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Evidence for a Rieske-type FeS center in the thermoacidophilic archaebacterium *Sulfolobus acidocaldarius*

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A high-potential iron–sulfur cluster with characteristics similar to a Rieske-type center was detected in the plasma membrane of *Sulfolobus acidocaldarius* by EPR spectroscopy. In the reduced form this center has g-values of $g_z = 2.031$, $g_y = 1.890$ and $g_x = 1.725$ ($g_{av} = 1.88$, rhombicity = 0.37) and its reduction potential at pH 7.4 was determined to be +325 ± 10 mV. The archaebacterial cluster exhibits some unique properties, in comparison to eubacterial and eukaryotic Rieske-type centers. First, the reduction potential is pH-dependent in the range from pH 6.7 to 8.2. Second, the typical inhibitor of Rieske FeS centers, DBMIB, had no effect on the g-values of this cluster. The center is reducible by both NADH and succinate in the presence of cyanide, an inhibitor of the terminal oxidases. The possible role of a Rieske-type center in an organism lacking any c-type cytochromes is discussed.

Archaebacteria; Extremophile; Reduction potential; EPR; Rieske-FeS; Iron–sulfur center

1. INTRODUCTION

Rieske FeS centers are unique in the group of the 2Fe–2S clusters. First, their average g-factor ($g_{av} = 1.90$–1.91) is significantly lower than those for the ferredoxin type clusters ($g_{av} = 1.94$–1.98) [1]. Second, their redox midpoint potential of either approximately +300 or +150 mV, depending on the physiological quinone type serving as an electron donor [2], is much higher than that of the ferredoxins (approx. −400 mV) [1]. The Rieske protein has been shown to be a constitutive part of the cytochrome bc_1 or b_6f complexes in various sources from the eubacterial and eukaryotic urkingdoms [1]. In the thermoacidophilicarchaebacterium *Sulfolobus acidocaldarius* many of the membrane bound components involved in the bioenergetic system have been purified and characterized [3–8]. Since this bacterium does not contain any soluble or membrane bound c-type cytochrome [9], the presence of a cytochrome bc_1 complex is obviously excluded. The following study, however, gives evidence for the presence of a high-potential iron–sulfur cluster with characteristics similar to a Rieske-type FeS center in the membrane of *S. acidocaldarius*. To our knowledge, this is the first example for the presence of a Rieske type FeS cluster in an archaebacterium.

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Abbreviation: DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone.

2. MATERIALS AND METHODS

The procedures for growth of *Sulfolobus* (*S.*) *acidocaldarius* (DSM 639) cells and membrane preparation have been described previously [8]. Potentiometric redox titrations were carried out at room temperature in 0.8 M KH_2PO_4, 10% (v/v) ethyleneglycol at pH 6.7–8.2 under anaerobic conditions according to Dutton [10] and as described in reference [11]. Membrane protein concentrations were 15–45 mg/ml.

The effect of DBMIB was tested by adding a solution of the reagent (final concentration: 5 mM) and sodium ascorbate (final concentration: 5 mM) to *S. acidocaldarius* membranes and incubating for 5 min at room temperature in the dark. Immediately afterwards the sample was poured into an EPR tube and frozen in liquid nitrogen. Membrane protein concentration was 23.4 mg/ml in 0.8 M KH_2PO_4, pH 7.2.

The concentrations of membrane protein were determined by a modified Biuret reaction [12].

EPR spectra were recorded with an X-band Bruker ESP 380 spectrometer equipped with an CF935 continuous-flow helium cryostat from Oxford Instruments.

3. RESULTS

The EPR-spectrum at 10K and 2.4 mW microwave power of *S. acidocaldarius* membranes reduced by sodium ascorbate is shown in Fig. 1. The resonances clearly indicate the presence of a high-spin ferric heme component with a g-value of about 6, which is in part due to the heme a_3 from cytochrome aa_3 [10]. The signal at $g = 4.3$ indicates the presence of low symmetry non-heme iron. In the $g = 2$ region, an intense resonance with a g-value of 2.08, presumably due to copper and further signals at $g \sim 2$, presumably due to the S3-center of the succinate-dehydrogenase [4] and to radicals are also observed. The resonances at $g = 1.89$ and

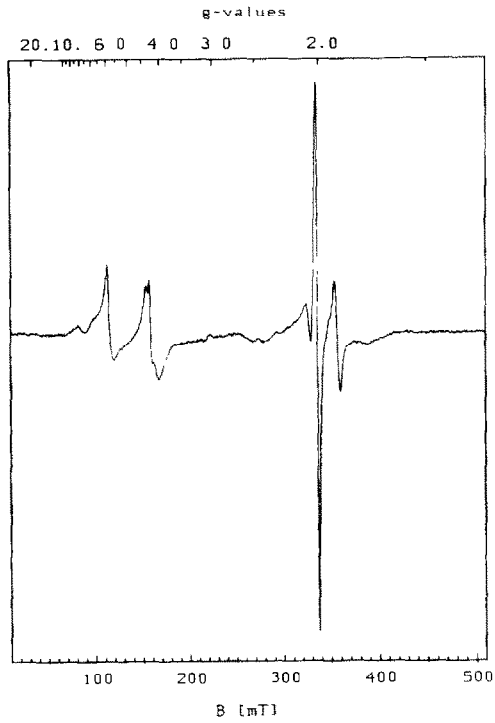

Fig. 1. EPR spectrum of *S. acidocaldarius* membranes reduced by sodium ascorbate (10 mM). Protein concentration was 58 mg/ml in 0.2 M KH_2PO_4, pH 7.0. Microwave frequency: 9.444 GHz, microwave power: 2.4 mW, modulation amplitude: 1 mT, temperature: 10K.

$g \sim 1.73$ have g-values very close to those of Rieske-type iron–sulfur clusters from various sources [1]. This signal was still detectable at 30K, but at higher temperatures linebroadening is observed, due to the increase in the electronic relaxation rate.

In order to measure the third g-value of the Rieske type iron–sulfur cluster, which was superimposed in the native membrane spectrum by the radical and S3-signals, it was necessary to measure the EPR spectrum at high temperature (30K) and very low microwave power (24 μW). The resulting spectrum clearly displays all three g-values of the cluster (Fig. 2, upper trace). The resonances could be simulated (Fig. 2, lower trace) with the following g-values and linewidths (in brackets): $g_z = 2.031$ (2.5 mT), $g_y = 1.890$ (5.5 mT) and $g_x = 1.725$ (23.5 mT). Power saturation studies at 8K and 20K (half-saturation power of 2 mW and 45 mW, respectively) indicate the presence of only one type of center in the membranes (data not shown).

The reduction potential of the iron–sulfur cluster and its pH dependence were determined by a series of EPR redox titrations of the membrane suspensions, performed in the region between pH 6.7 and 8.2 (Figs. 3 and 4). All titration curves could be simulated with simple $n = 1$ Nernst equations (Fig. 3). The reduction potential is pH dependent in the whole pH range studied (Fig. 4). A similar titration was performed at pH 7.5 with a partially purified cytochrome *bo* fraction from *S. acidocaldarius* [7], which also contains the iron–sulfur protein. This titration yielded a reduction potential of +340 mV for the detergent solubilized center, indicating that this center in the membrane-bound form or in solution has essentially the same redox behaviour.

Membranes of *S. acidocaldarius* were incubated with both NADH and succinate, in the absence and in the presence of potassium cyanide. The EPR spectra of these samples (Fig. 5) show clearly that only in the presence of an inhibitor of the terminal oxidases the Rieske-type center is significantly reduced by both substrates. No reduction of the Rieske-type protein was observed upon addition of KCN alone.

S. acidocaldarius membranes were also incubated with the typical inhibitor of Rieske type FeS clusters, DBMIB [13]. After incubation with the inhibitor and ascorbate, the EPR-spectrum of the cluster remained unaltered with respect to the control with ascorbate addition only (data not shown).

4. DISCUSSION

The EPR spectra of the high potential iron–sulfur cluster from the membranes of the thermoacidophilic

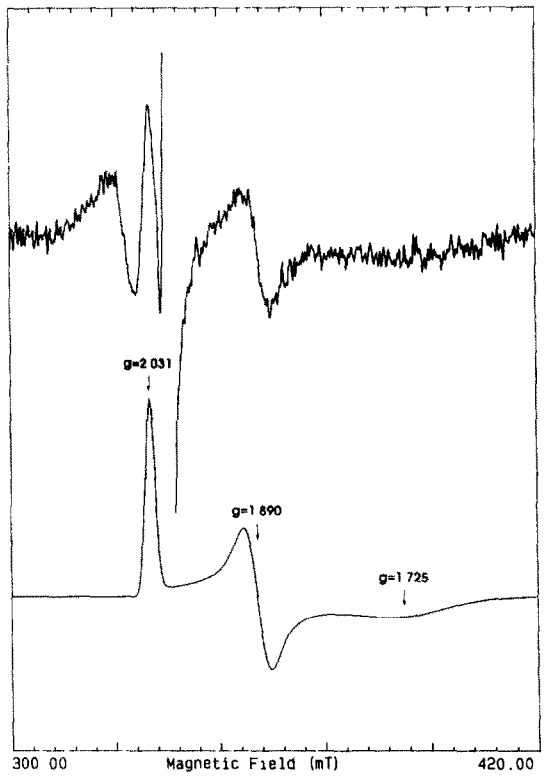

Fig. 2. Upper trace: EPR spectrum of *S. acidocaldarius* ascorbate (10 mM) reduced membranes. Protein concentration was 58 mg/ml in 0.2 M KH_2PO_4, pH 7.0. The radical signal at $g \sim 2.0$ was removed from the spectrum. Temperature 28K, microwave power 24 μW. Other conditions as in Fig. 1. Lower trace: Simulation of the spectrum using the following g-values and linewidths: $g_z = 2.031$, $g_y = 1.890$ and $g_x = 1.725$; 2.5 mT, 5.5 mT and 23.5 mT.

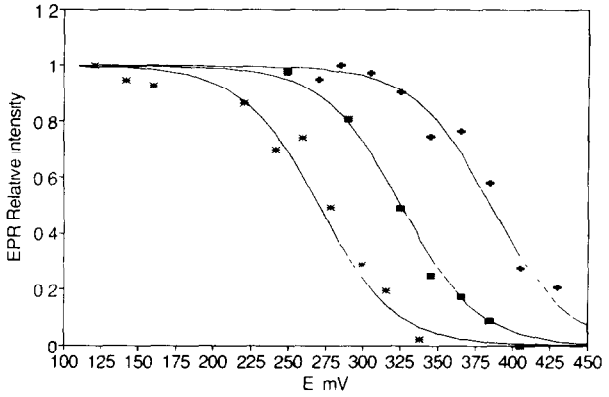

Fig. 3. EPR-redox titrations of *S. acidocaldarius* membranes at pH 6.7 (+), 7.4 (■) and 8.2 (★). EPR conditions as in Fig. 1; for further details, see section 2. The solid lines are Nernst equations for $n = 1$ processes, with E_o = 385 (+), 325 (■) and 270 mV (★).

archaebacterium *S. acidocaldarius* show characteristics similar to a Rieske-type iron–sulfur center. The *g*-values of the center are very similar to those of the known centers from the eubacterial and eukaryotic urkingdoms [1]. Furthermore, its reduction potential of about +345 mV at pH 7 is in the range of the values for the class of ubiquinone/plastoquinone oxidizing Rieske FeS centers [2].

The archaebacterial Rieske type FeS cluster, however, displays also significant differences with respect to all classes of Rieske centers. First, the EPR signals of the archaebacterial cluster are not sensitive to the classical Rieske type FeS inhibitor DBMIB, an analogue to ubiquinone/plastoquinone. This observation may be related to the fact that the respiratory system of *S. acidocaldarius* uses the structurally different benzothiophen quinone, caldariellaquinone [6]. Second, whereas all Rieske type FeS clusters so far studied show a pH dependence only above pH 8 [2], implying a pK_a value only above that pH, the pH dependence of the reduction potential of the archaebacterial cluster is observed in the physiological pH range.

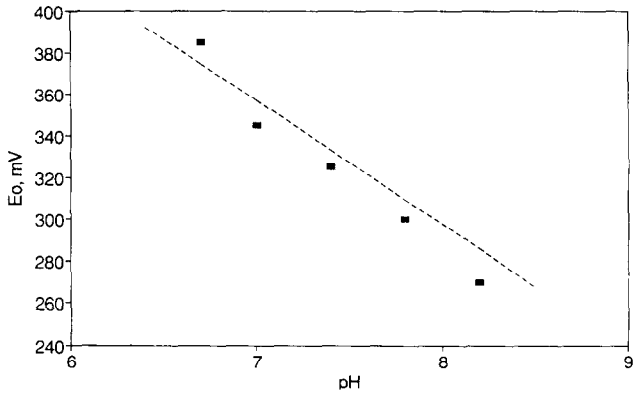

Fig. 4. pH dependence of the Rieske-type iron–sulfur center reduction potentials. A line with a slope of −59 mV/pH unit is displayed.

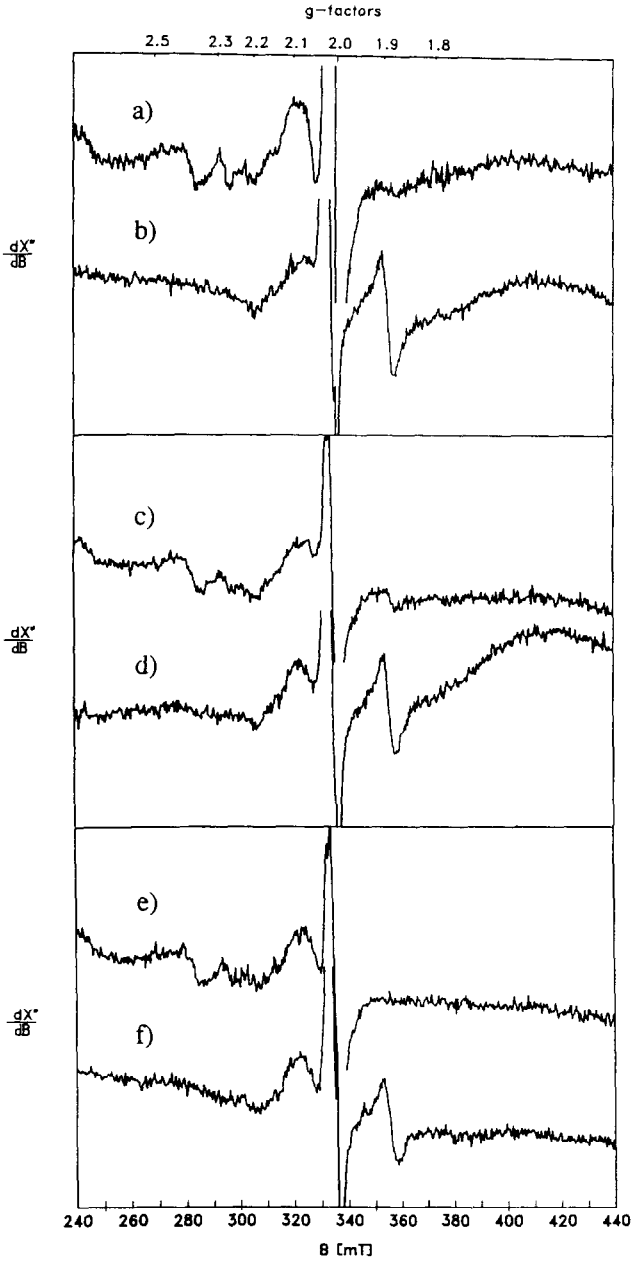

Fig. 5. Reduction of the Rieske-type iron–sulfur protein in isolated membranes of *S. acidocaldarius*. Membranes (32.5 mg/ml) were incubated for 12 min at 30°C in 25 mM Tris-HCl buffer, pH 7.5, with the following additions: (a) none; (b) 5 mM ascorbate; (c) 2 mM NADH; (d) 2 mM NADH and 5 mM KCN; (e) 2 mM succinate; (f) 20 mM succinate and 5 mM KCN. Following the incubation, the samples were transferred to EPR tubes and frozen in liquid nitrogen. EPR conditions as in Fig. 1, except for the temperature (15K) and microwave frequency (9.43 GHz).

The Rieske-type center from *S. acidocaldarius* can be reduced in intact membranes using the physiological electron donors NADH and succinate. Since the addition of cyanide is necessary to achieve this reduction under aerobic conditions it can be concluded that in spite of the very high reduction potential of the iron–

sulfur center, the electrons are preferentially transferred to oxygen via cytochrome aa_3. As succinate and NADH are almost equally capable of reducing the Rieske-type protein, it can be assumed that the physiological reductant within the membrane is caldariellaquinone, the putative electron acceptor of both NADH- and succinate dehydrogenases [3,4]. These results would be compatible with a simple linear electron transport system like that found in mitochondria. However, several observations clearly indicate that the electron-transfer chain in *S. acidocaldarius* is more complex. First, there are no membrane-bound cytochromes *c* nor any type of soluble cytochromes in this organism. Thus, the question of the physiological electron acceptor for this protein remains open for further investigations. Second, the purified cytochrome aa_3 from *S. acidocaldarius* has quinol oxidase activity [8], a strong indication for a direct electron flow from the quinol to oxygen via cytochrome aa_3. The SOX operon detected in *S. acidocaldarius* DNA [14] codes for more than only one single polypeptide contributing to the intact terminal oxidase of the aa_3 type and includes another cytochrome (presumably cytochrome a_{586}). However, no gene for a Rieske-type FeS protein was found. Nevertheless, the Rieske protein may be part of a terminal quinol oxidase or of a primitive ancestor of the eubacterial and eukaryotic bc_1 complexes, which would provide an alternative electron transport pathway leading to an as yet unidentified acceptor. The presence of a split electron transport pathway as found in many microorganisms is quite likely, since a *b*-type cytochrome was found in this organism, which might function as an alternative terminal oxidase [7].

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REFERENCES

[1] Trumpower, B.C. (1981) Biochim. Biophys. Acta 639, 129–155.
[2] Liebl, U., Pezennec, S., Riedel, A., Kellner, E. and Nitschke, W. (1992) J. Biol. Chem. 267, 14068–14072.
[3] Wakao, H., Wakagi, T. and Oshima, T. (1987) J. Biochem. (Tokyo) 102, 255–262.
[4] Moll, R. and Schäfer, G. (1991) Eur. J. Biochem. 201, 593–600.
[5] Lübben, M. and Schäfer, G. (1987) Eur. J. Biochem. 164, 533–540.
[6] Langworthy, T.A., Tornabene, T.G. and Holzer, G. (1982) Zentralbl. Bakteriol. Hyg. I Abt. Orig. C3, 228–244.
[7] Becker, M. and Schäfer, G. (1991) FEBS Lett. 291, 331–335.
[8] Anemüller, S. and Schäfer, G. (1990) Eur. J. Biochem. 191, 297–305.
[9] Anemüller, S., Lübben, M. and Schäfer, G. (1985) FEBS Lett. 193, 83–87.
[10] Dutton, P.L. (1978) Methods Enzymol. 54, 411–435.
[11] Anemüller, S., Bill, E., Schäfer, G., Trautwein, A.X. and Teixeira, M. (1992) Eur. J. Biochem. 210, 133–138.
[12] Watters, C. (1978) Anal. Biochem. 88, 695–698.
[13] Trebst, A., Harth, E. and Draber, W. (1970) Z. Naturforsch. 25b, 1157–1159.
[14] Lübben, M., Kolmerer, B. and Saraste, M. (1992) EMBO J. 11, 805–812.