Time-resolved chlorophyll fluorescence studies on photosynthetic mutants of *Chlamydomonas reinhardtii*: origin of the kinetic decay components

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**Abstract.** The room temperature chlorophyll fluorescence decay kinetics of photosynthetic mutants of *Chlamydomonas reinhardtii* have been measured as a function of Photosystem 2 (PS2) trap closure, DNB-induced quenching at FM, and time-resolved emission spectra. The overall decays have been analyzed in terms of three or four kinetic components where necessary. A comparison of the characteristics of the decay components exhibited by the mutants with the wild-type has been carried out to elucidate the precise origins of the different emissions in relation to the observed pigment-protein complexes. It is shown that a) charge recombination in PS2 is not necessary for the presence of long-lived decay components, b) there are two rapid PSI-associated emissions (τ = 30 and 150–200 ps), c) a slow PS1 decay is observed (τ = 1.73 ns) in the absence of PSI reaction centres, d) the two variable components (τ = 0.25–1.2 and 0.5–2.2 ns) observed in the wild-type arise from LHC2 and e) a rapid (τ = 50–250 ps) decay is associated with the PS2 core antenna (CP3 and CP4). These results show that the intact thylakoid membrane system is too complex to distinguish all of the individual kinetic components.

**Abbreviations:** Aexp — preexponential factor (Amplitude), chl — chlorophyll, DCMU — 3-(3,4-dichlorophenyl)-1,1-dimethyl urea, DNB — m, dinitrobenzene, FM — maximum chl fluorescence level, F0 — initial chl fluorescence level, Fv — variable chl fluorescence (FM–F0), LHC — light harvesting chl a/b protein complex, PS — photosystem, QA — primary stable electron acceptor of PS2

**Introduction**

The primary processes of photosynthesis are characterized by the efficient absorption of light energy, by a complex pigment-protein system embedded in the lipid matrix of the thylakoid membrane, and its subsequent rapid (ps) transfer to the reaction centres where the exciton is trapped and charge stabilization and separation take place. During exciton migration in the antenna, there is a finite probability that they will decay either by fluorescence or by various non-radiative decay processes before trapping can take
place. Photosynthesis is, however, highly efficient (Sun and Sauer 1971) and such energy losses constitute only minor deactivation pathways. Nevertheless, chlorophyll fluorescence has become an important and valuable tool in trying to understand better the energy transfer processes, about which much uncertainty still exists. One method by which this problem is currently being investigated is by the analysis of fluorescence decay kinetics using single photon counting coupled with low intensity picosecond laser excitation. The form of the overall in vivo fluorescence decay, exhibited by photosynthetic organisms containing the light harvesting chlorophyll a/b protein complex (LHC), is multi-exponential and can usually be well defined statistically by three exponential kinetic components (Gulotty et al. 1982, Haehnel et al. 1982, Haehnel et al. 1983, Karukstis and Sauer 1983) with lifetimes in the order of 50 ps, 200–850 ps and 450–2000 ps according to the state of the primary stable electron acceptor, QA*, of the photosystem 2 (PS2) reaction centre (Moya et al. 1986a). More recent studies suggest, however, that this is an oversimplistic model (Gulotty et al. 1985, Holzwarth et al. 1986, Hodges and Moya 1986) and that the overall decay contains at least four kinetic components with lifetimes of approximately 50 ps, 250 ps, 0.25–1.40 ns and 0.45–2.5 ns (Hodges and Moya 1986). Even a model composed of four distinct decays might still be inaccurate, but any further analysis using “intact” photosynthetic systems is probably limited by the real data (see Gulotty et al. 1985). This apparent complexity is not surprising since the photosynthetic light capturing apparatus is also extremely complex. It consists of two types of photosystems (1 and 2), each associated with their own light harvesting antenna polypeptides (e.g. LHC1 and LHC2)(see Barber 1986). The situation is further complicated by the reported structural heterogeneity of PS2 into α and β centres (Melis and Ow 1982), various heterogeneities on the electron acceptor side of PS2 like Q1/Q2 (Joliot and Joliot 1981), B/non-B (Lavergne 1982) and QM/QL (Horton and Croze 1979)(see review by Black et al. 1986) and perhaps by the phosphorylation state of the LHC2 (Barber 1986). This latter phenomenon is believed to control the energy distribution between the two photosystems (Barber 1986), and is highly dependent upon the background cation levels (Hodges and Barber 1984, Telfer et al. 1984). For a mechanistic description of the overall fluorescence decay it is necessary to assign each component to functional constituents of the photosynthetic system. It might be that the decay from the “intact” photosynthetic apparatus is too complex to fully describe in terms of individual kinetic components, and therefore it is probably necessary to analyze decays from simplified systems. One approach is to carry out the decay measurements on isolated pigment-protein complexes or thylakoid fragments devoid in one or more types of