Review

The *in vivo* and *in vitro* reconstitution of pigment-protein complexes, and its implication in acquiring a new system

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Abstract

Reconstitution is one of the most fundamental and powerful tools to investigate pigment-protein complexes, for example, light-harvesting complexes and reaction center complexes. Two reconstitution methods, *in vitro* and *in vivo*, have been applied to complexes. *In vitro* reconstitution methods were first developed using isolated proteins and pigments, and recently using over-expressed proteins. This method enables analysis of pigment binding, pigment stoichiometry, and protein flexibility when accepting extrinsic pigments, however, it has not yet been successfully applied to the core antenna system. *In vivo* reconstitution, which was developed using genetic modifications, is applicable even on core systems. In this Review, the *in vivo* reconstitution is mainly considered on the basis of the *in vitro* reconstitution, because the former was a recent development and will be expanded to many systems. When genes for a new pigment are acquired and expressed, the new pigment is incorporated into a pre-existing complex(es) and becomes functional when it is accepted by this complex(es), or abandoned if it is not. This process is postulated to occur during the evolutionary process(es) of antenna and reaction centers, and it is now possible to reproduce this evolutionary developmental pathway(s). Several examples of *in vivo* reconstitution are given and considered from the viewpoint of evolutionary implication with regards to the antenna and reaction centers.

Abbreviations: BChl – bacteriochlorophyll; Chl – chlorophyll; CP1 – P700-Chl a-protein complexes; CP43 and CP47 – core antenna complexes of PS II; LH – light-harvesting; LHC – light-harvesting complex; PS – photosystem; RC – reaction center

Introduction

The primary processes of photosynthesis are carried out by pigment-protein complexes such as photochemical reaction centers (RC) and antenna complexes. In these complexes, pigments (co-factors) convey photophysical and photochemical reactions, while protein moieties supply specific loci for these co-factors and vibrational motions to facilitate these reactions. The molecular structures of these complexes, including the co-factors, need to be strictly determined to drive these reactions, and recognition of co-factors by the protein moieties is one of the key factors involved in this structure–function relationship.

Recent analyses of the evolutionary aspects of photosynthesis clearly indicate continuity in reaction center complexes and other electron transfer
components between anoxygenic and oxygenic photosynthetic organisms (Blankenship 2002). The Photosystem (PS) I RC derives from green-sulfur bacteria and heliobacteria, whilst the PS II RC derives from purple bacteria and green filamentous bacteria. The cytochrome $bc_{1}$ complexes of photosynthetic bacteria are known to have evolved into the cytochrome $b_{6}f$ complexes of oxygenic photosynthetic organisms. The continuity of antenna complexes, however, remains unknown, but the principles of their constitution have been succeeded as follows: the PS I holds the antenna pigments in the RC complex, whilst in the PS II, RC and antenna complexes are separated; the antenna consists of a core and a peripheral antenna. The RC complex of purple bacteria is surrounded by a light-harvesting (LH) 1 complex, however, in oxygenic photosynthetic organisms LH 1 has been replaced by CP43/CP47.

It is not clear whether or not the complexes observed in currently existing organisms are the best machineries for their functions. It is possible that some of complexes currently used are fallen into a small pocket of development in the course of evolution. This hypothesis can be tested using reconstitution experiments, i.e., we might able to obtain a reconstituted complex better than the current one for a specific purpose.

Reconstitution is a useful fundamental technique for studying pigment-protein complexes. This method enables analysis of pigment binding, pigment stoichiometry, and protein flexibility when accepting extrinsic pigments. This last process can be realized by a less specific recognition, by incorporating pigments to the specific sites where intrinsic pigments bind. When new genes responsible for pigment biosynthesis are acquired and expressed, pigments might accumulate in cells. If a new pigment is free, that is, if it is not bound to an apo-protein, it might form an active oxygen species through photochemical reactions and therefore become harmful. Whereas when a new pigment becomes incorporated into a pre-existing protein(s), such as a pigment-protein complex, it might escape damage and acquire a new function within the cell. It is possible to experimentally examine this process, which is postulated to occur during the evolution of photosynthetic organisms, by introducing a gene(s) for pigment synthesis. This method can be termed ‘in vivo’ reconstitution in contrast to comparable ‘in vitro’ reconstitution methods.

New species that have acquired unique pigments have recently been reported, for example, chlorophyll (Chl) $d$ in cyanobacterium *Acaryochloris marina* (Miyashita et al. 1996, 1997), divinyl-Chl $a$ and divinyl-Chl $b$ in cyanobacterium *Prochlorococcus* sp. (Chisholm et al. 1988; Goericke and Ripeta 1992), and Zn-bacteriochlorophyll (BChl) $a$ in the aerobic photosynthetic purple bacterium *Acidiphilium rubrum* (Wakao et al. 1996). By comparing these with other organisms containing Chl $a$ or Mg-BChl $a$, it is possible to assign features of these organisms to ‘in vivo’ reconstitution. In this review, two reconstitution methods, namely *in vitro* and *in vivo* reconstitution, are discussed. The significance of the latter is discussed in detail from the evolutionary point of view of the antenna system of oxygenic photosynthetic organisms. Therefore, the readers are requested to refer another reviews on reconstitution of reaction center complexes of oxygenic photosynthesis and of photosynthetic bacteria (Scheer 2003) and of antenna system of photosynthetic bacteria (Loach and Parkes-Loach 1995).

The *in vitro* reconstitution of pigment-protein complexes

The definition of *in vitro* reconstitution is reconstitution using isolated or over-expressed proteins and purified pigments or their analogues. This method has been conducted with two types of pigment-protein complexes; the RC and the antenna complexes consisting of Chl and carotenoid molecules, both of which are necessary for *in vivo* functions.

The reconstitution of Chl in antenna complexes

**LHC I**

Light-harvesting complexes form the LHC superfamily, originally acquired as red algal PS I. *In vitro* reconstitution of the LHC I of the red alga *Porphyridium cruentum* revealed a clear indication of the evolutionary pathway of light-harvesting systems. It is known that this pigment-protein complex contains eight Chl $a$ and four zeaxanthin molecules (Wolfe et al. 1994). Grabowski et al. (2001) showed that by reconstituting Chl $b$, Chl $c$, or fucoxanthin, the LHC I of *P. cruentum* could bind these extrinsic pigments and perform energy