

Photosynthetic Light-Harvesting Systems Organization and Function

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ENERGY TRANSFER CALCULATIONS FOR TWO C-PHYCOCYANINS BASED ON
REFINED X-RAY CRYSTAL STRUCTURE COORDINATES OF CHROMOPHORES

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Photosynthetic antennas are capable of efficient light absorption and excitation energy transfer to the reaction centers over distances of several tens of nanometers. Light capture is realized by a variety of strongly absorbing chromophores embedded in a protein matrix. These chromophores have absorption maxima that are typically closely spaced. Energy transfer proceeds in a stepwise fashion among these chromophores. For these steps, two extreme mechanisms are generally considered (1). Weak exciton coupling is operative over short distances (typically $\leq 1\text{nm}$), and Förster transfer remains efficient over much longer distances (typically $\leq 6\text{nm}$). In both cases, orientation and distances among the chromophores are critical parameters, on which little information is available in most antenna systems.

C-Phycocyanin (PC) is one of the few cases where the necessary structural information is known. Based on the preliminary crystal structure of PC from the cyanobacterium Agmenellum quadruplicatum (2), rate constants for pairwise energy transfer and the derived sets of differential equations have been determined (3) by using exclusively the Förster model (4). The fair agreement with experimentally determined absorption and

Transfer Step	Coordinates			
	New		Old	
β-Subunit				
1B155 --> 1B84	34.4	(7.6)	28.5	(6.3)
$\alpha\beta$-Monomer				
1A84 --> 1B84	13.9	(10.6)	0.037	(0.028)
1B155 --> 1A84	2.15	(0.52)	3.44	(0.84)
$(\alpha\beta)_3$-Trimer				
3A84 --> 1B84	1527.	(1160.)	59.0	(44.8)
2B84 --> 1B84	8.8	(8.8)	46.2	(46.2)
2A84 --> 1B84	2.14	(1.62)	0.18	(0.138)
2B155 --> 1A84	2.4	(0.58)	16.9	(4.1)
2A84 --> 1A84	1.25	(1.25)	0.42	(0.42)
3B155 --> 1B84	0.82	(0.18)	6.2	(1.37)
2B155 --> 1B84	0.59	(0.131)	1.55	(0.35)
2B155 --> 1B155	0.29	(0.29)	0.37	(0.37)
3B155 --> 1A84	0.092	(0.023)	0.054	(0.0131)
$(\alpha\beta)_6$-Hexamer				
4A84 --> 1A84	391.	(391.)	185.4	(185.4)
6B155 --> 1B155	186.3	(186.3)	174.8	(174.8)
6B155 --> 1A84	68.8	(16.7)	20.7	(5.0)
6A84 --> 1B84	40.7	(30.9)	1.64	(1.24)
6B84 --> 1B84	10.6	(10.6)	10.9	(10.9)
6B155 --> 1B84	9.2	(2.04)	2.01	(0.45)
5B84 --> 1B84	9.0	(9.0)	53.6	(53.6)
4B84 --> 1B84	4.5	(4.5)	10.2	(10.2)
5B155 --> 1B84	3.8	(0.83)	1.11	(0.244)
4A84 --> 1B84	1.31	(0.99)	0.059	(0.045)
6A84 --> 1A84	0.94	(0.94)	0.58	(0.58)
4B155 --> 1B84	0.79	(0.176)	1.31	(0.29)
5A84 --> 1B84	0.72	(0.54)	0.044	(0.033)
4B155 --> 1A84	0.55	(0.134)	3.5	(0.85)
5B155 --> 1A84	0.52	(0.127)	0.0033	(0.0009)
5A 84 --> 1A84	0.37	(0.37)	0.134	(0.134)
5B155 --> 1B155	0.0112	(0.0112)	0.035	(0.035)
4B155 --> 1B155	0.0094	(0.0094)	0.045	(0.045)

fluorescence kinetic data suggested that this model might be sufficient to describe energy transfer in PC.

However, more recent experimental results (5-7) indicated a conflict. Whereas theory predicted transfer between the two chromophores of lowest energy, e.g. $\alpha 84$ and $\beta 84$, as the fastest process, wavelength-resolved decay kinetics suggested that transfer from the high-energy $\beta 155$ chromophore to the low-energy ones contributes strongly to the fastest resolved component of appx. 20-40 ps.

The recent refinement of the crystal structures of trimeric PC from *Mastigocladus laminosus* and hexameric PC from *A. quadruplicatum* (8), prompted us to reinvestigate this problem. The refined structures gave a much better definition of the chromophores, in particular of the previously only poorly resolved β -84 chromophore. This resulted in minor changes in the distances, but considerable changes in the orientation parameters. We have now recalculated the energy transfer and shown that the Förster mechanism may not be sufficient to describe the system, and that strong coupling has to be invoked at least for three symmetrically equivalent pairs of chromophores.

The rate constants for pairwise energy transfer according to Förster theory are listed in Table 1 ("new") for revised coordinates of PC from *A. quadruplicatum*. The rate constants for

Table 1: Rate Constants for Förster Excitation Transfer in PC

Rate constants calculated using the Förster inductive resonance transfer expression and spectroscopic overlap integrals, as described previously (3), but using a refractive index value $n=1.34$ (10). Results which are compared are based on the "old" (2) and "new" (8) coordinates. Rate constants for backward transfer are indicated in parenthesis. For increasing extent of aggregation, values from the top of the table are to be included in a cumulative manner. All other transfer constants are related by symmetry (2,8) to the ones shown.

M. lamosus, are very similar (not shown), in agreement with the very similar structures. The largest differences are calculated for rates involving chromophore $\beta 155$, whose conformation seems to be somewhat affected by crystal packing (9). As compared with the rate constants based on the previous structural data Table 1, "old"), changes up to a factor of 40 are observed. Although this increase was largest for some of the minor rate constants, some of the fastest and hence most important ones are affected as well. In aggregates, a rate of greater than 1ps^{-1} is calculated for transfer between adjacent α -84 and β -84 chromophores situated on different monomeric units. This rate is now by far the fastest, which is even more pronounced

Table 2: Excited State Exponential Decay Time Constants Calculated for Förster Excitation Transfer in C-Phycocyanin

	β -subunit	Monomer	Trimer	Hexamer
τ_1	24	21	0.37	0.36
τ_2	--	45	22	7.5
τ_3	1500	1500	1500	1500

Calculated values listed are time constants in ps for the fast (τ_1) and intermediate (τ_2) components of a three exponential analysis, with k_f input as 0.67ns^{-1} for the rate constant of radiative and non-radiative decay corresponding to τ_3 .

Table 3: Exciton Energies of Pairwise Interaction Calculated for Chromophores of C-Phycocyanin (only values $\geq 5\text{cm}^{-1}$ listed)

	Chromophores [V_{ij} , cm^{-1}]		Exciton energies calculated from the relation $V_{ij} = Dk/\epsilon R^3$ (11), with orientation factors, k , and interchromophore distances, R , taken from Schirmer, et al (8). Dipole strength (D , in Debye ²) determined from the spectroscopic deconvolution (3), with dielectric constant $n=1.34$ (10).
β -Subunit	1B155/1B84	7.3	
$(\alpha\beta)$ -Monomer	1A84/1B84	5.3	
$(\alpha\beta)_3$ -Trimer	1A84/2B84	56.0	
$(\alpha\beta)_6$ -Hexamer	1A84/4A84	32.0	
	1B155/6B155	23.8	
	1A84/6B155	10.8	
	1A84/6B84	9.1	

because the originally second fastest rate within the trimer (β -84 \rightarrow β -84) is strongly decreased.

The occurrence of these fast rates raised the possibility that other mechanisms of energy transfer must be considered. This is supported by calculations of exciton interactions using point-dipole approximation (table 3). Pairwise interactions are in particular substantial for α 84/ β 84 on adjacent monomers, and for α 84/ α 84 on different trimer disks in the hexamer. Similar results were reported using point-monopole approximation (12).

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