ELECTROCHEMICAL STUDIES ON RUBREDOXIN TYPE PROTEINS

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ABSTRACT: Rubredoxin (Rd), desulforedoxin (Dx), rubrerythrin (Rr) and desulfoferrodoxin (Dfx) are bacterial proteins containing the simplest iron-sulfur cluster found in biology: a mononuclear iron center coordinated by four cysteinyl sulfur atoms ($FeCys_4$ or Rd type centers). Although believed to be involved in electron transfer, their function in anaerobic bacteria is still unknown. The reversibility of redox reactions of these metalloproteins systems was studied by cyclic voltammetry, differential pulse voltammetry and square-wave voltammetry. Rd and Dx have been found to exhibit quasireversibility with $E^{o'}$s in the range -50 to +20 mV versus S.H.E..

Key words:
Iron-sulfur cluster, $FeCys_4$, rubredoxin, desulforedoxin, redox potential

Abbrebiations used:
Rd, rubredoxin; Dx, desulforedoxin; Rr, rubrerythrin, Dfx, desulfoferrodoxin; *D.*, *Desulfovibrio*

INTRODUCTION

The iron-sulfur proteins are distinguished from other non-heme iron proteins by having iron with at least partial sulfur coordination. This group includes proteins containing either mononuclear iron centers surrounded by four cysteinyl thiolate groups ($FeCys_4$ or Rd type centers) or clusters of iron bridged by inorganic sulfur and bound to the protein via amino acid side chains (in general SH groups from cysteinyl residues). The iron in these systems is in an approximately tetrahedral environment of sulfur atoms.

In this work we were concern with the class of iron-sulfur proteins that have mononuclear iron centers. Even being the simplest iron-sulfur cluster, $FeCys_4$ was found in two distinct structural arrangements (figure 1), due to the pattern of the iron ligating cysteine residues on the polypeptide chain.

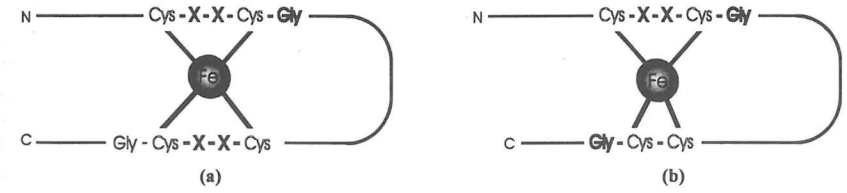

Figure 1 - Structural variability of mononuclear iron-sulfur clusters in biology:
(a) $FeCys_4$ center in Rd; (b) $FeCys_4$ center in Dx.

In rubredoxin, a monomeric iron-sulfur protein with a small size (circa 6 kDa), the cysteine pattern for the ligation to the iron, giving a tetrahedral geometry around the metal, is

- Cys - X - X - Cys - ... - Cys - X - X - Cys - .

In desulforedoxin, a small homodimeric protein (7.9 kDa) purified from the sulfate reducing bacterium *Desulfovibrio gigas*, this pattern is altered and two of the coordinating cysteines are in a consecutive position on the polypeptide chain imposing a distorted tetrahedral geometry around the metal

- Cys - X - X - Cys - ... - Cys - Cys - .

Rubrerythrin and desulfoferrodoxin are also iron-sulfur proteins containing $FeCys_4$ sites. In rubrerythrin a Rd like center was found together with a hemerythrin-type dinuclear iron center as prostetics groups. In desulfoferrodoxin was detected a Dx like center and a high-spin iron octahedrally coordinated with predominantly nitrogen and/or oxygen-containing ligands.

A variety of electrochemical studies were conducted in order to access the effect of the structural control of the polypeptide moiety on the redox properties of the metal site. Rd and Dx were analysed in different situations: native proteins, overexpressed proteins and chemically synthesised protein (in the case of Dx).

MATERIALS AND METHODS

Rubredoxins

Native proteins, *D. desulfuricans* (ATCC 27774) and *D. vulgaris* rubredoxins, were isolated as previously described [1,2].The gene encoding *D. vulgaris* Rd was cloned and the resulting protein, expressed in *E. coli*, was then purified (in col. with J. E. Wampler).

Desulforedoxins

Native protein was isolated from cell extracts of *D. gigas* as previously described [3]. The dsr encoding Dx was cloned and expressed in *E. coli* ([4] and in col. with F. Rusnak). In addition to the dimer form containing iron (Fe/Fe form), the overexpression in *E. coli* gives a second form containing equimolar amounts of zinc and iron (Fe/Zn form). Both forms were analyzed. A fourth structural variation of Dx was considerated for the electrochemical measurements: the 36 amino acid chain was synthesised and the iron reconstituted as previously described [5].

Electrochemistry

The working electrode (Glassy Carbon Electrode) was polished in an alumina slurry and washed thoroughly with water. No additional pretreatments was necessary. The other two electrodes, reference and counter electrodes (Saturated Calomel Electrode and platin wire, respectively) were only washed with destilled water before each experiment. Electrochemical data were recorded with a computer-interfaced AUTOLAB PSTAT 10 (ECO-Chemie) using a GEPES software (version 3.2, ECO-Chemie). Room temperature redox potentials are reported versus the standard hydrogen electroede and were determined at the potential at which the peak current occurred. Solutions of the various proteins (typically 2.5 mg/ml) were prepered in 10 mM Tris-HCl, pH 7.0, containing 0.1 M KNO_3, 1 mM $MgCl_2$ and 2 mM neomycine sulphate. Repeated cyclic voltammograms were taken of the various iron-sulfur proteins preparations at different scan rates. DPV voltammograms were obtained using a potential pulse of 25 mV and SWV voltammograms using a amplitude of 25 mV and a frequency of 10 Hz.

RESULTS AND DISCUSSION

Figure 2 shows CV and SWV electrochemical responses obtained for native Dx, representative of the metalloproteins under study.

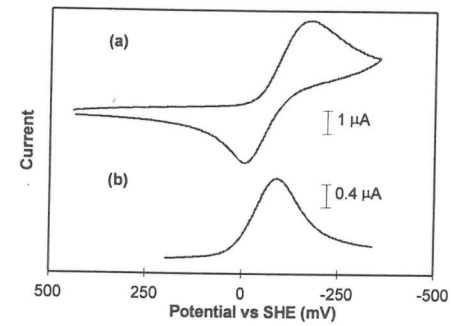

Figure 2 - Ciclic (a) and differential pulse (b) voltammograms of 2.5 mg/ml native Dx in 10 mM Tris-HCl, pH 7.0, containing 0.1 M KNO_3, 1 mM $MgCl_2$ and 2 mM neomycine sulphate. The scan rate of CV was 10 mV/sand the potential pulse of DPV was 25 mV; room temperature; working/reference/counter electrodes, Glassy Carbon/SCE/Pt electrodes.

In all cases examined, we observed a quasireversible response up to 25 mV/s.

Table 1 summarizes the results obtained by CV and DPV or SWV. In the same table the literature redox potentials obtained by an independent method (redox titrations followed by EPR measurements) are also indicated for comparison.

Table 1 - Half-wave potentials obtained for the various proteins using different electrochemical methods and values of oxidation-reduction potentials estimated by EPR redox titrations.

	CV	DPV	SWV	EPR (a)
Protein	**$E_{1/2}$ (mV)**	**$E_{1/2}$ (mV)**	**$E_{1/2}$ (mV)**	**E^{m} (mV)**
D. desulfuricans Rd (native)	- 8	+ 19	—	(b)
D. vulgaris Rd (native)	- 11	+ 9	—	(b)
D. vulgaris Rd (cloned in *E. coli*)	0	+ 18	—	(b)
D. gigas Dx (native)	- 10	- 75	—	- 35
D. gigas Dx (Fe/Fe form) (cloned in *E. coli*)	- 57	—	- 54	—
D. gigas Dx (Fe/Fe form) (cloned in *E. coli*)	- 17	- 13	- 13	—
Chemically Synthesised Dx	- 41	—	—	+ 5

(a) - The standard derivation of EPR redox titrations measurements is 15 mV
(b) - Usually, the redox potential range for rubredoxins is from 0 mV to -50 mV

The high resolved structural data now available for rubredoxin and desulforedoxin allows an accurate description of the iron center with a good definition of the S-Fe distances and coordination angles. These parameters are required for further analysis of the relationship between structure, function and reactivity, and are important for the control of electron transfer mechanisms and the control of the redox potential of the center. They are also essential for the establishment of correlations between these properties and the spectral characterization of both Dx and Rd.

The electrochemical determination of the redox potentials of rubredoxin type proteins enables a rapid method to access the value of the redox potential of the metal center. Also, a variety of proteins from different origins (native, overexpressed and chemically synthesized) were study. The work will be now extended to the association of these centers with other type of redox centers, as the indicated cases of Rr and Dfx. The chemical modification of these centers, obtained by replacing the native iron site in Rd and Dx by other metals (i.e. cobalt and nickel) will be also considered.

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ELECTROCHEMICAL STUDIES OF *Desulfovibrio desulfuricans* ATCC 27774 ALDEHYDE OXIDO-REDUCTASE (AOR)

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ABSTRACT - *Electrochemical studies on* Desulfovibrio desulfuricans *ATCC 27774 Aldehyde Oxido-Reductase (AOR) were carried out in order to optimize the conditions for the development of an enzymatic sensor for aldehydes. In presence of both aliphatic and aromatic aldehydes, AOR develops a catalytic activity with a characteristic Michaelis-Menten type behavior.*

KEY WORDS - Electrochemistry, Biosensors, Aldehydes, Aldehyde Oxido-Reductase.

INTRODUCTION

The sensitivity and the specificity of biological activities make enzymes a target for the development of enzyme electrodes. Biosensors (a special group of bioelectrodes) combine a sensitive layer based on biological components capable of detecting or responding to surrounding chemicals. The biological component translates the specific molecular recognition of the analyte into a signal that can be readily chemically or physically measured. In addition, enzyme immobilization is frequently used in the development of biosensors.

Aliphatic aldehydes are found in wastes waters which have a damage effect for the environment. On the other hand, aromatic aldehydes are present in several compounds of biological importance.

From nitrate-grown cells of *Desulfovibrio desulfuricans* ATCC 27774 (a sulfate reducing organism), a molybdenum-[iron-sulfur] containing aldehyde oxido-reductase (converting aldehydes into carboxylic acids) was isolated [1,2].

This enzyme has a significant potential for the use in the construction of biosensors, as it carries out a specific and well defined chemical transformation, is available in large amounts, can be purified by well established procedures and is quite stable in solution.