THERMODYNAMICS OF THE REDOX REACTION OF CYTOCHROMES *C* OF FIVE DIFFERENT SPECIES

Rimona MARGALIT and Abel SCHEJTER
Department of Biochemistry, Tel-Aviv University, Tel-Aviv, Israel

Received 18 December 1969

1. Introduction

The redox potential of cytochrome *c* has interested many investigators, because of its possible significance in understanding both the biological role played by this molecule, and also the relationship between its structure and physicochemical properties [1]. A better insight into this relationship can be gained through knowledge of enthalpy and entropy changes of the redox reaction. Such data were not available until recent calorimetric determinations by George et al. [2].

The evaluation of the heat change for several cytochromes *c*, made by applying the Van't Hoff isochore to equilibrium measurements at various temperatures, is reported in this paper.

2. Materials

Horse heart cytochrome *c* was a Sigma Chemical Co. preparation, purified on Amberlite CG-50 [3]; *Candida* cytochrome *c* was a crystallized sample from Sankyo Co. (Japan); Baker's yeast iso-1-cytochrome *c*, tuna heart and turkey heart cytochromes *c* were kind gifts of Dr. E.Margoliash. Analytical grade potassium ferrocyanide was from Riedel-De Haen AG (Seezle-Hannover); veronal and phosphate buffers were prepared from analytical grade reagents.

3. Methods

Spectroscopic measurements were made on a thermostated Cary 15 spectrophotometer; pH values were measured in a Radiometer 26 pH meter.

The equilibrium reaction studied was:

$$\text{Ferroctochrome } c + \text{ferricyanide} \rightleftharpoons \text{ferricytochrome } c + \text{ferrocyanide} \quad (1)$$

Since all the thermodynamic and potentiometric data for the hexacyanoferrate system, i.e.:

$$\text{Ferricyanide} + \tfrac{1}{2}H_2 \rightleftharpoons \text{ferrocyanide} + H^+ \quad (2)$$

are known [4], from equations (1) and (2) it is possible to obtain $E^{\circ\prime}$ and $\Delta F^{\circ\prime}$ values for the equilibrium:

$$\text{Ferricytochrome } c + \tfrac{1}{2}H_2 \rightleftharpoons \text{ferrocytochrome } c + H^+ \quad (3)$$

Oxidized cytochrome *c* was titrated with known amounts of ferrocyanide. The position of the equilibrium was estimated spectrophotometrically at 550 nm after each addition. Extinction coefficients at this wavelength for totally reduced and totally oxidized cytochrome *c* were estimated in excess sodium dithionite and potassium ferricyanide, respectively (table 1). The concentration of cytochrome *c* was estimated from the iron [5] or the amino acid content of its solutions.

The concentration of cytochrome *c* used in all the experiments was in the range of 5×10^{-5} M. The total ionic strength of the reaction mixture was $I = 0.006$, and was kept constant so as to remain in the Debye-Huckel region. The experiments at high ionic strength were performed in solutions of 0.22 M KCl; the total ionic strength of the solution was 0.23. Ion association [6, 7] was taken into account in calculating the results.

North-Holland Publishing Company – Amsterdam

Table 1
Molar extinction coefficients of cytochromes *c* at 550 nm.

Cytochrome *c* species	$\epsilon_{ox} \times 10^{-3}$	$\epsilon_{red} \times 10^{-3}$
Horse heart	8.50	29.8
Turkey heart	8.24	29.1
Tuna heart	9.00	29.6
Candida	8.80	31.6
Baker's yeast iso-1-cyt. *c*	8.50	29.8

4. Results

Fig. 1 represents a typical Van't Hoff plot of the experimental results obtained for *Candida* cytochrome *c*.

In table 2, the redox potentials and their equivalent free energy changes are listed together with the enthalpy and entropy changes estimated for reaction [3], for the cytochromes *c* of five different species. In the last column we have listed values of $\bar{S}^{\circ}_{red} - \bar{S}^{\circ}_{ox}$, the differences between the molar entropies of formation of the reduced and oxidized forms, estimated using the scale $\bar{S}^{\circ}_{H^+} = 0$.

5. Discussion

The value, $\Delta H^{\circ\prime} = -16.8 \pm 0.5$ kcal/mole, for the redox reaction of horse heart cytochrome *c* at $I = 0.01$ is in reasonable agreement with the calorimetric data, $\Delta H^{\circ} = -14.5 \pm 1.5$ kcal/mole.

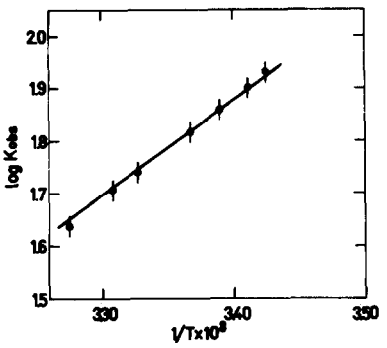

Fig. 1. Van't Hoff isochore of reaction (1). *Candida* cytochrome *c* in phosphate buffer, pH 7, total $I = 0.01$.

The Van't Hoff isochores are remarkably linear (fig. 1) indicating that the difference in heat capacities of the two forms is very small within the temperature range investigated, 20°C to 35°C. Our calculations assume that the difference between K_{obs} and K° is uniform through this temperature range.

The value of $\Delta H^{\circ\prime} = -10$ kcal/mole at $I = 0.23$ differs both from the value for myoglobin and hemoglobin at the same ionic strength, and from cytochrome *c* at low *I*.

These results may be explained by the following electrostatic considerations . Cytochrome *c* is a highly charged molecule with a net positive charge. The electrostatic repulsion between the charges at the surface

Table 2
Apparent redox potential and thermodynamic parameters of cytochromes *c*, myoglobin and hemoglobin, at 25°C, pH 6 to 7.

Hemoprotein	(I)	$\epsilon^{\circ\prime}$ (mV)	$\Delta F^{\circ\prime}$ (kcal/mole)	$\Delta H^{\circ\prime}$ (kcal/mole)	$\Delta S^{\circ\prime}$ (e.u.)	$\bar{S}^{\circ}_{R} - \bar{S}^{\circ}_{O}$ (e.u.)	Reference
Cytochrome c:							
Baker's yeast iso-1-cyt. *c*	(0.01)	261 ± 2	-6.0 ± 0.05	-17.1	-37.0	-21	this work
Candida	(0.01)	264 ± 2	-6.0 ± 0.05	-17.8	-39.0	-23	this work
Tuna heart	(0.01)	256 ± 2	-5.9 ± 0.05	-17.5	-39.0	-23	this work
Turkey heart	(0.01)	260 ± 2	-6.0 ± 0.04	-16.8	-36.0	-20	this work
Horse heart	(0.01)	261 ± 1	-6.0 ± 0.04	-16.8 ± 0.5	-36.0 ± 1.5	-20 ± 1.5	this work
Horse heart	(0.10)		-6.0	-14.5 ± 1.5	-28 ± 5	-12 ± 5	[2]
Horse heart	(0.23)	260	-6.0	-10.0	-13.0	+3.0	this work
Myoglobin	(0.25)	120	-2.8	-14.0	-38.0	-22	[8]
Hemoglobin	(0.25)	160	-3.7	-15.0	-38.0	-22	[8]

and the charge in the heme region is larger in the oxidized form, hence the molecule is more compact in the reduced state. At high ionic strength, these electrostatic repulsions are smaller due to the screening effect of counter ions, so that the difference between the two oxidation states is smaller.

Such salt effects should be distictly less in magnitude in the much less charge hemoglobin and myoglobin molecules; thus the parameters measured for these heme proteins at high salt concentration should be comparable with those of cytochrome *c* at low ionic strength. The difference in $\Delta F^{\circ\prime}$ (about 3 kcal/mole) is a consequence of enthalpy and entropy favoring the reduced state of cytochrome by 1–3, kcal/mole, and 10 e.u., respectively. These rather small differences may be misleading, since they include solvent effects as well as possible conformational changes. In fact, as results at low and high ionic strength tend to show, the contribution due to thawing of water molecules accompanying reduction of cytochrome *c* should tend to make $\Delta H^{\circ\prime}$ less negative, and $\Delta S^{\circ\prime}$ more positive. Therefore, the values observed do indicate that conformational changes may substantially contribute to $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$.

An important result of this investigation is the lack of difference between the thermodynamic parameters for the redox reaction of the cytochromes *c* of diverse species with widely different primary structures. In contrast large differences in enthalpy and entropy changes for ligand binding reactions of, eg. horse and yeast cytochrome *c* [9] are observed. If it is assumed that the heme environment, including the iron protein ligands, is the same in all these species, the observed similarity of thermodynamic parameters suggests that the conformations of reduced and oxidized cytochrome *c* of all the species investigated in this work, are of the same nature, regardless of variations in their primary structures.

References

[1] E.Margoliash and A.Schejter, Advan. Protein Chem. 21 (1966) 113.
[2] P.George, W.A.Eaton and M.Trachtman, Federation Proc. 27 (1968) 526.
[3] E.Margoliash and O.F.Walasek, in: Methods in enzymology, Vol. 10, eds. R.W.Eastabrook and M.E.Pullman (Academic Press, New York, 1967) p. 342.
[4] G.I.H.Hanania, D.H.Irvine, W.A.Eaton and P.George, J. Phys. Chem. 71 (1967) 2022.
[5] B.R.Cameron, Anal. Biochem. 11 (1965) 164.
[6] W.A.Eaton, P.George and G.I.H.Hanania, J. Phys. Chem. 71 (1967) 2016.
[7] I.M.Kolthoff and W.J.Tomsicek, J. Phys. Chem. 39 (1935) 945.
[8] P.George, G.I.H.Hanania and W.A.Eaton, in: Hemes and hemoproteins, eds. B.Chance, R.W.Eastabrook and T. Yonetani (Academic Press, New York, 1966) p. 267.
[9] I.Aviram and A.Schejter, J. Biol. Chem. 244 (1969) 3773.