Oligomerization and pigmentation dependent excitation energy transfer in fucoxanthin–chlorophyll proteins

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ABSTRACT

The ultrafast carotenoid to chlorophyll a energy transfer dynamics of the isolated fucoxanthin–chlorophyll proteins FCPa and FCPb from the diatom Cyclotella meneghiniana was investigated in a comprehensive study using transient absorption in the visible and near infrared spectral region as well as static fluorescence spectroscopy. The altered oligomerization state of both antenna systems results in a more efficient energy transfer for FCPa, which is also reflected in the different chlorophyll a fluorescence quantum yields. We therefore assume an increased quenching in the higher oligomers of FCPb. The influence of the carotenoid composition was investigated using FCPa and FCPb samples grown under different light conditions and excitation wavelengths at the blue (500 nm) and red (550 nm) wings of the carotenoid absorption. The different light conditions yield in altered amounts of the xanthophyll cycle pigments diadinoxanthin and diatoxanthin. Since no significant dynamic changes are observed for high light and low light samples, the contribution of the xanthophyll cycle pigments to the energy transfer is most likely negligible. On the contrary, the observed dynamics change drastically for the different excitation wavelengths. The analyses of the decay associated spectra of FCPb suggest an altered energy transfer pathway. For FCPa even an additional time constant was found after excitation at 500 nm. It is assigned to the intrinsic lifetime of either the xanthophyll cycle carotenoids or more probable the blue absorbing fucoxanthins. Based on our studies we propose a detailed model explaining the different excitation energy transfer pathways in FCPa.

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1. Introduction

Fucoxanthin–chlorophyll proteins (FCPs) are membrane intrinsic antenna proteins with three transmembrane α-helices found in diatoms (Bacillariophyceae) and brown algae. Their functions are light harvesting as well as protection against a surplus of light [1]. FCPs show homology to the light harvesting complex (LHC II) of higher plants mainly in helix 1 and 3 [2]. However, there are big differences in their pigmentation and pigment ratio. In FCPs chlorophyll (Chl) b is replaced by Chl a and the main carotenoid (Car) [3] is fucoxanthin (fx) instead of lutein in LHC II. FCPs also contain substoichiometric amounts of the xanthophyll cycle pigments diadinoxanthin (ddx) and diatoxanthin (dtx) [4,5]. The Chl:Car ratio is ~1 in FCPs, whereas LHCs contain much more Chl molecules than Cars (Chl:Car ~ 3:1) [6–8].

From Cyclotella meneghiniana two different FCP complexes were purified, which differ in their oligomeric states and polypeptide composition [9]. The trimeric FCPa consists of mainly 18 kDa proteins (Fcp2 polypeptides) and only small amounts of 19 kDa subunits (Fcp6 polypeptides) whereas FCPb contains only 19 kDa proteins (Fcp5) associated into higher oligomeric states (inset Fig. 1). There is no hard evidence for different functions of the two FCP complexes so far. However, there are first hints that FCPb is weakly bound to photosystem I whereas FCPa might be associated with photosystem II [10]. Although a molecular structure for the FCP complexes is not yet available, a preliminary model based on fluorescence emission spectra, ultrafast kinetic data and sequence analysis has been developed [2,11]. However, the data from ultrafast transient absorption measurements used for the model were obtained from samples containing all FCP complexes, i.e. no differentiation between FCPa and FCPb complexes concerning the excitation energy transfer (EET) could be made.

Biochemical studies show that the de-epoxidation of ddx to dtx correlates with the non-photochemical quenching (NPQ) of the chlorophyll fluorescence [12–14] as does the fluorescence yield in isolated FCPa complexes [15]. FCPa and FCPb were purified from high light (HL) and low light (LL) cultures and the amounts of the different pigments were determined (Fig. 2a) [6]. Under both light conditions FCPa binds slightly more fucoxanthin than FCPb. In FCPa and FCPb
samples the de-epoxidation ratio is increased under HL conditions by a factor of more than two, however, this effect is more pronounced in the FCPa samples. The polypeptide composition does not change with the growth conditions for FCPa whereas the amount of the 19 kDa subunits increases under HL conditions compared to the amount of the 18 kDa subunits for FCPa [6]. Recent studies on intact cells of C. meneghiniana and Phaeodactylum tricornutum indicated that there are two quenching sites located in two distinguishable FCP antenna systems [16]. According to the authors’ interpretation, one FCP subpopulation stays attached to photosystem II during quenching, whereas the other subpopulation is released and aggregates. The former FCP population was discussed to be most likely identical with the trimeric FCPa. However, this assignment still needs to be validated, since the measurements were not performed on isolated complexes.

In contrast to ddx and dtx the main carotenoid fx contains a carbonyl group that is responsible for its unique spectral behavior [17]. Like other carbonyl containing carotenoids such as peridinin and siphonaxanthin, fucoxanthin exhibits an intramolecular charge transfer state (ICT) in its excited state associated with the S1 (2A_g-like) state. This S1/ICT state is responsible for the unusual properties of these carotenoids [18,19]. The absorption maximum of fucoxanthin and the lifetime of the S1/ICT is highly solvent dependent and decreases from 60 ps in nonpolar solvents to 30 ps in polar solvents accompanied by an increasing excited state absorption (ESA) at 635 nm [17,19,20]. This S1/ICT state transfers energy to Chl a with more than 90% efficiency in case of the peridinin chlorophyll a protein (FCP) [21]. Like in different solvents the absorption maximum of fucoxanthin changes due to its environment in the protein. As a result of their specific position within the protein the individual fx molecules have different absorption maxima and are therefore called f_{red} and f_{blue} [3,22,23].

To test if there are any differences in the EET-pathways of FCPa and FCPb complexes, we used steady state fluorescence and ultrafast transient absorption spectroscopy selectively exciting the Car S2 state in a spectral region of common fx and ddx absorption (λ_{exc} = 500 nm) as well as in a region where solely fx absorbs (λ_{exc} = 550 nm) [22,24,25]. To investigate the role of ddx and dtx in the EET, samples isolated from HL and LL cultures were studied. We recorded transient absorption spectra probing not only in the visible but also in the near infrared (NIR) spectral region, to observe the ICT stimulated emission (SE) band of fucoxanthin (λ_{max} = 950 nm in polar solvents [19]).

2. Materials and methods

2.1. Sample preparation

FCPa and FCPb were isolated from the centric diatom C. meneghiniana as described earlier [6,15]. The cell cultures were grown under high light (HL, 145 µE m⁻² s⁻¹ of white light) as well as under low light (LL, 45 µE m⁻² s⁻¹) conditions for ten days. In brief, isolated thylakoid membranes were solubilized at 0.25 mg of Chl a/mL with 20 mM β-1,4-dodecyl maltoside [β-DDM, 41:1 (w/w)] β-DDM: Chl a ratio] for 20 min on ice, loaded on an ion exchange column [DEAE Toyopearl 650 S (Tosoh)] and FCP fractions were eluted using a NaCl gradient [15]. Fractions were pooled and run on a sucrose density gradient over night for further purification [15]. Purified FCP complexes were harvested and concentrated using Amicon filtration devices with cutoff of 30 kDa. All FCPs were diluted in a buffer (25 mM Tris, 2 mM KCl, 0.03% β-DDM, pH 7.4) and adjusted to an optical density of ~0.9 mm at 671 nm with a concentration of ~0.13 mg Chl a mL⁻¹. All experiments were performed at room temperature.

2.2. Spectroscopic methods

Steady state absorption spectra were recorded on a Jasco V670 spectrometer, the steady state emission and fluorescence excitation spectra were collected on a Perkin Elmer LS 50 luminescence spectrometer after excitation at 500 nm and at a fluorescence wavelength of λ = 675 nm, respectively. Excitation energy transfer efficiencies
were determined by recording the Chl a fluorescence quantum yield after excitation of the Car S2 state. The emission spectra of all samples were recorded under identical conditions after excitation at λ_ex = 500 nm and λ_ex = 550 nm. The spectra were corrected with the fluorimeter detection function. The integrated fluorescence intensities and the respective absorption values of the test and the reference samples with increasing concentrations were then used to calculate the fluorescence quantum yield of Chl a and accordingly the energy transfer efficiencies in the different FCP samples.

The time resolved measurements using the femtosecond pump/probe technique were performed on a setup described before [26]. In brief, the pulse source for the time resolved experiments was a Clark CPA 2001 femtosecond laser system operating at a central wavelength of 775 nm with a repetition rate of 1 kHz. The excitation pulses with a central wavelength of 500 nm and 550 nm, respectively and a pulse energy of ~20 nJ were generated by a noncollinear optical parametric amplifier (NOPA) and focused in the sample with a focal diameter of ~100 µm. The sample was probed with super continuum white light generated in a sapphire substrate (polarization parallel to excitation) in the visible region from 450 nm to 730 nm as well as in the near infrared (NIR) spectral range from 870 nm to 1090 nm. The white light pulses were split into two beams serving as probe and reference. The transient absorption signal is dispersed and afterwards detected using two 42 segment diode arrays (multi-channel detection) providing a spectral resolution of 8 nm and a resolution of 5 ¥ 10^-4 absorbance units. To prevent degradation of the sample the cuvette was moved in two dimensions. To monitor the stability of the samples steady state absorption spectra were measured before and after the time resolved measurements.

2.3. Data analysis

Before the global analysis the data were corrected for dispersion of the white light passing through the sample. For the quantitative data analysis we used a kinetic model that describes the data as a sum of exponential decays. A Marquart downhill algorithm optimizes n global time constants (τ_i) for all wavelengths simultaneously with wavelength dependent amplitudes A_i(λ) for each component. Our model function assumes Gaussian pump and probe pulses with a (1/e) cross correlation width τ_cc:

$$\Delta A(t, \lambda) = \sum_{i=1}^{n} A_i(\lambda) \cdot \exp \left( \frac{t^2}{4\tau_i^2} - \frac{t}{\tau_i} \right) \cdot \frac{1}{2} \left( 1 + \text{erf} \left( \frac{t}{\tau_{cc}} - \frac{t_{cc}}{2\tau_i} \right) \right).$$

The n wavelength dependent fit amplitudes A_i(λ) represent the decay associated spectra (DAS) for each decay time constant. In this definition an infinite time constant is equal to the maximum time constant in our experiments (~1.5 ns).

3. Results

3.1. Steady state spectroscopy

For the steady state characterization absorption (Fig. 1, solid line), emission (dotted line) and excitation (dash-dotted line) spectra of HL-FCPa from C. meneghiniana were recorded. The main absorption features are due to the electronic transitions of the Chls. The Soret band and the Q_b band of Chl a are observable at 440 nm and at 670 nm, respectively, while the weak band around 460 nm and the shoulder at 635 nm belongs to the Soret band and the Q_b band of Chl c. The signatures in the spectral region between 480 nm and 570 nm originate from the S_0→S_2 absorption of the carotenoids. The exact band position of the particular carotenoid hereby specifically depends on the number of conjugated double bonds and the environment in the protein. Nevertheless, the solvent dependence of the absorption maximum of the carotenoids points to a spectrally broad characteristics for the fx molecules, whereas the xanthophyll pigments ddx and dtx mainly absorb around 500 nm [3,22].

To investigate the EET characteristics of the carotenoids to Chl a an excitation spectrum was recorded. For this purpose the fluorescence properties upon excitation at 500 nm were determined in a first step. The respective emission spectrum shows the Chl a fluorescence band at 675 nm accompanied by a further vibrational band located around 740 nm. Fig. 1 compares the obtained excitation (λ_ex = 675 nm) and absorption spectrum. The close similarity of both indicates the energy transfer from the carotenoids to Chl a as main pathway. For wavelengths between 535 and 600 nm excitation and absorption spectra perfectly resemble each other, whereas the excitation spectrum exhibits lower intensities at λ < 535 nm. This shows that the energy transfer efficiency of the carotenoids absorbing around 550 nm is higher compared to the ones absorbing around 500 nm. This may lead to the conclusion that ddx and dtx are less involved in the EET.

Fig. 2 shows the quantum yields of Chl a fluorescence after excitation at 500 nm and 550 nm as a measure of the energy transfer efficiencies of the carotenoids for FCPa and FCPb under HL and LL conditions. The obtained values for the complexes extracted from HL and LL cultures are the same within the error margins. On the contrary differences are found for FCPb and FCPa samples as well as for the different excitation wavelengths. FCPb samples show a reduced fluorescence quantum yield compared to the FCPa samples. Regarding the excitation wavelength dependence higher fluorescence quantum yields are obtained after excitation at λ_ex = 550 nm. This further confirms the assumption that a lower fraction of carotenoids transfers energy to Chl a upon excitation at λ_ex = 500 nm.

3.2. Transient absorption dynamics in FCPs — general band assignment

For the transient absorption measurements FCPa and FCPb samples isolated from HL and LL cultures were excited at 500 nm and 550 nm, respectively. Excitation at 500 nm leads to the population of the S_2 excited state of fx, ddx and dtx molecules, whereas upon pumping at 550 nm only fucoxanthin molecules are excited. Although significant differences for the two oligomeric states and excitation wavelengths are recorded, comparable spectral signatures are visible in all measurements. For a general band analysis the transient data of HL-FCPa probed in the visible (Fig. 3, panel a) and NIR (panel b) spectral region after excitation at λ_ex = 500 nm are illustrated in color coded 2D plots. Region A around 500 nm shows the GSB of the carotenoids which is followed by the ESA of the Chl a molecules (region B). In region C (540–700 nm) a broad ESA band depicts the carotenoid S_1→S_0 excited state absorption. This feature is superimposed by the long living GSB and SE of the Q_b band of Chl a around 670 nm (region D) [11]. Between 880 nm and 1090 nm the S_0→S_2 ESA of the carotenoids is visible at very early times (region E) [27]. In region F at wavelengths above 950 nm a weak negative band attributed to the S_1/ICT SE of fucoxanthin appears and decays within the first few picoseconds [19].

3.3. Comparison of HL and LL samples

To investigate the influence of the pigment ratios (Fig. 2a) on the EET, in a first step the HL and LL data sets of FCPa and FCPb complexes after excitation at 500 nm and 550 nm were compared. It turned out that no significant differences are obtained for these samples. This can be unambiguously confirmed in the exemplary transients depicted in Fig. 4. Slight alterations in the intensity of the signals are due to minor changes in the white light spectrum of the probe pulse. The
subsequent discussion therefore focuses on the analyses of the FCPa and FCPb samples and the excitation wavelength dependence.

3.4. Dynamics of FCPa and FCPb

In a comparison of the data sets obtained for FCPa and FCPb differences in the transient absorption spectra are clearly visible. Fig. 4 shows the individual transients at selected probing wavelengths upon excitation at 500 nm. The differences between FCPa and FCPb obtained from $\lambda_{\text{exc}}=550$ nm (data not shown) are analog to those presented for $\lambda_{\text{exc}}=500$ nm. The kinetic traces at $\lambda_{\text{probe}}=486$ nm show at early times the GSB of the carotenoids which is followed by a long living Chl a ESA after 3 ps. For a probing wavelength of 566 nm the rapid rise of the carotenoids ESA is visible which decays within 40 ps into the weaker ESA signal of Chl a. The strong negative signal at $\lambda_{\text{probe}}=678$ nm corresponds to the Qy-GSB and the SE of Chl a, that rises until $\sim 10$ ps, stays constant until $\sim 200$ ps and decays with a time constant beyond the observation time of the experiment. The comparison of the FCPa and FCPb dynamics shows a faster recovery of the Car ground state bleach for FCPb ($\lambda_{\text{probe}}=486$ nm). Also the Car ESA signal amplitude is considerably higher in FCPa than in FCPb, while the Chl a ESA signals exhibit almost the same intensity ($\lambda_{\text{probe}}=566$ nm). In the Chl a GSB/SE region around 678 nm we observe a faster rise and an immediate decay of this signal for the FCPb sample compared to FCPa.

The lifetimes and the corresponding DAS derived from the global fit analysis of the transient absorbance data in the visible spectral region are depicted in Fig. 5. They were obtained from a combined global fit analysis for the visible and NIR region. After $\lambda_{\text{exc}}=500$ nm five time constants were required for an optimized description of the data of both samples. The smallest decay time constant is not shown since it is in the range of the time resolution of the measurements. The values for $\tau_5$ are comparable with those of Chl a fluorescence lifetime measurements by time correlated single photon counting [28], but are treated as infinitive time constants in this analysis.

The comparative analysis of FCPa and FCPb shows, that both the spectral shape and the values of the time constants are similar for $\tau_2$, $\tau_3$ and $\tau_5$. Deviations are observed for $\tau_4$. In the case of FCPa the contribution of the ground state recovery of the carotenoids is still visible around 475 nm and also the decay characteristics of Car ESA around 560 nm is more pronounced than in the FCPb sample. In contrast, the GSB/SE signal from Chl a at 678 nm dominate the DAS of FCPb. In the NIR region (Fig. 4, $\lambda_{\text{pr}}=1000$ nm) the $S_2-S_0$ ESA signal decays with a time constant $\tau_4 \sim 200$ fs for both samples. The $S_1$/ICT SE band decays with 2.6 ps ($\lambda_{\text{exc}}=500$ nm) in the case of HL-FCPb and has shorter lifetimes for HL-FCPa ($\lambda_{\text{exc}}=500$ nm: $2.0$ ps). The entire data set showed no evidence for a slow $S_1$/ICT channel of fx transferring energy to Chl a, since the $S_1$/ICT SE signal decays within a few picoseconds.

3.5. HL-FCPa — excitation wavelength dependence

The transient absorption changes of HL-FCPa measured after excitation at $\lambda_{\text{exc}}=500$ nm and $\lambda_{\text{exc}}=550$ nm are shown in Fig. 3 and Fig. 6. The color coded 2D plots (Fig. 3) give an overview on the observed differences in the transient absorbance data, whereas selected kinetic traces normalized for optical density and excitation energy (top) as well as spectra for different delay times (bottom) are shown in Fig. 6.

The two excitation wavelengths result in noticeable differences in the transient absorption spectra of FCPa and less pronounced in the data of FCPb. The most pronounced effects are found in the Car GSB region (Fig. 3, region A) around 500 nm and in the carotenoid ESA region (region C), mainly around 560 nm. Upon excitation at $\lambda_{\text{exc}}=550$ nm a faster ground state recovery of the carotenoids is observed ($\lambda_{\text{pr}}=486$ nm, Fig. 6). Also the ESA around 566 nm is less pronounced and exhibits a faster decay compared to the data obtained after $\lambda_{\text{exc}}=500$ nm. Furthermore, the carotenoid ESA at 638 nm decays faster for $\lambda_{\text{exc}}=550$ nm compared to $\lambda_{\text{exc}}=500$ nm. Both kinetic traces level off at different offsets that belong to the ESA of Chl a. In contrast, the traces at 678 nm associated to the Chl a GSB and SE show almost the same shape for both excitation wavelengths. In the NIR spectral region the $S_2-S_0$ ESA (Fig. 3, region E) is restricted to wavelengths below 1000 nm for $\lambda_{\text{exc}}=550$ nm. Due to the excitation of different fx molecules and the ddx/dtx $S_2$ state this signal is clearly observed up to 1080 nm at $\lambda_{\text{exc}}=500$ nm. The $S_1$/ICT signal (region F) is stronger in case of $\lambda_{\text{exc}}=550$ nm compared to the $\lambda_{\text{exc}}=500$ nm data.

The observed features of the single transients are nicely reflected in the transient spectra in Fig. 6 (bottom). At a delay time of
τ₀ = 200 fs the main spectral feature above 540 nm is the ESA of the carotenoids (mainly fx and a slight contribution of ddx/dtx for λₑₓ𝑐 = 500 nm) and the ground state bleach below 530 nm. Even at this early delay time the contribution of the Chl a bleaching around 675 nm is already visible, indicating a fast S₂-mediated energy transfer channel from fx to Chl a [11,29,30]. At longer delay times, the carotenoid ESA decays and the Chl a bleach around 675 nm and the Chl a ESA around 480 nm grow in simultaneously. The excitation wavelength dependence is clearly observable in the spectra for τ₀ = 3 ps (Fig. 6, bottom) as an additional ESA band around λₚ₀ᵣₜₑᵣₑₑ = 560 nm and the remaining GSB around 475 nm for λₑₓ𝑐 = 500 nm. These features become even more apparent in a double difference spectrum of both excitation wavelengths taken after 3 ps (inset Fig. 6). The spectrum after 25 ps consists mainly of the Chl a contributions.

Whereas a satisfactory fit of the FCPa data upon λₑₓ𝑐 = 500 nm required five time constants (τ₁ < 150 fs, τ₂ = 0.6 ps, τ₃ = 2.6 ps, τ₄ = 25 ps and τ₅ = infinite) a comparable fit of the λₑₓ𝑐 = 550 nm data resulted in an insignificant DAS of τ₄. Thus, only four time constants (τ₁ < 150 fs, τ₂ = 0.9 ps, τ₃ = 4.2 ps and τ₅ = infinite) were sufficient to achieve a good approximation of the data. The fit amplitude of τ₄ = 25 ps upon λₑₓ𝑐 = 500 nm clearly shows the spectral features of the S₁–S₂ transition of ddx/dtx and/or fₓblue around 560 nm.

Fig. 4. Individual transients of HL-FCPa (●), LL-FCPa (○), HL-FCPb (△) and LL-FCPb (▽) measured at different probing wavelengths after excitation at 500 nm and normalized for optical density and excitation energy. The lines denote the obtained fit curves in a global fit analysis. GSB: ground state bleach, ESA: excited state absorption, SE: stimulated emission.

Fig. 5. Decay associated spectra and time constants (λₑₓ𝑐 = 550 nm in parentheses) derived from the global fit analysis of HL-FCPa (black) and HL-FCPb (grey) excited at λₑₓ𝑐 = 500 nm (→) and λₑₓ𝑐 = 550 nm (→→). The shortest time constant is not shown. Please note, that for HL-FCPa excited at λₑₓ𝑐 = 550 nm no fit amplitude for τ₄ is shown, since four time constants were sufficient to fit the data.

Fig. 6. Top: Transients at selected probing wavelengths for HL-FCPa after excitation at λₑₓ𝑐 = 500 nm (black) and λₑₓ𝑐 = 550 nm (grey) normalized for optical density and excitation energy. Bottom: Transient absorption spectra of HL-FCPa measured at different delay times after excitation at λₑₓ𝑐 = 500 nm (black) and λₑₓ𝑐 = 550 nm (grey). The difference (λₑₓ𝑐 = 500 nm minus λₑₓ𝑐 = 550 nm) between the spectra taken after 3 ps is shown in the inset.
In contrast, for FCPb five time constants are necessary for a good fit for both excitation wavelengths ($\lambda_{\text{exc}} = 500$ nm: $\tau_1 < 150$ fs, $\tau_2 = 0.4$ ps, $\tau_3 = 2.0$ ps, $\tau_4 = 19$ ps and $\tau_5 = \infty$; $\lambda_{\text{exc}} = 550$ nm: $\tau_1 = 150$ fs, $\tau_2 = 0.7$ ps, $\tau_3 = 3.0$ ps, $\tau_4 = 27$ ps and $\tau_5 = \infty$). The DAS of $\tau_2$, $\tau_3$ and $\tau_5$ resemble each other, however there are differences in the spectral shape for $\tau_a$. Upon excitation at $\lambda_{\text{exc}} = 500$ nm there is a contribution from the carotenoids around 560 nm (Fig. 5). Even though this contribution is smaller compared to FCPa, it is clearly visible compared to the DAS of FCPb for $\lambda_{\text{exc}} = 550$ nm, that has only spectral features of Chl a like the DAS for $\tau = \infty$.

4. Discussion

The goal of this study is to obtain a detailed view on the photo-dynamics of different FCP complexes after Car excitation with respect to the excited carotenoids and to the oligomerization state of FCP. It is a typical feature of light harvesting systems that the pigment composition and the strong electronic coupling between them lead to a complex photodynamic behavior of fast, competing reactions. In all FCPs the internal conversion from the $S_2$ state to the $S_1$/ICT state occurs very fast as for all Cars [27,31]. Nevertheless, excitation energy is also transferred directly from the $S_2$ state to Chl a as can be seen in the time traces <150 fs and the 2D-spectra, where the Chl a GSB and SE signal is almost instantaneously present.

In a comparison between the transient absorption spectra of HL and LL samples of both FCPa and FCPb no pronounced differences are visible (Fig. 4). The fX/Chl a ratio, that is important for the EET, is not strongly affected by varying the light conditions (Fig. 2a). Hence the transient absorbance data of the complexes indicate that the increased de-epoxidation of ddx to dtx in the HL cultures has no effect on the spectral shape for $\lambda_{\text{exc}} = 550$ nm. The $\tau_a = 27$ ps lifetime in FCPb should be comparable to $\tau_d = 25/19$ ps (FCPa/FCPb) obtained from the 550 nm excitation data, however, the time traces and fit amplitudes of the $\tau_d = 27$ ps time constant (FCPb, $\lambda_{\text{exc}} = 550$ nm) do not show the characteristic contribution of $f_{\text{blue}}$ and ddx/dtx around 560 nm (Fig. 5). The DAS of FCPb excited at 550 nm shows the spectral characteristics of Chl a like the DAS for $\tau = \infty$, indicating that the associated process can be attributed to the decay of the Chl a GSB/SE signal around $\lambda_{\text{probe}} = 678$ nm. All the differences might refer to different functions of the two protein complexes within the photosynthetic apparatus or at least to specific interaction with one of the photosystems [10,16], although we are not able to make an assignment based on our transient absorption measurements.

None of the results contained spectral signatures indicative for active involvement of Chl c in the excitation energy transfer from Car to Chl a, i.e. the fx molecules transfer the energy directly to the Chl a molecules. Additional transient absorption measurements upon direct excitation of Chl c (at $\lambda_{\text{exc}} = 630$ nm, data not shown) also resulted in spectra without characteristic spectral signature of Chl c, indicating a direct and rapid EET from Chl c to Chl a as already suggested from measurements of a mixture of FCP complexes [11].

4.1. Conclusion

The model depicted in Fig. 7 was constructed in order to demonstrate the different reaction pathways for the different excitation wavelengths in FCPa. Due to the similarities in the transient absorption

Fig. 7. Schematic model describing the energy transfer in FCPa upon excitation at two different wavelengths. The solid arrows show the main energy transfer channels, wavy arrows represent nonradiative relaxation processes and the dashed arrows depict the fluorescence of Chl a.
data of FCPa and FCPb the model might also be applied to FCPb with slight modifications. It is based on schematic models for LHC from *Amphidinium carterae* [24] and for FCPs from *C. meneghiniana* obtained from former transient absorption measurements [11]. The data obtained from the two excitation wavelengths revealed that two types of fucoxanthins (fxx-red and fxx-blue) are contained in the protein, each transferring excitation energy via their S2 and their relaxed as well as unrelaxed S1/ICT state to Chl a. To test if there is any EET from ddx/dtx to Chl a we plan further experiments with FCPs containing specifically altered amounts of ddx/dtx.

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