Analyzing the Role of Pressure and Temperature on Biological Electron Transfer Reactions: Cytochrome *c* as a Model System

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Abstract: Horse heart cytochrome *c* serves as a test case for analyzing the role of temperature and pressure on electron transfer since its temperature- and pressure-dependent reduction potentials are known. Both the magnitude and direction of experimental reduction potential changes observed in cytochrome *c* as a function of temperature and pressure were reproduced empirically using a numerical solution to the Poisson−Boltzman equation. It is proposed based on these calculations and analysis of the X-ray crystal structures in both oxidation states that the heme iron is screened more efficiently from surface charges in the oxidized state.

Over half of the earth's biosphere is oceanic, a diverse habitat containing a wide range of temperatures and pressures. Hyperthermophilic microorganisms, for instance, have been isolated near marine hydrothermal vents at temperatures exceeding 100 °C and depths >3 miles (≈450 atm).[1−3] These remarkable microorganisms show little growth below 75 °C. Much of the global biosphere exists at pressures near 400 atm,[4] sufficient conditions to inhibit growth in non-adapted microorganisms.[5] In light of these facts, it is of interest to examine the role of temperature and pressure on biological electron transfer pathways, a mechanism by which virtually all living organisms obtain energy. A fundamental aspect in the thermodynamic analysis of biological electron transport pathways is the measurement of formal equilibrium reduction potentials ($E^{\circ\prime}$s). To date, the role of temperature and pressure on $E^{\circ\prime}$s of electron transport proteins has not received much attention. Horse heart cytochrome *c* serves as a test case for analyzing the role of temperature and pressure on electron transfer since its temperature- and pressure-dependent reduction potentials have been reported (-0.7 mV/°C[6] and 0−0.02 mV/atm[7 a,b]).

Reduction potential changes observed in both temperature-controlled[8,9] and pressure-controlled[7,10] electrochemical experiments have been attributed to differences in solvent−solute interactions between oxidation states of an electron transfer molecule. Temperature-controlled electrochemical studies probe, among other things, entropy changes that accompany electron transfer reactions, and these changes have been attributed to differences in the ordering of solvent molecules around a particular oxidation state. Similarly, pressure-controlled electrochemical studies probe the volume changes that accompany electron transfer reactions, and these changes have also been attributed to the reorientation and tighter packing of additional molecules around a particular oxidation state. Thus, temperature and pressure influence the stability of a particular oxidation state by altering solute−solvent interactions that stabilize electronic charge.

Theoretical Procedure

The formal reduction potential ($E^{\circ\prime}$) of an electron transfer protein is a measure of the electrostatic work required to bring an electron from an infinite distance to its electron transfer center, and thus represents the summation of all its electrostatic interaction energies. Electrostatic interaction energies between charged particles in a homogenous medium can be analytically calculated by Coulomb's law:

$$E = \sum qq/\epsilon r \tag{1}$$

where q is charge, ϵ is the dielectric constant, and r is the distance between charges. Electrostatic models, which are distinguished by the treatment of these parameters,[11,12] have been moderately successful at quantitatively reproducing experimental data, including a recent study of recombinant yeast cytochrome *c*.[13] DELPHI (Copyright Columbia University, 1987), a commercially available and widely used continuum dielectric model, is used here to reproduce experimental changes in reduction potential as a function of temperature and pressure. This program calculates electrostatic interaction energies in proteins by numerically solving the Poisson−Boltzman equation, which allows charge density (q) and the dielectric constant (ϵ) to vary in space. It has been proposed[14,15] that temperature- and pressure-dependent reduction potentials of biomolecules represent changes in electrostatic interaction energies, due to changes in atomic partial charges, charge separation, or charge environment according to the following equations:

$$\Delta E_T = \Delta(\sum qq/\epsilon r)_T \tag{2}$$

$$\Delta E_P = \Delta(\sum qq/\epsilon r)_P \tag{3}$$

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Table 1. Comparison between Calculated and Experimental Changes (mV) in Reduction Potential for Cytochrome *c* as a Function of Temperature and Pressure

	calcd change for different protein dielectric constants[a]		
	$\epsilon_{oxidized} = 5$ $\epsilon_{reduced} = 5$	$\epsilon_{oxidized} = 7.5$ $\epsilon_{reduced} = 5$	exptl change
$(\Delta E°/\Delta T)_P$[b]	+36	−29	−26 (ref 6)
$(\Delta E°/\Delta P)_T$[c]	−2	+1	+ 1 (ref 7a)
			+ 5 (ref 7b)

[a] See text for additional parameters used in calculations. [b] Values were calculated for tuna cytochrome *c* from 25 to 70 °C, and they were measured for horse cytochrome *c* over the same temperature range. [c] Values were calculated for tuna cytochrome *c* from 1 to 200 atm, and they were extrapolated for the same pressure range from measurements of horse cytochrome *c*. The discrepancy between published values for the reduction potential of cytochrome *c* has been attributed to differences in experimental conditions and in the pressure dependence of the reference electrode.[7a]

These parameters are likely to be interrelated and influenced by temperature and pressure to various degrees, but experimental evidence suggests that electronic charge and charge separation are not as likely to be as temperature and pressure dependent as is the dielectric constant.[15] Temperature- and pressure-controlled electrochemical experiments thus provide a direct method for measuring relative changes in the dielectric constant surrounding electron transfer centers in biomolecules.

Differences in electrostatic interaction energies of tuna cytochrome *c*, which is structurally related to horse heart cytochrome *c*,[16] were calculated using DELPHI on a Silicon Graphics INDY work station. Partial atomic charges for amino acids[17] and heme[18] were assigned to coordinates of tuna cytochrome *c* as determined from X-ray crystal structures for both oxidation states.[19] Hydrogen atoms and solvent water molecules were not explicitly included in the calculations. The solvent was assigned an ionic strength of 0.1, and a dielectric constant as a function of temperature and pressure.[20] The protein was assigned a variable dielectric constant as described below. The resolution of the calculations was 0.84 grids per angstrom.

Results and Discussion

Calculated and experimental changes in reduction potential as a function of pressure and temperature are listed in Table 1. The change in reduction potential resulting from an increase in temperature from 25 to 70 °C due to the change in the dielectric constant of the solvent ($\epsilon_{solvent} = 80 \rightarrow 64$, and $\epsilon_{protein} = 5$) was calculated to increase 36 mV. Notably, it would be predicted from the discussion above that the reduction potential of cytochrome *c* would increase in response to an increase in temperature (or decrease in pressure). On the basis of the X-ray crystal structure of this protein, the positively charged heme−iron would "feel" the positively charged solvent exposed amino acid side chains surrounding the heme more strongly as the dielectric constant of the solvent decreases. This electrostatic repulsion would shift the equilibrium to favor the formation of the reduced electron transfer center, thereby lowering the electrostatic interaction energy and increasing the reduction potential of the heme−iron.

Experimentally, however, it is observed that the reduction potential of horse heart cytochrome *c* *decreases* 26 mV over the temperature range from 25 to 70 °C.[6] The heme is apparently shielded from the positively charged heme crevice as the temperature is raised, despite the fact that the dielectric constant of the solvent is lower. Indeed, an analysis of the X-ray crystal coordinates of tuna cytochrome *c* revealed that the oxidized heme iron was shielded by an additional water molecule from positively charged amino acid side chains surrounding the heme crevice.[19] It was also noted from previous electrostatic calculations[21] that electrostatic interactions between the protein and the oxidized heme−iron were less favorable than those of the reduced heme. In this study, the additional shielding of the oxidized heme by water was empirically represented by a larger dielectric constant for the oxidized protein ($\epsilon_{oxidized}$), and results from these electrostatic calculations are listed in Table 1. By using a larger dielectric constant for the oxidized protein, the temperature-dependent reduction potential of horse heart cytochrome *c* can be theoretically reproduced. It was calculated $\Delta E°' = -29$ mV for tuna cytochrome *c* using $\epsilon_{oxidized} = 7.5$ and $\epsilon_{reduced} = 5$ for the protein, and taking into account the change in the dielectric constant of the solvent ($\epsilon_{solvent} = 80 \rightarrow 64$), for an increase in temperature from 25 to 70 °C.

Similarly, by accounting for the change in the dielectric constant of the solvent from 1 to 200 atm, it was calculated that the reduction potential of tuna cytochrome *c* decreased −2 mV using protein dielectric constant $\epsilon_{protein} = 5$ and increased +1 mV using protein dielectric constants $\epsilon_{oxidized} = 7.5$ and $\epsilon_{reduced} = 5$. The later value is in reasonable agreement with previously published values of horse heart cytochrome *c* as listed in Table 1.[7a,b] The positive change in reduction potential of cytochrome *c* accompanying an increase in pressure has been attributed to the release of water upon reduction, which results in a smaller protein volume.[7a] In order to explain in terms of electrostatic interaction energies the observed positive pressure-dependent reduction potential of cytochrome *c*, the dielectric constant of the oxidized protein must be higher. As discussed above for the temperature-dependent reduction potential of this protein, a higher dielectric constant for the oxidized protein may result from an additional water molecule(s) in close proximity to the oxidized heme−iron.

Empirically derived values of the protein dielectric constant used in these electrostatic calculations reproduce with reasonable accuracy both magnitude and direction for the experimentally measured temperature- and pressure-dependent reduction potentials of horse heart cytochrome *c* as listed in Table 1. In both cases, it is suggested that the dielectric constant for the oxidized protein is higher than the reduced protein due to the association of an additional water molecule to the oxidized heme−iron. It is recognized that other protein dielectric constants may also reproduce the observed changes in reduction potential for cytochrome *c*. The importance of the empirical approach described in this study, however, is its ability to model at a molecular level the relative changes in electrostatic interaction energies of cytochrome *c*, and possibly other biomolecules, as a function of pressure and temperature.

Conclusion

An empirical approach was used to analyze the influence of temperature and pressure on the equilibrium electron transfer reaction of cytochrome *c*. Solute-solvent interactions of cytochrome *c*, as well as other biological electron transfer centers,

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will be directly influenced by temperature and pressure, and these interactions will alter electrostatic interaction energies. These interactions may be accounted for by changes in the dielectric constant of both the solute and solvent. The relative change in the dielectric constant of proteins as a function of temperature and pressure may be empirically derived from electrostatic calculations. Future investigations should determine whether temperature and pressure/reduction potential profiles of electron transfer proteins provide a direct and powerful method for probing changes in electrostatic interaction energies.

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