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The redox thermodynamics of microperoxidase are dependent on the solvent medium

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Received (in Cambridge, UK) 20th September 2002, Accepted 16th December 2002
First published as an Advance Article on the web 15th January 2003

The redox thermodynamics of the heme undecapeptide, microperoxidase have been examined in aqueous buffer and in glycerol. The change in $E^{\circ\prime}$ on transition from water to glycerol is dominated by the change in $\Delta S^{\circ\prime}$.

The protein matrix, degree of metal co-factor exposure, ligand binding, and solvent medium have all been considered as pertinent to the analysis of factors contributing to the reduction potential of redox proteins.[1] One experimental method for seeking to understand the influence of these factors, is to extract thermodynamic information for the reduction of redox centres from the variation of formal reduction potentials ($E^{\circ\prime}$) with temperature. In this manner, the influence of the entropic ($\Delta S^{\circ\prime}_{rc}$) and enthalpic ($\Delta H^{\circ\prime}_{rc}$) components of $E^{\circ\prime}$ can be gauged.[1,2] This analysis is garnered from the recognition that the thermodynamic compartmentalization of $E^{\circ\prime}$ allows a comparative study of the components which, together, determine the redox potential and therefore help elucidate the controlling mechanisms.

Microperoxidase (MP-11) is a proteolytic fragment of cytochrome c containing the heme group and amino acids 11–21 of the parent protein. MP-11 has a highly exposed iron protoporphyrin ring system with Fe^{III} coordinated on the proximal side by the imidazole ring of His-18 of the peptide backbone. The heme distal site is exposed to the solvent medium and is usually coordinated by a water molecule.[3] The high degree of solvent exposure renders MP-11 a suitable model for examination of the influence of both the solvent medium and the peptide matrix on the redox potential.

To date, studies of the redox potentials of proteins in aqueous solution have utilised either native and/or point mutated proteins, or modified active centre model systems.[4] The approach presented here examines the thermodynamic changes that occur when microperoxidase is transferred from an aqueous to an organic medium. To our knowledge, this represents the first examination of heme-peptide redox thermodynamics in an organic solvent.

Cyclic voltammetry was used to determine the temperature dependence of $E^{\circ\prime}$ for both immobilised MP-11 (MP-11_{IM}) and solution phase imidazole ligated MP-11 (MP-11-Imid).[5] Immobilisation avoids the solubility problems normally associated with proteins in organic solvents.[6] The value of $E^{\circ\prime}$ for MP-11_{IM} in aqueous solution (Table 1) is in agreement with that obtained previously,[7] while in glycerol, the 30 mV increase in $E^{\circ\prime}$ is in broad agreement with the increased values obtained for MP-11 in DMSO and MP-11_{IM} in acetonitrile and ethanol.[8]

As the temperature is increased, $E^{\circ\prime}$ remains essentially constant in aqueous solution, while increasing substantially in glycerol (Fig. 1). The electrochemical and thermodynamic data derived from these data are summarised in Table 1. Over the temperature range 5–35 °C, the values of $\Delta H^{\circ\prime}_{rc}$ (21 kJ mol^{-1}) and $\Delta S^{\circ\prime}_{rc}$ (−19 J mol^{-1} K^{-1}) obtained for MP-11-Imid (solution phase) were in reasonable agreement with those obtained previously (13 kJ mol^{-1} and −18 J mol^{-1} K^{-1}, respectively).[9,10]

For MP-11_{IM} in aqueous buffer, $\Delta H^{\circ\prime}_{rc}$ was 35.5 kJ mol^{-1}, indicating that the Fe^{III} state of the immobilised form is more stable. $\Delta S^{\circ\prime}_{rc}$ decreased dramatically relative to solution phase data, from 49[10] to 4.8 J mol^{-1} K^{-1}. This decrease may be ascribed to the restricted mobility of the tethered MP-11_{IM}, with concomitant alteration of the solvent shell structure about the heme of MP-11_{IM}. On immersion of MP-11_{IM} in glycerol, $\Delta H^{\circ\prime}_{rc}$ increased by *ca*. 50%, while there was a dramatic, twenty fold, increase in $\Delta S^{\circ\prime}_{rc}$. The combined effect of these changes produces the observed increase in $E^{\circ\prime}$ of 30 mV.

The thermodynamic component most pertinent to the change in reduction potential ($\Delta E^{\circ\prime}$) observed in the solvent transition can be determined from eqn. (1):

Table 1 Thermodynamic data for MP-11_{IM} reduction in aqueous buffer and glycerol

Solvent	$E^{\circ\prime}$(V)[ab]	$\Delta H^{\circ\prime}_{rc}$ (kJmol^{-1})[c]	$\Delta S^{\circ\prime}_{rc}$ (J$K^{-1}mol^{-1}$)[c]	$-\Delta H^{\circ\prime}_{rc}/F$ (V)	$T\Delta S^{\circ\prime}_{rc}/F$ (V)[b]
Glycerol	−0.323	54.4	77.2	−0.563	0.238
Aqueous	−0.353	35.5	4.8	−0.368	0.015

[a] Errors are ±0.009 V and ±0.003 V for glycerol and aqueous medium respectively. [b] T = 298K. [c] Average errors for $\Delta H^{\circ\prime}s_{rc}$ and $\Delta S^{\circ\prime}_{rc}$ in glycerol are 1.2 kJ mol^{-1} and 8.0 J mol^{-1} K^{-1}, respectively, and in aqueous medium are 0.3 kJ mol^{-1} and 2.6 J mol^{-1} K^{-1}, respectively.

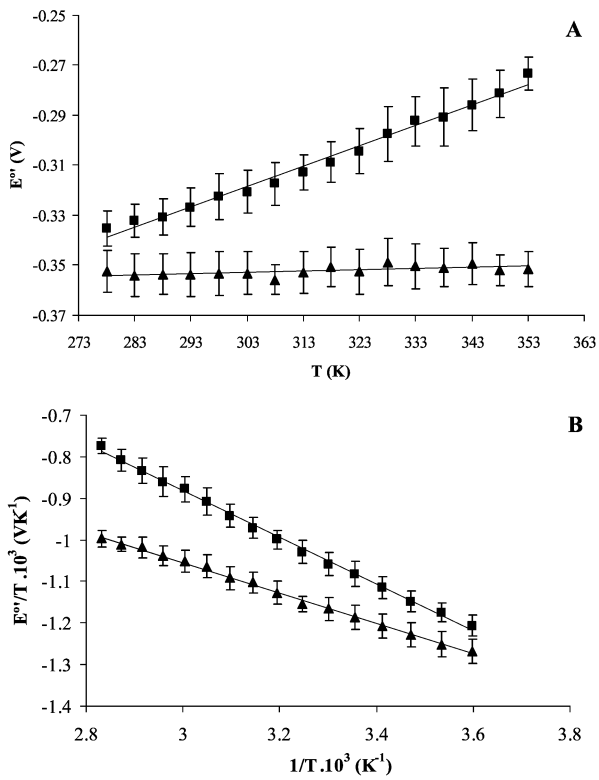

Fig. 1 The variation of formal reduction potential ($E^{\circ\prime}$) for MP-11_{IM} electrodes in glycerol (■) and aqueous buffer (▲) (A) and $E^{\circ\prime}/T$ *vs.* $1/T$ plots prepared from the same data (B).

DOI: 10.1039/b209202f

$$\Delta E^{\circ\prime} = \frac{\Delta\Delta H^{\circ\prime}_{fr}}{F} + \frac{T\Delta\Delta S^{\circ\prime}_{fr}}{F} \quad (1)$$

Here, $\Delta E^{\circ\prime}_{glycerol\text{-}aqueous} = -0.195 + 0.226 = 0.031$ V,[11] indicating that, though $E^{\circ\prime}$ is a function of both enthalpic and entropic components, the determining component of $\Delta E^{\circ\prime}$ on transition from aqueous buffer to glycerol switches from enthalpic to entropic control and, that $E^{\circ\prime}$ is a function of the solvent medium to which the redox centre is exposed.

While the transition from water to glycerol renders heme iron reduction enthalpically less favourable (increasing the stability of Fe^{III}), this is countered by the increased entropic component of $E^{\circ\prime}$. The enthalpy of reduction is a function of a range of effects including the nature of the axial ligand(s), the net charge (of both the heme and the peptide), extent of the hydrogen bond network, and the degree of solvent exposure.[10,12] The observed increase in reduction enthalpy observed is the net result of a number of counteracting factors: a) displacement of water by glycerol from the distal ligand site; such a displacement would be expected to stabilise the Fe^{III} state; b) the presence of a more hydrophobic heme environment in glycerol which would favour reduction, reducing $\Delta H^{\circ\prime}_{rc}$; c) the formation of hydrogen bonds between glycerol and both the heme and the peptide backbone of MP-11, modulating the reduction enthalpy (either positively or negatively depending on the extent and location of hydrogen bonds relative to the heme). At present, it is not possible to quantify the effect, if any, of each of these components. The resonance Raman[5] (Fig. 2) and visible spectra of MP-11_{IM} indicate that only minor changes in the structure of the heme of MP-11_{IM} occur in glycerol, the nature of which may be due to hydrogen bonding of glycerol to MP-11_{IM} and/or interactions between glycerol and the heme iron.

In spite of the substantial increase in $\Delta H^{\circ\prime}_{rc}$, the change in $E^{\circ\prime}$ is determined by $\Delta S^{\circ\prime}_{rc}$. Reduction entropies of redox proteins are considered to be due to solvent induced reorganisation effects, alteration of solvent dielectric about the metal redox centres, and the influence of ligation.[13] Glycerol is a polar organic solvent, which is capable of stripping water from the hydration layer of proteins[14] and in the same fashion would be expected to dehydrate MP-11. The solvation sphere about the redox centre is a significant contributor to $\Delta S^{\circ\prime}_{rc}$. The decrease in positive charge allows for a decrease in the ordering of the solvation sphere, increasing $\Delta S^{\circ\prime}_{rc}$.[12] Such an effect would be more pronounced in glycerol, a less polar solvent than water, mimicking the effect of the hydrophobic pocket which exists about the heme centre of the parent protein, cytochrome c, whose large positive $E^{\circ\prime}$ (0.262 V) can be partially attributed to water exclusion from the heme.[15]

These results demonstrate that while $E^{\circ\prime}$ of MP-11_{IM} is a function of both enthalpic and entropic terms, it is $\Delta S^{\circ\prime}_{rc}$ which controls $\Delta E^{\circ\prime}_{glycerol\text{-}aqueous}$. Since the polarity of the heme environment plays a crucial role in determining $E^{\circ\prime}$,[1,16] experiments are in progress to determine $\Delta\Delta H^{\circ\prime}_{rc}$ and $\Delta\Delta S^{\circ\prime}_{rc}$ of MP-11 and its parent protein, cytochrome c, in other solvents.

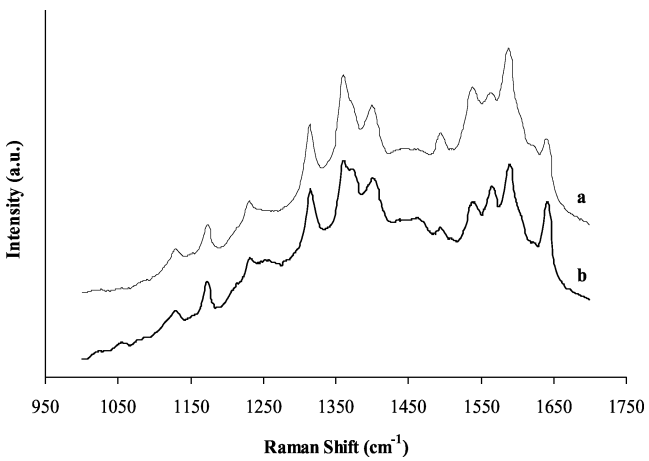

Fig. 2 Resonance Raman spectra of MP-11_{IM} exposed to: (a) aqueous buffer, and (b) glycerol at 298 K. Spectra are the average of five scans.

Funding from the Department of Chemical and Environmental Sciences and helpful discussions with Professor G. Barletta are gratefully acknowledged.

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