Photosynthesis Research **61:** 53–64, 1999.

Regular paper

Spectral resolution of low-energy chlorophylls in Photosystem I of *Synechocystis* sp. PCC 6803 through direct excitation

Erin M. Gill[1,3] & Bruce P. Wittmershaus[2,*]
[1]*Department of Physics and Astronomy, Arizona State University, Tempe, AZ 85287, USA;* [2]*School of Science, Pennsylvania State University: Erie, The Behrend College, Station Rd, Erie, PA 16563-0203, USA;* [3]*Present address: NIST M.S. 815.03, 325 Broadway, Boulder, CO 80303, USA;* **Author for correspondence (e-mail: bpw2@psu.edu; fax: +1-814-898-6213)*

Received 2 September 1998; accepted in revised form 21 May 1999

Key words: antenna chlorophylls, cyanobacteria, energy transfer, long-wavelength emission

Abstract

We have measured fluorescence spectra from Photosystem I (PS I) on a PS II-less mutant of the cyanobacterium *Synechocystis* sp. PCC 6803 at room temperature as a function of excitation wavelength. Our data show a gradual enhancement of long-wavelength fluorescence at 710 nm as the excitation wavelength is increased from 695 to 720 nm. This verifies the presence of low-energy chlorophylls (LE Chls), antenna Chls with energy levels below that of the primary electron donor, P700. The change in fluorescence with excitation wavelength is attributed to the finite time it takes for equilibration of excitations between the bulk and LE Chls. The spectra were deconvoluted into the sum of two basis spectra, one an estimate for fluorescence from the majority or bulk Chls and the other, the LE Chls. The bulk Chl spectrum has a major peak at 688 nm and a lower amplitude vibrational band around 745 nm and is assumed independent of excitation wavelength. The LE Chl spectrum has a major peak at 710 nm, with shoulders at 725 and 760 nm. The relative amplitude of emission at the vibrational side bands increases slightly as the excitation wavelength increases. The ratio of the fluorescence yields from LE Chls to that from bulk Chls ranges from 0.3 to 1.3 for excitation wavelengths of 695 to 720 nm, respectively. These values are consistent with a model where the LE Chls are structurally close to P700 allowing for direct transfer of excitations from both the bulk and LE Chls to P700.

Abbreviations: CCD – charge-coupled device; Chl – chlorophyll; LHC – Light-harvesting complex; LE Chl – low-energy chlorophyll; PS – Photosystem; *Synech.* – *Synechocystis* sp. PCC 6803

Introduction

The light-harvesting antenna chlorophylls (Chls) of Photosystem I (PS I) are unusual in that they typically include a small number of Chls with energy levels below that of the reaction center, P700. Once thought to be an artifact (Brody 1958; Butler 1961; Holzwarth 1991), the existence of these low-energy Chls (LE Chls) at physiological temperatures is now supported by steady-state and time-resolved measurements (Mukerji and Sauer 1989, 1990; Turconi et al. 1993; Woolf et al. 1994; Hastings et al. 1994, 1995a, b; DiMagno et al. 1995; Croce et al. 1996; White et al. 1996; Karapetyan et al. 1997; Pålsson et al. 1998; Wittmershaus et al. 1998). The role of LE Chls in PS I is unclear but they may act to extend the absorption range and work as a 'moat' to localize excitations near P700 to maximize the trapping rate (van Grondelle and Sundstrom 1988; Werst et al. 1992; Trissl 1993; Laible et al. 1994; Trinkunas and Holzwarth 1996; White et al. 1996; Beddard 1998).

The X-ray structure of PS I from the cyanobacterium *Synechococcus elongatus* reveals an oval bowl arrangement of at least 83 antenna Chls in almost

two-fold symmetry about the electron transfer chain (Krauss et al. 1996; Schubert et al. 1997). Though there is some preference for alignment of the Chls close to the center of the complex (Schubert et al. 1997), their orientations appear more random (Beddard 1998) compared to the highly ordered structures found in some other antenna protein complexes (MacDermott et al. 1995; Karrasch et al. 1995). The four Chls of the electron transfer system near P700 may act in a secondary role as part of the excitation-transfer process (Krauss et al. 1996; Schubert et al. 1997). Two of the closest antenna Chls to P700 , defined as **cC** and **cC**′ (Schubert et al. 1997), appear uniquely arranged within the structure as 'connecting' molecules for excitation transfer to P700 (Krauss et al. 1996; Schubert et al. 1997). They are symmetrically located on opposing sides of the electron transfer system in closer proximity to the general antenna Chl network than any other Chls. White et al. (1996) and Beddard (1998) propose that **cC** and **cC**′ may be LE Chls.

There are several spectral Chl-components associated with PS I. PS I has an absorption maximum around 680 nm attributed to the bulk Chls, which make up >80% of the total Chl (van Grondelle et al. 1994; Fromme 1996). A small shoulder in the 700 to 710-nm region is attributed to absorption by the LE Chls and is organism dependent (van Grondelle et al. 1994; Woolf et al. 1994; DiMagno et al. 1995; Turconi et al. 1996; Pålsson et al. 1996, 1998). The room temperature fluorescence lifetime and yield from PS I is independent of the redox-state of P700 and has peaks around 690 nm and usually at 703 to 730 nm (Holzwarth 1991; Wittmershaus et al. 1992; Karapetyan et al. 1997; Trissl 1997). The relative size and peak location of the long-wavelength component is dependent on the organism (Wittmershaus et al. 1992, 1998; Turconi et al. 1993; van Grondelle et al. 1994; DiMagno et al. 1995; Pålsson et al. 1995, 1998; Croce et al. 1996). *Spirulina platensis*, for example, has its longest emission peak at 760 nm (Shubin et al. 1995) while there is no LE Chl emission present in *Gloeobacter violaceus* (Koenig and Schmidt 1995). PS I core-complexes from many organisms appear to be similar, yet extrapolation of results from one type to another should only be made in consideration of possible spectral differences (Wittmershaus et al. 1998). There is evidence indicating spectral heterogeneity in the LE Chls (Trissl 1993; Turconi et al. 1993; Hastings et al. 1994; Croce et al. 1996; Pålsson et al. 1996, 1998; Wittmershaus et al. 1998). This heterogeneity is not evident in the context of their role in excitation transfer where they appear to act as a single pool (Woolf et al. 1994; Hastings et al. 1995a, b; Wittmershaus et al. 1998).

Efficient excitation transfer between the bulk and LE Chls is indicated by steady-state and time-resolved measurements (Werst et al. 1992; Wittmershaus et al. 1992, 1998; Holzwarth et al. 1993, 1996; Turconi et al. 1993, 1996; Woolf et al. 1994; van Grondelle et al. 1994; Hastings et al. 1994, 1995a, b; DiMagno et al. 1995; Pålsson et al. 1995; Shubin et al. 1995; Croce et al. 1996; White et al. 1996; Karapetyan et al. 1997). Time-resolved measurements have resolved inter-antenna transfer equilibration times of 3.5 to 12 ps between the bulk and LE Chls and trapping times by P700 from 19 to 50 ps (Turconi et al. 1993, 1996; Hastings et al. 1994, 1995a, b; DiMagno et al. 1995; Pålsson et al. 1995; White et al. 1996; Karapetyan et al. 1997). Theoretical modeling supports the transfer of excitations to P700 as trap or transfer-to-the-trap limited (Laible et al. 1994; van Grondelle et al. 1994; Valkunas et al. 1995; Trinkunas and Holzwarth 1996; White et al. 1996; Trissl 1997; Beddard 1998).

The strategy of our work is to enhance LE Chl fluorescence over that of the bulk Chls by directly exciting the LE Chls. The goals are to detect fluorescence from the LE Chls, form estimates of their spectral characteristics, and gain information as to their role in the excitation transfer network. The rapid rate of inter-antenna excitation transfer has been used to support the idea that excitations in the antenna Chls reach an equilibrium distribution before trapping occurs (Trissl 1993; Trissl et al. 1993; Turconi et al. 1993; Hastings et al. 1994, 1995a, b; Fromme 1996). This is based on the assumption that the excitations, once created, are transferred between antenna Chls so fast that the system reaches equilibrium quickly compared to the rate of loss processes, the major one being trapping by P700. If this assumption were true, the fluorescence spectrum of PS I would be independent of the wavelength of excitation. Through our experiments using direct excitation of the LE Chls we show that the dependence on excitation wavelength of the fluorescence spectrum from PS I at room temperature indicates the antenna Chls do not reach equilibrium fast enough to ignore the initial distribution of excited states.

When using the term, equilibrium, we mean the equilibrium of excited states between different Chls in the antennae network, most notably between the bulk and LE Chls. We assume that complete thermal equilibrium has occurred for all initially excited Chl