Minireview

A comparative structural and functional analysis of cyanobacterial plastocyanin and cytochrome c₆ as alternative electron donors to Photosystem I

Photosystem I reduction in cyanobacteria

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Abstract

Plastocyanin and cytochrome c₆ are two soluble metalloproteins that act as alternative electron carriers between the membrane-embedded complexes cytochromes b₆f and Photosystem I. Despite plastocyanin and cytochrome c₆ differing in the nature of their redox center (one is a copper protein, the other is a heme protein) and folding pattern (one is a β-barrel, the other consists of α-helices), they are exchangeable in green algae and cyanobacteria. In fact, the two proteins share a number of structural similarities that allow them to interact with the same membrane complexes in a similar way. The kinetic and thermodynamic analysis of Photosystem I reduction by plastocyanin and cytochrome c₆ reveals that the same factors govern the reaction mechanism within the same organism, but differ from one another. In cyanobacteria, in particular, the electrostatic and hydrophobic interactions between Photosystem I and its electron donors have been analyzed using the wild-type protein species and site-directed mutants. A number of residues similarly conserved in the two proteins have been shown to be critical for the electron transfer reaction. Cytochrome c₆ does contain two functional areas that are equivalent to those previously described in plastocyanin: one is a hydrophobic patch for electron transfer (site 1), and the other is an electrically charged area for complex formation (site 2). Each cyanobacterial protein contains just one arginyl residue, similarly located between sites 1 and 2, that is essential for the redox interaction with Photosystem I.

Abbreviations: Cyt – cytochrome c₆; Kₐ – equilibrium constant for complex formation; kₘₐₙ – bimolecular rate constant; kₑₜ – electron transfer rate constant; kₒₛₑₜ – pseudo-first-order rate constant; Kₐ – equilibrium constant for complex rearrangement; Pc – plastocyanin; pI – isoelectric point; PS I – Photosystem I; ΔVₑₜ – electrostatic potential for protein–protein interaction; WT – wild-type; ΔGₑₜ, ΔHₑₜ and ΔSₑₜ – changes in activation free energy, enthalpy and entropy, respectively, for the overall reaction; ΔΔGₑₜ – difference between the activation free energy changes of PS I reduction by WT and mutant donor protein

Introduction

In photosynthetic organisms, the transfer of electrons from cytochromes b₆f to Photosystem I (PS I) – which are both membrane-embedded complexes – is performed by the soluble metalloproteins cytochrome c₆ (Cyt) and plastocyanin (Pc). Pc is replaced by Cyt in cyanobacteria and green algae under copper starvation. The interchangeability of these two proteins thus depends on copper availability; in fact, the expression level of the genes encoding the metalloproteins is regulated by copper concentration (Merchant et al. 1991;
In higher plants, it had been widely accepted that Pc is the only electron carrier and so Cyt has been evolutionarily eliminated from plant chloroplasts. However, Gupta et al. (2002) and Wastl et al. (2002) very recently reported the characterization of a functional Cyt-like protein in plants that can replace Pc in the photosynthetic electron transfer process.

At first glance, the structures of Pc and Cyt are so much different (see below) that it is difficult to explain how they are able to interact with the same redox partners and how they can play the same physiological role (see Navarro et al. 1997; Hope 2000; De la Rosa et al. 2002, for reviews). The following question should thus be addressed: are equivalent areas there in Pc and Cyt?

In the last years, we have carried out a comparative structural and functional analysis of PS I reduction by Pc and Cyt isolated from a wide variety of organisms, namely plants, green algae and cyanobacteria (Hervás et al. 1992, 1995, 1996). Pc and Cyt react with PS I following a similar kinetic model and exhibit similar rate constants when they are both isolated from the same organism, but vary from one another. In order to determine and identify the equivalent interaction surface areas in Pc and Cyt, we also performed a site-directed mutagenesis study of these two proteins isolated from two cyanobacteria, *Synechocystis* and *Anabaena*, whose Pc and Cyt molecules are both almost neutral or highly basic, respectively (De la Cerda et al. 1997, 1999; Molina-Heredia et al. 1999, 2001). A number of Pc mutants from *Prochlorothrix*, which represents a diverse group of cyanobacteria, were further investigated (Navarro et al. 2001b).

In this minireview, we summarize the main experimental data that allowed us to conclude that Pc and Cyt possess two equivalent interaction areas: site 1 (or hydrophobic patch) for redox interaction with their membrane partners, and site 2 (or electrically charged region) for complex formation. Such structural similarities of Pc and Cyt at the surface level, in spite of their so great different internal structures, can thus explain their physiological exchangeability. This is indeed an excellent case model for the analysis of the structural and functional evolution of macromolecules. Kinetic and thermodynamic analyses of PS I reduction by wild-type (WT) and mutant metalloproteins are discussed.

### Structural features

Pc is a very well characterized protein, which consists of a single polypeptide chain (ca. 100 amino acids) with a copper atom. Its crystal and NMR structures have been solved in a great number of organisms, namely plants, green algae and cyanobacteria. At the Protein Data Bank, there are by now more than 30 structures of Pc: WT and mutant, oxidized and reduced forms, holo- and apoprotein, mercury-substituted species, as well as molecules at varying pH values and forming complex with cytochrome *f*. All Pc structures exhibit a similar folding (Figure 1), that is, a β-barrel formed by eight β strands along with a small α-helix. The redox center, which is typical of Type I blue copper proteins, is formed by one metal atom and four ligands (His37, Cys84, His87 and Met92) in tetrahedral coordination (Figure 1). The imidazol ring of His87 is the only solvent-exposed ligand, which has been proposed to be involved in the electron transfer from cytochrome *f* and to PS I (see Sigfridsson 1998, for a review). These four residues, as well as those forming the hydrophobic patch that surrounds His87, are highly conserved (Figure 2).

Cyt, in its turn, is likewise a monomeric protein, with 80–90 amino acids and a heme group. Its 3D structure was first solved by X-ray diffraction in the green alga *Monoraphidium braunii* (Frazão et al. 1995) and *Chlamydomonas reinhardtii* (Kerfeld et al. 1995). Up to now, four other structures of Cyt from green algal (Schnackenberg et al. 1999), red algal (Yamada et al. 2000) and cyanobacterial (Beissinger et al. 1998; Sawaya et al. 2001) sources have been determined. In all cases, Cyt is a typical Class I c-type cytochrome, mainly formed by four α-helices and a heme group, which is covalently bound to the polypeptide chain by thio-ether bonds at cysteines 15 and 18. The axial ligands to the iron atom are His19 and Met61 (Figure 1). The heme porphyrin ring is buried in a large protein cleft, with only the propionates and rings C and D being in part accessible to solvent. The comparison of Cyt amino acid sequences reveals that the heme-binding motif (CXXCH), some of the residues forming the hydrophobic pocket (Phe11, Tyr79, Trp89) and the loop containing Met61 (the distal axial ligand) are highly conserved (Figure 2).

Despite their differences in primary and secondary structures, folding pattern and redox center, Pc and Cyt possess a number of physicochemical features in common: the molecular mass is ca. 10 kDa, the midpoint redox potential value is ca. 350 mV at pH 7, and...