Unusual Temperature Dependence of Fluorescence caused by Aggregation

VU University Amsterdam

Low-Temperature Fluorescence of CP43 and CP47 Complexes from *Synechocystis* PCC 6803

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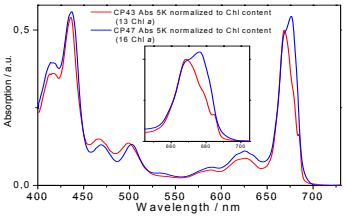
ABSTRACT

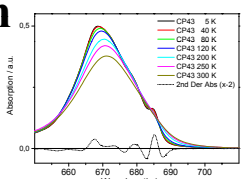
The absorption and fluorescence emission of His-tagged CP43 and CP47 antenna complexes isolated from *Synechocystis* PCC 6803 have been studied as a function of temperature between 4 K and RT, with the aim to detect differences with the corresponding spectra of these (non-His-tagged) complexes from spinach. When the complexes were diluted in glycerol solution with sufficient detergent (0.09% *β*–DM for His-tagged complexes) the 4 K emission spectrum of CP47 showed a blue-shifted main emission band compared to that of spinach (685.2 nm versus 690 nm), while that of CP43 showed a red-shifted main emission (684.9 nm versus 682.8 nm). The red shift of the latter is caused by a 2 nm red-shift of the narrow absorption band that is characteristic for CP43. The temperature dependence of the fluorescence quantum yield displayed a similar behaviour for all these complexes, i.e. an about two-fold increase of the yield upon cooling from RT to 4 K, which is typical for monomeric chlorophyll-protein complexes. Dilution in glycerol solution with smaller amounts of detergent (e.g., 0.03% for the His-tagged complexes) resulted in an unexpectedly strong temperature dependence of the fluorescence quantum yield, a very low yield at room temperature, red-shifts of the peaks at 4 K and even stronger red-shifts upon increasing the temperature (for CP47). We attribute all these characteristics to aggregation: if the excitations migrate among several complexes with inhomogeneously broadened red-most absorption bands, it will be easier to find a red form (explaining the red-shift at 4 K), and because uphill energy transfer will become more probable at higher temperatures, the effective domain size will increase (resulting in an even further shift to the red) as well as the possibility to meet a quencher (resulting in a lower fluorescence quantum yield). A similar behaviour was observed earlier for the light harvesting complex LHCII (1).



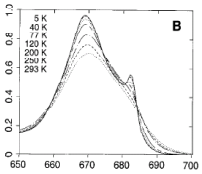
Syn. CP43 and CP47 Absorption at 5 K

Absorption

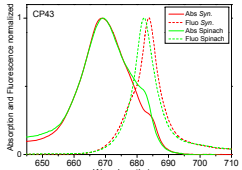
Temperature Dependence & Comparison with spinach



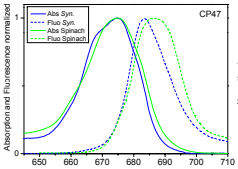
Temp. Depend. of Absorption for *Syn.* CP43 with 0.03 % *β*–DM



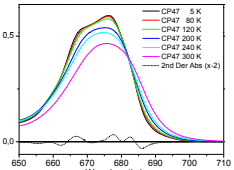
Temp. Depend. of Absorption for Spinach CP43 with 0.03 % *β*–DM (2)



CP43 Absorption and Fluorescence at 80 K: *Syn.*(0.09 % *β*–DM) vs. Spinach



CP47 Absorption and Fluorescence at 80 K: *Syn.*(0.09 % *β*–DM) vs. Spinach

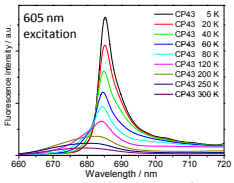


Temp. Depend. of Absorption for *Syn.* CP47 with 0.03 % *β*–DM

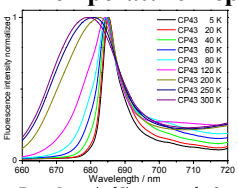
Fluorescence

Temperature Dependence & Comparison with spinach

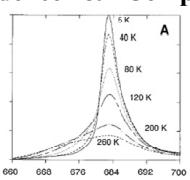
The samples prepared with 0.03 % *β*–DM are aggregated !!!!



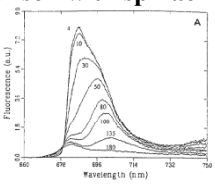
Temp. Depend. of Fluorescence for *Syn.* CP43 with 0.03 % *β*–DM



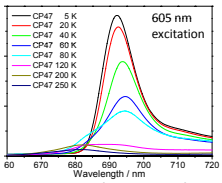
Temp. Depend. of Fluorescence for *Syn.* CP43 with 0.03 % *β*–DM normalized



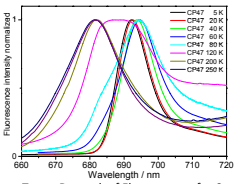
Temp. Depend. of Fluorescence for Spinach CP43 with 0.03 % *β*–DM (2)



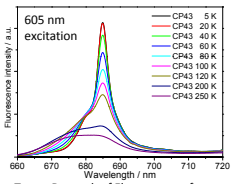
Temp. Depend. of Fluorescence for Spinach LHCII aggregates



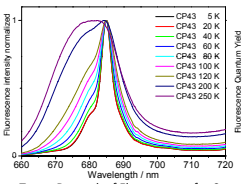
Temp. Depend. of Fluorescence for *Syn.* CP47 with 0.03 % *β*–DM



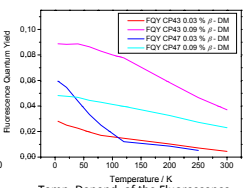
Temp. Depend. of Fluorescence for *Syn.* CP47 with 0.03 % *β*–DM normalized



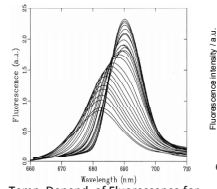
Temp. Depend. of Fluorescence for *Syn.* CP43 with 0.09 % *β*–DM



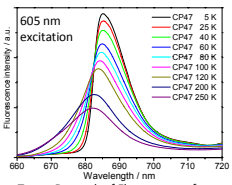
Temp. Depend. of Fluorescence for *Syn.* CP43 with 0.09 % *β*–DM normalized



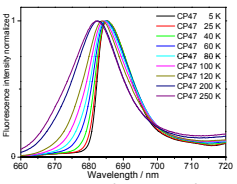
Temp. Depend. of the Fluorescence Quantum Yield



Temp. Depend. of Fluorescence for Spinach CP47 with 0.03 % *β*–DM (3)

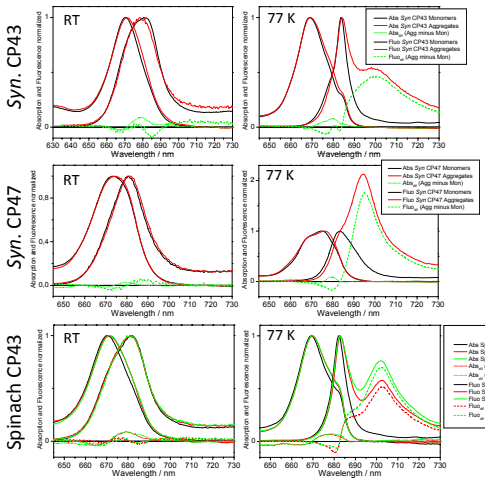


Temp. Depend. of Fluorescence for *Syn.* CP47 with 0.09 % *β*–DM



Temp. Depend. of Fluorescence for *Syn.* CP47 with 0.09 % *β*–DM normalized

Fluorescence upon aggregation



	Abs_{max} / nm	$Fluo_{max1}$ / nm	$Fluo_{max2}$ / nm	Stoke's shift		$Fluo_{max1}$ - $Fluo_{max2}$
Syn. CP43 mon	669	684		15		
Syn. CP43 agg	669	684	699	15	30	15
Syn. CP47 mon	675	683.5		8.5		
Syn. CP47 agg	676.5	684	694.5	7.5	18	10.5
Spinach CP43 mon	669.5	682.3		12.8		
Spinach CP43 agg	670	683	702	13	32	19
Spinach CP43 agg	670	683	702	13	32	19
Spinach LHCII trim	675.5	678		2.5		
Spinach LHCII agg	676.5	680	693.5	3.5	17	13.5
Spinach LHCII agg	676.5	680	694	3.5	17.5	14

The data is explained by: possible ET among complexes in the aggregate which is not possible in the solubilized (monomeric) complexes; homogeneous and inhomogeneous broadening and heterogeneity of the sample (which could also be explained by switching among different energetic states of the individual complexes present in the sample ensemble (5))

The fluorescence features attributed to aggregation have been shown to be present also in non-aggregated complexes (4)

The core antennas could also be fluorescence quenchers *in vivo* !!!!!

CP43 monomers

F680 amplitude and position constant over 5-120 K range

It arises from "blue" complexes which does not contain red states

F685 amplitude decreases with temperature

It arises from "red" complexes

CP43 aggregates

F680 not present due to energy transfer among complexes in the aggregate

F685 amplitude decreases strongly with temperature

It arises from the red-most complexes

CP47 monomers

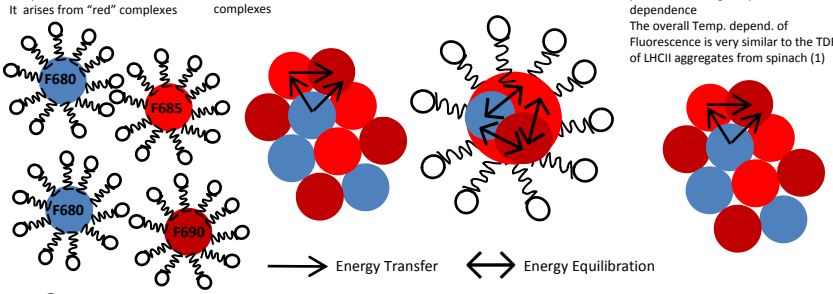
The temperature dependence of Fluorescence is explained by thermal equilibration of excitations over different spectral forms

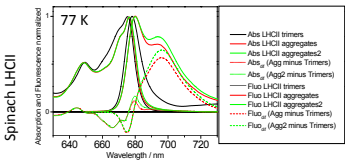
CP47 aggregates

F685 appears around 60-80 K when the thermal energy is sufficient to populate this state

F692 (5K) and F695 (60-80 K) redshifts with increasing temperature and presents a strong temperature dependence

The overall Temp. depend. of Fluorescence is very similar to the TDF of LHCII aggregates from spinach (1)





References

1. Ruban, A. V., *et al.*, (1995), *Photochem. Photobiol.*
2. Groot, M. L., *et al.*, (1995), *Biophys. J.*
3. Groot, M.-L., *et al.*, (1999), *Biophys. J.*
4. Ilioaia, C., *et al.*, (2008), *J. Biol: Chem.*
5. Krüger, T. P. J. , *et al.*, (2010), *Biophys. J.*