Concerning the Binding Site on Plastocyanin for its Natural Redox Partner Cytochrome f

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From pH effects, competitive inhibition, and Cr^{III} modification, it is concluded that cytochrome f binds at the negative patch on plastocyanin incorporating residues 42–45.

Single Cu (type 1) plastocyanin is one of a number of metalloproteins involved in electron transport between photosystems II and I in the chloroplast of higher plants.[1] Inorganic complexes have been used extensively as probes for redox reactivity, recent experiments having indicated two sites on plastocyanin at which electron transfer can occur.[2] From n.m.r. line-broadening, it has been demonstrated that the negatively charged complex $Cr(CN)_6^{3-}$ exhibits specificity for a site in the vicinity of His 87 (the so-called north site), while positively charged complexes such as Cr(1,10-phenanthroline)$_3^{3+}$, and $Cr(NH_3)_6^{3+}$, interact at a site close to Tyr 83, which is adjacent to a negative patch incorporating residues 42—45 (the east site),[3,4] Figure 1. It has also been shown by competitive inhibition that cytochrome c, a protein carrying an overall 8+ charge in the reduced state, reacts at the east site.[5] Having tested a number of different approaches, we are now in a position to investigate and comment on the much more important question of the reactivity of plastocyanin

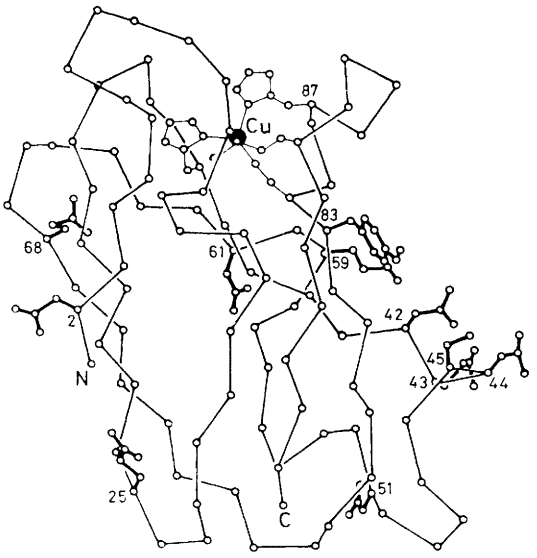

Figure 1. The structure of plastocyanin as reported by Freeman.[7]

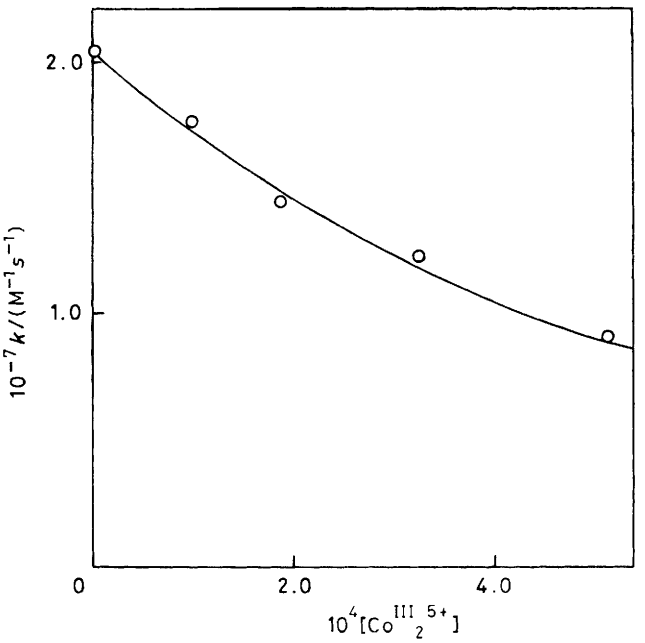

Figure 2. The competitive inhibition of the plastocyanin PCu^{II} (5.0×10^{-6} M) oxidation of cytochrome f(II) (5.0×10^{-7} M) by redox inactive $(NH_3)_5Co{\cdot}NH_2{\cdot}Co(NH_3)_5^{5+}$ at 10 °C, pH 7 (Tris–HCl), $I = 0.20$ M (NaCl).

with its natural partners cytochrome f (reductant) and P700 (oxidant). Here we report experiments concerned with the determination of the reaction site for cytochrome f on plastocyanin in aqueous solution.

The blue Cu protein plastocyanin (m.wt. 10 500) occurs in all higher plants (99 amino acids), and green and blue-green algae.[6] Freeman and colleagues have determined the structure of poplar plastocyanin to 1.6 Å resolution.[7] The reduction potential of 370 mV (pH 7) lies between that of cytochrome f (360 mV) and P700 (520 mV).[8] Cytochrome f (m.wt. 30 000, *ca.* 280 amino acids) has a substantial overall negative charge at pH 7 (pI 5.5).[9] An assessment of its reactivity with a range of inorganic complexes has been completed in this laboratory. Following experiments using plastocyanin, PCu^{II}, modified at 42—45 by attachment of a single Cr^{III}, Farver *et al.* have suggested that oxidation by P700 occurs at the east site, and reduction by cytochrome f (by implication) is at the north site.[10] However, a study by Burkey and Gross[11] involving P700 oxidation of PCu^{I}, modified by ethylenediamine attachment at 42—45, reveals that there is very little decrease in K_m as a result of modification. This result is contrary to the assignment of Farver *et al.*[10] We therefore decided to attempt to resolve this difference for the plastocyanin reaction with cytochrome f using three different approaches established in our previous studies.

The methods used include, (a) reaction of PCu^{II} modified by attachment of Cr^{III} (procedure as described by Farver *et al.*[10]) with cytochrome f(II), (b) competitive inhibition of the PCu^{II} oxidation of cytochrome f(II) using a 5+ redox inactive complex known to associate at the negative patch on plastocyanin,[5] and (c) pH studies to ascertain whether the protein acid dissociation pK_a observed for the reduction of PCu^{II} by cytochrome c(II) (4.9) and $Ru(NH_3)(pyridine)^{2+}$ (pK_a 5.0), and known to be a characteristic of the east site,[5] is also observed in the cytochrome f(II) reduction of PCu^{II}. All the studies described are with plastocyanin from parsley leaves,[12] and cytochrome f from cabbage,[9] sources which we have used successfully in other studies.

The Cr modification of PCu^{II} at 42—45 produces a marked inhibition of the reaction with cytochrome f(II). At 10 °C and pH 8.0 (10^{-2} M Tris–HCl), $I = 0.20$ M (NaCl), the rate constant of 2.1×10^{7} M^{-1} s^{-1} for reduction of native PCu^{II} is decreased by 36%. At lower pH's of 5.1 and 5.8 [10^{-2} M 2-(*N*-morpholino)ethane sulphonic acid (MES)/NaOH], 65% decreases are observed. Moreover attaching two Cr^{III}'s to PCu^{II} (modification procedure[10] repeated) a 90% decrease is observed. Assuming the second Cr binds at or near to 42—45, these results clearly suggest an involvement of the east site of plastocyanin in the reaction with cytochrome f.

Competitive inhibition studies were carried out with the 5+ redox inactive $Co^{III}{}_2$ complex $(NH_3)_5Co{\cdot}NH_2{\cdot}Co(NH_3)_5^{5+}$, which is known to associate strongly with PCu^{II} ($K = 2.2 \times 10^{3}$ M^{-1} at 10 °C, pH 7.5, $I = 0.2$ M) at the east site. Rate constants decreased significantly with increasing amounts of $Co^{III}{}_2$, Figure 2. We have not found this complex to be similarly effective in other studies involving cytochrome f, and conclude that the inhibition indicates an involvement of the east site of plastocyanin.

Niwa *et al.*[13] have published a pH profile of rate constants for the reduction of PCu^{II} with cytochrome f(II). This gives a pK_a of 4.9 (our calculation), which agrees well with values observed for the cytochrome c(II) and $Ru(NH_3)_5(pyridine)^{2+}$ reductions of PCu^{II}. We have repeated the cytochrome f study and obtained a pK_a of 5.1 in good agreement.

All three approaches described therefore give a firm indication of involvement of the east site of plastocyanin in the reaction with cytochrome f, a result contrary to the suggestion of Farver *et al.*[10] Our results are of considerable further interest because the responses observed with cytochrome f(II) as reductant clearly suggest that either there is a positive patch on cytochrome f, or that electrostatic considerations are of secondary importance in the interaction of these two partners. Although cytochrome f carries an overall negative charge and is, for example, strongly held on a DEAE cellulose column, it does have 19—24 lysines and 7—11 arginines (amino-acid compositions of charlock[9] and spinach[14]), and its reactions with negatively charged inorganic complexes suggest a functional positively charged region. One possibility is that this is located in the vicinity of an exposed heme edge

of cytochrome f. The negative patch on plastocyanin may of course be a recognition site which is part of a much broader contact region between the two proteins.

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