THE REDOX POTENTIAL OF CYTOCHROME c:
ION BINDING AND OXIDATION STATE AS LINKED FUNCTIONS

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1. Introduction

In a previous communication we described the results of a thermodynamic study on the redox potential of cytochrome c of five different species [1]. In the present report, we deal with the ionic strength and ion binding effects on the redox properties of horse cytochrome c.

2. Materials

Horse heart cytochrome c, obtained from Sigma Chemical Company or from Boehringer, was purified on Amberlite CG50. All other reagents were of analytical grade of purity.

3. Methods

The experimental methods to estimate equilibrium constants were described elsewere [1].

4. Results

In table 1, the observed equilibrium constants for reaction (1):

ferrocytochrome c + ferricyanide $\rightleftharpoons$
ferricytochrome c + ferrocyanide,   (1)
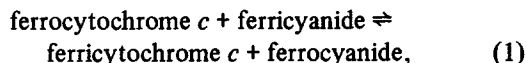
at pH 7.0 and 25°, are listed for a series of increasing ionic strengths, I, obtained in two different ways: one, by raising the concentration of the tris-cacodylate

Table 1
The effect of ionic strength on the equilibrium constant for reaction (1).

$I \times 10^3$	K_{obs} binding medium	$I \times 10^3$	K_{obs} non-binding medium
3.37	65	3.07	60
4.30	60	3.84	69
5.13	57	5.74	81
5.90	56	6.64	88
7.14	52	8.91	99

Cytochrome c, 2×10^{-5} M; potassium ferrocyanide, 1×10^{-4} M, 25°, pH 7.0. Non-binding medium 4 to 12×10^{-3} M tris-cacodylic acid buffer; binding medium; 4×10^{-3} M tris-cacodylic acid buffer, 1 to 5×10^{-3} M sodium chloride.

buffer , and the other, by keeping it constant and adding the required amounts of sodium chloride. In the first case, where only non-binding ions are present, the equilibrium constants increase with increasing I, while the opposite is true in the presence of increasing concentrations of the binding ions.

Table 2
The effect of sodium chloride on the equilibrium constant of reaction (1) at 25°, pH 7.0 and $I = 0.004$. Buffer: 2×10^{-3} M tris-cacodylic acid.

$[Cl^-] \times 10^4$	K_{obs}
2.00	63
3.02	66
3.98	70
5.01	75

A second type of experiment is shown in table 2. At pH 7.0 and 25°, a constant I of 0.004 was obtained by appropriate mixtures of tris-cacodylate buffer and sodium chloride. The equilibrium constants increase with increasing concentrations of the binding ions, indicating that the presence of the latter displaces the equilibrium towards the oxidized form of cytochrome c.

5. Discussion

The results presented above indicate that in the non-binding medium provided by the tris-cacodylate buffer, cytochrome c behaves at low I in a typical Debye-Hückel fashion. The data of table 1 after appropriate conversion to redox potentials [1], can be represented graphically (fig. 1) in terms of the expanded Debye-Hückel equation, as follows:

$$E^{\circ}_{\text{obs}} (\text{volt}) = 0.274 - 0.336 \frac{\sqrt{I}}{1 + 6\sqrt{I}} \qquad (2)$$

The factor 6 is obtained by assuming the distance of closest approach between cytochrome c and the ions in the solution to be 18.5 Å, according to crystallographic estimation of the size of the protein [2]. From the negative value of the slope it is estimated that

there is a decrease in the net charge of the molecule upon reduction, from +8.3 to +7.6, in good agreement with the amino acid composition of horse cytochrome c.

For the other set of results of table 1, with sodium chloride, equation (3) applies:

$$E^{\circ}_{\text{obs}} (\text{volt}) = 0.225 + 0.775 \frac{\sqrt{I}}{1 + 6\sqrt{I}} \qquad (3)$$

The value of the slope indicates under these conditions a net increase of charge upon reduction from 5.0 to 7.6. It should be noticed that in the experiments of table 1 the concentration of sodium ions was of the order of 4×10^{-3} M, namely, one order of magnitude higher than the concentration of cations provided by the ferri- and ferrocyanide, while in the experiments of table 2 the concentration of sodium ions was of the same order of magnitude as that contributed by the redox reagent. Since it is known that ferric cytochrome c binds chloride anions [3], this striking inversion of the slope can be understood if it is assumed that the anion of the medium binds the oxidized but not the reduced form of cytochrome c. Thus, the overall redox reaction in the presence of chloride can be written:
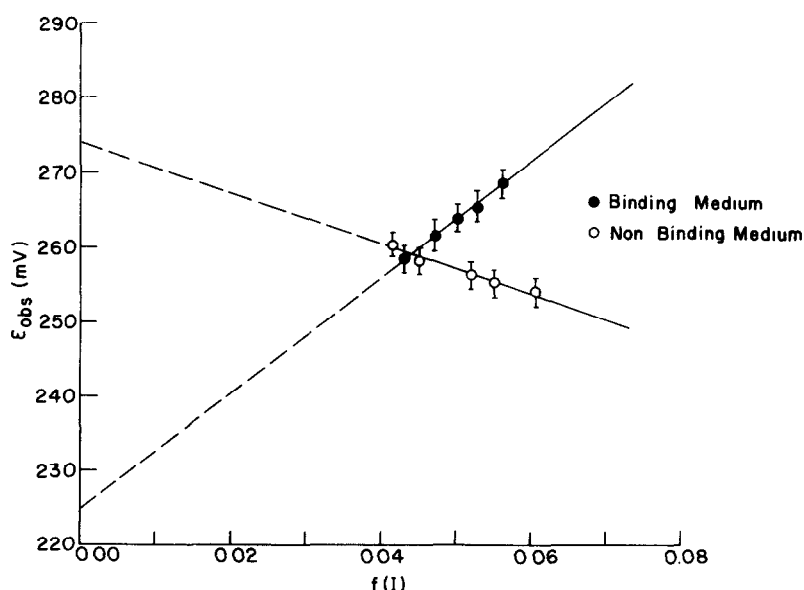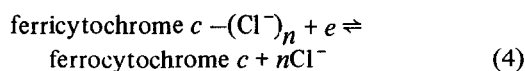
$$\text{ferricytochrome } c \, –(\text{Cl}^-)_n + e \rightleftarrows$$
$$\text{ferrocytochrome } c + n\text{Cl}^- \qquad (4)$$

Fig. 1. The effect of ionic strength on the redox potential of cytochrome c in non-binding medium (equation (2)) and binding medium (equation (3)).

Straightforward mathematical treatment leads to the following expression:

$$\log [K_{obs} - K_{NB}] = n \log [Cl^-] +$$
$$+ [\log K_{NB} - \log K_L^{ox}] \qquad (5)$$

where K_{obs} is the observed equilibrium constant for the reaction (1) in which all species, bound and not bound, are included; K_{NB} is the same constant measured at the same conditions in non-binding medium, and K_L^{ox} is the instability constant of ferricytochrome $c-(Cl^-)_n$. The results of table 2 are plotted according to equation (5) in fig. 2. From fig. 2, the value of n can be estimated as 1.6 while
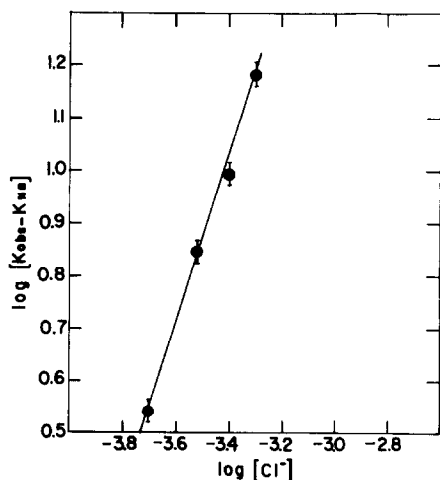
the binding constant of chloride to ferric cytochrome c is evaluated as $K = 5 \times 10^5$ M^{-1}. Preliminary experiments with another binding anion, phosphate, lead to similar results. The value of 1.6 for the net change of charge was obtained in experiments using low concentrations of sodium chloride, while with large concentrations a value of 2.6 was obtained. This difference can be explained if it is assumed that one cation binds ferrocytochrome c with an affinity at least one order of magnitude smaller than that of the anion for ferricytochrome c. The final expression thus becomes:

$$\text{ferricytochrome } c - (Cl^-)_2 + Na^+ + e^- \rightleftharpoons$$
$$\text{ferrocytochrome } c - (Na^+) + 2 \, Cl^- \qquad (6)$$
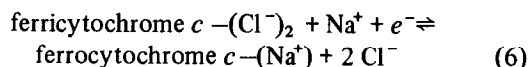
In conclusion, the oxidation states of cytochrome c and the binding of ions to the protein are an example of thermodynamically linked functions [4].

It is also worth noticing that because oxidation state and ion binding are linked functions of cytochrome c, this molecule is able to play a central role in oxidation linked ion translocation, with all its important corollaries [5].

Fig. 2. The effect of chloride concentration on the value of $\log [K_{obs} - K_{NB}]$ (equation (5)).

References

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