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KINETIC STUDIES ON THE REACTIONS OF IRON-SULPHUR PROTEINS

V. THE EFFECT OF REDOX-INACTIVE CHROMIUM(III) COMPLEXES ON THE OXIDATION OF THE REDUCED FORM OF THE BIFUNCTIONAL *CLOSTRIDIUM PASTEURIANUM* 8Fe FERREDOXIN BY COBALT(III) COMPLEXES *

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The effect of redox-inactive chromium(III) complexes on the kinetics of the two-equivalent oxidation of reduced *Clostridium pasteurianum* 8Fe ferredoxin, which contains two [4Fe-4S] clusters, each one-electron active, with the oxidants $Co(NH_3)_6^{3+}$, $Co(acac)_3$ and $Co(edta)^-$ has been studied at 25°C, pH 8.0 (Tris-HCl), $I = 0.10$ M (NaCl). All the reactions give a single kinetic step which can be accounted for in terms of statistically related biphasic schemes, in which the bifunctional fully reduced protein 8Fe(rr) reacts at twice the rate of the semi-reduced form 8Fe(or). Binding of the chromium(III) complexes (K_{Cr}, M^{-1}), $Cr(NH_3)_6^{3+}$ (212), $Cr(en)_3^{3+}$ (318) and $(en)_2Cr \cdot \mu(OH, O_2CCH_3) \cdot Cr(en)_2^{4+}$ (326) to the protein completely inhibits oxidation by $Co(NH_3)_6^{3+}$ ($k_{Cr} = 0$). With $Co(edta)^-$ the binding of $Cr(NH_3)_6^{3+}$ (221) produces an increase in rate whereas with $Co(acac)_3$ partial inhibition is observed. The results are discussed in terms of reaction by all reagents within a common functional zone in the vicinity of one or both clusters.

Introduction

The 8Fe bacterial ferredoxins have a single polypeptide chain of approx. 55 amino acids (mol. wt. 6000) [2] and contain two [4Fe-4S] clusters each of which are spectroscopically similar to the single [4Fe-4S]clusters in the ferredoxins from *Bacillus stearothermophilus* and *Bacillus polymyxa* [3,4]. An X-ray crystal structure determination for the 8Fe ferredoxin from *Peptococcus aerogenes* shows the two distorted cubane-like clusters approx. 12 Å apart [5]. A variety of physico-chemical measurements along with similarities in amino acid sequences and conservation of cysteine (SR) residues leaves little doubt that other 8Fe ferredoxins including that from *Clostridium pasteurianum* have very similar structural features [6]. It has been demonstrated that the two clusters function independently with no evidence for cooperativity [7–9]. Reduction potentials for the one-electron active [4Fe-4S] clusters at approx. −0.4 V are very similar to those for the 2Fe ferredoxins [10].

Previous kinetic studies on the oxidation of reduced 2Fe [11,12] and reduced 8Fe [13] proteins have been reported. The aim of the present investigation is to obtain further information regarding kinetic behaviour patterns and to examine the effects of redox-inactive chromium(III) complexes on reactivity with cobalt(III) oxidants of varying charge type. Different oxidation states of the 8Fe protein are denoted by 8Fe(oo), 8Fe(or) and 8Fe(rr).

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Experimental Procedure

Ferredoxin. The isolation of 8Fe(oo) ferredoxin from *Cl. pasteurianum* (as supplied by the PHLS Centre for Applied Microbiology and Research, Porton, U.K.), generation of the reduced form 8Fe(rr) (using sodium dithionite) and adjustment of pH (Tris-HCl) were carried out as described previously [13].

Complexes. The following complexes were prepared by literature procedures and characterized by comparison of ultraviolet-visible absorption spectra, peak positions λ, in nm (ε, $M^{-1} \cdot cm^{-1}$), with previously reported literature values [11–13]: hexaamminecobalt(III) chloride, $[Co(NH_3)_6]Cl_3$ [14], 473 (57.1), 339 (46.4); sodium ethylenediaminetetraacetatocobaltate(III) tetrahydrate, $Na[Co(edta)] \cdot 4H_2O$ [15], 535 (320); tris(acetylacetonato)cobalt(III), $Co(acac)_3$ [16], 590, 257 ($3.4 \cdot 10^4$); hexaamminechromium(III) chloride, $[Cr(NH_3)_6]Cl_3$ [17], 462 (40), 350 (33); tris(ethylenediamine)chromium(III) chloride trihydrate, $[Cr(en)_3]Cl_3 \cdot 3H_2O$ [18], 457 (73), 351 (63); μ-hydroxo-μ-acetatobis((bisethylenediamine)chromium(III)) perchlorate dihydrate, $[(en)_2Cr \cdot \mu(OH, O_2CCH)_3) \cdot Cr(en)_2](ClO_4)_4 \cdot 2H_2O$ [19], 505 (209), 378 (100).

Stoichiometry. This was as determined previously with $Co(NH_3)_6^{3+}$ as oxidant [1],

$$8Fe(rr) + 2Co(III) \rightarrow 8Fe(oo) + 2Co(II) \quad (1)$$

and is assumed to be the same for the reactions with $Co(edta)^-$ or $Co(acac)_3$ as oxidant [13]. All absorbance changes at 420 nm with oxidant in excess of the 2:1 ratio were consistent with the 8Fe(rr) ($\epsilon = 1.1 \cdot 10^4\ M^{-1} \cdot cm^{-1}$) to 8Fe(oo) ($\epsilon = 2.7 \cdot 10^4\ M^{-1} \cdot cm^{-1}$) change.

Kinetics. Reactions were monitored on a Dionex stopped-flow spectrophotometer. The oxidant was always in large (greater than 10-fold) excess of protein. For the $Co(NH_3)_6^{3+}$ oxidation (which exhibits limiting kinetics) [13] a relatively low concentration of $Co(NH_3)_6^{3+}$ was required so that the condition $K[Co(III)] \ll 1$ applied (K is for association of protein and Co(III)). Treatment of data was as previously described [11–13].

Results

A single-stage absorbance (A) change was observed in all reactions, where first-order rate constants, k_{obs} (Table I), were as defined by the rate law Eqn. 2:

$$\ln(A_\infty - A_t) = k_{obs}t + \text{constant} \quad (2)$$

First-order plots of $\ln(A_\infty - A_t)$ against t were linear for three to four half-lives. For the Cr(III) blocking of the $Co(NH_3)_6^{3+}$ reaction the sequence in Eqns. 3 and 4 applies:

$$P + Cr(III) \overset{K_{Cr}}{\rightleftharpoons} P, Cr(III) \quad (3)$$

$$P + Co(NH_3)_6^{3+} \rightarrow \text{products} \quad (4)$$

where P represents the protein and $Co(NH_3)_6^{3+}$ is unable to oxidize the adduct P, Cr(III). A good fit is obtained to the dependence, Eqn. 5 (Fig. 1):

$$\frac{k_{obs}}{[Co(III)]} = \frac{k}{1 + K_{Cr}[Cr(III)]} \quad (5)$$

with three different Cr(III) complexes, where $k (= Kk_{et})$ is the second-order rate constant in the

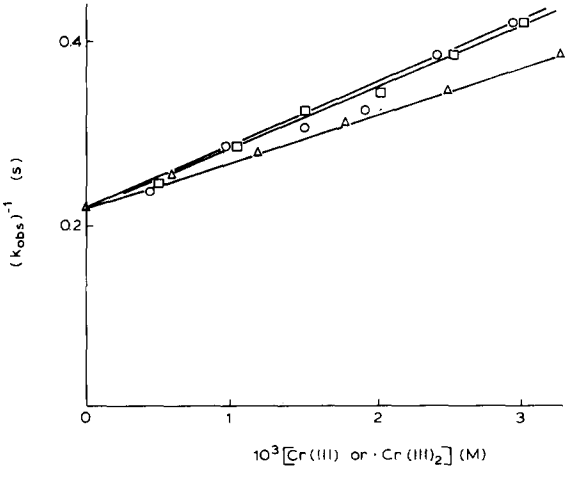

Fig. 1. The dependence of first-order rate constants k_{obs} on [Cr(III)] for the blocking of the $Co(NH_3)_6^{3+}$ ($1.0 \cdot 10^{-4}$ M) oxidation of 8Fe(rr) with $Cr(NH_3)_6^{3+}$ (△) $Cr(en)_3^{3+}$ (□), and $(en)_2Cr \cdot \mu(OH, O_2CCH_3) \cdot Cr(en)_2^{4+}$ (○), at 25°C, pH 8.0 (Tris), $I = 0.10$ M (NaCl).

TABLE I

FIRST-ORDER RATE CONSTANTS, k_{obs} (25°C), FOR Co(III) OXIDATION OF *CLOSTRIDIUM PASTEURIANUM* 8Fe FERREDOXIN IN THE PRESENCE OF REDOX INACTIVE Cr(III) COMPLEXES, pH 8.0 (Tris-HCl), $I = 0.10$ M (NaCl)

1. (a) $[Co(NH_3)_6^{3+}] = 1.0 \cdot 10^{-4}$ M							
$[Cr(NH_3)_6^{3+}](M)(\times 10^3)$	: 0,	0.58,	1.18,	1.79,	2.51,	3.29	
k_{obs} (s^{-1})	: 4.6,	3.9,	3.6,	3.2,	2.9,	2.6	
(b) $[Co(NH_3)_6^{3+}] = 1.6 \cdot 10^{-4}$ M							
$[Cr(NH_3)_6^{3+}](M)(\times 10^3)$	: 0,	0.73,	1.34,	1.94,	2.65,	3.51	
k_{obs} (s^{-1})	: 7.4,	5.7,	5.2,	4.4,	4.1,	3.8	
2. $[Co(NH_3)_6^{3+}] = 1.0 \cdot 10^{-4}$ M							
$[Cr(en)_3^{3+}](M)(\times 10^3)$	: 0,	0.48,	1.02,	1.50,	2.03,	2.55,	3.02
k_{obs} (s^{-1})	: 4.6,	4.1,	3.5,	3.1,	2.9,	2.6,	2.4
3. $[Co(NH_3)_6^{3+}] = 1.0 \cdot 10^{-4}$ M							
$[Cr(III)_2](M)(\times 10^3)$ [a]	: 0,	0.47,	0.99,	1.51,	1.94,	2.44,	2.99
k_{obs} (s^{-1})	: 4.6,	4.2,	3.5,	3.3,	3.1,	2.6,	2.4
4. $[Co(edta)^-] = 8.0 \cdot 10^{-4}$ M							
$[Cr(NH_3)_6^{3+}](M)(\times 10^3)$	0,	0.58,	1.00,	1.65,	2.09,	2.69,	3.23
k_{obs} (s^{-1})	: 8.8,	12.5,	14.7,	18.1,	18.9,	20.9,	22.5
5. $[Co(acac)_3] = 2.2 \cdot 10^{-4}$ M							
$[Cr(NH_3)_6^{3+}](M)(\times 10^3)$	: 0,	0.50,	1.0,	1.5,	2.0,	2.5	
k_{obs} (s^{-1})	: 7.3,	7.0	6.7,	6.4,	6.2,	6.0	
$[Co(acac)_3] = 5.0 \cdot 10^{-4}$ M							
$[Cr(NH_3)_6^{3+}](M)(\times 10^3)$	: 0,	0.50,	0.75,	1.0,	1.5,	2.0,	2.5
k_{obs} (s^{-1})	:16.7,	15.9,	15.6,	15.2,	14.7,	14.2,	13.7
$[Co(acac)_3] = 7.5 \cdot 10^{-4}$ M							
$[Cr(NH_3)_6^{3+}](M)(\times 10^3)$	: 0,	0.50,			1.5,		2.5
k_{obs} (s^{-1})	:25.0,	23.7,			21.7,		20.8

[a] $(en)_2Cr \cdot \mu(OH, O_2CCH_3) \cdot Cr(en)_2^{4+}$.

absence of Cr(III). For the binuclear complex, [Cr(III)] in this equation should be replaced by $[Cr(III)_2]$. At 25°C, pH 8.0, $I = 0.10$ M (NaCl), $k = 4.6 \cdot 10^4\ M^{-1} \cdot s^{-1}$, and K_{Cr} values (in M^{-1}) are 212 ± 15 for $Cr(NH_3)_6^{3+}$, 318 ± 29 for $Cr(en)_3^{3+}$ and 326 ± 27 for the $Cr(III)_2$ complex.

With $Co(edta)^-$ and $Co(acac)_3$ as oxidant there is no restriction regarding Co(III) concentration, since limiting kinetics are not observed [13]. On addition of $Cr(NH_3)_6^{3+}$, rate constants for

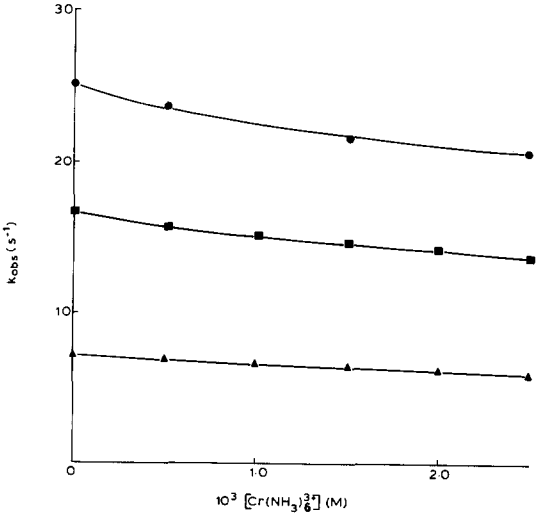

Fig. 2. The variation of first-order rate constants, k_{obs}, with $[Cr(NH_3)_6^{3+}]$ for the $Co(acac)_3$ oxidation of 8Fe(rr) (approx. $0.5 \cdot 10^{-5}$ M), at 25°C, pH 8.0 (Tris), $I = 0.10$ M (NaCl). Concentrations of $Co(acac)_3$ $2.2 \cdot 10^{-4}$ M (▲), $5.0 \cdot 10^{-4}$ M (■), and $7.5 \cdot 10^{-4}$ M (●).

$Co(edta)^-$ increase and conform well to Eqn. 6:

$$k_{obs} - k[Co(III)] = \frac{(k_{Cr} - k)K_{Cr}[Cr(III)][Co(III)]}{1 + K_{Cr}[Cr(III)]} \quad (6)$$

which is derived with the inclusion of Eqn. 7 in addition to Eqns. 3 and 4.

$$P, Cr(III) + Co(III) \xrightarrow{k_{Cr}} \text{products} \quad (7)$$

Values of k_{Cr} ($5.1 \cdot 10^4$) and K_{Cr} (221 M^{-1}) are obtained.

In the presence of $Cr(NH_3)_6^{3+}$, rate constants for $Co(acac)_3$ decrease, as shown in Fig. 2. Substitution of data into Eqn. 5 yields a value $K_{Cr} = 88$ M^{-1}. Alternatively, a scheme involving Eqns. 3, 4 and 7 may be invoked as for $Co(edta)^-$, in which $k_{Cr} < k$. Rate constants give a good fit to Eqn. 8:

$$\frac{k_{obs}}{[Co(III)]}(1 + K_{Cr}[Cr(III)]) = k + k_{Cr}K_{Cr}[Cr(III)] \quad (8)$$

which is an alternative form of Eqn. 6 and requires insertion of a known value for K_{Cr}. Using the previously determined value $K_{Cr} = 212$ M^{-1}, plots of the left-hand side of Eqn. 8 against [Cr(III)] are linear (Fig. 3). At 25 °C, pH 8.0, $I = 0.10$ M (NaCl), $k_{Cr} = (1.75 \pm 0.10) \cdot 10^4$ $M^{-1} \cdot s^{-1}$.

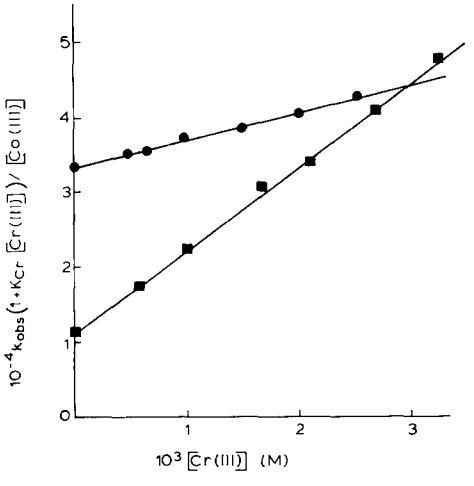

Fig. 3. Oxidation of 8Fe(rr) by $Co(acac)_3$ (●) and $Co(edta)^-$ (■). Dependence of rate constants k_{obs} on $[Cr(NH_3)_6]^{3+}$ according to Eqn. 8, using $K_{Cr} = 212$ M^{-1}.

Discussion

The bifunctional nature of the protein and manner of the oxidation of 8Fe(rr) through 8Fe(or) to 8Fe(oo) are of continuing interest in the context of this paper. As previously indicated [13], the single-stage kinetic process which is observed for the two-electron charge with one-equivalent oxidants is consistent with statistical kinetics (Eqn. 9):

$$\underset{\epsilon_1}{8Fe(rr)} \xrightarrow{k_1} \underset{\epsilon_2}{8Fe(or)} \xrightarrow{k_2} \underset{\epsilon_3}{8Fe(oo)} \quad (9)$$

where the requirements are that $\epsilon_2 - \epsilon_1 = \epsilon_3 - \epsilon_2$ and $k_1 = 2k_2$. Deviations of greater than 20% from these conditions are not apparent [13]. Absorbance changes can accordingly be expressed as in Eqn. 10:

$$A_\infty - A_t = 2(\epsilon_2 - \epsilon_1)C_0 e^{-k_2 t} \quad (10)$$

where C_0 is the initial concentration of 8Fe(rr), and the rate constant k_2 is as defined in Eqn. 9. Values of k listed in Table II correspond, therefore, to the conversion of monofunctional reactant 8Fe(or) to 8Fe(oo) and rate constants k_1 for the reaction of 8Fe(rr) are obtained upon multiplication by 2. Different schemes, as depicted in Fig. 6 of Ref. 13, apportioning this statistical factor to either K (for precursor adduct formation) or k_{et} (for electron transfer) have been considered. The 8Fe proteins exhibit remarkable intramolecular symmetry, with the first and second halves of the amino acid sequences being closely related [6]. Similar aspects are consequently generated upon rotation of 180°. One possibility, therefore, is that, for example, in the reactions of $Co(NH_3)_6^{3+}$, two approximately equivalent reaction sites on the protein are utilized, one close to each [4 Fe-4 S] cluster. The strong structural similarities between $Co(NH_3)_6^{3+}$ [15] and $Cr(NH_3)_6^{3+}$ [16] lead us to expect that these cations will compete for identical locations on the protein surface. In studies with parsley 2 Fe ferredoxin, a single specific site for the binding of these species was favoured, since a single $Cr(NH_3)_6^{3+}$ was found to completely block reduction by $Co(NH_3)_6^{3+}$ [12]. Furthermore, that this effect was not due merely to partial quenching

TABLE II

SUMMARY OF DATA FOR THE Co(III) OXIDATION OF THE 8Fe FERREDOXIN, IN THE PRESENCE OF REDOX INACTIVE Cr(III), AT 25°C, pH 8.0 (Tris-HCl), $I = 0.10$ M (NaCl)

Oxidant	Cr(III) complex	K_{Cr} (M^{-1})	k ($M^{-1} \cdot s^{-1}$) ($\times 10^{-4}$)	k_{Cr} ($M^{-1} \cdot s^{-1}$) ($\times 10^{-4}$)
$Co(NH_3)_6^{3+}$	$Cr(NH_3)_6^{3+}$	212	4.6 [a]	[b]
	$Cr(en)_3^{3+}$	318	4.6 [a]	[b]
	$Cr(III)_2$ [c]	326	4.6 [a]	[b]
$Co(edta)^-$	$Cr(NH_3)_6^{3+}$	212 [d]	1.1 [e]	5.2
$Co(acac)_3$	$Cr(NH_3)_6^{3+}$	212 [d]	3.3 [f]	1.75

[a] As determined previously [11], no Cr(III) present.
[b] No contribution.
[c] $(en)_2Cr \cdot \mu(OH, O_2CCH_3) \cdot Cr(en)_2^{4+}$.
[d] As determined for the $Cr(NH_3)_6^{3+}$ blocking of the $Co(NH_3)_6^{3+}$ reaction.
[e] Previous value $1.1 \cdot 10^4$ $M^{-1} \cdot s^{-1}$ [11].
[f] Previous value $3.1 \cdot 10^4$ $M^{-1} \cdot s^{-1}$ [11].

of the high overall negative charge carried by the native protein seemed likely in view of the complete inhibition also observed with the lower charged oxidants $Co(NH_3)_5Cl^{2+}$ and $Co(NH_3)_5C_2O_4^+$. It is reasonable to extend these arguments to the present study. Thus, in the case of two equivalent reaction sites being utilized, bound $Cr(NH_3)_6^{3+}$ (one at each site) effects k_1 and k_2 to the same extent. Significantly, a square dependence on [Cr(III)] would not be introduced, since the effect of [Cr(III)] on k_2 only would be manifest in Eqn. 10. Alternatively, schemes involving a single $Co(NH_3)_6^{3+}/Cr(NH_3)_6^{3+}$-binding site and a situation invoking either similar electron-transfer routes from each cluster or rapid electron exchange between the two clusters [22] may be envisaged. In the remaining discussion, no further distinction is made between these possibilities and the protein molecule is treated as a mono-functional system.

The oxidation of 8Fe(rr) by $Co(acac)_3$ and $Co(edta)^-$ in the presence of $Cr(NH_3)_6^{3+}$ can be satisfactorily accounted for by including the additional reaction step (Eqn. 7) involving k_{Cr}. All instances in which $Cr(NH_3)_6^{3+}$ is used give a satisfactory fit to Eqn. 8 using the value $K_{Cr} = 212$ M^{-1} at 25°C. Attempts to fit the $Co(acac)_3$ data to Eqn. 5 results in a quite different value of K_{Cr} (88 M^{-1}). We cannot rule out the possibility that reaction with $Co(acac)_3$ is unaffected by binding of $Cr(NH_3)_6^{3+}$ to the site used by $Co(NH_3)_6^{3+}$, but totally inhibited by $Cr(NH_3)_6^{3+}$ binding at a lower affinity site. However, from the value of K_{Cr} (221 M^{-1}) obtained by fitting data for $Co(edta)^-$ to Eqn. 6, it can be concluded that $Co(NH_3)_6^{3+}$ and $Co(edta)^-$ are influenced by binding of $Cr(NH_3)_6^{3+}$ at a common site. It seems somewhat less likely that $Co(acac)_3$ would be unaffected by this association yet completely inhibited by less favourable $Cr(NH_3)_6^{3+}$ binding at an alternative site. The simplest explanation is that $Cr(NH_3)_6^{3+}$ affects all three reactions by binding in the vicinity of a common electron-transfer point, i.e., a highly localized region providing a lead-in group, possibly a cysteinyl or bridging sulphur, at which an electron may enter or leave the protein. The model that results from consideration of the available data invokes a 'functional zone' as shown schematically in Fig. 4, in which the cationic reagents such as $Co(NH_3)_6^{3+}$ or $Cr(NH_3)_6^{3+}$ bind at a negatively charged site that is very near (but does not comprise) the electron-transfer point. Thermal motion would, however, provide the means for refining the mutual orientation of redox partners within this adduct to promote electron transfer. It is likely that this might explain the high values of

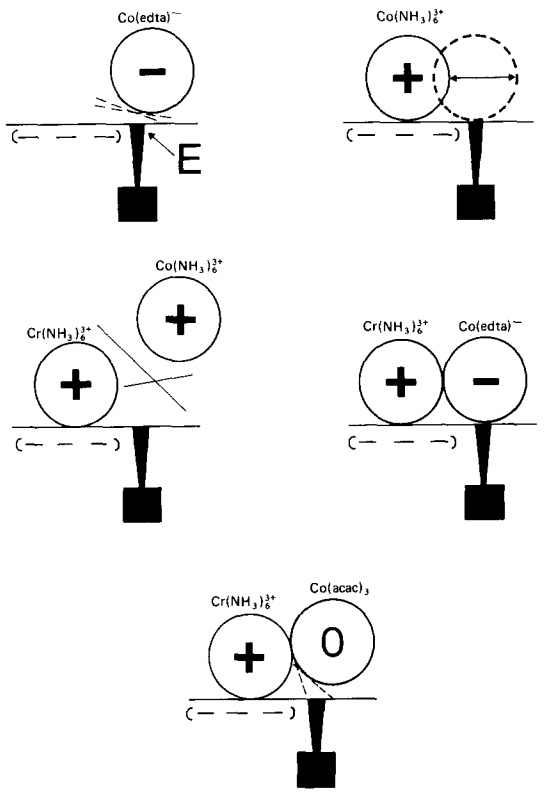

Fig. 4. Schematic representation depicting the response of the functional zone to small inorganic reagents. The charge carried by these is shown as +, −, or 0, + generally being a cation of the type $Co(NH_3)_6^{3+}$ or $Cr(NH_3)_6^{3+}$. Adverse interactions ⋰ (partial) or ✕ (prohibitive) with incoming reagents are indicated. The following are considered: (a) No bound cation. The incoming anionic reagent, e.g., $Co(edta)^-$, is partially repelleted by negative charge near the electron-transfer point (E). (b) Bound cationic oxidant, e.g., $Co(NH_3)_6^{3+}$. Thermally promoted excursions provide frequent encounter with electron-transfer point. (c) Bound cationic inhibitor, e.g., $Cr(NH_3)_6^{3+}$. Incoming cationic oxidant repelled. (d) Bound cation, e.g., $Cr(NH_3)_6^{3+}$. Approach of anionic oxidant more favourable than in a. (e) Bound cation, e.g., $Cr(NH_3)_6^{3+}$. Access of neutral oxidant is restricted due to adverse (most likely steric) interactions with cation.

$\Delta H_{et}^{\ddagger}$ observed with $Co(NH_3)_6^{3+}$ (15.3 kcal · mol^{-1}) and $Co(en)_3^{3+}$ (16.1 kcal · mol^{-1}) [13]. In the case of protein complexes with redox-inactive $Cr(NH_3)_6^{3+}$, direct attack by $Co(NH_3)_6^{3+}$ at the electron-transfer point would also be unfavourable in view of the adverse electrostatics operative in the size-restricted functional zone. However, such modification of the electrostatic character within this area would be expected to produce a favourable response with regard to anionic reagents such as $Co(edta)^-$. At this point we emphasise once again that the favoured binding site for $Co(NH_3)_6^{3+}/Cr(NH_3)_6^{3+}$ must lie close to but not actually on the point at which electron transfer is mediated, otherwise physical restriction of $Co(edta)^-$ is likely to outweigh the electrostatic enhancement. The partial blocking by $Cr(NH_3)_6^{3+}$ of the oxidation with $Co(acac)_3$ ($k_{Cr} < k$) conforms well to this model. As expected there is no electrostatic enhancement, instead bound $Cr(NH_3)_6^{3+}$ restricts the accessibility of the neutral reagent to the electron-transfer point.

With $Co(NH_3)_6^{3+}$ as oxidant the effect of three different Cr(III) complexes was investigated. The variations in K_{Cr} in going from $Cr(NH_3)_6^{3+}$ (212 M^{-1}) to $Cr(en)_3^{3+}$ (318 M^{-1}) to $(en)_2Cr \cdot \mu(OH, O_2CCH_3) \cdot Cr(en)_2^{4+}$ (326 M^{-1}) are mild, the latter surprisingly so. This pattern is to be compared with that observed for the $Co(NH_3)_6^{3+}$ and $Pt(NH_3)_6^{4+(3+)}$ (at pH 8.0, a 3+ species predominates owing to acid dissociation, pK_a 7.2 [23]) oxidations of 8Fe(rr) when association constants $K = 446$ and 2400 M^{-1}, respectively, were reported [12]. Of further interest is comparison with the trend established for reduced parsley 2 Fe ferredoxin with the oxidants $Co(NH_3)_5Cl^{2+}$ (194 M^{-1}), $Co(NH_3)_6^{3+}$ (998 M^{-1}), $Pt(NH_3)_6^{4+(3+)}$ (21000 M^{-1}) and $(NH_3)_5Co \cdot NH_2 \cdot Co(NH_3)_5^{5+}$ (i.e., $Co(III)_2^{5+}$) (24600 M^{-1} at 7°C). Clearly, the binding capability of $Cr(III)_2^{4+}$ falls short of expectations based upon the above figures, a feature which could be accounted for by the diminished positive charge density. Another possibility is that an 'end-on' aspect of $Cr(III)_2^{4+}$ is 'seen' by the protein, decreasing the effective charge to 2+, the stronger association of $Co(III)_2^{5+}$ with 2 Fe ferredoxin arising from a 'sideways-on' interaction.

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