Existence of Specific Binding Sites in 1:1 Oxidations of Reduced Parsley 2-Fe Ferredoxin with Inorganic Complexes

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Summary Competition studies using redox active and inactive inorganic complexes have indicated that single unique binding sites are effective in individual reactions, and for the series of reactions investigated selection and utilization of different sites varies with the charge and/or ligand type on the complex.

PLANT ferredoxins are redox active metalloproteins, M.W. *ca.* 10,500, containing the iron–sulphur cluster $[Fe_2S_2{}^*(SR)_4]^{n-}$, where SR is a cysteine residue of the peptide chain, and the two S* atoms are acid-labile sulphides which bridge the Fe atoms.[1] The proteins are involved in a variety of processes, notably photosynthesis, where ferredoxin reduced by chloroplasts serves to reduce a wide range of different compounds.[2] Reduced ferredoxin, $[Fe_2S_2{}^*(SR)_4]^{3-}$, is one-electron active with a redox potential in the region $-0{\cdot}42$ V.[3] Mössbauer and ENDOR spectroscopy have demonstrated that the reduced form, which we write as $Fe^{II,III}$, contains localised Fe^{II} and Fe^{III} oxidation states.[4] Plant ferredoxins have negative overall charges which it is estimated, based on amino-acid composition, may be as high as -17 at pH values in the range 7—9.[5]

The kinetics of one-electron oxidations of parsley ferredoxin, $[Fe_2S_2(S^*R)_4]^{3-}$, with Co^{III} complexes, equation (1),

$$Fe^{II,III} + Co^{III} \longrightarrow Fe^{III,III} + Co^{II} \quad (1)$$

have been investigated. With the 3+ oxidant $[Co(NH_3)_6]^{3+}$ the kinetics are consistent with the reaction sequences (2) and (3), involving protein–complex association followed by electron transfer within the adduct.[6] Thus, on varying the concentration of oxidant, present in large (> 10-fold) excess, over the range $(0{\cdot}5—4{\cdot}0) \times 10^{-3}$ M, first-order rate constants k_{obs} conform to equation (4). At 25 °C, pH

$$Fe^{II,III} + Co^{III} \overset{K}{\rightleftharpoons} Fe^{II,III},Co^{III} \quad (2)$$

$$Fe^{II,III},Co^{III} \overset{k_{et}}{\longrightarrow} Fe^{III,III} + Co^{II} \quad (3)$$

$$k_{obs} = Kk_{et}[Co^{III}]/(1 + K[Co^{III}]) \quad (4)$$

7·0—9·0 (Tris buffer), $I = 0{\cdot}10$ M (NaCl), values of K (998 l mol^{-1}) and k_{et} (19·2 s^{-1}) are obtained. With oxidants of lower charge $[Co(NH_3)_5Cl]^{2+}$, $[Co(NH_3)_5C_2O_4]^+$ and $[Co(acac)_3]$,† as well as $[Co(edta)]^-$† and $[Co(C_2O_4)]^{3-}$, we believe equations (2) and (3) also describe the reaction sequence, but K is smaller so that $K[Co^{III}] << 1$, and the kinetics conform to a second-order rate law over a wide range of $[Co^{III}]$. However, the simple concept of protein–complex formation is limited, in that further definition is necessary regarding the number of binding sites available to any one oxidant, and whether the same binding site is effective for a range of oxidant types.

Studies with the redox-inactive complex $[Cr(NH_3)_6]^{3+}$ have provided further insight into these questions, and helped define an experimental approach. By selecting a relatively low Co^{III} concentration, such that $K[Co^{III}] << 1$ and second-order kinetics apply, it was possible to test the effect of varying amounts of $[Cr(NH_3)_6]^{3+}$ from $0{\cdot}5 \times 10^{-3}$ to $3{\cdot}0 \times 10^{-3}$ M. With $[Co(NH_3)_6]^{3+}$ as oxidant first-order rate constants k_{obs} conform to equation (5), where

$$k_{obs}/[Co^{III}] = Kk_{et}/(1 + K_{Cr}[Cr^{III}]) \quad (5)$$

Kk_{et} is the rate constant with no added $[Cr(NH_3)_6]^{3+}$. It is concluded that $[Cr(NH_3)_6]^{3+}$ ($K_{Cr} = 476$ l mol^{-1}) associates with the protein as in equation (2), blocking the reaction with $[Co(NH_3)_6]^{3+}$. Since a single $[Cr(NH_3)_6]^{3+}$ completely blocks the reaction only one binding site is operative in the $[Co(NH_3)_6]^{3+}$ oxidation.

With the complexes $[Co(NH_3)_5Cl]^{2+}$ and $[Co(NH_3)_5C_2O_4]^+$ a single $[Cr(NH_3)_6]^{3+}$ likewise completely blocks the reaction, with association constants $K_{Cr} = 462$ and 467

† Abbreviations, acac=acetylacetonate and edta=ethylenediamine-*NN'*-tetra-acetate are used.

1 mol^{-1}, respectively, and there can be little doubt that the same site is being utilized by all three Co^{III} oxidants. The extrapolated rate constant at high $[Cr(NH_3)_6]^{3+}$ concentration is in each case zero, a point which we wish to emphasize. However, with the neutral complex $[Co(acac)_3]$, see Figure, the presence of associated $[Cr(NH_3)_6]^{3+}$ leaves the rate unaffected. This complex therefore utilizes a different site from that used by the positively charged Co^{III} ammine oxidants.

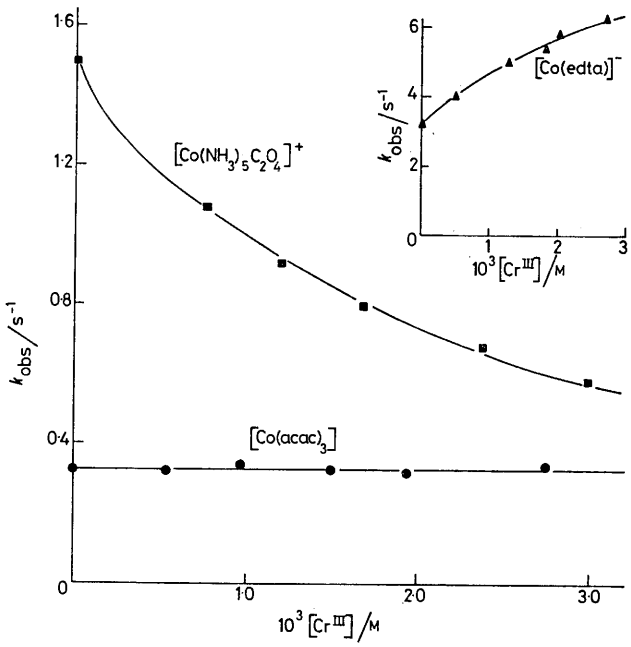

FIGURE. The effect of redox inactive $[Cr(NH_3)_6]^{3+}$ on the first-order rate constants, k_{obs}, for the oxidation of reduced parsley ferredoxin, $[Fe_2S_2{}^*(SR)_4]^{3-}$, with the cobalt(III) complexes; $2{\cdot}5 \times 10^{-4}$ M $[Co(NH_3)_5C_2O_4]^+$ (■), $8{\cdot}4 \times 10^{-5}$ M $[Co(acac)_3]$ (●), and (inset) $4{\cdot}0 \times 10^{-4}$ M $[Co(edta)^-]$ (▲) at 25 °C, pH 8·0 (Tris buffer), I = 0·10 M (NaCl).

With the negatively charged oxidant $[Co(edta)]^-$ rate constants actually increase with $[Cr(NH_3)_6]^{3+}$ present, see insert to Figure, consistent with the protein–Cr complex adduct being more reactive than the protein itself. From the fit of data, K_{Cr} = 459 l mol^{-1}, so that once again $[Cr(NH_3)_6]^{3+}$ is effective from the site employed by the positively charged ammine oxidants. The presence of the $[Cr(NH_3)_6]^{3+}$ is unlikely to be affecting the redox potential in view of the absence of any response with $[Co(acac)_3]$. Therefore it is concluded that the oxidant $[Co(edta)]^-$ reacts at a site in close proximity to the bound $[Cr(NH_3)_6]^{3+}$ which is favourably influenced by the 3+ charge, or at a site more distant from the $[Cr(NH_3)_6]^{3+}$ which has induced a favourable conformation change. Any similar study of the influence of $[Cr(NH_3)_6]^{3+}$ on the $[Co(C_2O_4)_3]^{3-}$ oxidation was precluded by precipitation which is observed on mixing these two complexes.

These experiments demonstrate that for reactions of a plant ferredoxin one and only one site is utilized by the positively charged oxidants $[Co(NH_3)_6]^{3+}$, $[Co(NH_3)_5Cl]^{2+}$, and $[Co(NH_3)_5C_2O_4]^+$. Different sites (or just possibly a single site) are moreover used by the neutral $[Co(acac)_3]$ and negatively charged $[Co(edta)]^-$ oxidants. At this stage it is not clear whether charge and/or ligand type is most important and further experiments are proposed to check this point. The use of inorganic complexes as blocking reagents as illustrated in this study can also be envisaged for protein–protein reactions as a means of defining binding sites.

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