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Review

Water soluble chlorophyll binding protein of higher plants: A most suitable model system for basic analyses of pigment–pigment and pigment–protein interactions in chlorophyll protein complexes

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ABSTRACT

This short review paper describes spectroscopic studies on pigment–pigment and pigment–protein interactions of chlorophyll (Chl) *a* and *b* bound to the recombinant protein of class IIa water soluble chlorophyll protein (WSCP) from cauliflower. Two Chls form a strongly excitonically coupled open sandwich dimer within the tetrameric protein matrix. In marked contrast to the mode of excitonic coupling of Chl and bacterio-Chl molecules in light harvesting complexes and reaction centers of all photosynthetic organisms, the unique structural pigment array in the Chl dimer of WSCP gives rise to an upper excitonic state with a large oscillator strength. This property opens the way for thorough investigations on exciton relaxation processes in Chl–protein complexes.

Lifetime measurements of excited singlet states show that the unusual stability towards photodamage of Chls bound to WSCP, which lack any protective carotenoid molecule, originates from a high diffusion barrier to interaction of molecular dioxygen with Chl triplets.

Site selective spectroscopic methods provide a wealth of information on the interactions of the Chls with the protein matrix and on the vibronic structure of the pigments.

The presented data and discussions illustrate the great potential of WSCP as a model system for systematic experimental and theoretical studies on the functionalizing of Chls by the protein matrix. It opens the way for further detailed analyses and a deeper understanding of the properties of pigment protein complexes.

Contents

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Introduction

The functionalizing of pigments in excitation energy transfer (EET) and electron transfer (ET) is of central relevance for the underlying mechanisms of solar radiation exploitation as unique Gibbs free energy source of the biosphere through the process of photosynthesis. This goal is achieved in a perfect manner by incorporation of suitable chromophores into protein matrices. During evolution two classes of pigment–protein complexes have developed with distinctly different functions: (i) antenna systems and (ii) reaction centers (RCs). These operational units perform the light driven processes of photosynthesis in a controlled and highly efficient manner. The pigments of the antenna complexes (for reviews, see Green and Parson, 2003; Renger, 2008) absorb light and transfer the electronic excitation energy via radiationless EET to the photochemically active pigment(s) of the RCs where the transformation into electrochemical free energy takes place (for a review, see Renger, 2010). Fig. 1a schematically summarizes the functions of antenna systems and RCs and illustrates that the spectral and photochemical properties of these operational units are determined by pigment–pigment and pigment–protein interactions (Fig. 1b). Accordingly, detailed knowledge on these features is the prerequisite for a deeper understanding of the underlying mechanisms of antenna systems and RCs. A great variety of different spectroscopic methods and theoretical approaches are available to analyse the two types of interactions in pigment–protein complexes (for reviews, see Messinger et al., 2009). Unfortunately, the interpretation of experimental data obtained on antenna complexes and RCs is rather complicated because all of the known systems contain a set of multiple pigments in each subunit, thus giving rise to pigment–pigment interactions of different strengths and spectral congestion with pronounced inhomogeneous pigment–protein interaction. It is therefore highly desirable to find simple model systems that permit straightforward spectral analyses. An ideal system for detailed studies on pigment–protein interactions should contain only one chromophor bound to the protein matrix. Likewise, a complex with well defined binding of two chromophores (either excitonically or weakly coupled) would be optimal for analyzing the details of pigment–pigment interactions. Pigment–protein complexes with these properties are rare in photosynthetic organisms.

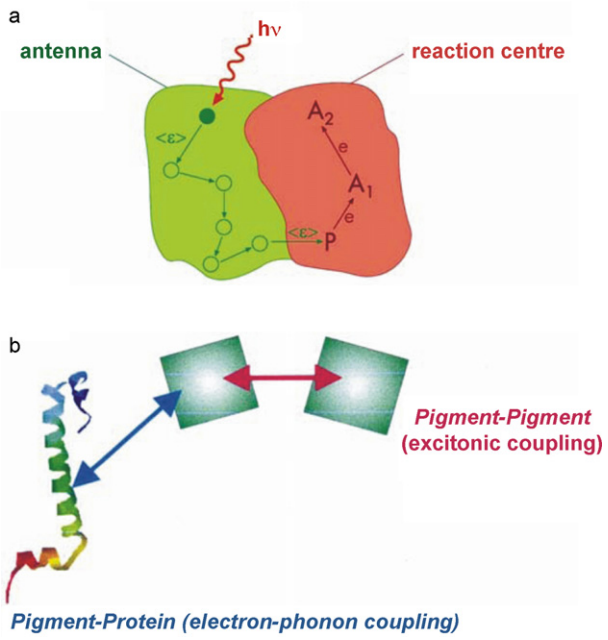

Fig. 1. Schematic representation of antenna systems and reaction centers (panel a) and of pigment–pigment and pigment–protein interactions (panel b).

Our recent studies revealed that the recombinant water soluble chlorophyll binding protein (WSCP) reconstituted with either chlorophyll (Chl) *a* or Chl *b* alone or mixtures of both offers an almost perfect model system for studies on both pigment–pigment and pigment–protein interactions. The present review summarizes the experimental and theoretical results to illustrate the potential of WSCP as a model system for thorough analyses on pigment–protein complexes.

Water soluble chlorophyll proteins in plants

Numerous higher plants (*Brassicaceae*, *Polygonaceae*, *Chenopodiaceae* and *Amaranthaceae*) contain, in addition to the pigment protein complexes that are involved directly in the primary light reactions of photosynthesis, a water-soluble Chl-binding protein (WSCP) not bound to thylakoids. This protein, mostly present in a tetrameric form, binds Chls *a* and *b* and, *in vitro*, also binds some Chl derivatives. Its affinity to Chls is high enough that it is able to extract these pigments efficiently from isolated thylakoid membranes (Satoh et al., 1998; Schmidt et al., 2003). The physiological role of WSCP is not yet clarified. It is neither part of the photosynthetic antenna system nor does it contribute to the function of the RCs. The induction of WSCP synthesis under drought- (Downing et al., 1992), salt- (Reviron et al., 1992), and heat- (Annamalai and Yanagihara, 1999) stress or upon leaf detachment (Nishio and Satoh, 1997) suggests a protective function and/or participation in the degradation/repair cycle of the photosystems.

WSCPs can be assigned to two classes: class I WSCPs exhibit a significant red shift upon illumination while class II WSCPs do not undergo photoconversion (for a review see Satoh et al., 2001). Within class II two types (IIa and IIb) exist which differ in their Chl content: Class II WSCPs form tetrameric complexes with the Chl content ranging from 1 to 4 Chls per tetramer (Satoh et al., 2001). Native as well as reconstituted recombinant class IIa WSCP (from cauliflower) binds mostly two Chls (Satoh et al., 1998; Schmidt et al., 2003) and class IIb WSCP (from *Lepidium virginicum*) four Chls per tetramer (Horigome et al., 2007).

Compared to all antenna complexes and RCs the WSCPs are characterized by unique properties which are most suitable as a model system for detailed studies on pigment–pigment and pigment–protein interactions and the interplay of the latter with the pigment–protein coupling: (i) each polypeptide subunit binds no more than one chlorophyll molecule, (ii) the size of each WSCP subunit of about 20 kDa (Nishio and Satoh, 1997) is comparable with the size of LHCII monomers and related members of the LHC family of antenna complexes of plants, all of which bind several pigments per subunit (for reviews see Green and Parson, 2003; Renger, 2008), (iii) two chlorophylls in the tetramer are excitonically coupled and (iv) WSCPs are the only Chl binding proteins known so far that do not contain carotenoids (Cars) (Schmidt et al., 2003).

Native class IIa WSCP from cauliflower binds Chl *a* and Chl *b* at a stoichiometric ratio of about 6:1 (Murata et al., 1971). The recombinant class IIa WSCP is of great advantage as model system because it can be reconstituted with different chromophores, in our studies either with Chl *a* or Chl *b* alone, thus leading to samples containing exclusively homo-dimers, or with mixtures of both pigments, giving rise to ensembles containing both homo- and heterodimers.

Spectral properties and theoretical modelling of the pigment–pigment interaction in class IIa WSCP

Fig. 2 shows the ground state absorption spectra of recombinant cauliflower WSCP reconstituted with Chl *a*/Chl *b* ratios of 2.6:1