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SELECTIVITY OF THE INTERACTION BETWEEN CYTOCHROME *c* AND TRANSITION METAL ION COMPLEXES OF EDTA AND RELATED LIGANDS

INTRODUCTION

The monohaem electron-transfer protein, mitochondrial cytochrome *c*, has a highly positively charged surface that binds anionic inorganic redox reagents at a variety of sites. Some of these sites are kinetically important for the accompanying electron-exchange reactions and they may be identified by the use of NMR spectroscopy [1-3] in combination with the X-ray crystal structures [4]. The experimental procedure is straightforward. First, the perturbations introduced into the NMR spectra of cytochrome *c* by the paramagnetic reagents are measured. Second, assigned NMR resonances are grouped into those unaffected by the added reagent and those perturbed by it with the second group being rated according to the extent of the perturbation. Third, the pattern of resonance perturbation is compared with the X-ray structure and the probable binding sites located.
Using this procedure we have identified three anion binding sites on cytochrome *c* close to its exposed haem edge [1-3]. These sites bind the reagents $[Fe(CN)_6]^{3-}$ and $[Fe(edta)(H_2O)]^-$ but they do so with differing relative affinities that are not determined solely by the overall charge of the reagent but by its chemical composition and structure. We have investigated these factors by varying the nature of metal-polyaminocarboxylate (pac) complexes and in the present communication we report that subtle differences between the pac complexes significantly affect their binding preferences on cytochrome *c*.

RESULTS AND DISCUSSION

In Fig. 1 the paramagnetic difference NMR spectra (PDS) for binding of $[Fe(edta)(H_2O)]^-$ and $[Fe(dtpa)]^{2-}$ to ferricytochrome *c* are shown. The $[Fe(dtpa)]^{2-}$ PDS is similar to the previously reported $[Cr(CN)_6]^{3-}$ PDS [1] and significantly different from the $[Fe(edta)(H_2O)]^-$ PDS (Fig. 1). The PDS for the edta and dcta complexes of Mn(III) are similar to that for $[Fe(edta)(H_2O)]^-$ but the PDS for the heedta complex of Fe(III) is similar to the $[Fe(dtpa)]^{2-}$ PDS (not shown).
Most of the spectral perturbations revealed by the PDS are caused by binding of the paramagnetic reagent to three sites on the surface of cytochrome *c*. These sites are illustrated in Fig. 2. The different resonance patterns of the PDS result from a change in the relative affinities of the three sites for the different reagents. $[Fe(dtpa)]^{2-}$ binds at sites 2 and 3 with about the same affinity but $[Fe(edta)(H_2O)]^-$ has a greater affinity for site 2 than for site 3. This selectivity is not caused simply by the change in net charge of the reagent. From the range of compounds studied the following points emerge:

1) Trinegative, spherically symmetric complexes, such as $[Cr(CN)_6]^{3-}$, have a marked preference for site 3.
2) Reduction in the net negative charge of spherically symmetric complexes reduces the selectivity of the sites.
3) Some asymmetric complexes have a preference for site 2.

The reasons why some asymmetric complexes prefer site 2 to site 3 are not entirely clear. In solution, $[Fe(edta)(H_2O)]^-$ may have an unligated carboxylate group whereas the dtpa and heedta complexes do not. Perhaps by interacting directly with a lysine residue of the protein, a free carboxylate assists binding at site 2. Also, site 2, unlike site 3,

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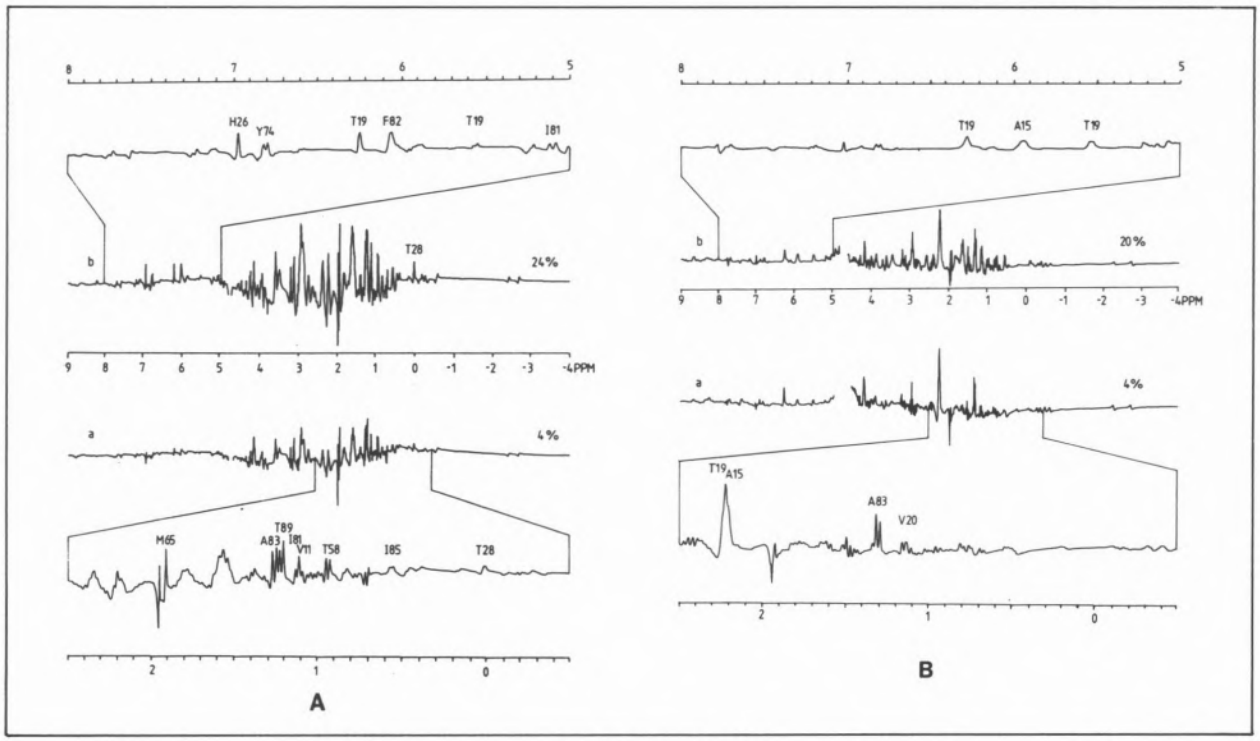

Fig. 1

300 MHz NMR paramagnetic difference spectra (PDS) for the binding of $[Fe(dtpa)]^{2-}$ *to 4 mM horse ferricytochrome* c *at pH* 5.3 and 27°C (A) and* $[Fe(edta)(H_2O)]^{-}$ *to 4 mM tuna ferricytochrome at pH* 5.5 and 27°C (B).*

Experimental details for obtaining such spectra have been reported elsewhere [1-3]. The percentage figures are mol %. Horse and tuna cytochromes *c* show the same binding preferences at their main binding surfaces.

Resonance labels are: V11, Val-11; A15, Ala-15; T19, Thr-19; V20, Val-20; H26, His-26; T28, Thr-28; Y58, Thr-58; M65, Met-65; Y74, Tyr-74; I81, Ile-81; F82, Phe-82; A83, Ala-83; I85, Ile-85; T̂89, Thr-89.

Complexes studied in the present work are the 1:1 Fe(III) and Mn(III) complexes of: dtpa, diethylenetriamine-*N,N,N',N',N''*-pentaacetic acid; edta, ethylene-*N,N,N',N'*-tetraacetic acid; dcta, *trans*-1,2-diaminocyclohexane-*N,N,N',N'*-tetraacetic acid; heedta, N-(2-hydroxyethyl)ethylenediamine-*N,N',N'*-triacetic acid

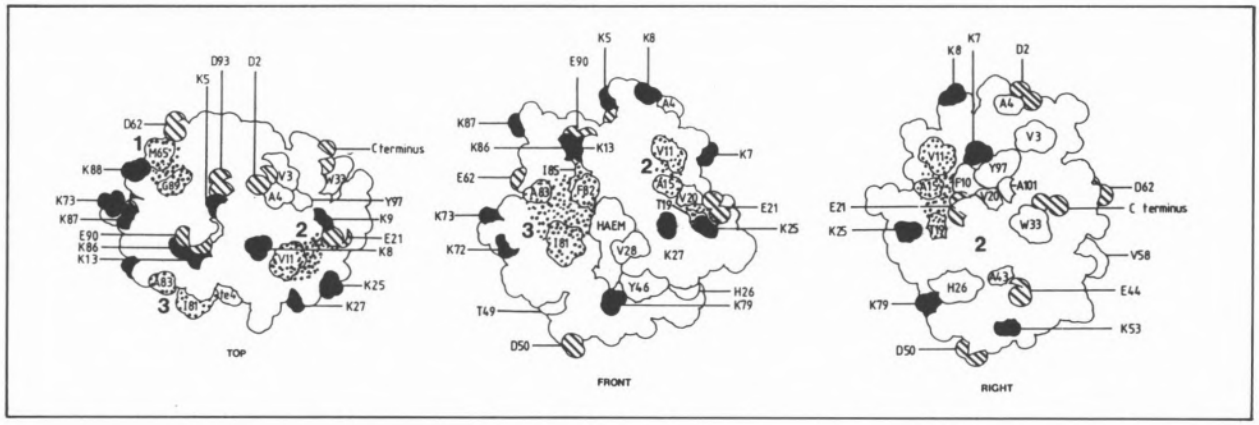

Fig. 2

Representation of the three major anion binding sites on cytochrome c.

The space-filling diagrams for tuna cytochrome *c* illustrate the positions of negatively charged groups (striped) and positively charged groups (solid). Important residues with assigned NMR resonances are shown in outline. Top, front and right refer to the conventional orientation of cytochrome *c* [4]. The stippled residues are those affected by low concentrations of the paramagnetic reagents and 1-3 refer to the binding sites

includes a protein carboxylate (Fig. 2), Glu-21, and it is possible that this plays an important role either by binding to the central metal ion of the reagent or by hydrogen bonding to its ligated H_2O. Whatever the exact cause of the selectivity these studies demonstrate that proteins do have the capacity for selecting between similar complexes. They also emphasize the need for care in interpreting reactivity parameters for redox reactions between proteins and small molecules especially in cases where there appears to be more than one possible reaction pathway, as with cytochrome *c*. Using chemically modified lysine derivatives of cytochrome *c* we hope to unravel the observed binding selectivities.

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NMR STUDIES OF A MONOHEME CYTOCHROME FROM *WOLINELLA SUCCINOGENES*, A NITRATE RESPIRING ORGANISM

An ascorbate reducible monoheme *c*-type cytochrome (8.2 KDa, $E'_o \sim +100$ mV) was purified from the nitrate "respiring" organism *Wolinella succinogenes* (VPI 10659). The optical spectrum in the ferro and ferric forms are typical of a *c*-type heme coordination. The oxidized state shows the 695 nm band taken as indicative of methionyl axial coordination, but additional optical bands are also observed at 619 nm and 498 nm (shoulder) reminiscent of the absorption bands of cytochrome *c'* [1]. These peculiarities of the optical spectrum prompted us to study this situation of spin-equilibrium by nuclear magnetic resonance (NMR) spectroscopy.

The NMR spectrum of the reduced state is shown in Fig. 1. The heme mesoproton resonances (9.88, 9.59, 9.30 and 9.25 ppm) and the resonances originated from the bound axial methionine ($S\text{-}CH_3$ at -3.72 ppm and methylene protons at -3.85, -1.66 and -0.70 ppm) are readily discernible.