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Energy transfer in photosystem II is slow

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Introduction

Photosystem II (PSII) is a complicated system, both from a structural and an energetic point of view. One of the outstanding problems is how to describe the trapping of excitation energy, which in dim light occurs predominantly through the process of charge separation. For a long time the trapping in PSII has been described with trap-limited models with varying levels of sophistication (Schatz et al., 1988; Barter et al., 2001), in which it is assumed that spatial excited-state equilibration in PSII occurs much faster than the process of charge separation. We recently concluded from different types of experiments that excited-state equilibration in LHCII (and thus in PSII) is rather slow (Barzda et al., 2001; van Amerongen & van Grondelle, 2001). A detailed understanding of the mechanism of trapping in PSII is also important for understanding the process of nonphotochemical quenching in PSII, which amongst others is determined by where and how long excitations reside in specific subunits of PSII.

Theory

In order to describe the excitation trapping we use a simplified model, which is sufficiently detailed for our purposes. We assume that irreversible charge separation takes place in the reaction center (RC) with rate constant k_{CS} . After excitation of PSII, the longest decay time is the trapping time τ_{trap} and it is in good approximation given by (Van Grondelle & Somsen, 1999):

$$\tau_{trap} = \tau_{mig} + \tau_{del} + \tau_{CS}$$

τ_{mig} reflects the spatial equilibration time of excitations in the antenna, τ_{del} the delivery time, describing excitation energy transfer from the antenna to the RC. The sum $\tau_{mig} + \tau_{del}$ is also known as the first-passage time and reflects the spatial equilibration between the antenna and RC. The parameter τ_{CS} is the charge separation time, which is given by the following equation:

$$\tau_{CS}^{-1} = \frac{e^{-E_1/(k_B T)}}{\sum_i e^{-E_i/(k_B T)}} k_{CS}$$

where the summation is taken over all pigments of PSII and $i = 1$ indicates the primary electron donor. E_i is the (Q_y) excited-state energy of pigment i , T is the absolute temperature and k_B is Boltzmann's constant. For instance, in the case of 150 isoenergetic pigments,

including the primary donor, $\tau_{trap} = 450$ ps would imply that the rate of charge separation k_{CS} equals $(3 \text{ ps})^{-1}$. A variation on this model is the radical-pair equilibrium model (Schatz et al., 1988) in which the first charge-separated state can lead to charge recombination, after which excitation energy transfer to the antenna is possible, leading to nonexponential decay of the excited-state/fluorescence. For trap-limited models it is assumed that $\tau_{mig} + \tau_{del}$ can be neglected. However, here we show that spatial equilibration is not negligibly fast and is essential for describing the trapping process.

Discussion

Most of the pigments in PSII are bound to the major light-harvesting complex LHCII, a trimeric complex, 4 of which are approximately present per RC (Jansson, 1994). The 3.4 Å crystal structure shows the locations of 12 chlorophylls (Chl's), 7 of which are presumably Chl *a*, the remaining ones being Chl *b* (Kühlbrandt et al., 1994). Two of the 3-4 xanthophylls were also revealed. Most of the down-hill energy transfer from xanthophyll and Chl *b* to Chl *a* occurs within 1 ps and within a couple of ps this transfer is complete (Visser et al., 1996; Gradinaru et al., 1998a; Gradinaru et al., 2000). Further transfer within LHCII (or to other) complexes occurs between Chl *a* molecules. While excitation energy transfer from xanthophylls and Chl *b* towards Chl *a* can easily be observed because the pigments are spectrally very different, transfer between Chl *a* molecules is difficult to monitor, especially at room temperature where the absorption spectra are very similar or at least overlap to a large extent and spectral equilibration takes at most a few ps (Kwa et al., 1992).

More details can be observed at cryogenic temperatures (transfer times range from 100 fs to over 10 ps) but energy transfer from one monomer to another cannot be discerned because the spectra of the various monomers are virtually identical. Thus no significant differences in the fs-ps kinetics can be observed for monomers (Gradinaru et al., 1998a) and trimers (Visser et al., 1996). Spatial equilibration can be studied with the use of ps singlet-singlet annihilation measurements in which intense laser pulses create more than one excitation per complex. When two excitations 'meet' each other during their diffusion process through the complex, one excitation is lost, an event that can easily be monitored as a decrease in signal intensity. The rate of annihilation for trimers at room temperature was found to be $(24 \text{ ps})^{-1}$ by Barzda et al. (2001), close to the results of Bittner et al. (1994). Because this rate reflects the simultaneous diffusion of two excitations, it is easily seen that the spatial equilibration time for one excitation in a trimer is ~ 48 ps.

The equilibration per trimer was also estimated from straightforward modeling (van Amerongen & van Grondelle, 2001), using the crystal structure of LHCII, the pigment assignment of Remelli et al. (1999), and the results from previous fs-ps pump-probe and polarized steady-state measurements. The equilibration time was estimated to be ~ 30 ps. Given the approximate nature of the modeling (it was for instance assumed that all Chl *a* molecules are isoenergetic), perfect agreement between these results would have been fortuitous, but both results indicate relatively slow equilibration.

The assumption that all Chl *a* molecules are isoenergetic is not realistic (Hemelrijk et al., 1992) and the question arises how the different spectral forms are distributed over LHCII. This question was addressed by Remelli et al. (1999). Comparison of the pigment composition and the absorption spectrum of wild-type reconstituted LHCII and of reconstituted LHCII with selectively mutated Chl-binding sites led to the conclusion that excitations tend to be located at the stromal side for two reasons: The number of Chl *a* molecules at the stromal side is higher (5 vs. 2 at the luminal side) and the average excited-state energy is lower. Taking the pigment identities as determined by Remelli et al. (1999), it was also concluded by van Amerongen and van Grondelle (2001) that the average excited-

state energy of the stromal Chl *a* molecules is lower. This conclusion was entirely based on the results of previous ultrafast and polarized steady-state experiments. At the stromal side, excitations are expected to be preferentially located near the outside of the complex, instead of near the center, for the same reasons (3 vs. 2 Chl *a* molecules are located at the outside and their excited-state energies are lower on average (Remelli et al., 2000 van Amerongen and van Grondelle, 2001). This might lead to a preference for intertrimer rather than intratrimer transfer.

Singlet-singlet annihilation studies have also been performed on lamellar aggregates of LHCII (Barzda et al., 2001). The rate constant for annihilation (normalized per trimer) was found to be $(16 \text{ ps})^{-1}$, corresponding to a spatial equilibration rate of $(32 \text{ ps})^{-1}$ per trimer. This is faster than found for individual trimers, whereas the opposite would be the case for a regular lattice of isoenergetic pigments, i. e. the normalized rate per trimer would slightly increase with increasing lattice size (Barzda et al., 2001). Therefore, these findings are in line with the idea that excitations tend to be at the periphery of LHCII.

From these results a rough estimate can be made for the contribution of LHCII to τ_{mig} of the entire antenna of PSII. Given the fact that on average ~ 4 LHCII trimers are present per RC, the corresponding spatial equilibration time is $\sim 4 \times 32 \text{ ps} = 128 \text{ ps}$. In addition to LHCII, the PSII antenna also consists of CP24, CP26, CP29 (all probably structurally similar to monomeric LHCII). For CP29 one transfer time over 10 ps is observed at 77 K (Gradinaru et al. 1998b), like for LHCII, and the core antennae CP43, CP47 show besides many subps transfer steps, also transfer components of many ps (F. de Weerd et al., see these proceedings). Thus we estimate that τ_{mig} is on the order of 150 ps or more. Many different results have been published on the overall trapping time for PSII and the best estimates range from 280 to 480 ps (Roelofs et al., 1992; Gilmore et al., 1996; Vasil'ev, 1998). It is therefore clear that τ_{mig} is not negligible. Of course, the above picture is extremely simplified, because the LHCII distribution is very heterogeneous.

Also τ_{del} cannot be ignored. The recent crystal structure of the PSII core reveals that the closest distances between Chl's in the antenna proteins CP47 and CP43 (here called linker pigments) and those of the RC are significantly larger than within CP47 and CP43 themselves (or within LHCII). Given the approximate R^{-6} dependence for the transfer rate between two pigments (with R being the inter-pigment distance) it can be anticipated that the inverse transfer rate from a linker pigment to the RC (k_{del}) is much larger than the inverse average transfer rate between neighboring pigments in the antenna. In that case τ_{del} can be approximated by $N/(zk_{del})$, where N is the number of Chl *a* molecules per RC (assumed to be isoenergetic) and z is the number of linker pigments. Taking reasonable numbers of $N = 150$, $z = 6$ and $k_{del} = (10 \text{ ps})^{-1}$, we arrive at a value of $\tau_{del} = 150 \text{ ps}$. Although the estimates for τ_{mig} and τ_{del} (both $\sim 150 \text{ ps}$) are only approximate, it seems safe to assume that the first-passage time cannot be neglected, and the trapping in PSII is at least partially diffusion-limited. This is in agreement with a recent study of Jennings et al. (2000) in which the same conclusion was reached. Going back to our original calculations where a value of $\tau_{trap} = 450 \text{ ps}$ would imply that $k_{CS} = (3 \text{ ps})^{-1}$ in the trap-limited case, we now conclude that a value of $k_{CS} = (1 \text{ ps})^{-1}$ would probably be more realistic, taking $\tau_{mig} + \tau_{del} \sim 300 \text{ ps}$. A similar value for the rate of charge separation (or even faster) was recently proposed by Dekker and van Grondelle (2000), based on ultrafast spectroscopy studies on isolated RC's.

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