ELECTROCHEMICAL STUDIES ON DESULFOREDOXIN AND DESULFOFERRODOXIN, TWO $FeCys_4$ CONTAINING PROTEINS

Carla S.Ascenso, Maria João Lima, Frank Rusnak, Isabel Moura and José J.G. Moura

Departamento de Química, Centro de Química Fina e Biotecnologia
Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa
2825 Monte de Caparica, Portugal

ABSTRACT: Desulforedoxin (Dx) and Desulfoferrodoxin (Dfx) contain both a simple iron-sulfur center with a single iron atom coordinated to four S-cysteinyl residues in a distorted tetrahedral geometry. Different spectroscopic tools (UV-vis, EPR and Mössbauer) were used to characterize the metal center in these two proteins. The use of electrochemical methods to study the pH dependence of the redox potentials of these $FeCys_4$ centers showed that there is a pH dependent process in the alkaline region. The structural origin of this effect will be discussed.

Key words: $FeCys_4$ center, Rd and Dx type centers, desulforedoxin, desulfoferrodoxin, redox potentials

Abbreviations used: Rd, rubredoxin; Dx, desulforedoxin; Dfx, desulfoferrodoxin; *D.*, *Desulfovibrio*

INTRODUCTION

The most characterized non-heme iron center in biology is the one present in Rubredoxin, the simplest member of the iron-sulfur class of metalloproteins [1]. Rubredoxin (Rd) contains a high-spin iron atom with tetrahedral coordination by four cysteinyl sulfur atoms. A structural variant of this center is found in Desulforedoxin (Dx), also a small protein (homo-dimer with 36 aminoacids per subunit). Each monomer of Dx has one iron atom bound to four cysteinyl residues [2-4]. The metal binding motif in Rd consists of two pairs of cysteinyl sulfur ligands with two aminoacids (aa) separating the cysteines of each pair (Scheme I). The aa sequence of the polypeptide chain of Dx has an unusual sequence of cysteine residues: the two final cysteines are in consecutive positions [5,6]. These adjacent cysteines (Cys 28 and 29) impose distortions on the ideal tetrahedral coordination. Dx and Rd centers appear also as distinct domains in association with other types of centers. Desulfoferrodoxin (Dfx), a single polypeptide chain (14 kDa) provides the binding motif for the Dx-type center (center I) and for another mononuclear octahedral iron center with N/O ligands and also a cysteine ligand (center II) [2,7]. The N-terminal fragment, containing centre I, was recently cloned and expressed in *E. coli* (our unpublished results). Another case is Rubrerythrin (Rh), a dimer of 21 kDa, containing (per subunit) one Rd centre and a binuclear iron cluster, similar to the one found in Hemerythrin [2]
Comparison of Rd, Dx and N-terminal Dfx aa sequences are indicated in Scheme I.

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SCHEME I

Rd (gigas): – Tyr(4) Val(5) Cys (6) ——— Cys (9) —— Cys (39) —— Cys (42) – Ala(44) —
Dx: – Tyr(7) Lys(8) Cys (9) ——— Cys (12) —— Cys (28) ····· Cys (29) – Glu(31) —
Dfx N-term: – Tyr(7) Lys(8) Cys (9) – His (11) Cys (12) —— Cys (28) ····· Cys (29) – Glu(31) —

a) Sequences are aligned in terms of relevant conserved aa residues
b) Numbers refer to individual aa sequences

The 3D structure of Rd and Dx are known at high resolution [1,6], however, their biological role is still unknown. The difficulty with the definition of a function for these simple iron-sulfur proteins is due to their redox potentials value, too high to be implicate in the metabolism of anaerobes. Using voltammetric technics, we pretend to collect more information about the redox properties of these centers.

MATERIALS AND METHODS

Proteins

All proteins used in this work were recombinant proteins, proved to have physical and spectroscopic properties identical to those observed in the respective native proteins. The genes encoding *D.gigas* Dx and *D.vulgaris* Dfx were cloned from genomic DNA using the polymerase chain reaction, expressed in *E.coli* and purified to homogeneity [8, and our unpublished results]. Dfx center I was also studied using a second protein form: a N-terminal fragment of Dfx (Dfx N-term) containing only the Dx-type center. This Dfx N-term was obtained by overexpression in *E.coli* of Dfx encoding gene until the Met(36) residue and then purified.

Electrochemical measurements

A two-compartment cell was used, contained a glassy carbon electrode as working electrode, a Ag/AgCl (3 M KCl) reference electrode and a platinum wire auxiliary electrode. The working electrode was polished for 5 min in an alumina (0,075 μm) slurry, sonicated to remove all aluminum oxide particles, washed with water and dried after each measurement. Oxygen was purged from the solutions by bubling Argon gas for 15 min directly into the cell before recording the experimental data. Voltammograms were recorded using a computer-interfaced Autolab PSTAT10 potenciostat/galvanostat running with a GPES version 3.2 software (EcoChemie). Typical values for the SWV parameters used in this work are: square-wave amplitude 50 mV; step potential 3 mV; frequency 8 Hz. All potentials are given against normal hydrogen electrode (NHE). Experiments were carried out at room temperature.
Protein solutions with a final concentration of 600 μM were prepared in 50 mM buffer containing 0.1 KNO_3, 2 mM neomycine sulphate and 1 mM $MgCl_2$. Neomycine sulphate was used to promote the direct electrochemistry between the protein and the electrode and Mg^{2+} was added in solution, probably to decrease repulsion between the very negative proteins and the electrode [9]. The pH dependence was measured using citric acid-K_2HPO_4, tris-HCl, HEPES and CAPS as buffers, all at a final concentration of 50 mM. The pH values were adjusted before and confirmed after each measurement.

RESULTS

The electrochemical responses of the two proteins results in well defined voltammograms, as shown in Figure 1. A typical CV response for Dx and a SWV response of Dfx are illustrated.

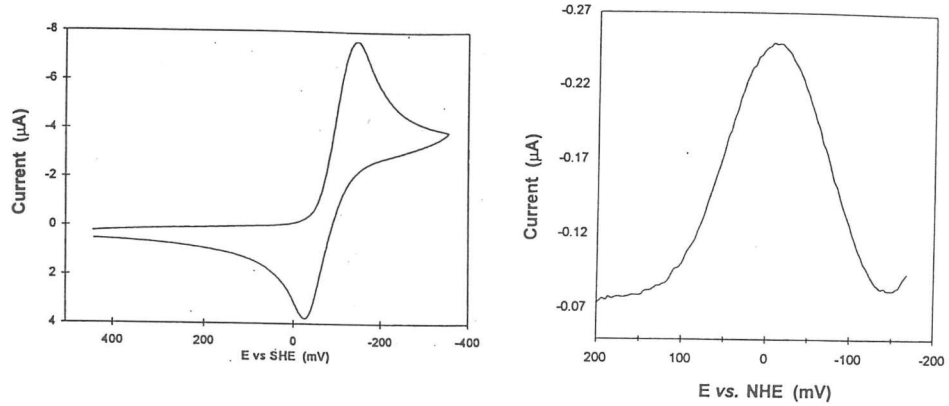

Figure 1 - Typical electrochemical responses of the studied proteins, obtained in the conditions described in material in methods section. (a) - cyclic voltammogram (CV) of Dx in Tris-HCl pH 7.6. The scan rate was 10 mV/s. (b) square wave voltammogram(SWV) of Dfx in citric acid pH 6.5.

The pH dependence of the redox potential was investigated for Dx, Dfx and also for Dfx N-term form, in a pH range from 4.5 to 10.5. Table 1 compiles the pK values associated with the pH dependent redox transitions.

Table 1 - pH dependence of the midpoint potential parameters obtained by fit of the equation 1 to the data points

	$pK_{ox\,2}$	$pK_{red\,1}$	E_m (pH 7.0) / mV	$E_{low\,pH}$ / mV	$E_{high\,pH}$ /mV
Dfx	9.3	5.4	- 4	381	- 144
Dfx N-term	8.6	4.3	+ 45	295	- 170
Dx	9.0	4.5	+ 17	366	- 162

The pK values determined are very similar to the ones previously reported (9.2 and 5.3) [10].
pKa values extracted after fitting the experimental data with equation

$$E_m = E_{low\,pH} - 0.06\ \log\frac{K_{ox\,1} + [H^+]}{K_{red\,1} + [H^+]} + E_{high\,pH} - 0.06\ \log\frac{K_{ox\,2} + [H^+]}{K_{red2} + [H^+]}$$

CONCLUSIONS

Unlike *D.gigas* rubredoxin, the redox potentials of Dfx, Dx and Dfx N-term are pH dependent. The presence of an ionizable residue (histidine) between two cysteinyl ligands detected in the

primary sequence of Dfx and Dfx N-term was suspected to be the cause of the pH dependent redox potential. Since Dx does not contain such an homologous residue, but also show a pH dependent redox potential, led us to the search for another conserved ionizable residue.Tyrosine in position 7 could be a plausible candidate, but is also conserved in the Rd sequence. Lys8 and Glu31 are conserved residues in the vicinity of the metal center and are most probably the ionizable sites that influence the pH dependence observed. In D.gigas Rd the equivalent position to Glu31 is replaced by an Ala (residue 44). In Rubredoxins the aa sequence position equivalent to Lys 8 is occupied with Val or Ala residues.

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COMPARATIVE STUDY OF THE REDOX PROPERTIES OF PLATINUM, PALLADIUM, COPPER AND IRON CAMPHOR DERIVED COMPLEXES

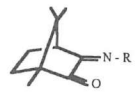
M. Fernanda N.N. Carvalho [a], Armando J.L. Pombeiro [a] and Rudolf Herrmann[b]

[a] Centro de Química Estrutural, Complexo I, Instituto Superior Técnico, Av. Rovisco Pais, 1096 Lisboa codex, Portugal

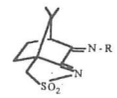
[b] Institut für Organisch-Chemie und Biochemie, Technische Universität München, Lichtenbergstrabe 4, D-85474 Garching, Germany

We have previously prepared several camphorimine complexes using metal sites such as $\{PdCl_2\}$ [1], $\{FeCl_2\}^+$, [2] or $\{CuCl_2\}$ [2] and studied their redox properties by cyclic voltammetry.

We now report the electrochemical behaviour of some new platinum complexes, Table 1, and compare their electronic properties with those of Pd(II), Fe(III) or Cu(II) derived from camphorimine species of type (A) or (B).



(A)



(B)

Table 1 -Cyclic voltammetric data[a] for $[PtCl_2L_2]$ (L=A, R=Ph or NMe_2) and $[PtCl_2L]$ (L=B, R=NMe_2) complexes, and for the free camphorimine ligands (L).

COMPLEX (ligand type) / R group	$E^{ox}_{1/2}$	$E^{red}_{1/2}$	L type / R	E^{ox}_p	E^{red}_p
(A) / Ph	1.45	-1.00[b]	(A) / Ph [3]	1.86	-1.54
(A) / NMe_2	1.34[c]	-1.10	(A) / NMe_2 [2]	1.48	—
(B) / NMe_2	1.36[d]	-1.40	(B) / NMe_2 [2]	1.54	-1.42

[a] Values in V ± 20 mV *vs.* SCE measured (at 200 mV s^{-1}) at a Pt wire electrode, in 0.2 M $[NBu_4][BF_4]$ / CH_2Cl_2, using $[Fe(\eta^5\text{-}C_5H_5)_2]^{0/+}$ ($E^{ox}_{1/2}$=0.54 V) as internal reference. [b] Another wave is detected at $E^{red}_{1/2}$ =-1.25 V. [c] Irreversible wave (E^{ox}_p); another wave is detected as a shoulder at E^{ox}_p =1.20 V. [d] Another wave is detected at $E^{ox}_{1/2}$=1.68 V.

The results were obtained by cyclic voltammetry and controlled potential electrolysis commonly in 0.2 M $[NBu_4][BF_4]$/ CH_2Cl_2 solutions, but in some cases in THF, using Pt wire or Pt gauze working electrodes, respectively. All the potential