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Analysis of the disk-to-disk energy transfer processes in C-phycocyanin complexes by computer simulation technique

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Abstract

Based on the crystal structure and spectral properties of C-phycocyanin (C-PC) from cyanobacteria, models for complexes with 2 and 3 C-PC hexamer disks were built and the energy transfer dynamic properties were studied by the use of stochastic computer simulation approach. In addition, an experimental parameter of 0.056 ps^{-1}, corresponding to a time constant of 18 ps, derived from the previous time-resolved measurement, was used for simulation of the energy transfer process from the three terminal symmetrically equivalent β_{84} chromophores of the core-linked disk to an α_{84} chromophore of the allophycocyanin (APC) core. The simulation showed: (*1*) The disk-to-disk energy transfer can be as fast as several picoseconds. (*2*) The energy transfer efficiencies from the first disk to the core would depend on the length of the rod (*i.e.* the number of disks). Efficiencies of 0.95, 0.87, and 0.75 were found for the rods with 1, 2 and 3 hexamer disks, respectively. (*3*) The energy transfer along a rod in a native phycobilisome (PBS) is probably very close to the one-way manner. It is the core of PBS that makes the excitation energy be transferred fast in a nearly one-way manner.

Additional key words: allophycocyanin; dynamics; kinetics; model; phycobilisome.

Introduction

The light-harvesting system of algae plays an important role in the absorption of solar energy for photosynthesis. In recent years, great progress has been made in the elucidation of the structure and function of the light-harvesting system of algae. On the other hand, up to now, the high-resolution crystal structures were limited to some isolated phycobiliproteins, while detailed kinetics and dynamic properties of energy transfer between these isolated proteins are still not clear, especially those in a PBS of cyanobacteria. For example, it is still difficult to determine experimentally the kinetic properties of the disk-to-disk transfer in a C-PC rod. In this work, models for C-PC complexes with 2 and 3 hexamer disks were built based on the crystal structure data (Duerring *et al.* 1991) and spectral properties of individual chromophore of C-PC (Glazer 1985, Debreczeny *et al.* 1993). The energy transfer processes were simulated by the use of stochastic computer simulation approach. Besides, by the use of experimental parameter of 0.056 ps^{-1}, a model PBS was re-built and the energy transfer rates and efficiencies and the dynamic properties were obtained. For understanding the model, Fig. 1 shows the relative positions of the chromophores within a 3-disk rod and an APC core.

Methods

The models for the C-PC complexes: By applying the crystal structure data with 0.166 nm resolution (Duerring *et al.* 1991) and by shifting the co-ordinates of the first disk by one or two cell lengths, we determined the position and orientation of all the chromophores in a 2-disk or 3-disk C-PC complex. In a previous paper, 18 ps time constant was derived from time-resolved fluorescence measurement of the rod-core complex and was ascribed to the energy transfer from the three terminal symmetrically equivalent β_{84} chromophores of the core-linked disk

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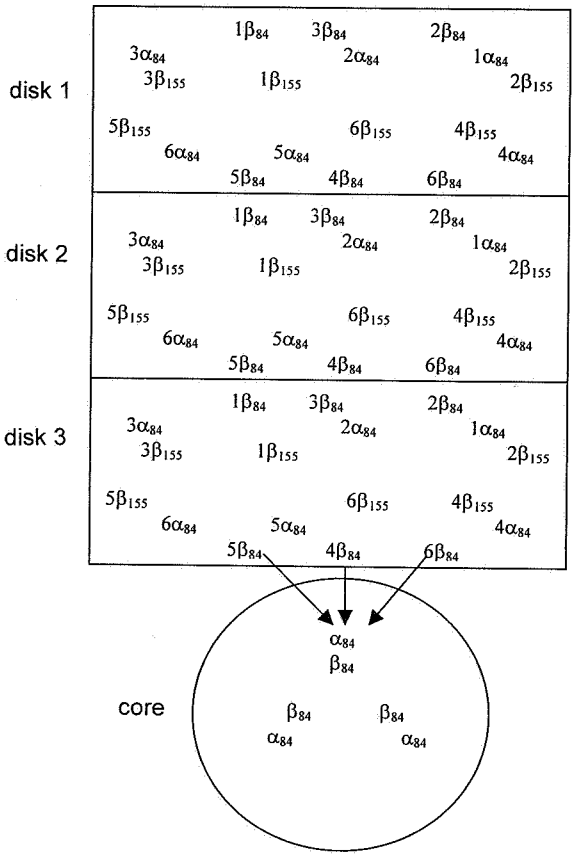

Fig. 1. Relative positions of chromophores within a 3-disk rod and an APC core.

to an α_{84} chromophore of the allophycocyanin (APC) core (Zhang *et al.* 1997). Therefore, an energy transfer rate of 0.056 ps^{-1} was used as experimental parameter.

The spectroscopic data of individual chromophore in different disks of a rod: To find the spectral properties of individual chromophores of a C-PC monomer has long been a difficult problem. Fortunately, Debreczeny *et al.* (1993) determined those successfully by using the cpcB/C155S mutant of an alga. The absorption and fluorescence values of that study are used in our work. Spectral properties of the C-PC hexamers are different depending on their sequence order in a rod (as well as on the bound linker polypeptides) (Glazer 1985). Absorption and fluorescence maxima used for building the models of C-PC complexes in this work are listed in Table 1 (taken from Glazer 1985).

The stochastic computer simulation approach: The method was published originally in 1977 (Gillespie 1977) and was described in detail for application in study of energy transfer processes in C-PC and model phycobilisomes (Zhao *et al.* 1994, 1995a,b).

Table 1. The peak wavelength of the spectra in different disks of C-PC (from Glazer 1985). Disk 3 is nearest to the core of PBS and disk 1 is farthest from it.

	Disk 1	Disk 2	Disk 3
λ^{A}_{max} [nm]	620.0	622.5	622.5
λ^{F}_{max} [nm]	643.0	648.0	652.0

Table 2. The rate constants for energy transfer in a C-PC hexamer (ns – 1). The time constant for fluorescence emission is taken as 1.5 ns.

k_T	path	k_T	k_T	path	k_T
2.8	$1\beta_{155} \leftrightarrow 1\beta_{84}$	13.5	71.2	$\beta_{84}(1) \leftrightarrow \beta_{84}(2)$	88.6
4.3	$1\alpha_{84} \leftrightarrow 1\beta_{84}$	6.9	9.0	$\alpha_{84}(1) \leftrightarrow \alpha_{84}(2)$	11.8
512.2	$1\alpha_{84} \leftrightarrow 2\beta_{84}$	814.8	9.7	$\beta_{84}(2) \leftrightarrow \beta_{84}(3)$	78.6
55.2	$1\beta_{155} \leftrightarrow 6\beta_{155}$	55.2	8.8	$\alpha_{84}(1) \leftrightarrow \alpha_{84}(2)$	10.1
149.6	$1\alpha_{84} \leftrightarrow 4\alpha_{84}$	149.6			
8.3	$1\beta_{155} \leftrightarrow 6\alpha_{84}$	27.1			
11.5	$1\alpha_{84} \leftrightarrow 6\beta_{84}$	18.5			

The energy transfer rates were calculated based on Forster dipole-dipole resonance mechanism (Foster 1967), which is reasonable for describing the energy transfer processes in the monomers and trimers of C-PC (Debreczeny and Sauer 1995, Debreczeny *et al.* 1995). The fluorescence quan-tum yields for α_{84}, β_{84}, and β_{155} chromophores obtained by Debreczeny *et al.* (1993) were only 0.23, 0.19, and 0.25, respectively, which is far less than used in their previous papers (Debreczeny and Sauer 1995, Debreczeny *et al.* 1995), *i.e.* 0.72, 0.69, and 0.72. In our work, the former group of values was applied. The calculated rate constants for important paths are listed in Table 2, while those for the less important paths are omitted. For disk-to-disk energy transfers, only these paths from $\beta_{84}(1)$ to $\beta_{84}(2)$ and from $\alpha_{84}(1)$ to $\alpha_{84}(2)$ are important for linking the two disks. The Arabic numerals in the brackets are used for denoting the disk numbers in a rod. The energy transfer rates (as well as the time constants for single-step energy trans-fers) are much lower than those used before (Sauer and Scheer 1988) because much smaller fluorescence quantum yields are used in our work.

Results and discussion

Energy transfer within a C-PC hexamer disk: In an isolated C-PC hexamer disk, the excitation energy is finally lost through fluorescence emission by the way of the chromophores of α_{84}, β_{84}, and β_{155}. Their calculated fluorescence fractions were 34.0, 54.6, and 11.4 %, respectively. The time constants for single-step energy transfers can be separated into three groups depending on which one of the three types of chromophores plays the role of a donor. The three time constants were 1.00, 1.87, and 10.20 ps for the single-step transfers with α_{84}, β_{84}, and β_{155} as the donor, respectively. The averaged transfer times through a certain path listed in Table 3 can be used to estimate the relative importance of the path. In the table, $1\beta_{155} \leftrightarrow 1\beta_{84}$ and $1\alpha_{84} \leftrightarrow 1\beta_{84}$ stand for the energy transfer paths in a monomer of C-PC. Therefore, there are 6 equivalent paths in a disk. There are also six equivalent paths for $1\alpha_{84} \leftrightarrow 2\beta_{84}$, the fast-transfer pair. Each of $1\alpha_{84} \leftrightarrow 4\alpha_{84}$, $1\beta_{155} \leftrightarrow 6\beta_{155}$, $1\alpha_{84} \leftrightarrow 6\beta_{155}$, and $1\alpha_{84} \leftrightarrow 6\beta_{84}$ represents three equivalent paths for linking the two trimers.

Table 3 shows that the averaged number of transfer times in a fast-transfer pair, such as $1\alpha_{84} \leftrightarrow 2\beta_{84}$, is as much as 70, which means that it is most probable to find the excitation energy on the two related chromophores. The α_{84} is most important for energy transfer between the two trimers while β_{155} plays only one tenth of the role.

Energy transfer in C-PC complexes: We assume that only the chromophores in disk 1 could accept photons in a C-PC complex. To observe the energy transfer dynamics, disk-to-disk energy transfer is assumed in two manners, *i.e.* in partially reversible way and in one-way. For the former, excitation energy can be transferred in two directions, *i.e.* both from disk 1 to disk 2 (forward transfer) and from disk 2 to disk 1 (back transfer) are possible, while for the latter only the forward transfers (from disk 1 to disk 2) are permissible.

The complex with two C-PC hexamer disks: The calculated time constants are 1.0, 1.7, and 10.0 ps for single-step transfers with α_{84}, β_{84}, and β_{155} as the donors, respectively, similar to those in one disk. The averaged transfer times through each path are listed in Table 4.

In a C-PC complex, the excitation energy will spend most of the time to travel forward and back in a fast-transfer pair (more than 30 times) as well as between the disks (more than 5 times). On the other hand, when the disk-to-disk transfers are assumed to be one-way, the averaged transfer times in a fast-transfer pair of disk 1 dramatically decrease (down to 2.5), while those of the disk 2 increase up to 69. The averaged transfer times from disk 1 to disk 2 could be used to evaluate the disk-to-disk energy transfer efficiency, which can be estimated as 3 (0.2890+0.0293) % = 95.5 % because three symmetrically equivalent paths are involved in energy transfer between disks.

Table 3. The averaged transfer times through the paths within a hexameric disk.

Times	Transfer path	Times	Times	Transfer path	Times
0.383	$1\beta_{155} \leftrightarrow 1\beta_{84}$	0.378	1.540	$1\beta_{155} \leftrightarrow 6\beta_{155}$	1.540
0.581	$1\alpha_{84} \leftrightarrow 1\beta_{84}$	0.595	0.710	$1\beta_{155} \leftrightarrow 6\alpha_{84}$	0.760
69.770	$1\alpha_{84} \leftrightarrow 2\beta_{84}$	69.800	1.578	$1\alpha_{84} \leftrightarrow 6\beta_{84}$	1.581
12.830	$1\alpha_{84} \leftrightarrow 4\alpha_{84}$	12.830			

Table 4. Averaged number of the transfer times through the paths in a 2-disk system. R stands for partially reversible transfer, S for one-way transfer.

Times Disk 1 R	S	Disk 2 R	S	Transfer path	Times Disk 1 R	S	Disk 2 R	S
0.170	0.012	0.216	0.379	$1\beta_{155} \leftrightarrow 1\beta_{84}$	0.178	0.024	0.191	0.341
0.277	0.021	0.316	0.582	$1\alpha_{84} \leftrightarrow 1\beta_{84}$	0.268	0.021	0.335	0.583
31.450	2.500	38.300	68.850	$1\alpha_{84} \leftrightarrow 2\beta_{84}$	31.430	2.460	38.200	68.710
5.766	0.450	7.022	12.610	$1\alpha_{84} \leftrightarrow 4\alpha_{84}$	5.766	0.450	7.022	12.610
0.702	0.115	0.813	1.465	$1\beta_{155} \leftrightarrow 6\beta_{155}$	0.702	0.115	0.813	1.465
0.363	0.063	0.400	0.709	$6\alpha_{84} \leftrightarrow 1\beta_{155}$	0.311	0.025	0.398	0.690
0.691	0.036	0.832	1.520	$1\alpha_{84} \leftrightarrow 6\beta_{84}$	0.699	0.060	0.869	1.560
		5.22(R)	0.00(S)	$\beta_{84}(1) \leftrightarrow \beta_{84}(2)$	5.35(R)	0.289(S)		
		0.41(R)	0.00(S)	$\alpha_{84}(1) \leftrightarrow \alpha_{84}(2)$	0.454(R)	0.0293(S)		

To illustrate the energy transfer dynamics between disks, Fig. 2 shows the excitation population entering into disk 2 from disk 1 with time (the partially reversible transfer model and the one-way model are denoted R and S, respectively). In Fig. 3, the excitation decay in disk 1 and the rise in disk 2 are shown for the two transfer models. Disk-to-disk transfer kinetics can be easily observed from the one-way model.

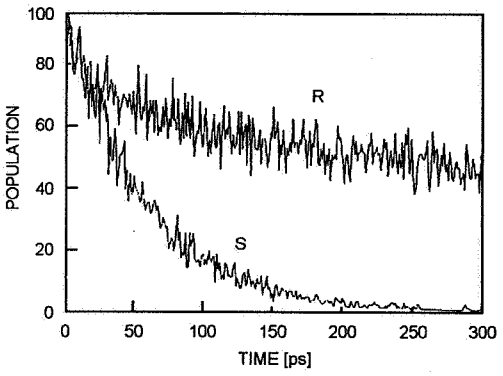

Fig. 2. Population distribution of excitation energy entering into disk 2 from disk 1 with time. R stands for the partially reversible and S for the one-way model.

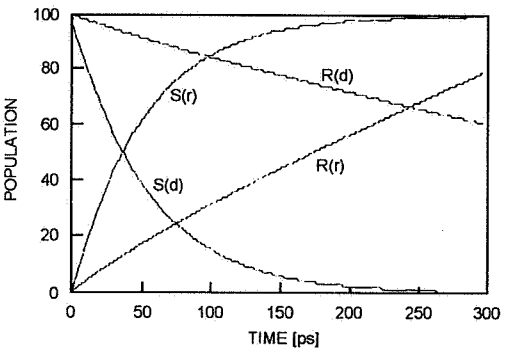

Fig. 3. The excitation decay in disk 1 and rise in disk 2. S(r) and S(d) stand for the rise and decay in one-way model, R(r) and R(d) for those in the partially reversible model.

Fig. 2 shows that the maximal population on disk 2 appeared within the first picoseconds, which indicates the energy transfer between disks is very fast. The "population distribution" of the excitation energy accounts how many photons (excitation energy) enter the next disk at different time intervals. Physically, it is similar to the rise time obtained from time-resolved spectra, however, the time at which the maximal population appears is longer than the rise time which is usually obtained by exponential fit of a rising curve instead of the peak value.

The complex with 3 C-PC hexamer disks: Fig. 4 shows the excitation population entering into disk 2 and 3 with time and Fig. 5 shows the decay and rise of excitation energy in the disks for the two different models. The kinetic properties for the excitation decay and rise are easily understandable considering the different transfer dynamics in the two models. In fact, in a real C-PC complex, the energy transfer is neither one-way nor completely reversible. The partially reversible way most likely reflects the real energy transfer processes in the C-PC complexes. However, in a native PBS, a central core located between the PBS rod and thylakoid membrane plays the role of an energy trap, which makes energy transfer more directional from the periphery C-PC disks to the central core of PBS.

C-PC complexes with 1-3 disks and a core: To simulate energy transfer processes in an intact PBS, the models for complexes with 1-3 hexamer-disks and an abstract core are built. In the models, the rate constant for energy transfer from the terminal disk of a rod to the core is replaced by an experimental parameter of 0.056 ps^{-1}. In addition, the energy transfer is assumed to be one-way from the disk to the core. The assumption is basically reasonable considering large spectral difference between C-PC and APC.

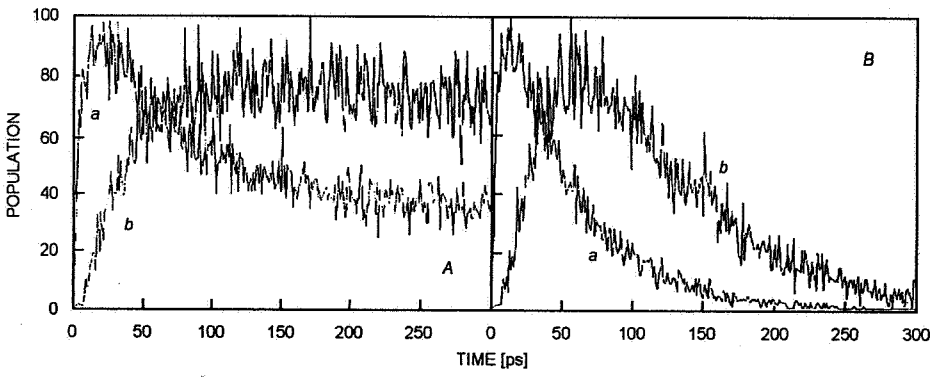

Fig. 4. Population distribution of excitation entering the disk 2 from disk 1 (*a*) and entering disk 3 from disk 2 (*b*) with time for three-disk complex and partially reversible model (*A*), and for three-disk complex and one-way model (*B*).

Energy transfer properties: Summation of the excitation population from the disks into the core can be an estimation of the energy transfer efficiencies, which were 0.9489, 0.8711, and 0.7533 for the complexes with 1, 2,

and 3 disks and a core, respectively. In fact, the absolute values of the efficiencies were sensitive and influenced by the pre-determined fluorescence lifetime, quantum yields of fluorescence, and so on. Observing the averaged transfer times can help to understand the energy transfer properties in the complexes with a trap, which most probably is a proper description of a native PBS. For example, when a trap exists with the C-PC complexes, the averaged transfer times in the fast-transfer pairs (Table 5) are far lower than those without the trap (see Table 4).

In a 2-disk complex, the averaged transfer times are 0.8856 and 0.6051 for the forward and back transfers

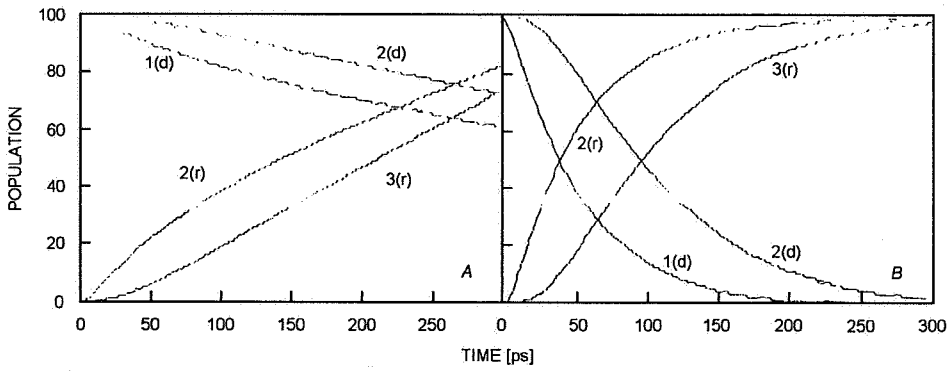

Fig. 5. Excitation decay in disk 1 [1(d)] and disk 2 [2(d)] as well as excitation rise in disk 2 [2(r)] and disk 3 [3(r)] in a 3-disk complex and partially reversible model (*A*) or in a 3-disk complex but one-way model (*B*).

Table 5. Averaged transfer times in a fast-transfer pair of the complexes with a trap.

Complex		$1\alpha_{84} \leftrightarrow 2\beta_{84}$	$2\beta_{84} \leftrightarrow 1\alpha_{84}$
1-disk		3.6702	3.7243
2-disk	disk 1	5.9895	6.0396
	disk 2	4.2082	4.4298
3-disk	disk 1	7.6547	7.7563
	disk 2	6.2369	6.4546
	disk 3	3.7082	3.9138

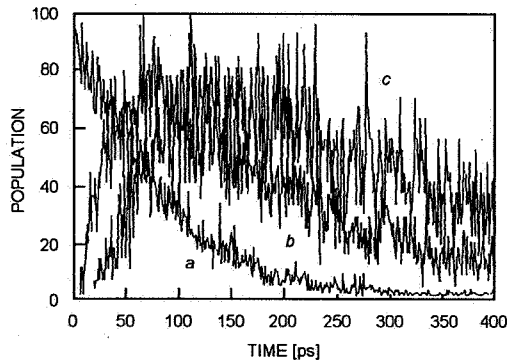

Fig. 6. Population distribution of excitation energy entering into the core with time for the C-PC complexes with 1 to 3 disks and a core. *a*: one disk; *b*: two disks; *c*: three disks.

between disks, respectively, which indicates that energy transfers in the complex are indeed partially reversible. However, by quantitative comparing of the values of transfer times in the fast-transfer pairs in Tables 4 and 5, it can be concluded that energy transfer in a C-PC complex with a trap is much closer to that shown by the one-way model.

Excitation population entering into the core with time: Fig. 6 shows the population of excitation entering into the core with time. The maximum population appeared in the core at roughly 5, 67, and 100 ps for the complexes with 1, 2, and 3 disks, respectively. The last two values are similar to the results for native PBS measured by time-resolved spectroscopy (Suter and Holzwarth 1987, Yamazaki *et al.* 1988), while the first value is much smaller than the measured one (45 ps for a phycobilisome with one-disk rods – Gillbro *et al.* 1983). The difference is most probably induced by the methodology. In fact, in time-resolved spectra of PBS, the time constant was determined based on measured fluorescence rising time of the terminal acceptor of the core, while in this work it is the time at which the excitation enters into the core which is calculated. Therefore, the difference most probably reflects the time needed for energy transfer within the core. The wide spread and large fluctuation of the population in the 2-disk and 3-disk models are due to the probability features of energy transfers in a multiple-chromophore system.

Fig. 7 shows the population distribution of excitation energy entering into the disks and the core, respectively. The maximum distribution appeared at about 10 ps in disk 2 and at roughly 60 ps in the core for the 2-disk complex (Fig. 7*A*). In Fig. 7*B*, the time for the maximum population is about 15 ps in disk 2, about 100 ps in disk 3, and 110 ps in the core. Hence the time needed for disk-to-disk energy transfer depends on which pair of disks is considered, *i.e.* the time needed for energy transfer from disk 1 to disk 2 is not the same as that from disk 2 to disk 3. In this work only the chromophores in disk 1 were excited with a δ-radiation pulse at time zero, *i.e.* at time zero the excitation probability of a chromophore in disk 1 was 1, while those in disks 2 and 3 were zero. On the

other hand, a chromophore in disk 2 could become excited only by accepting energy from disk 1, therefore, the time for excitation of the chromophores in disk 2 would spread wider. In addition, back transfers would make the distri-bution even wider. The dynamic properties of the disk-to-disk energy transfer may most likely reflect the details of real system.

Conclusion: Determination of crystal structures of nearly 10 phycobiliproteins in high-resolution make "molecular level" study possible. However, how to establish an entire model of the light-harvesting system of algae based on the structure information is still another matter. Based on the structure data and available spectral properties, some results and concepts of the energy transfer kinetics and dynamics in the models of C-PC complexes were obtained from computer simulation in this work: (*1*) Energy transfer between the disks should be very fast (as several ps). The real transfer behaviour should be a partially reversible way, while the reversibility degrees could be evaluated quantitatively by averaged numbers of the forward and the back transfer times. (*2*) When the core is linked to the C-PC complexes, the disk-to-disk energy transfer dynamics approach the one-way manner. The core is mainly responsible for the directional character and the high efficiency of energy transfers in a native PBS. (*3*) The time constants for disk-to-disk energy transfer in different disk pairs are not necessarily the same due to the different excitation probabilities of the chromophores in the disks. In fact, the time constant for the transfer from disk 2 to disk 3 must be longer than that from disk 1 to disk 2 because the chromophores in disk 1, located on the periphery of PBS, should be excited in much more probability.

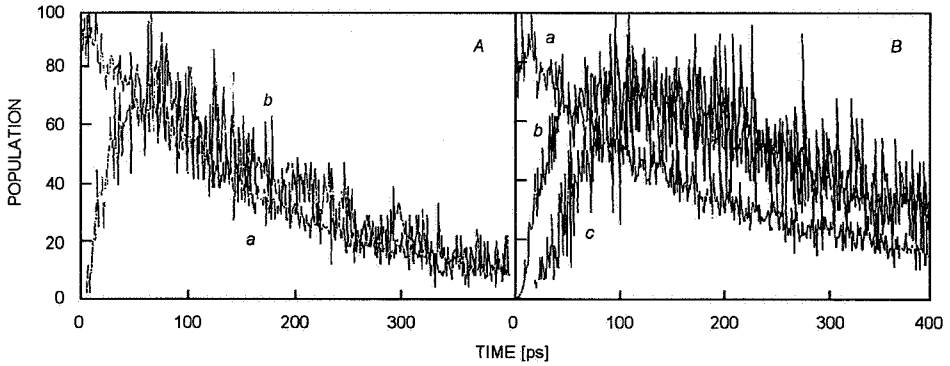

Fig. 7. Population distribution of excitation energy entering into disk 2 from disk 1 (*a*) and into the core from the disk 2 (*b*) with time for the 2-disk (*A*) or 3-disk (*B*) complex with a core.

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