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Short Communication

Chlorosoma: How can it contribute to photosynthesis of green bacteria?

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Numerous kinetic data for excitation energy decay in bacteriochlorophylls as well as extremely short lifetimes of excitations in dominating chlorosoma pigment exclude the possibility of an efficient migration of this energy from chlorosoma to the main intra-membrane photosystem of some green bacteria. Author discusses the main purpose and molecular mechanism which account for this inconsistency in huge, 3D chlorosoma and substantiates the conditions of cell growing under which this energy migration from chlorosoma may become efficient.

Key words: Green bacteria, chlorosoma, energy migration and trapping.

INTRODUCTION

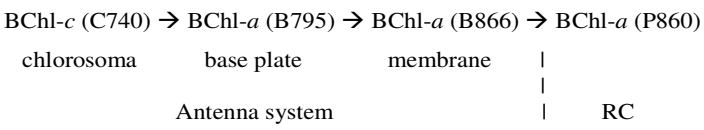
Green bacteria have light-harvesting organelles – chlorosomes located on the cytoplasmic side of the inner cell membranes. In sulfur bacteria chlorosomes may have up to 10.000 - 20.000 bacteriochlorophyll (BChl) *c* (or *d, e*) molecules which are enveloped into thin lipid capsule (Blankenship et al., 1995; Blankenship, Matsuura 2003). BChl-*c* concentration in chlorosomes is extremely high, its pigments are not bound to proteins, but aggregated themselves, therefore the red shift of their absorption bands, up to 720 - 750 nm is reasonably attributed to pigment-pigment interactions (Van Grondelle et al., 1994). So called base plates are located between chlorosoma and photosynthetic membrane (Blankenship et al., 1995; Blankenship and Matsuura, 2003). It contains BChl-*a*, carotenoids and proteins. A modest red shift of its absorption peak to about 795 nm suggests that these molecules are apparently monomers like B800 ones in purple bacteria.

Many green bacteria contain up to 1000 - 2000 BChl-*c* (in chlorosoma), 30 – 50 BChl-*a* (in base plate) and about 60 - 70 BChl-*a* molecules (in membrane) per reaction center (RC) (Blankenship and Matsuura, 2003). This pigment system seems to represent a classical type of cascade-like array, which should funnel excitations from the light-harvesting antenna to the RC traps.

The lifetime of photoexcited BChl-*c* was first measured in green bacterium Chloribium limicola as, $\tau^* = 35 \pm 15$ ps (Borisov et al., 1977). It was supposed in this work that such short lifetime reflects an efficient transfer of electronic excitations (EEs) to the major BChl-*a* in the membrane. Later this lifetime was measured with greater precision and often turned to be within 10 - 15 ps (see Holzwarth et al., 1990; Miller et al., 1991; Lin et al., 1991; Savikhin et al., 1994) and reviews (Blankenship et al., 1995; Van Grondelle et al., 1994). Many researchers made efforts to prove the activity of the above presented model in EE funneling, either by revealing the fluorescence of the main BChl-*a* B866, induced by photoexcitation of chlorosoma C740 pigment, or by monitoring the increase of C740 lifetime caused by disconnecting of chlorosoma from the main BChl-*a* apparatus, but without real success. Several non evident suppositions were suggested in literature pursuing the aim to reconcile such data with the above presented migration model.

Below a hypothetical notion is suggested which apparently explains the reasons of these troubles and

Abbreviations: BChl, bacterio/chlorophyll; **EE,** electronic excitation.



formulates the conditions in which chlorosoma pigment may contribute to the net photosynthesis of green bacteria.

Strong non-photochemical excitation quenching in 3D chlorosoma

What kind of mechanism may be responsible for EE quenching in chlorosoma pigment? The answer is borrowed from physics. It was demonstrated in classical experiments by Nobel prize winner J. Perrin and S. Vavilov with rhodamin and methilene blue that their fluorescence emissions were quenched by about two orders when these molecules have formed associates, mostly dimers (Monograph Terenin, 1967). Later many similar works appeared. Chlorophylls are not exceptions in this virtue. Fluorescence lifetime (τ_{fl}) of BChl-*a* dissolved in a number of organic solutes occurred to be within 2 - 4 ns. In photosynthetic tissues it is much shorter, for example in RC special pairs of purple bacteria the rate constant for the sum of wasteful losses approximates to $(0,3\ ns)^{-1}$ (Shuvalov, 2000) although these BChl pairs are not real dimers, but closely positioned molecules. The mechanism of such EE quenching was well established. When chromophores of dyes make direct contacts, their outer orbits got distorted. This effect has two consequences: (a) a tremendous increase of singlet-triplet intercombination rate constant, (b) the splitting of the lower singlet excited level into "*n*" ones (*n* - the number of interacting molecules) hampers greatly EE deactivation into oscillations. Both these mechanisms increase greatly the sum of EE deactivation constants especially in higher molecular associates which phenomena manifest themselves in a strong decrease of EE lifetime.

In photosynthesis, the fine structures of photosystems of all known bacteria, alga and plants are organized by specific transmembrane polipeptides. They arrange Chl and BChl antenna complexes in such a way, that although having the majority of closest intermolecular distances within 9 - 12 Á, they have no direct contacts. In order to illustrate the importance of this unique virtue of transmembrane proteins, one should remember that such imterchromophore spacing corresponds to mean concentrations of 0.1 - 0.03 M/l (!) while in solutions, the formation of dimers starts at concentrations ~ $3\ 10^{-4}$ M/l. The only exception is chlorosoma of green bacteria in which BChl-*c* molecules apparently form direct contacts. It was proved in Betti et al. (1982) that in non polar solutions "…Chl-*c* forms aggregates whose spectra resemble those in chlorosomes". It is appropriate to refer to unpublished experiments which the author has performed in late professor Tumerman' laboratory in which in slightly acidic aqueous solutions, Chl-*a* molecules readily adsorbed on the surface of ZnO crystal powder and on fine-dispersed drops of stearine.

In the beginning its fluorescence lifetime was about 3.5 ns, but it drop drastically, when the adsorbed dye molecules molecules formed more than 2 - 3 layers on the surfaces of these bearers. Apparent explanation: Chl-*a* molecules were well separated from each other in a single or two layers on hydrophobic surfaces, because the dimensions of their p-electron clouds (by definition they envelop 90% electron density) are 14 × 15 Á and no quenching centers were formed. It appears likely that 1 - 2 next Chl layers also could keep some kind of pseudo-crystalline structure with low quenching ability.

In the light of above said, it seems reasonable to come to the conclusion. The efficient EE migration is hardly possible from the multilayer BChl-*c* complexes quenched at the rate $(10 – 15\ ps)^{-1}$ like we often observe in huge chlorosoma.

DISCUSSION

But why could such huge chlorosama appear? The following explanation may be suggested. When green bacteria get into the media with some unfortunate parameters, the weakly persistent structure in the C740 outer layers cannot survive and hence get broken. So, a number of quenching centers appear in it which leads to a decrease of the portion of EEs delivered from it to the base plate and then to the major B866 antenna. This diminution of the EE income activates the natural regulatory mechanism to synthesize additional BChl-*c* molecules but in such situation, they only increase the mass of heaving up quenchers on the outer side of the chlorosoma. This leads to a further quenching of EEs in it and thus decreases C740 lifetime to the level of 10 - 15 ps. Possibly redox titration of chlorosoma (both *in vivo* and in isolated ones, as it was demonstrated in some works (Karapetian et al., 1980; Wang et al., 1990; Blankenship et al., 1993) renders such an example. Such redox titrations down to Em about - 1.46 V (Blankenship et al., 1993) produce such unfavorable conditions that the fine structure of chlorosoma got damaged which leads to a creation of numerous quenching centers especially in the outer layers of BChl-*c*. If this notion is reasonable, the fluorescence lifetime of BChl-c should be high in intact chlorosoma and should fall down to the unfortunately low value in the course of titration. This version requires lifetime control: the value of C740 fluorescence lifetime at least of 2 - 3 hundreds of picoseconds should be used as the criterion of chlorosoma intactness.

Possibly, enormously huge chlorosoma are specific for widespread regime of cell growing: in pursuit for more biomass, scientists keep up bacteria to very high optical densities. Thus, in the final days, most shadowy cells get enormously weak light. As a result, such cells continue to synthesize surplus of BChl-*c* unless its outer layers in chlorosoma loses its crystalline structure and produce many quenching associates. Guessing, the energy migration from chlorosoma to B795 may be rather high if green bacteria are collected from the reactor before their optical density exceeds ~ 0.5 OD.

Excitons in chlorosoma

One may reasonably expect that light absorbed by C740 creates excitons (Blankenship and Matsuura, 2003; Van Grondell et al., 1994) which expands over tens of chlorosoma molecules, provided they do have crystalline structure. Bearing in mind that the transition dipoles of C740 are parallel within 15 - 20^{o} (Betti et al., 1982; Fetisova et al., 1987; Van Amerongen et al., 1988; Griebenov et al., 1991) and assuming high degree of their crystallinity, the values of efficient dipoles of such excitons may be considerably greater than that of monomer BChl-*c* molecule. The rate constant values between chlorosoma C740 and C795 of the baseplate may thus increase close to the vector sum of individual C740 molecules involved. Unfortunately, even 30-fold increase of these rate constants can not help to elevate considerably the efficiency of C740 → C795 migration, given the distance between C740 and C795 exceeds 45 - 50 Å. It should be also noted, that the values of EE lifetime of the order of 10 - 15 ps apparently excludes the presence of good crystalline structure in C740.

Base plate

As it concerns the base plates of green bacteria, the efficiency of EE delivery from their BChl-a B795 to the major BChl-a, B866 must be high, because there exist two important advantages as compared with EE transfer from chlorosoma.

(a) Contrary to huge chlorosoma the usual ratio of B795 to B866 is only 0.5 - 0.7. Thus, the entropy barrier of about 70-90 meV, which exists in chlorosoma with 1000 – 2000 BChl-*c* per about 60 B866 is eliminated. This circumstance ensures 20 - 40-fold gain in the migration constants from its B795 to the major B866.
(b) As in B800 monomers of purple bacteria, the rate constant of wasteful losses in monomers B795 must be of similar order ~ $(1.5 - 2\ ns)^{-1}$ [compare with $(\sim 15ps)^{-1}$ in some chlorosoma], which makes 100 - 150-fold gain in favor of EE delivery from the base plate pigment to B866.
Our modeling within the use of T. Ferster' theory proved that the efficiency of $B795^{*}$ → B866 EE transfer reaches 70 and 50% for the distances between C795 and B866 pigments as long as 45 Å and 48 Å respectively.

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