Biochimica et Biophysica Acta, 1102 (1992) 85–90

BBABIO 43691

Reactivity of cytochromes *c* and *f* with mutant forms of spinach plastocyanin

Sandeep Modi [a], Margareta Nordling [b], Lennart G. Lundberg [b,c], Örjan Hansson [b] and Derek S. Bendall [a]

[a] *Cambridge Centre for Molecular Recognition, Department of Biochemistry, University of Cambridge, Cambridge (UK),* [b] *Department of Biochemistry and Biophysics, Chalmers University of Technology and University of Göteborg, Göteborg (Sweden) and* [c] *Cell Biology, Preclinical R&D, Astra-Hässle AB, Mölndal (Sweden)*

(Received 28 January 1992)

Key words: Cytochrome *f*; Cytochrome *c*; Electron transfer; Plastocyanin

The reduction of plastocyanin by cytochromes *c* and *f* has been investigated with mutants of spinach plastocyanin in which individual, highly conserved surface residues have been modified. These include Leu-12 and Phe-35 in the 'northern' hydrophobic patch and Tyr-83 and Asp-42 in the 'eastern' acidic patch. The differences observed all involved binding rather than the intrinsic rates of electron transfer. The Glu-12 and Ala-12 mutants showed small but significant decreases in binding constant with cytochrome *c*, even though the cytochrome is not expected to make contact with the northern face of plastocyanin. These results, and small changes in the EPR parameters, suggested that these mutations cause small conformational changes in surface residues on the eastern face of plastocyanin, transmitted through the copper centre. In the case of cytochrome *f*, the Glu-12 and Ala-12 mutants also bound less strongly, but Leu12Asn showed a marked increase in binding constant, suggesting that cytochrome *f* can hydrogen bond directly to Asn-12 in the reaction complex. A surprising result was that the kinetics of reduction of Asp42Asn were not significantly different from wild type, despite the loss of a negative charge.

Introduction

The recent development of expression systems for the small blue copper protein, plastocyanin [1–4], has provided a valuable tool for study of the molecular details of its interaction with its native reaction partners (cytochrome *f* and Photosystem I in the photosynthetic electron transport chain). Site-directed mutagenesis of the pea gene has recently led to the first conclusive evidence that the side chain of Tyr-83, which lies in the middle of the elongated acidic patch on the 'eastern' face of plastocyanin (see Fig. 1), is directly involved in binding to cytochrome *f* and forms part of the main route of electron transfer to the copper atom [4]. Conversely, reaction with Photosystem I has been shown by mutation of spinach plastocyanin at Leu-12 to involve binding at the 'northern' hydrophobic patch [5]. The copper ligand, His-87, lies in the centre of this patch, and is likely to provide a direct route for electron transfer to P700, although evidence for this is not yet available.

Cytochrome *c*, whose three-dimensional structure is well known [6], has frequently been studied as a model electron donor to plastocyanin [7] in place of the natural donor cytochrome *f*, whose structure has still to be determined [8]. In both cases a dominating feature of the reaction is the involvement of complementary charges on the two proteins, negative on plastocyanin and positive on the cytochrome, despite the fact that cytochrome *f* is overall a neutral or weakly acidic protein. The reaction with cytochrome *c* is rapid, although not as rapid as with cytochrome *f*. Comparison of the reactions with the two donors should provide an interesting insight into the origins of the specificity for the native donor.

In the present paper we report parallel studies of the kinetics of electron transfer from cytochrome *f* and cytochrome *c* to a series of spinach plastocyanins in which individual highly conserved surface residues have been modified. This is the first report of the effects of modifying residues at the northern end of the molecule on the reactivity with the cytochromes. Phe-35 has been changed to Tyr-35 and Leu-12 to Glu-12, Ala- 12

Correspondence to: D.S. Bendall, Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QW, UK.

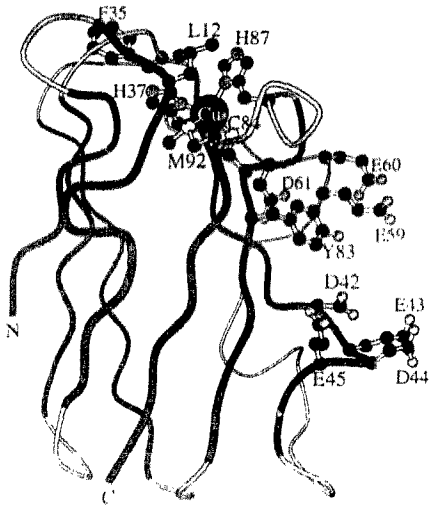

Fig. 1. Structure of plastocyanin. The program MOLSCRIPT [20] was used to draw the α-carbon backbone, the Cu ligands and selected side-chains. On the 'eastern' face are shown the two acidic patches, residues 42–45 and 59–61, and Tyr 83. In the 'northern' hydrophobic patch are shown the Cu-ligand His-87, Leu-12 and Phe-35. Coordinates were taken from the Brookhaven Database for poplar plastocyanin with the substitution of Glu-45 (as in spinach plastocyanin) for Ser-45. β-Strands are shaded.

or Asn-12. The first of these had no significant effect on the kinetics, but the last (Leu12Asn) gave the surprising result that the rate with cytochrome *f*, but not cytochrome *c*, was markedly stimulated. On the eastern face of the molecule, Tyr-83 has been changed to Phe-83 and the most highly conserved of the acidic residues, Asp-42, has been changed to Asn-42. Unexpectedly, the latter had no significant effect on rates of electron transfer.

Materials and Methods

Site-directed mutagenesis and mutant plastocyanin proteins

Mutant and wild-type proteins were obtained by expression of the mutated spinach plastocyanin gene in *E. coli*. The expression vector pUG101t$_r$ was constructed as described in Refs. 1, 5. The mutant genes were amplified in two steps by the polymerase chain reaction according to Landt et al. [9] with alterations described in Ref. 5. Transformed *E. coli*, strain TG1 [1], was grown in a fermenter [10] and proteins were purified according to Refs. 1, 5. The purity of proteins was confirmed by SDS-PAGE and measurement of the absorbance ratio A_{278}/A_{597}.

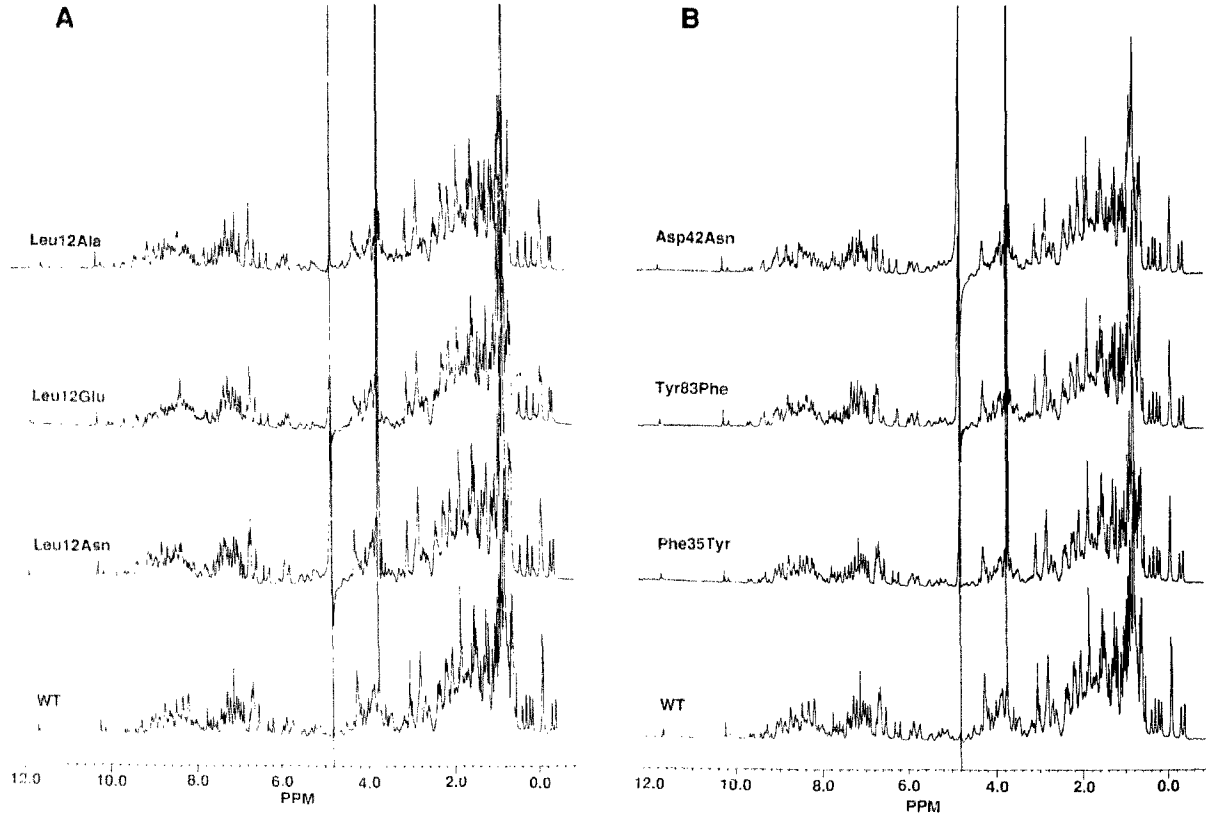

Fig. 2. ^{1}H-NMR spectra of wild type and mutant spinach plastocyanins.

Other proteins

Horse-heart cytochrome *c* (Type VI) was obtained from Sigma Chemicals and purified further by passage through columns of Sephadex G-100 and CM-cellulose [11]. Cytochrome *f* was purified from leaves of oil-seed rape, as described by Gray [12,13]. Protein concentrations were determined from the following absorption coefficients: reduced horse heart cytochrome *c*, $\epsilon_{550\ nm} = 2.76 \cdot 10^4\ M^{-1}\ cm^{-1}$; reduced oil-seed rape cytochrome *f*, $\epsilon_{554\ nm} = 2.6 \cdot 10^4\ M^{-1}\ cm^{-1}$; oxidised plastocyanin, $\epsilon_{597\ nm} = 4.7 \cdot 10^3\ M^{-1}\ cm^{-1}$.

Spectroscopic measurements

^{1}H-NMR spectra of reduced plastocyanins in 50 mM phosphate buffer (pH 6.0) at 300 K were recorded with the 500 MHz Bruker AM spectrometer of the Cambridge Centre for Molecular Recognition. 1,4-Dioxan was used as an internal reference (3.74 ppm). X-band EPR spectra were recorded with a Bruker ER 200D-SRC spectrometer equipped with an Oxford Instruments ESR-9 helium flow cryostat at 20 K. Visible absorption spectra were recorded with a Perkin-Elmer Lambda 9 spectrophotometer. Association constants (K_A) for cytochrome *c* were obtained by measuring changes in absorbance (ΔA) at 410 nm on titration of cytochrome *c* (50.62 μM) with a varying concentration of plastocyanin (0 to 260 μM) in a buffer containing 10 mM phosphate, 90 mM NaCl (pH 6.0) using quartz cells of pathlength 2 mm at 300 K [14]. Association constants for cytochrome *f* were obtained similarly, except that the concentration of cytochrome *f* was 10.8 μM, that of plastocyanin ranged from 0 to 60 μM, and 10 mm quartz cuvettes were used [4].

Kinetic measurements

Electron transfer from reduced cytochrome *c* and reduced cytochrome *f* to oxidized plastocyanin was monitored at 417 nm and 421 nm, respectively, with an Applied Photophysics stopped-flow spectrophotometer (SF.17MV). For measurement of the second-order rate constant, k_2, the concentrations of cytochrome *c* and cytochrome *f* were 0.8–0.9 μM and 0.1–0.2 μM, respectively, and oxidation of cytochrome *c*/*f* was carried out by addition of a 10-fold excess of oxidized plastocyanin in a buffer containing 10 mM phosphate, 90 mM NaCl (pH 6.0) at 300 K. The observed first order rate constant was divided by the plastocyanin concentration to give k_2.

The rate of binding of plastocyanin and cytochrome *c*/*f* was measured by following the increase in absorbance of oxidized cytochrome *c*/*f* at 410 nm in the stopped-flow spectrophotometer at 300 K [4,14]. Oxidized cytochrome *c*/*f* (10–12 μM) and oxidized plastocyanin (40–50 μM) were dissolved in 10 mM phosphate, 90 mM NaCl (pH 6.0).

TABLE I

EPR and ^{1}H-NMR parameters for mutant and wild-type plastocyanins

$A_\parallel$ and $g_\parallel$ denote the hyperfine splitting and *g*-value, respectively, of the low-field peak in the plastocyanin first-derivative EPR spectrum. The estimated error ranges are ± 0.05 mT for $A_\parallel$ and ± 0.005 for $g_\parallel$.

Plastocyanin	$A_\parallel$ (mT)	$g_\parallel$	Chemical shift (ppm) His-37-N$^\epsilon$H
Wild type	6.4	2.24	11.65
Leu12Asn	6.2	2.24	11.82
Leu12Glu	5.9	2.24	11.76
Leu12Ala	5.5	2.25	11.47
Tyr83Phe	6.4	2.24	11.68
Asp42Asn	6.4	2.24	11.70
Phe35Tyr	6.3	2.24	11.66

Results

As a basis for understanding the kinetic results described below, the conformation of all mutant plastocyanins was examined by ^{1}H-NMR. 1D spectra of the mutant proteins in H_2O are compared with that of the wild-type in Fig. 2. The assignments used are based on those of Driscoll et al. [15], but the chemical shifts are slightly different from those originally reported because of the different conditions of our measurements. In all the Leu-12 mutants the upfield resonance of the C$^\delta$-methyl protons at 0.29 ppm is clearly missing (Fig. 2A). Small shifts in the region of the backbone amide proton of Leu-12 at 6.66 ppm are also apparent. In the downfield region there are shifts in the resonances of His-37-N$^\epsilon$H at 11.65 ppm (Table I), Asn-38 backbone NH at 10.12 ppm, Ser-85 backbone NH at 9.66 ppm and, most surprisingly, of the backbone NH of Tyr-83 (9.41 ppm). Overall, however, the close similarity between the spectra of the mutant proteins and the wild-type shows that no change in tertiary structure can have occurred.

In the case of Phe35Tyr it is possible to discern that the peak at 5.73 ppm due to the backbone NH of Phe-35 is replaced by a new peak slightly downfield, presumably that of Tyr-35 (Fig. 2B). In Tyr83Phe the backbone NH of Tyr-83 at 9.41 ppm has been replaced by a new peak slightly upfield which is likely to be that of Phe-83. In this mutant there is a noticeable shift of the Gln-88-N$^\epsilon$ proton (6.30 ppm). In the crystal structure of poplar plastocyanin [16] the side chain of Gln-88 was found to lie close to that of Tyr-83. Resonances of Asp-42 occur in crowded regions of the spectrum and so the changes in Asp42Asn will require 2D spectra for satisfactory analysis; a new peak has appeared at about 6.65 ppm which may be attributable to the side chain N$^\delta$H. In this mutant there has also been a noticeable downfield shift of the backbone NH of Tyr-83; these two residues are closely associated in the three-dimensional structure. Again, the spectra provide no evi-

dence for major change in tertiary structure of any of the mutant proteins.

EPR spectra provide a sensitive test of the ligand structure of the copper atom in plastocyanin. The distorted tetrahedral conformation is responsible for unique spectral properties such as the intense blue colour and the narrow hyperfine splitting in the EPR spectrum [17]. The values for the EPR parameters of the mutant and wild-type proteins are listed in Table I. These parameters are related to both the symmetry of the ligand atom arrangement around the metal ion and the covalency of the Cu^{2+}-ligand bond. The results show that there is very little change in the ligand structure in any of the mutant proteins, although there is evidence for slight distortion compared to the wild-type in Leu12Ala and Leu12Glu.

The absorption spectra of the mutant proteins in the visible region were indistinguishable from that of the wild-type protein. The possibility of changes in the absorption coefficient of the oxidized protein was tested for by titration with ascorbate in the presence of a trace of *N,N'*-tetramethyl-*p*-phenylenediamine. The small differences observed were within the experimental error of the method (±2%) and have been ignored in the calculation of rate constants. Small changes in redox potential of the Leu-12 mutants have been reported previously [18], but these have not been considered further here because the significant differences described below lie in the association constant (K_A) rather than the intrinsic rate of electron transfer (k_f).

For analysis of the kinetics of reduction of plastocyanin a simple model was used, as described previously [4]. It is assumed that oxidized plastocyanin and reduced cytochrome form a reaction complex, $[PC_{ox} \ldots Cyt_{red}]$ in which electron transfer takes place:

$$PC_{ox} + Cyt_{red} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} [PC_{ox} \ldots Cyt_{red}] \overset{k_f}{\rightleftharpoons} [PC_{red} \ldots Cyt_{ox}] \rightleftharpoons PC_{red} + Cyt_{ox}$$

Under our conditions, in which $K_A[PC_{ox}] \ll 1$ ($K_A = k_a/k_{-a}$), the overall second order rate constant (k_2) depends on the partial reactions according to the following equation:

$$\frac{1}{k_2} = \frac{1}{k_a} + \frac{1}{K_A k_f}$$

K_A, k_a and k_2 were measured as described in Materials and Methods, and the values obtained were used for calculation of k_f. The results are shown in Table II. The second order rate constant (k_2) for reduction of wild-type plastocyanin by cytochrome *f* is about 12-times larger than by cytochrome *c*, and these values are very close to those reported for the reactions with pea plastocyanin under the same conditions [4,14].

At first sight the rate constants with cytochrome *c* as donor show little variation (Table II). However, the Leu12Glu and Leu12Ala mutants have smaller values of k_2 by factors of 0.69 and 0.60, respectively, which are largely due to decreases in the affinity constant K_A (the small changes in the intrinsic rate constant, k_f, are not significant). Moreover, although the Leu12Asn mutant shows no change in k_2, there is a small but significant decrease in K_A, compensated for by a change in k_f, although the latter difference is not in itself significant. There are no significant changes from

TABLE II

Kinetic parameters for reduction of spinach plastocyanin by cytochromes c and f

Values are given as mean ± S.D. ($n = 4$).

Plastocyanin	k_2 ($\times 10^{-6}$) ($M^{-1} s^{-1}$)	K_A (M^{-1})	k_a ($\times 10^{-6}$) ($M^{-1} s^{-1}$)	k_{-a} ($\times 10^{-3}$) (s^{-1})	k_f ($\times 10^{-3}$) (s^{-1})
Cytochrome *c* as donor					
Wild type	3.49 ± 0.07	1280 ± 27	22.7 ± 0.4	17.7 ± 0.7	3.22 ± 0.26
Leu12Asn	3.47 ± 0.04	1130 ± 20	22.3 ± 0.5	19.7 ± 0.8	3.64 ± 0.28
Leu12Glu	2.41 ± 0.04	898 ± 30	18.2 ± 0.6	20.2 ± 1.3	3.09 ± 0.37
Leu12Ala	2.09 ± 0.05	748 ± 11	17.0 ± 0.7	22.7 ± 1.3	3.18 ± 0.40
Tyr83Phe	3.45 ± 0.06	1250 ± 29	22.4 ± 0.5	17.9 ± 0.8	3.26 ± 0.29
Asp42Asn	3.47 ± 0.09	1260 ± 28	22.3 ± 0.8	17.7 ± 1.0	3.26 ± 0.41
Phe35Tyr	3.50 ± 0.12	1253 ± 25	22.7 ± 0.7	18.1 ± 0.9	3.30 ± 0.40
Cytochrome *f* as donor					
Wild type	41.7 ± 1.1	10109 ± 168	44.9 ± 1.0	4.44 ± 0.17	58 ± 30
Leu12Asn	157 ± 8.0	34765 ± 499	186 ± 9.0	5.30 ± 0.33	29 ± 15
Leu12Glu	9.35 ± 0.05	2257 ± 18	10.0 ± 0.20	4.43 ± 0.12	64 ± 22
Leu12Ala	24.6 ± 0.6	5891 ± 83	26.6 ± 1.0	4.51 ± 0.23	55 ± 36
Tyr83Phe	5.19 ± 0.05	1358 ± 16	5.60 ± 0.08	4.12 ± 0.11	52 ± 13
Asp42Asn	40.6 ± 1.0	9791 ± 211	43.7 ± 1.2	4.46 ± 0.22	58 ± 33
Phe35Tyr	41.6 ± 0.6	9942 ± 24	45.0 ± 0.9	4.53 ± 0.10	55 ± 19

wild type for the Phe35Tyr mutant, and for the two mutants involving the eastern face of the molecule, Tyr83Phe and Asp42Asn.

By contrast, there are some striking variations in k_2 with cytochrome *f* as donor. It is again noticeable that Leu-12 has an important influence. While Glu or Ala in this position decrease the rate constant because of weaker binding, Asn has the remarkable effect of stimulating it nearly 4-fold as a result of stronger binding. Another surprising result is that there is no significant change with Asp42Asn, despite the loss of a negative charge. Tyr83Phe shows an 8-fold decrease in k_2 which is entirely the result of a smaller K_A; this confirms the result already reported for pea plastocyanin [4]. None of the mutants shows a significant change in the intrinsic rate constant for electron transfer.

Discussion

The results described above will be discussed in relation to the ways in which plastocyanin interacts with the two cytochromes. There are two basic facts to be borne in mind. The first is that the main route for electron transfer from the haem iron of either cytochrome to the copper atom of plastocyanin involves Tyr-83 of the latter protein [4,14]. The second is that none of the mutants showed a significant change in the intrinsic rate constant of electron transfer, so that the effects observed can be explained almost entirely by changes in binding. An assumption made is that the value of K_A measured by Soret band enhancement is appropriate for electron transfer. This has been discussed previously [14].

It is convenient to consider the interaction with cytochrome *c* first, because this is a small protein of known structure. A recent modelling study of this interaction suggests that as the two proteins approach each other electrostatic forces would tend to rotate the molecules into a number of energetically favoured relative orientations [19]. All of these involve an approach of the exposed haem edge of cytochrome *c* and the surrounding groups of lysine residues towards the acidic residues concentrated on the eastern side of plastocyanin. These preferred orientations generate no contact between the northern end of plastocyanin and the surface of cytochrome *c*, and approach of cytochrome *c* towards the northern end would be hindered by the projecting loop of residues 88–91 of plastocyanin, as well as being energetically unfavourable. In terms of this model it is, at first sight, surprising that loss of one negative charge close to Tyr-83 in the Asp42Asn mutant led to no significant change in binding energy. However, the electrostatic calculations referred to above consider the precollision orientations rather than those after docking, because as the proteins come into contact other factors come into play, such as side-chain rearrangements and Van der Waals forces. Modelling of possible structures of the complex suggests that it may play a less significant role in the reactive conformation of the complex because Asp-42 projects less far from the surface of plastocyanin than Glu-43 and Asp-44.

The above discussion leads to the conclusion that the northern end of plastocyanin is not involved in the productive conformation of the complex with cytochrome *c*. Nevertheless, all three mutations involving Leu-12 gave small decreases in the value of K_A. The side chain of Leu-12 lies close to that of the copper ligand, His-87, in the crystal structure. These observations suggest, therefore, that modification of the side chain at position 12 causes small, indirect conformational changes in residues on the eastern side of the molecule that are directly involved in binding to cytochrome *c*. This effect might be relayed via the ligand cage around the copper, which would be consistent with the observation that the NMR spectra show shifts in the position of the His-37-N$^{\epsilon}$H resonance (Fig. 1A, Table I) and with the small changes in EPR parameters (Table I). Phe35Tyr shows no such NMR shift and no change in K_A. The conformation changes discussed here should not be large enough to disrupt the tertiary structure of the protein. Their characterisation will require further studies by 2D ^{1}H-NMR methods.

When cytochrome *f* was the reductant it remained true that changes in k_2 in the mutant proteins were entirely due to changes in K_A, but there were some much larger effects than observed with cytochrome *c*. With Leu12Ala, k_2 and K_A were decreased proportionately in the same ratio, whichever donor was used, which might be explained by the indirect conformational effect discussed above. With Leu12Glu and Leu12Asn, however, there were much larger effects with cytochrome *f*. With the former protein, binding was more than 4-fold weaker, whereas with the latter it was nearly 4-times stronger. These large effects suggest that there may be a direct interaction between cytochrome *f* and this part of the plastocyanin surface, in addition to the well-established interaction with the eastern acidic face. The simplest explanation is that, in the appropriate position, cytochrome *f* contains an acidic residue which is capable of acting as a hydrogen bond acceptor for Asn-12, or of repelling the negative charge of Glu-12. It is interesting to note that there is no change in binding with Phe35Tyr despite the introduction of the phenolic OH, which would be capable of hydrogen bonding, in a position fairly close to that of Leu-12; this emphasises the specific nature of the interaction between plastocyanin and cytochrome *f*.

On the eastern side of the molecule the loss of the negative charge in Asp42Asn had no effect on binding to cytochrome *f*. As with cytochrome *c*, lack of direct contact might be at least part of the explanation, but

ignorance of the structure of cytochrome *f* allows an alternative to be suggested, which is that the introduction of a hydrogen bond between Asn-42 and cytochrome *f* compensates for the loss of the negative charge. There is a stronger case for the existence of a hydrogen bond involving the OH of Tyr-83 in the reactive conformation of the complex, as shown by the 8-fold decrease in the value of K_A in Tyr83Phe; this confirms the result already reported for pea plastocyanin [4].

The above results suggest that the superiority of cytochrome *f* over cytochrome *c* as an electron donor to plastocyanin lies partly in the nature of the binding reaction and partly in the intrinsic rate constant of electron transfer. With the Phe-83 mutant, in which the sole difference from the wild-type is the loss of a hydrogen bond between the two proteins, the second-order rate constant with cytochrome *f* is only slightly faster than with cytochrome *c*. Although with this mutant K_A differs little between the two proteins, the on and off reactions are slower with cytochrome *f*, and a consequence of this is that the encounter complex has a longer lifetime. With all the forms of plastocyanin studied, however, the intrinsic rate constant of electron transfer is substantially greater for cytochrome *f* than for cytochrome *c*, suggesting that the former forms a more favourable configuration in the complex.

Acknowledgements

This work was supported by grants from the SERC Molecular Recognition Initiative (UK) and the Swedish Natural Science Research Council. We would like to thank Professor Tore Vänngård for advice and encouragement. We are also grateful to Dr Roland Aasa and Mr Kalle Sigfridsson for assistance with obtaining EPR spectra, to Mr Simon Young for help with the purification of plastocyanin mutant proteins and to Dr P.J. Kraulis for help with the MOLSCRIPT drawing of plastocyanin.

References

1 Nordling, M., Olausson, T. and Lundberg, L.G. (1990) FEBS Lett. 276, 98–102.
2 Last, D.I. and Gray, J.C. (1989) Plant Mol. Biol. 12, 655–666.
3 Last, D.I. and Gray, J.C. (1990) Plant Mol. Biol. 14, 229–238.
4 He, S., Modi, S., Bendall, D.S. and Gray, J.C. (1991) EMBO J. 10, 4011–4016.
5 Nordling, M., Sigfridsson, K., Young, S., Lundberg, L.G. and Hansson, Ö. (1991) FEBS Lett. 291, 327–330.
6 Dickerson, R.E. (1980) Sci. Am. 242, 99–110.
7 Sykes, A.G. (1990) Structure and Bonding 75, 175–224.
8 Martinez, S.E., Szczepaniak, A., Smith, J.L. and Cramer, W.A. (1991) Biophys. J. 59, 524a.
9 Landt, O., Grunert, H.-P. and Hahn, U. (1990) Gene 96, 125–128.
10 Pascher, T., Bergström, J., Malmström, B.G., Vänngård, T. and Lundberg, L.G. (1989) FEBS Lett. 258, 266–268.
11 Brautigan, D.L., Ferguson-Miller, S. and Margoliash, E. (1978) Methods Enzymol. 53, 128–164.
12 Gray, J.C. (1978) Eur. J. Biochem. 82, 133–141.
13 Gray, J.C. and Phillips, A.L. (1982) in Methods in Chloroplast Molecular Biology (Edelman, M., Hallick, R.B. and Chua, N.-H., eds), pp. 917–932, Elsevier, Amsterdam.
14 Modi, S., He, S., Gray, J.C. and Bendall, D.S. (1992) Biochim. Biophys. Acta1101, 64–68.
15 Driscoll, P.C., Hill, H.A.O. and Redfield, C. (1987) Eur. J. Biochem. 170, 279–292.
16 Guss, J.M. and Freeman, H.C. (1983) J. Mol. Biol. 169, 521–563.
17 Malkin, R. and Malmström, B.G. (1970) Adv. Enzymol. 33, 177–244.
18 Kyritsis, P., Lundberg, L.G., Nordling, M., Vänngård, T., Young, S., Tomkinson, N.P. and Sykes, A.G. (1991) J. Chem. Soc. Chem. Commun, 1441–1442.
19 Roberts, V.A., Freeman, H.C., Olson, A.J., Tainer, J.A. and Getzoff, E.D. (1991) J. Biol. Chem. 266, 13431–13441.
20 Kraulis, P.J. (1991) J. Appl. Crystallogr. 24, 946–950.