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Thermodynamic Parameters of pH-dependent Optical Transition in Bovine Heart Cytochrome c Oxidase

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ABSTRACT: Cytochrome c oxidase was prepared according to the method of Yonetani (1961). The pH-induced optical transition in the oxidised (resting) enzyme was studied between 400 and 500 nm at different temperatures. The changes in enthalpy, entropy and free energy for the transition were determined to be -37.89 ± 6.20KJ/mole, - 158.16 ± 8.20 JK^{-1} $mole^{-1}$ and -41.60KJ/mole respectively. The results are discussed in terms of the binding of a strong ligand (possibly histidine) to the distal side of an already pentacoordinate high spin cytochrome a_3.

INTRODUCTION

Detergent-Solubilized cytochrome c oxidise is known to exhibit pH-dependent optical transition. The enzyme isolated from bovine heart, using the method of Yonetani (1961), exhibits a wavelength maximum at 418 nm at pH 7.0, which shift to 430 nm upon increasing the pH, with an apparent pKa of 8.0 at 20°C (Gullo *et al.*, 1993). This optical transition is also associated with enhanced rate of intramolecular electron transfer between cytochromes a and a_3 within the enzyme. A similar bathochromic shift in the Soret spectrum of myoglobin has also been reported at high pH, which is attributable to the low spin transition associated with the binding of hydroxyl group to the six axial coordination position of its already pentacoordinate constituent haem (Palmer, 1983).

The optical and kinetic variability in cytochrome c oxidase are, however, thought to be due to the structural heterogeneity within the binuclear cytochrome a_3/CuB centre. The presence or absence of a bridging ligand between cytochrome a_3 and CuB, which is responsible for the antiferromagnetic superexchange interaction (Gullo *et al.*, 1983) is thought to be responsible for the observed differences in the rates of electron transfer and ligand binding to the binuclear centre (Moody *et al.*, 1991, and Wilson *et al.*, 1994). Cytochrome a_3 is, however, known to be pentacoordinate with histidine 419 in the proximal co-ordination sphere (Hosler *et al.*, 1993). The dissociation of histidine 419 from the proximal pocket and the binding of another intrinsic ligand on the distal side (while cytochrome a_3 remain essentially pentacoordinate with the reversal of distal and proximal side), is also another explanation proposed for the differences observed in the rate of reduction of the binuclear centre (Brunori *et al.*, 1994). However, the exact molecular basis of the pH-dependent optical transition in cytochrome c oxidase, though attributable to the binuclear centre, is poorly understood. Coupled with this is the paucity of thermodynamic data associated with the transition. The aim of this work is to evaluate the thermodynamic determinants of this transition with a view to gaining some insight into the nature of this pH-induced optical effect of far-reaching functional significance.

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EXPERIMENTAL

Bovine heart cytochrome c oxidase was prepared according to the method of Yonetani (1961), the final pellet was dissolved in 20 mM phosphate buffer, pH 7.4, containing 1% tween-80. The concentration of the enzyme was determined from the difference in absorbance at 605 nm using the difference in extinction coefficient of 22.00 $mM^{-1}cm^{-1}$ per functional unit (Brunori *et al.*, 1983).

The enzyme was diluted to a concentration of 10 µM (total haem) in 0.1 M phosphate, in the pH range between 6 and 10, containing 1% tween-80. The different enzyme preparation were incubated in a thermostand water bath at the different temperatures studied for 3 hours prior to recording their absorption spectra. All spectra were recorded using Cary 5E spectrophotometer interfaced with a personal computer for data capture and storage. The ΔA416-430 were determined, at the different temperatures, as a function of pH and fitted to Henderson-Hasselbalch equation using the nonlinear Enzfitter (Cambridge) functional minimization software from which the pKa's at the different temperatures were determined.

ANALYSIS OF RESULTS

The results were analyzed using the equation:

$$pKa = \frac{\Delta S}{2.303R} - \frac{\Delta H}{2.303RT},$$

derived from the application of Erying equation to the forward and reverse reactions with the assumption that the reactions are kinetically adiabatic or, at least, have nearly identical transmission coefficients. All symbols in the equation have their usual thermodynamic connotations. The changes in entropy and enthalpy were determined, therefore, from the intercept and slope of the linear graph of pKa as a function of reciprocal absolute temperature, respectively.

RESULTS AND DISCUSSION

Figure 1 illustrates the spectra of oxidised, dithionite-reduced and reduced-CO species of bovine heart cytochrome c oxidase. The oxidised is characterized by a Soret absorption peak centred around 418 nm which is bathchromically shifted to 445 nm upon dithionite reduction. Addition of CO to the reduced enzyme resulted in a hypochromic shift of the absorption band to 430 nm. These spectroscopic signatures, which are attributable to in-plane polarized * transitions, are in agreement with the results of other workers (Feiffer *et al.*, 1992, and Yonetani, 1960). Coupled with this is that our enzyme preparation catalyzed the oxidation of ferrocytochrome c as determined using stopped-flow spectrophotometry (data not shown), with a maximum turnover number of 160 electrons per second per mole aa_3 (Abubakar, 1994).

The absorption band, in the oxidized enzyme, centred around 418 nm is known to be composite due to the absorbance contributions of both cytochromes a and a_3 constituents of the enzyme. Spectral deconvolution using second derivative spectroscopy (Copeland, 1993) has revealed that the absorption due to cytochromes a_3 and a are centred around 416 and 429nm respectively. However, since only cytochrome a_3 is known to be pentacoordinate (Brunori *et al.*, 1994 and Hostler *et al.*, 1993) and, therefore, receptive to exogenous ligands, then the spectral changes associated with the binding of such ligands may be expected to be due predominantly, to cytochrome a_3. Accordingly, the spectral changes observed upon the incubation of the oxidized bovine heart enzyme (Figure 2) under 1 atmosphere CO may be attributable, exclusively, to the low spin character engendered by CO binding to cytochrome a_3 (see for example Nicholls, 1976). A similar bathochromic shift in the spectrum of cytochrome a_3 is also observed upon the ligation of CN to oxidized cytochrome a_3 (Wilson *et al.*, 1994; Palmer, 1993). In all these cases, the absorption peak due to cytochrome a is not visibly shifted and persists at 429 nm. We wish to posit, therefore, that all the spectral shifts in cytochrome c oxidase with composite absorption band centred at 430 nm, including pH-induced shifts, and in which cytochromes a remain essentially ferric are due to spectral transitions in cytochrome a_3 (see for example Moody *et al.*, 1991).

A typical plot of the dependence of Δ A416-430nm as a function of pH is shown in Figure 3. This optical transition, involving spectral change in cytochrome a_3, exhibited a pKa of 7.35 ± 0.098 at 20°C. This value which is based on curve fitting involving a single acid/base group is quite close to the pKa of the imidazole group of histidine and agrees, fairly well, with the value reported by other workers (Gullo *et al.*, 1993). A plot of pKa as a function of reciprocal temperature (Figure 4) gave a straight line (correlation coefficient = 0.97) from which the value of the enthalpy change was calculated to be -87.89 ± 6.2 KJ/mole with a corresponding entropy change of -158.16 ± 8.2 $JK^{-1}mole^{-1}$. The overall change in free energy, at 20°C, was calculated to be -41.60KJ/mole indicating that the transition is a thermodynamically downhill process with the decrease in enthalpy more than

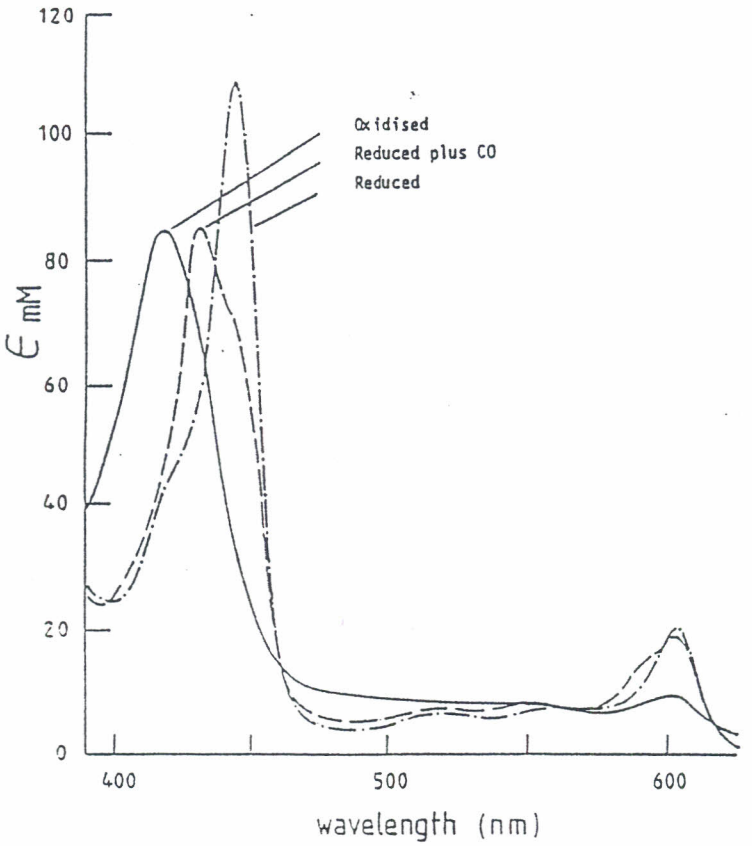


Figure 1: Absorption Spectra (expressed as the wave length dependence at mM absorbance Coefficient) of oxidised, dithionite reduced and reduced - Co bovine heart cytochrome c oxidase in 20 mm phosphate buffer, f.4 containing 2% Cholate at 18°C.

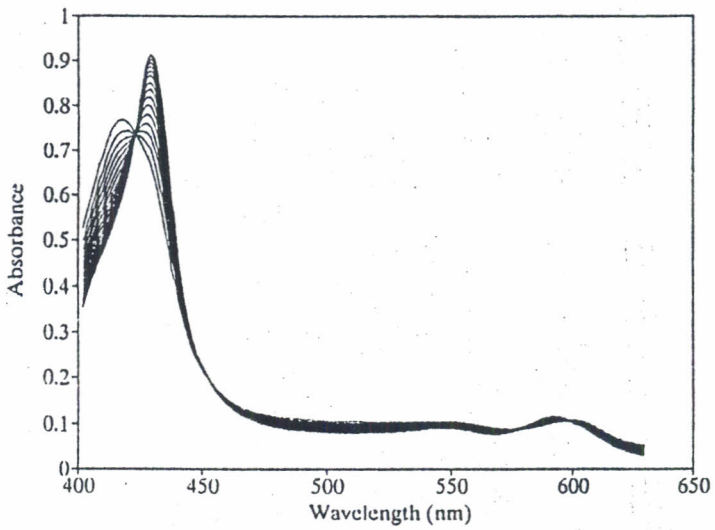
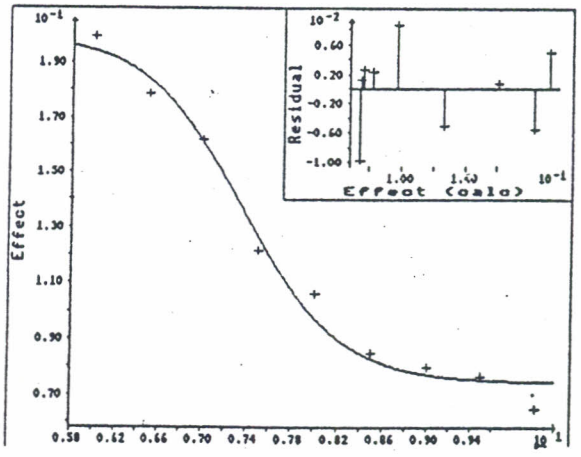


Figure 2: Spectra changes observed upon incubation of oxidized bovine heart cytochrome c oxidase with CO with 20mm phosphate buffer, pH 7.4.

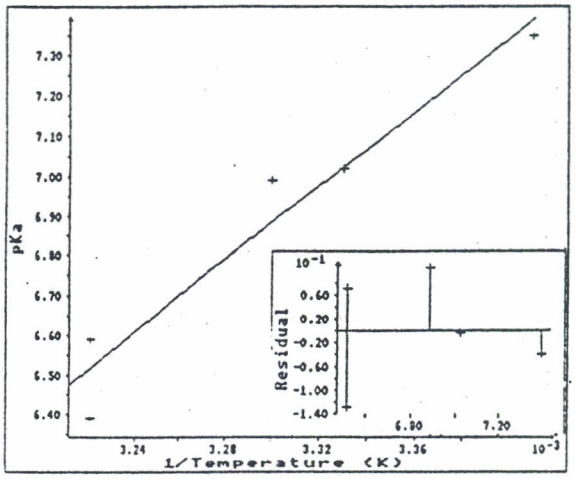


pH titration

pKa determination
Simple weighting

Variable	Value	Std. Err.
pKa	7.35225E+00	9.80751E-02
Lower limit	1.99614E-01	6.16039E-03
Upper limit	7.42501E-02	3.54072E-03

Figure 3: pH titration curve of bovine heart cytochrome c oxidase at 20°C.



pKa V 1/T (K)
Linear Regression
Simple weighting

Variable	Value	Std. Err.
Gradient	4.59004E+03	6.63975E+02
Intercept	-8.2608E+00	2.18898E+00

1/Temperature (K)	pKa	Calculated
1 3.41000E-03	7.35000E+00	7.39127E+00
2. 3.33000E-03	7.02000E+00	7.02406E+00
3. 3.30000E-03	6.99000E+00	6.88636E+00
4. 3.22000E-03	6.59000E+00	6.51916E+00
5. 3.22000E-03	6.39000E+00	6.51916E+00

Figure 4.

compensating for the unfavorable isothermally unavailable energy term ($T\Delta S$). This favourable enthalpy may not be explained, exclusively, in terms of a single deprotonation event which is generally thought to be associated with unfavourable enthalpy change (Wilson and Greenwood, 1995). We are of the opinion, therefore, that this deprotonation is accompanied by a ligand binding event at the binuclear centre which off-sets the unfavourable enthalpy usually associated with reactions involving deprotonation. The interpretation of the unfavourable entropy change associated with this transition in terms of the changes in the geometry of the binuclear centre is, however, less straight forward because of the difficulty in assessing the contribution of the solvent effect to the total entropy change.

The 418 to 430 nm optical transition induced by pH and its associated increase in intramolecular electron transfer in cytocrhome a_3 (see for example Aitken, 1995) may, in our opinion, be rationalized in terms of the binding of imidazolate, a strong field ligand, to one of the axial positions of an already pentacoordinate ferricytochrome a_3 to induce a low spin transition. Such a transition, in cytochrome c oxidase, is always associated with a 418 to 430 nm optical transition (Nicholls, 1978) and electron transfer to and from low spin haem centres is facile because of the relatively little electronic reorganisation needed prior to the electron transfer (Cheesman *et al.*, 1991 and Marcus and Sutin, 1985). However, a model invoking upon the labile coordination chemistry of the binuclear centre which involves ligand exchange, possibly coupled to the proton pumping function of the enzyme, has already been proposed (Woodruff, 1993). In this model, the enhanced rate of intramolecular electron transfer to the binuclear centre,within the enzyme, has been attributed to the ligation of the otherwise dissociated histidine 419 to the proximal pocket of a pentacoordinate cytochrome a_3 (Brunori *et al.*, 1994), thereby obviating the need for the slow through-space electron transfer to this centre from the cytochrome a/CuA centre. In conclusion, although explanations have been proferred to account for the variability in the rate of reduction of the binuclear centre, the simplest explanation for the observed optical and electron transfer properties of the enzyme, in our opinion, is the dissociation of the bridging (strong) ligand from CuB and its subsequent ligation to the distal side of cytochrome a_3 as discussed elsewhere (in this volume) with histidine 419 already coordinated to the proximal side (see Hosler *et al.*, 1993) as suggested above and supported by the thermodynamic data presented herein. However, unless the question of the full structure of the enzyme is resolved by x-ray crytallography, at least to 2 Å resolution, the ligation state of the binuclear centre and hence the molecular mechanism of the dioxygen reduction and proton pumping can only be conjectured.

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