REDOX STUDIES ON RUBREDOXINS FROM SULPHATE AND SULPHUR REDUCING BACTERIA

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1. Introduction

Rubredoxins are present in aerobe as well as in anaerobe organisms and are characterized by the absence of labile sulphur and the presence of 1 iron atom linked in a tetrahedral arrangement to the sulphur cysteine residues.

Rubredoxins from sulphate reducing bacteria (*Desulfovibrio sp.*) and sulphur reducing bacteria (*Desulfuromonas acetoxidans*) have been isolated [1–5]. Here we report the oxidation–reduction properties of 3 rubredoxins isolated from *Desulfovibrio gigas* (NCIB 9332), *Desulfovibrio salexigens* (strain British Guiana 8403) [6] and *Desulfuromonas acetoxidans* (strain 5071).

Potentiometric titrations in the presence of oxidation–reduction mediators, followed by EPR measurements were used to determine the mid-point reduction potentials of the active centres.

2. Methods

Rubredoxins from *D. gigas, D. salexigens* and *Drm. acetoxidans* were purified as in [1,5,6]. Anaerobic oxidation–reduction titrations of the iron centre of rubredoxins were carried out as detailed in [7] using an apparatus similar to that designed in [8].

The rubredoxins in 100 mM Tris–HCl (pH 8.4) were poised at different potentials in the presence of oxidation–reduction mediators, all at 70 μM, as listed in [9]. The potential was adjusted by addition of small amounts of dithionite (0.2 M in Tris–HCl (pH 9.0)) or ferricyanide (0.2 M) solution. The protein concentration, as estimated from the extinction coefficients at visible wavelengths [4,6,10] was 50 μM.

After equilibration at a fixed potential, a sample was transferred into an EPR tube under argon pressure and immediately frozen at 77 K for posterior EPR quantification.

EPR spectra were recorded in a Bruker ER-200 tt spectrometer at 77 K.

3. Results and discussion

The mid-point oxidation–reduction potentials were estimated from measurements of the sharp and intense EPR signal at $g = 4.3$ observed at 77 K, which is typical of the oxidized form of rubredoxins [11]. The spectra observed in the oxidized form of the rubredoxins under study were similar to those observed for other rubredoxins [9,12].

The intensity of the signal was plotted as a function of the oxidation–reduction potential as shown in fig.1 and the experimental data were fitted with calculated Nernst curves for $1e^-$ reduction. The mid-point potentials determined are: –46 mV for *Drm. acetoxidans* Rb, –31 mV for *D. salexigens* Rb and +6 mV for *D. gigas* Rb. The estimated error for these values is ± 10 mV.

A mid-point potential of –57 mV for *Clostridium pasteurianum* Rb was reported by optical measurements at pH 7.0 [13].

Abbreviations: EPR, electron paramagnetic resonance; MCD, magnetic circular dichroism; Rb, rubredoxin

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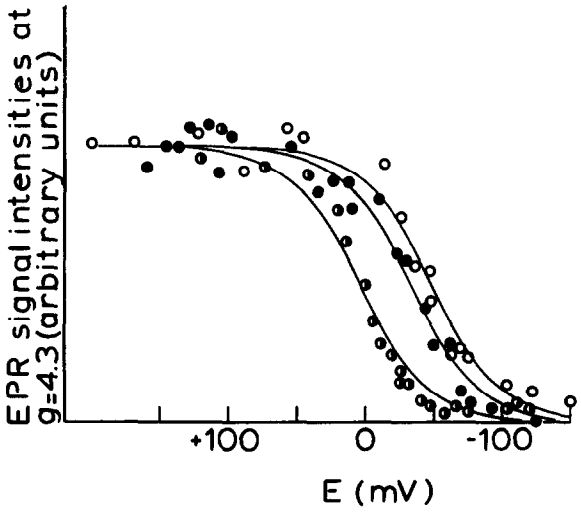

Fig.1. Redox titration curves. EPR signal intensities at $g = 4.3$ are plotted versus redox potencial from *D. gigas* Rb (⊙), *D. salexigens* Rb (●) and *D. acetoxidans* Rb (○) in 0.1 M Tris–HCl (pH 8.4). Signal intensities were adjusted to give the same maximum signal height. The theoretical curves shown in figure were calculated from the Nernst equation.

The comparison of the amino acid compositions of Rb from sulphate and sulphur reducing organisms as well as from *C. pasteurianum* Rb is presented in table 1. The overall distribution of the amino acids is rather similar but differences can be observed in particular when the charged residues are considered. The primary structures of *D. gigas* Rb, *D. vulgaris* Rb and *C. pasteurianum* Rb are known [14–16], showing that 31 residues are unchanged when the three amino acid sequences are compared, with remarkably conservative positions for cysteine, proline and aromatic residues. Also the polypeptide chains in the close proximity of the iron binding site are homologous.

The X-ray structures known so far (*D. vulgaris* Rb [17] and *C. pasteurianum* Rb [18]) show that the structural parameters of the active centre are not significantly altered. This fact is supported by other spectroscopic techniques: ultraviolet–visible spectroscopy – *C. pasteurianum* Rb [13], *D. gigas* [9], *D. Norway* Rb [4], *D. vulgaris* Rb and *D. salexigens* Rb [19], *Drm. acetoxidans* Rb [6], EPR – *C. pasteurianum* Rb [11], *D. gigas* Rb [9], *D. vulgaris* Rb,

Table 1
Comparison of the amino acid composition of rubredoxins

	C. pasteurianum [16]	*D. gigas* [15]	*D. salexigens* [5]	*D. desulfuricans* (*Berre S*) [3]	*Drm. acetoxidans* [6]	*D. vulgaris* [15]	*D. Norway* [4]
Lys	4	6	3	4	2	4	5
His	0	0	0	0	0	0	0
Arg	0	0	0	0	0	0	0
Trp	2	1	nd	1	nd	1	nd
Asp	11	8	8	7	8	7	13
Thr	3	2	2	2	2	3	4
Ser	0	2	1	2	2	2	0
Glu	6	4	7	8	4	3	5
Pro	5	5	5	6–7	5	6	5
Gly	6	5	6	6	6	6	7
Ala	0	4	3	6	4	4	5
Cys	4	4	4	4	4	4	4
Val	5	3	2	5	4	5	6
Met	1	1	1	1	2–3	1	1
Ile	2	2	0	2	2	0	0
Leu	1	1	2–3	0	1	1	1
Tyr	3	3	2	3	3	3	4
Phe	2	2	2	3	2	2	2
Total	55	52	48–49	60–61	51–52	52	62

D. salexigens Rb and *Drm. acetoxidans* Rb [19]; MCD – *C. pasteurianum* Rb [20] and *D. gigas* Rb [21].

The differences observed in the values of the oxidation–reduction potentials for the studied rubredoxins may result from modulation of structural features on the active centre. For a small molecule like rubredoxin the dissimilarities in the number of charged groups indicated above, may be determinant in the constrains imposed by the polypeptide chain on the iron centre, as well as in the specificity of protein–protein interactions. The importance of the differences in charged residues was used [22] to explain the high specificity of the NADH-H* rubredoxin oxido-reductase from *D. gigas* for the rubredoxin of the same organism, when compared with rubredoxins from other species (*D. vulgaris* and *C. pasteurianum*).

The possibility for the same oxidation reduction centre to vary its redox potential in a wide range is as well known for other proteins, e.g., [4 Fe, 4 S] ferredoxins [23,24] and heme proteins [25].

It is interesting to point out here than our recent studies (in preparation) on the interaction between cytochrome c_3 and other proteins show that the midpoint oxidation–reduction potential of *D. gigas* Rb is altered in the presence of *D. gigas* cytochrome c_3, becoming ~35 mV more positive.

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