J|A|C|S
COMMUNICATIONS

Published on Web 02/24/2004

Pseudospecificity of the Acidic Patch of Plastocyanin for the Interaction with Cytochrome *f*

Katsuko Sato,[†] Takamitsu Kohzuma,[‡] and Christopher Dennison*,[†]

School of Natural Sciences, Bedson Building, University of Newcastle upon Tyne, Newcastle upon Tyne, NE1 7RU, UK, and Department of Materials and Biological Science, Faculty of Science, Ibaraki University, Mito, Ibaraki 310-8512, Japan

Received August 28, 2003; E-mail: christopher.dennison@ncl.ac.uk

The interaction between electron transfer (ET) proteins is usually transient in nature, which is essential for physiological function.[1,2] The specificity of such reactions is maintained without compromising the need for a rapid flow of electrons.[2] Long-range electrostatic forces enhance association and preorient the partners, whereas hydrophobic interactions (which are effective over shorter distances) are important for reactive encounter complex formation.[1] The reaction between cytochrome *f* (cyt *f*) and plastocyanin (PCu), which forms part of the photosynthetic ET chain in plants, green algae, and cyanobacteria, has been widely studied.[3−9] The structure of the complex formed by the proteins from higher plant sources has been determined[6] (see Figure 1A). The patch of acidic residues on the surface of the higher plant PCus around the conserved Tyr83 interact with a number of basic amino acids on cyt *f*. The His87 ligand, which protrudes through a hydrophobic patch on PCu, approaches the heme of cyt *f*, providing the ET pathway. The surface properties of PCu vary depending on the type of organism from which the protein originates (see Figure 1B).[14,15] In green algae (for example *Ulva pertusa*), the acidic patch is more diffuse, while in the novel PCu from the fern *Dryopteris crassirhizoma*, very few acidic residues are found around Tyr83 and an arc of aspartates and glutamates are located on the periphery of the hydrophobic patch. The latter surface feature is smaller in the *D. crassirhizoma* protein than in any other PCu.[16] Cyanobacterial PCus (such as that from *Synechococcus*) possess very few charged surface residues and have a more extensive hydrophobic patch than other PCus.[16,17] To assess the importance of the extent and location of the acidic patch for physiological function we have investigated the ionic strength dependence of the ET reaction between a variety of PCus and a higher plant cyt *f*.

The kinetics of the oxidation of turnip cyt *f*[18] by PCus from a number of sources[19] has been studied.[22] In all cases, linear plots of first-order rate constants against PCu(II) concentration are obtained (data not shown) and the slopes provide the second-order rate constant k_2.[23] The influence of ionic strength on k_2 was determined for all four PCus in the range I ≈ 0.02−2.58 M, and the results obtained are shown in Figure 2. The PCu and cyt *f* from higher plant sources react rapidly at low ionic strength[9] due to favorable electrostatic attraction between the oppositely charged surfaces on the proteins. As the ionic strength is increased, the surface charges are shielded, and thus a decrease in k_2 is observed. The absence of an acidic patch, as in the cyanobacterial PCus, has a dramatic effect on the interaction with the cyt *f*,[9] and at low ionic strength, the k_2 value for *Synechococcus* PCu is 2 orders of magnitude smaller than that for the higher plant protein. The rise in k_2 as the ionic strength is increased is due to the presence of a couple of basic residues on the surface of the *Synechococcus* PCu, which hinder association

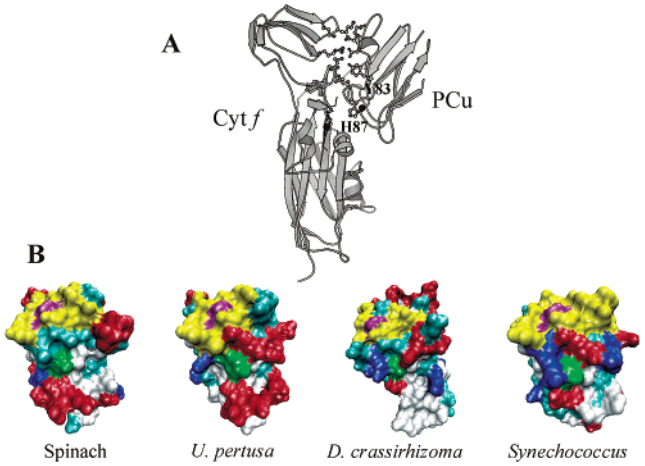

Figure 1. (A) Structure of the complex of spinach PCu with turnip cyt *f* (PDB entry 2pcf).[6] The His87 ligand of PCu is indicated as is its Tyr83 residue and surrounding acidic patch. A number of basic residues on the surface of cyt *f* are also included. The iron of cyt *f* and the copper of PCu are both shown as black spheres. (B) The surface properties of PCus from spinach (PDB entry 1AG6),[10] *U. pertusa* (PDB entry 1IUZ),[11] *D. crassirhizoma* (PDB entry 1KDJ),[12] and *Synechococcus* (PDB entry 1BXU)[13] in which the exposed imidazole ring of His87 is shown in purple and the surrounding hydrophobic patch is yellow. The acidic and basic residues are red and dark blue, respectively, polar residues are cyan, and Tyr83 is green.

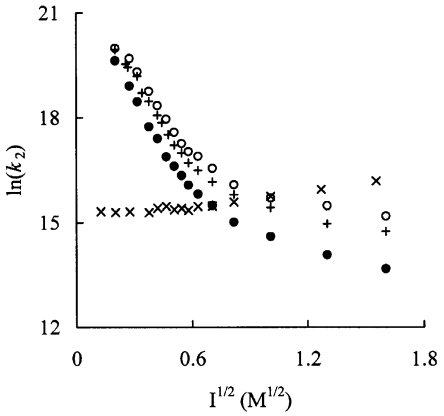

Figure 2. Ionic strength dependence of ln(k_2) for the oxidation of turnip cyt *f* by spinach (+), *U. pertusa* (○), *D. crassirhizoma* (●), and *Synechococcus* (×) PCu(II)s.

with the plant cyt *f* at low ionic strength.[9] The larger k_2 value at high ionic strength for the cyanobacterial protein, as compared to all of the other PCus (see Figure 2), can be attributed to its more extensive hydrophobic patch (see Figure 1B).

The acidic patch of the green algal PCu from *U. pertusa*[24] is more diffuse than the corresponding region of the spinach protein

[†] University of Newcastle upon Tyne.
[‡] Ibaraki University.

10.1021/ja038188k CCC: $27.50

(see Figure 1B). This has very little effect on the interaction with turnip cyt *f* (see Figure 2). At low ionic strength, the curve is virtually identical to that of spinach PCu, and it remains similar with increasing ionic strength.

The fern PCu from *D. crassirhizoma*[25] has an almost identical k_2 value for the reaction with the higher plant cyt *f* at low ionic strength as the spinach and *U. pertusa* proteins (see Figure 2). The repositioning of the acidic patch in this protein (see Figure 1B) therefore does not have a deleterious effect on the interaction with cyt *f*. Thus, the long-range electrostatic attractions, which are key to rapid encounter complex formation,[1,26,27] prevail and lead to a complex that is as efficient at performing the physiological function as the higher plant complex. As the ionic strength is increased, a similar decrease in k_2, as seen for the higher plant and green algal PCus, is observed. Thus, shielding of charge on the surface of the fern protein also disrupts the interaction with cyt *f*. At very high ionic strength, the k_2 value for *D. crassirhizoma* PCu is approximately half the value observed for the spinach protein. On the basis of observations made for the cyanobacterial PCus under these conditions, this is due to diminished hydrophobic interactions, consistent with the less extensive hydrophobic patch in the fern protein (see Figure 1B).[16]

This study demonstrates that an acidic patch on the surface of eukaryotic PCus is essential for the interaction with its physiological electron donor cyt *f*. When this region is absent, as in the *Synechococcus* PCu, the association with cyt *f* is dramatically diminished at low ionic strength.[9] Small changes in this region of PCu (as in the green algal proteins) have a minimal effect on the interaction with cyt *f*. This influence is considerably smaller than that seen in studies on mutants in which changes are made in the acidic patch.[4] However, in many of these variants not only are acidic residues removed but the replacements are basic. The remarkable finding from the present studies is that the relocation of the acidic patch (as in the fern PCu) has very little influence on the k_2 value for the reaction with cyt *f* (the structure of the complex formed will presumably be different from that shown in Figure 1A). The acidic patch on the *D. crassirhizoma* PCu is therefore still able to effectively enhance association with cyt *f*. Thus, the only requirement of a PCu to enable it to interact efficiently with a eukaryotic cyt *f* in vitro is that it has a hydrophobic patch close to the active site, which has acidic residues at its periphery. These conclusions are consistent with the fact that a number of soluble ET proteins are able to interact with various partners.[2,27,28] Thus, the need to maintain the efficient flow of electrons along an ET chain requires the protein−protein interactions involved to be pseudospecific (not highly specific).[28]

Acknowledgment. We are grateful for financial support from Newcastle University and Universities UK (for an ORS award to K.S.). We thank Professor Peter Schürmann (Université de Neuchâtel, Switzerland) for providing the spinach PCu gene.

References

(1) Bendall, D. S. In *Protein Electron Transfer*; Bendall, D. S., Ed.; Bios Scientific: Oxford, 1996; pp 43−64.
(2) Crowley, P. B.; Ubbink, M. *Acc. Chem. Res*. **2003**, *36*, 723−730.
(3) Qin, L.; Kostić, N. M. *Biochemistry* **1993**, *32*, 6073−6080.
(4) Kannt, A.; Young, S.; Bendall, D. S. *Biochim. Biophys. Acta* **1996**, *1277*, 115−126.
(5) Soriano, G. M.; Ponamarev, M. V.; Piskorowski, R. A.; Cramer, W. A. *Biochemistry* **1998**, *37*, 15120−15128.
(6) Ubbink, M.; Ejdebäck, M.; Karlsson, B. G.; Bendall, D. S. *Structure* **1998**, *6*, 323−335.
(7) Illerhaus, J.; Altschmied, L.; Reichert, J.; Zak, E.; Herrmann, R. G.; Haehnel, W. *J. Biol. Chem*. **2000**, *275*, 17590−17595.
(8) De Rienzo, F.; Gabdoulline, R. R.; Cristina Menziani, M.; De Benedetti, P. G.; Wade, R. C. *Biophys. J*. **2001**, *81*, 3090−3104.
(9) Schlarb-Ridley, B. G.; Bendall, D. S.; Howe, C. J. *Biochemistry* **2003**, *42*, 4057−4063.
(10) Xue, Y. F.; Okvist, M.; Hansson, Ö.; Young S. *Protein Sci*. **1998**, *7*, 2099−2105.
(11) Shibata, N.; Inoue, T.; Nagano, C.; Nishio, N.; Kohzuma, T.; Onodera, K.; Yoshizaki, F.; Sugimura, Y.; Kai, Y. *J. Biol. Chem*. **1999**, *274*, 4225−4230.
(12) Kohzuma, T.; Inoue, T.; Yoshizaki, F.; Sasakawa, Y.; Onodera, K.; Nagatomo, S.; Kitagawa, T.; Uzawa, S.; Isobe, Y.; Sugimura, Y.; Gotowda, M.; Kai, Y. *J. Biol. Chem*. **1999**, *274*, 11817−11823.
(13) Inoue, T.; Sugawara, H.; Hamanaka, S.; Tsukui, H.; Suzuki, E.; Kohzuma, T.; Kai, Y. *Biochemistry* **1999**, *38*, 6063−6069.
(14) Sato, K.; Kohzuma, T.; Dennison, C. *J. Am. Chem. Soc*. **2003**, *125*, 2101−2112.
(15) Hervás, M.; Navarro, J. A.; Díaz, A.; Bottin, H.; De la Rosa, M. A. *Biochemistry* **1995**, *34*, 11321−11326.
(16) The hydrophobic patches of spinach and *U. pertusa* PCus are made up of 10 nonpolar residues. In the fern PCu, the hydrophobic patch consists of only 7 residues. In the *Synechococcus* protein, this is expanded to 12 hydrophobic amino acids.
(17) De Rienzo, F.; Gabdoulline, R. R.; Menziani, M. C.; Wade, R. C. *Protein Sci*. **2000**, *9*, 1439−1454.
(18) The soluble domain of cyt *f* from turnip was purchased from Sigma and passed down a CM sepharose column prior to use in kinetic studies. Protein concentrations were determined using $\epsilon_{421} = 195\ \mathrm{mM^{-1}\ cm^{-1}}$ for reduced cyt *f*.
(19) All of the PCus were isolated and purified as described previously.[14] Protein concentrations were determined for the oxidized proteins using the following extinction coefficients (ϵ values): $\epsilon_{597} = 4700\ \mathrm{M^{-1}\ cm^{-1}}$ (spinach),[20] $\epsilon_{595} = 4900\ \mathrm{M^{-1}\ cm^{-1}}$ (*U. pertusa*),[21] $\epsilon_{590} = 4700\ \mathrm{M^{-1}\ cm^{-1}}$ (*D. crassirhizoma*),[12] and $\epsilon_{601} = 4900\ \mathrm{M^{-1}\ cm^{-1}}$ (*Synechococcus*).[14]
(20) Ejdebäck, M.; Young, S.; Samuelsson, A.; Karlsson, B. G. *Protein Expression Purif*. **1997**, *11*, 17−25.
(21) Sasakawa, Y.; Onodera, K.; Karasawa, M.; Im, S. C.; Suzuki, E.; Yoshizaki, F.; Sugimura, Y.; Shibata, N.; Inoue, T.; Kai, Y.; Nagatomo, S.; Kitagawa, T.; Kohzuma, T. *Inorg. Chim. Acta* **1998**, *283*, 184−192.
(22) Oxidation of cyt *f*(II) by the various PCu(II)s was monitored at 421 nm on an Applied Photophysics SX.18MV stopped-flow reaction analyzer at 25 °C with the proteins in 20 mM Tris pH 7.5. All rate constants were obtained under pseudo-first-order conditions [with PCu(II) in greater than 10-fold excess using cyt *f*(II) concentrations of typically 0.1 μM] and are an average of at least five determinations using the same solutions. In all cases, linear plots of first-order rate constants against [PCu(II)] were obtained. For the ionic strength dependence studies the appropriate amount of NaCl (BDH, AnalaR) was added to the buffer to give total ionic strengths in the range $I = 0.016-2.576$ M.
(23) Interpretation of the results described herein is based on a kinetic model described previously.[1,9] The second-order rate constant (k_2) for the oxidation of cyt *f*(II) by PCu(II) has been defined as: $k_2 = k_{on}k_f/(k_{off} + k_f)$, where k_{on} is the rate of association of the two proteins, k_{off} is the rate of dissociation before the ET reaction has taken place, and k_f is related to k_{ET}. It has been demonstrated[9] that for the reaction between PCu and cyt *f*, $k_f \gg k_{off}$ and thus $k_2 = k_{on}$. As a consequence of this kinetic model, variations in the reduction potentials of the PCus should not influence k_2.[9] However, the reduction potentials of spinach and *D. crassirhizoma* PCus are identical (this indicates that the different distribution of charged residues does not seem to influence the reduction potentials of PCus).
(24) The acidic patch of higher plant PCus can be divided into two regions: the upper and the lower acidic patches. The upper acidic patch is made up of residues E59, E60, and D61, whereas the lower acidic patch consists of D42, E43, D44, and E45 (D51 is also found in the vicinity of the lower acidic patch). In green algal PCus, two residues are deleted in the region of the upper acidic patch, resulting in a single carboxylate (an Asp in the *U. pertusa* protein) replacing the Ser58 to Glu60 sequence of the higher plant proteins. The residue Glu85 (numbering as in higher plant PCus) partly compensates for the loss of acidic residues in this region of the green algal proteins. The *U. pertusa* PCu also lacks one of the normal lower acidic patch residues (E45) but has an additional aspartate at position 53. These features result in the acidic patch being more diffuse in the green algal protein (see Figure 1B).
(25) Ferns form a division of the seedless vascular plants that are among the oldest terrestrial organisms known.
(26) Sheinerman, F. B.; Novel, R.; Honig, B. *Curr. Opin. Struct. Biol*. **2000**, *10*, 153−159.
(27) McLendon, G.; Hake, R. *Chem. Rev*. **1992**, *92*, 481−490.
(28) Williams, P. A.; Fülöp, V.; Leung, Y. C.; Chan, C.; Moir, J. W. B.; Howlett, G.; Fergusson, S. J.; Radford, S. E.; Hajdu, J. *Nat. Struct. Biol*. **1995**, *2*, 975−982.

JA038188K