are the complicated polymers containing dozens or perhaps hundreds of monomeric molecules.

It can be seen from Table I that under solution emission excitation into PChl absorption band ($\lambda = 460\text{nm}$) the fluorescence polarization data of individual PChl and Chl aggregates are considerably different. But in mixed associates the high degree of fluorescence polarization excited under the same conditions and measured in both acceptor and donor emission bands remains constant up to $C_{\text{Chl}} = 1 \cdot 10^{-6} \text{ M/l}$ (Fig.4). This regularity is also typical for other donor-acceptor pairs. Consequently, the formation of mixed associates does not change the ordered structure of donor matrix. In addition the high degree of the acceptor (Chl and BChl) fluorescence polarization being excited under these conditions due to the energy transfer from the donor component of aggregate points to the high degree of molecular organisation of donor and acceptor parts in

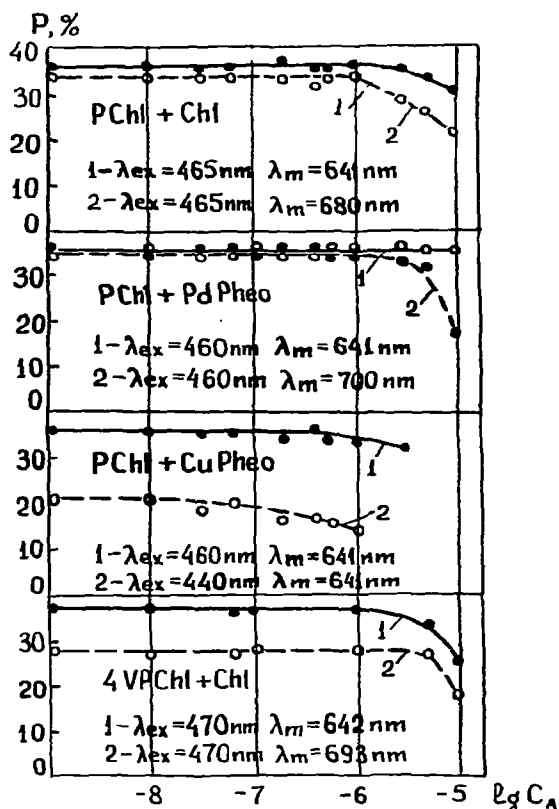


Figure 4. Plot of luminescence polarisation degree of pigment mixed associates in water-dioxane mixture 4:1 on acceptor concentration

mixed complexes. Eventually, these data show that energy migration in such systems occurs from the lowest excited electronic state of donor component.

Discussion

Let us consider the physical reasons for a different quenching ability of investigated acceptors. The

process of transfer and excitation trapping in mixed associates may be presented as follows: 1) the migration along the donor matrix (the probability F_1); 2) the transfer from matrix molecules to acceptor molecules (the probability F_2). It should be noted that for Chl, CuPh and BChl the energy difference between first excited electronic levels of donor (PChl) and acceptor is substantially greater than kT . Therefore, the usual back transfer from the Boltzmann vibrational level of the acceptor excited electronic state to the donor is hardly probable for these systems.

The low limit of the transfer probability from PChl matrix molecules to the acceptor (F_2) may be estimated by Forster-Galanin theory [4,5] taking into account the distance between molecules in aggregate $R \approx 10 \text{ \AA}$ and using donor characteristics listed in Table I. Band overlap factors for the donor-acceptor pairs investigated were taken from [6]. Taking into account the high degree of molecular ordering in the complex we took the value of the orientation factor equal to $K^2=4$. Thus, the corresponding value of F_2 was calculated as $F_2 > 3,5 \cdot 10^{12} \text{ sec}^{-1}$. The energy migration probability F_1 may be estimated from CD spectra of PChl long-wavelength aggregates. Near to symmetrical the splitting in CD spectra of these aggregates permits us to estimate the exciton interaction energy in such systems $V_{12} \approx 75 \text{ cm}^{-1}$ which corresponds to an "excitation transfer time" in matrix $\tau_i \approx 8 \cdot 10^{-14} \text{ sec}$. From these data the probability of energy transfer along the donor component of complex was found to be $F_1 \approx$

$\approx 1,2 \cdot 10^{13} \text{ sec}^{-1}$. The estimates cited are in a good agreement with analogous values we obtained directly from experimental data on investigation the energy transfer from donors with low luminescence efficiency[2]

Such high values of excitation transfer probabilities permit us to explain the discovering of energy migration for systems in which the overlap of donor luminescence spectra and acceptor absorption is extremely small (PChl \rightarrow BChl). But in contrast to the other acceptors (Chl, PdPh, CuPh) the efficiency of excitation trapping is the smallest for system PChl \rightarrow BChl due to bad conditions for the resonance dipole-dipole interaction between transition dipole moments of donor and acceptor molecules. Probably the latter factor is the physical reason of the weak influence of BChl molecules on absorption and CD spectra of PChl in mixed aggregates in comparison with the other acceptors.

It is known that molecules of CuPh do not possess measurable fluorescence because of the presence of unpaired d -electron in central Cu ion resulting in the probability deterioration of intersystem crossing into the triplet state [7]. Therefore, it may be supposed that the greatest quenching action of CuPh in comparison with Chl and PdPh is connected not only with energy transfer but with the perturbing action of Cu ion of CuPh molecule on nonradiative transition probabilities in PChl molecules surrounding acceptor molecule in mixed associate. In order to check this assumption the luminescence quenching of Chl aggregate by Cu-protophytylin (CuPPn) in mixed aggregates was investi-

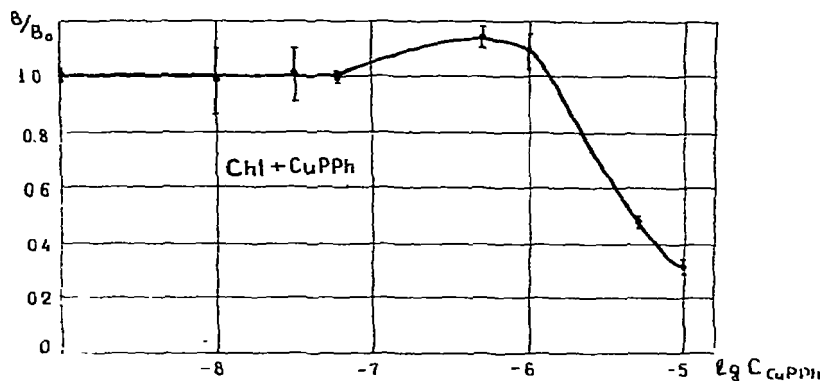


Figure 5. Luminescence relative quantum yield of Chl as function of CuPPh concentration in mixed aggregates. $C_{\text{Chl}} = 10^{-5} \text{ m/l} = \text{const}$

gated. According to the energetic position of excited state levels CuPPh is the potential energy donor for Chl. Fig.5 shows that appreciable Chl fluorescence quenching by CuPPh molecules is observed only under equimolar concentrations of these pigments in mixed complex. When the ratio between Chl and CuPPh molecules is equal to 1000:1 no quenching effect is found while in the case of PChl \rightarrow CuPh pair under the same ratio of component concentrations the PChl luminescence quenching makes up 20% (Fig.3).

Table I shows that the excitation deactivation probability in CuPh molecule is equal to 10^{13} sec^{-1} while for Chl and PdPh these values are considerably

smaller (10^{10} sec⁻¹ and 10^{11} sec⁻¹, correspondingly, . In that case so far as the energy migration probabilities along donor matrix (F_1) and directly to the acceptor component (F_2) of mixed associate are comparable with the vibrational relaxation probability in complex organic molecules ($W \sim 10^{12} - 10^{13}$ sec⁻¹) the low trapping efficiency of PChl excitation by Chl and PdPh molecules in comparison with CuPh may be explained by the existence of the back before-relaxation energy transfer from Chl and PdPh to PChl. Such back energy migration must take place before or during the process of Boltzmann distribution of vibrational energy in acceptor excited electronic state. The energy transfer from PChl to CuPh is essentially irreversible due to the high excitation deactivation probability in the acceptor.

Our use of artificial mixed aggregates of chlorophyll and its derivatives with varied concentration of acceptors is the necessary link to account for the behavior of photosynthetic systems, in which trapping excitation energy out of a large array of pigment molecules is an essential step in their function.

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