THE TEMPERATURE DEPENDENCE OF THE REDOX POTENTIAL OF HORSE HEART CYTOCHROME c IN SODIUM CHLORIDE SOLUTIONS

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Summary

The $E^{0\prime}$ values for the conversion of horse heart cytochrome c from the oxidized to the reduced form as a function of temperature have been measured in 0.10 M NaCl, 0.10 M sodium phosphate, pH 7.0 solutions in H_2O and D_2O. In H_2O, the decrease in the $E^{0\prime}$ value is linear with increasing temperature up to 42°C. Above this temperature, the decrease is again linear but with a much greater slope. In D_2O solutions, however, this biphasic behavior was not observed but instead a single line was obtained over the temperature range studied (25°C to 50°C). These results are interpreted in terms of the ability of NaCl to cause a destructuring of the bulk H_2O above 42°C but not in the more stable D_2O (Kreishman, Foss, Inoue and Leifer (1976) Biochemistry, 15, 5431-35). This decrease in water structure results in a shift in the equilibrium to the larger oxidized form as indicated by the decrease in $E^{0\prime}$.

INTRODUCTION

Recent studies (1-3) of the main ionic species in HCl-H_2O solutions have shown that there are two main species present, namely, the unhydrated ion (Cl^-) and the trihydrate ($Cl(H_2O)_3^-$). Thermodynamic analysis of the equilibrium $Cl^- + 3H_2O \rightleftharpoons Cl(H_2O)_3^-$ showed a marked discontinuity in the entropy change for the hydration reaction. The decrease in entropy above 42°C was larger than below. These results were interpreted in terms of a change in the extent of structuring of the bulk water. In the presence of sodium chloride, the bulk water is apparently more structured below 42°C than above this temperature. This postulated change in bulk water structure at 42°C was found to be consistent with the degree of self-association of purine as measured by vapor pressure osmometry and proton magnetic resonance spectroscopy. The strength of the hydrophobic interaction between purine molecules decreased above 42°C in the presence of NaCl (1). It was speculated that this decrease

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in the strength of the hydrophobic interaction might cause destabilization of vital cellular components and thus cause death in higher forms of life above 42°C (1).

In order to assess the importance of this effect on a more complex biological system, we have measured the redox potential for horse heart cytochrome c (cyt c) in saline solutions as a function of temperature. Cyt c is well known as a component in the electron transport chain (4). Any change in the solvent structure and the state of hydration of the Cl^- ion should be reflected in the redox potential of cyt c since (*i*) the larger oxidized form would be favored over the smaller reduced form in a less structured solvent (5) and (*ii*) the equilibrium may be shifted due to changes in the concentrations of the ions involved in binding to cyt c (6,7).

EXPERIMENTAL

Methods The optically transparent thin layer cell is a redesigned version of the minigrid-microscope slide cell which has been reported previously (8,9). Detailed description will be reported elsewhere (10). A gold minigrid (120 lines per inch, Buckbee-Mears Co., St. Paul, Minn.) was sandwiched between two clear plexiglass sheets. The plexiglass sheets (1 x 3 inches) were machined to incorporate auxiliary and reference (S.C.E.) electrodes. The sheets were separated by 2 mm wide strips of 2 mil pressure sensitive Teflon tape (Dilectrix Corp., Farmingdale, N.Y.). The 1 x 2 cm gold grid working electrode defined an electrochemical cell volume of ca. 40 µl.

Potentials were applied to the cell by a potentiostat of conventional operational amplifier design. The temperatures were measured with a thermistor embedded in the plexiglass in close proximity to the optical beam. A Fluke 8000A digital multimeter or Digitec 261C DVOM was used to measure potentials and thermistor resistance.

Thermal regulation was accomplished by encasing the spectroelectrochemical cell between two water circulating type heating blocks with openings in

each to allow the optical beam to pass. The water was circulated and thermostatted with a Haake model FJ constant temperature circulator.

Optical measurements were made employing a Harrick RSS-B spectrometer (Harrick Scientific Co., Ossining, N.Y.) and spectra were recorded on a Houston Instruments Model 2000 x-y recorder.

$E^{0'}$ values for cytochrome c were measured at varying temperatures using a previously described spectropotentiostatic technique with a gold minigrid optically transparent thin layer electrode (8,9). Thermal equilibrium was established by allowing the cell to equilibrate with the thermostat for 30 minutes before each spectropotentiostatic measurement. This procedure gave temperature control to better than 0.2°C during the course of each experiment.

Materials. All H_2O solutions were prepared at 20°C to pH 7.00 with 0.1 M phosphate buffer. (Na_2HOP_4, analytical reagent grade, Mallinkrodt; $NaH_2PO_4 \cdot H_2O$ certified A.C.S. Fisher Scientific.) NaCl (Suprapur E. M. Laboratories) or NaI (Fisher Scientific) was added to make the solutions 0.10 M in halide. D_2O solutions were prepared at 20°C to pD = 7.00 by the same procedure. 2-6-Dichlorophenolindophenol was obtained 99% pure from Fluka, Columbia Organic Chemicals, Columbia, S.C., and was used without further purification. The horse heart cytochrome c (Type VI, 95-100% pure, Sigma Chemical Co., St. Louis, Mo.) was also used without further purification. D_2O of Gold Label grade was obtained from Diaprep (Aldrich Chemical Co., Milwaukee, Wis.).

RESULTS AND DISCUSSION

The $E^{0'}$ values for cyt c in the various solutions as a function of temperature are summarized in Figure 1. For the D_2O plus NaCl and H_2O plus NaI solutions, a linear relationship between $E^{0'}$ and T was obtained throughout the temperature range studied. In contrast, the data for the H_2O plus NaCl could best be fit by two lines. One linear region was obtained for temperatures up to 42° C, as has been previously reported by Margalit and Schejter (5). Above 42°C, however, a distinct change in slope occurs. No evidence of protein denaturation or aggregation was detectable by either ultraviolet or proton mag-

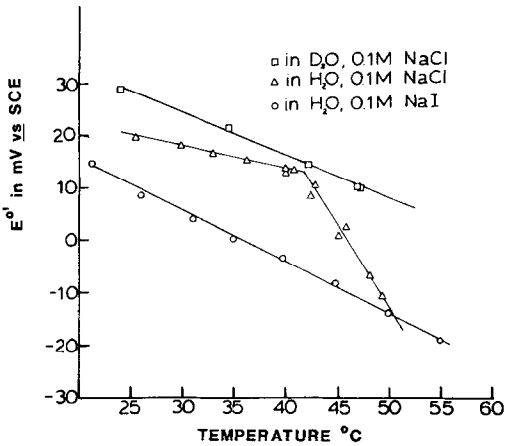

Fig. 1 The variation of $E^{0}{}'$ for cytochrome c as a function of temperature. Solutions contained 0.1 M sodium halide and 0.1 M sodium phosphate at pH or pD 7.0. The values of $E^{0}{}'$ have not been related to the zero potential of the hydrogen electrode since the temperature dependence of the SCE relative to the hydrogen electrode has not been determined.

netic resonance spectroscopy for the H_2O plus NaCl solution from 25°C to 50°C.

Since the change in slope of the temperature dependence occurs only in the H_2O plus NaCl solution at 42°C, the data can be interpreted in terms of the previously noted chloride induced structure changes in bulk H_2O (1-3) and possibly in terms of changes in the state of hydration of the chloride ion (1,11).

Since the oxidized form of cyt c is larger than the reduced form (5), as the solvent structure decreases, the $E^{0}{}'$ value will decrease due to a shift in the equilibrium to the larger oxidized form. Above 42°C in NaCl-H_2O solutions where the bulk water is less structured, the decrease in the $E^{0}{}'$ value is even more marked. This effect of solvent structure on the $E^{0}{}'$ temperature dependence is consistent with the changes observed in the D_2O solution. Since the only difference between the two samples is the strength of solvent hydrogen bonding, the lack of biphasic behavior in D_2O reflects the inability of

NaCl to disrupt the more structured bulk D_2O (1). Recent studies have shown that specific ion binding may be involved in the reduction process, and it is postulated that, upon reduction, the oxidized form releases a chloride ion and the reduced form binds a cation (6,7). This is given by the following equation:

$$cyt^{III}\underline{c} - Cl_x^- + yM^+ + e^- \rightleftarrows cyt^{II}\underline{c} - M_y^+ + xCl^-$$

Thus any change in the concentrations of the ions will affect $E^{0\prime}$. If the binding of the Cl^- ion is specific, the state of hydration of the ion would be important. It has been postulated that the unhydrated ion is bound specifically and a correction must be made in the $E^{0\prime}$ value to give a truer value of $E^{0\prime}$ for a reaction involving ion binding (11). If this correction is applied to the temperature dependence of $E^{0\prime}$, only part of the observed decrease in $E^{0\prime}$ above 42°C can be accounted for. Therefore, most of the temperature dependence of $E^{0\prime}$ is due to bulk solvent structural changes.

In their paper, Kreishman *et al.* suggested that higher forms of life could not exist at temperatures much above 42°C because some component of the cell may become nonfunctional due to the solvent structure change in a saline environment (1). Cyt *c* may be such a component. If the E^0 value for cyt *c* varies as much on the surface of the mitochondrial membrane as it does in aqueous saline solution, the delicate balance in redox potentials of the various components of the electron transport chain may be altered to such an extent above 42°C that this vital system of the cell is no longer functional.

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