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Review

Archaeal complex II: 'classical' and 'non-classical' succinate:quinone reductases with unusual features

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Abstract

Reversible succinate dehydrogenase (SDH) activities have been ubiquitously detected in organisms from the three domains of life. They represent constituents either of respiratory complexes II in aerobes, or of fumarate dehydrogenase complexes in anaerobes. The present review gives a survey on archaeal succinate:quinone oxidoreductases (SQRs) analyzed so far. Though some of these could be studied in detail enzymologically and spectroscopically, the existence of others has been deduced only from published genome sequences. Interestingly, two groups of enzyme complexes can be distinguished in Archaea. One group resembles the properties of SDHs known from bacteria and mitochondria. The other represents a novel class with an unusual iron–sulfur cluster in subunit B and atypical sequence motifs in subunit C which may influence electron transport mechanisms and pathways. This novel class of SQRs is discussed in comparison to the so-called 'classical' complexes. A phylogenetic analysis is presented suggesting a co-evolution of the flavoprotein-binding subunit A and subunit B containing the three iron–sulfur clusters.

Keywords: Archaeon; Succinate:acceptor oxidoreductase; Complex II; Evolution; Succinate dehydrogenase

1. General introduction

In the central metabolism of all organisms the four-carbon compounds play an essential role. The succinate–fumarate couple deserves special interest as a key redox system in catabolic as well as anabolic functions of the citric acid cycle and related pathways. The standard reduction potential of +0.03 V reflects the small change of free energy of only 5.79 kJ/mol and results in easy reversibility of the reaction under most metabolic conditions. Thus, this couple can serve as an electron accepting device under anaerobic conditions as well as an electron source to the respiratory chain of aerobes. The flavin-catalyzed primary reaction step represents a key mechanism for introduction of aliphatic carbon double bonds by direct hydrogen abstraction, whereas the product fumarate is a key molecule for the introduction of carbon–oxygen bonds simply by water addition. Accordingly, it has even been hypothesized that both succinate and fumarate were already essential com-

Abbreviations: DCPIP, 2,6-dichlorophenol-indophenol; EPR, electron paramagnetic resonance; FRD, fumarate reductase; HdrB/D, heterodisulfide reductase subunit B/D; PMS, phenazine methosulfate; Q, quinone; QFR, quinol:fumarate reductase; SQR, succinate:quinone oxidoreductase; SDH, succinate dehydrogenase; TMPD, *N*,*N*,*N*′,*N*′-tetramethyl-*para*-phenylenediamine; TFR, thiol:fumarate reductase; TfrB, thiol:fumarate reductase subunit B

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pounds in prebiotic systems of organic molecules initiating the origin of life and metabolic circles [1]. On that basis it appears conceivable that reaction pathways involving the fumarate–succinate couple have been conserved throughout organismic evolution, and enzymes catalyzing their interconversion are found ubiquitously including the Archaea [2].

Independent of whether the reaction succinate+Q ⇄ fumarate+QH_2 is involved in oxidative or in reductive metabolism it is normally associated with membranes – mitochondrial membranes, or microbial plasma membranes – as an essential characteristic. The functional basis for that is the use of lipid soluble quinones (Q)/quinols residing in membranes as electron acceptors or donors, respectively. Thus, succinate:Q reductases (SQR) and quinol:fumarate reductases (QFRs) presumably evolved from a common primordial precursor and therefore possess a high degree of similarity with respect to their overall composition and cofactor requirement. However, reducing equivalents are exchanged via a series of iron–sulfur and heme centers with electron transport systems within the membrane. It is that part of the enzyme complexes – SQR and QFR – where essential topological and structural differences can occur as will be discussed below. Previous comprehensive reviews [3–5] could refer only to very few examples of archaeal succinate dehydrogenases (SDHs). The rapid development of extremophile research and the completion of microbial genome projects has now paved the way towards a comparative consideration of archaeal SQRs.

In the present paper a comprehensive description of archaeal SDHs isolated so far will be given. In the first part the focus will be on their functional and structural properties, while their genomic organization and phylogenetic relations are discussed in the second part. Specially addressed questions will be whether or not distinct characteristics of archaeal SDHs can be derived, and if so, what their physiological function in the respective organisms may be.

2. The archaeal domain

Archaea represent the so-called third kingdom of

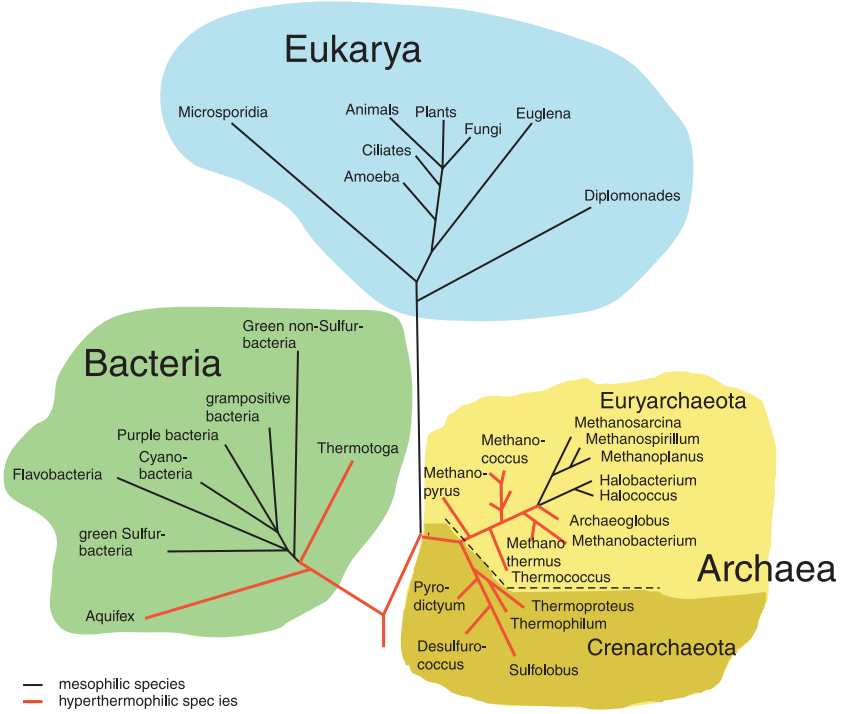

Fig. 1. The three domains of life. The simplified phylogenetic tree, redrawn according to [45], is based on 16S rRNA sequences and shows the two major archaeal branches (Crenarchaeota and Euryarchaeota) in more detail. For simplicity only the names of orders or genera but not of single species are given.

organisms as illustrated by the simplified phylogenetic tree of Fig. 1. It is based on the evolution of 16S rRNA [6]. The archaeal domain itself splits into two major branches, the Crenarchaeota and the Euryarchaeota. The latter comprise all methanogenic and the halobacterial genera, whereas the former include the majority of thermoacidophilic sulfur-metabolizing Archaea. Commonly Archaea inhabit extreme environments with respect to salinity, pH, pressure, and temperature, either as a single environmental condition or in a combination of these (for a review see e.g. [7]). Many species belong to the hyperthermophiles as indicated by the red lines in the scheme of Fig. 1. Interestingly, even at the lowest branches of this tree microaerophilic and also obligate aerobic species are located which possess SQRs. However, only a few examples of protein chemically and functionally characterized archaeal SDHs or fumarate reductases (FRDs) are known. Strong reasons for this situation are the limited accessibility of biological material mainly imposed by the extreme growth conditions, low growth rates, and/or oxygen sensitivity or instability at low (i.e. 'normal') salt concentrations of purified protein complexes. In contrast, a vast amount of sequence data is available from genome data banks which will be included in the following considerations.

3. Functional properties of archaeal SQRs

3.1. Archaea with SDH activity

Succinate-stimulated respiration of cells or isolated plasma membranes has been reported for *Halobacterium salinarum* more than three decades preceding classification of this organism as an Archaeon [8,9]. However, the enzyme or an intact complex II of the respiratory chain has never been isolated in purified form.

A systematic search for components of archaeal respiratory chains including membrane-residing SDHs was initiated with the investigation of the hyperthermoacidophilic Crenarchaeon *Sulfolobus*.

Actually, succinate-induced respiration of *Sulfolobus acidocaldarius* cells was first shown from our laboratory [10,11], and isolation of a detergent-soluble protein complex catalyzing succinate:acceptor reductase activity was achieved [12]. It allowed the first operon of an archaeal SQR to be sequenced [13] revealing several unusual differences as compared to known SDHs.

Table 1 summarizes archaeal organisms which either have since been shown to exhibit membrane-bound SDH activity, or from which enriched or purified SDH preparations were described. It has to be

Table 1
Comprehensive collection of data on archaeal SDH and FRD complexes

Organism	Membr. SDH activity	SDH isolated	Number and mass of subunits (kDa)	Heme *b* present	Membr. anchor?	Cys motif subunit C	Predomin. Q
T. acidophilum	+	−	63.1, 27.5, 14.8, 13.9	+	+	−	TK
N. pharaonis	g	+	69.1, 33.5, 14.3, 13.3	+	+	−	MK
H. salinarum	+	+	66.9, 33.6, 15.2, 13.1	+	?	−	MK
A. fulgidus	g	−	63.2, 27.2, 14.2, 13.1	+	+	−	MK
M. thermoautotrophicum	g(+)	−	58, 50	−	sol.*	+	(Mph)
M. jannaschii	g(+)	−	60.4, 56.1	−	sol.*	+	(Mph)
S. acidocaldarius	+	+	63.1, 36.5, 32.2, 14.1	−	−	+	CK, SK
S. solfataricus P2	+	−	62.5, 36.8, 32.1, 14.2	−	−	+	CK, SK
Sulfolobus sp. strain 7	+	+	66, 37, 33, 12	−	?	n.d.	CK
S. metallicus	+	−	n.d.	n.d.	?	n.d.	CK?
A. ambivalens	+	+	62.9, 36.4, 34.1, 14.0	−	−	+	CK
P. aerophilum	g	−	64.8, 27.1, 15.8, 12.8	?	?	−	MK
A. pernix	g	−	64.4, 34.7, 18.2, 12.9	?	?	−	CK?

The first column indicates whether the respective enzyme activity has been measured in membrane preparations (+) or has only been concluded from genetic data (g). The molecular masses have been calculated from accessible genomic data. n.d. = not determined; sol. = enzyme is soluble, cytosolic, * = Thiol:FRD. The electron-accepting Qs are: TK, *Thermoplasma* Q; CK, *Caldarialla* Q; MK, menaquinone; Mph, methanophenazine; SK, sulfolobusquinone; a '?' in this column indicates 'assumed' by phylogenetic relations of the respective organism. All listed properties were extracted from the references cited in the text of this article.

stressed that activities with solubilized SDH complexes usually were determined with artificial electron acceptors; only in two cases reasonable activities with a Q as terminal acceptor were reported: the preparations from *Sulfolobus* sp. strain 7 [14], and from *Acidianus ambivalens* [15]. A common characteristic of most reported activities is their sensitivity towards malonate as a competitive inhibitor. Other detailed kinetic data are scarcely available. More precisely reported are electron paramagnetic resonance (EPR) spectroscopic characteristics of the iron–sulfur centers; this has been achieved because in some cases the high abundance of the complex allowed direct measurements of the S1, S2, and S3 centers in native membranes [16,17].

The organisms of Table 1 are ordered into Euryarchaeota and Crenarchaeota according to their phylogenetic position. The discussion of individual SQR preparations follows below in the same order.

3.2. Specific archaeal SQR properties

3.2.1. The SQR from Halobacterium salinarum (previously H. halobium)

The extremely halophilic, aerobic Euryarchaeon *H. salinarum* thrives optimally at salt concentrations up to 4 M NaCl at neutral pH and 37°C [18]. Already 40 years ago, the presence of an SDH activity could be shown for this Archaeon. Surprisingly, about 80% of the total succinate-phenazine methosulfate (PMS) reductase activity was found in the cytoplasmic fraction [19]. In subsequent investigations, also a membrane-associated succinate oxidase was described [9]. In 1981, an SDH activity as well as a succinate-reducible membrane-bound *b*-type cytochrome were reported in *H. salinarum* [20]. As in the case of *H. salinarum*, the succinate-PMS/2,6-dichlorophenol-indophenol (DCPIP) reductase activity was mostly detected in the soluble fraction, indicating an only loose attachment of the enzyme to the cytoplasmic membrane. The activity of the soluble enzyme was inhibited by malonate and oxaloacetate. The K_M for succinate was determined as 3 mM at 3 M NaCl. In a followup study, the apparent molecular mass of the SDH was estimated as 90 kDa [21]. The salt optimum for the succinate-PMS/DCPIP reductase activity was in the range of 2–3 M KCl. The K_M for succinate in the membrane fraction was determined as 0.7 mM, significantly lower than the respective value for the solubilized enzyme (2.3 mM). The spectroscopic investigation of *H. salinarum* membranes gave evidence for the presence of four different *b*-type hemes with reduction potentials of +261, +160, +30 and −153 mV, respectively [22]. None of these, however, was attributed to the SDH, although both low-potential cytochromes might serve as possible candidates for hemes b_H and b_L of SDH. In the most recent report on *H. salinarum* SDH, inhibitor studies were performed [23]. The catalytic activity of the membrane-bound enzyme could be totally blocked by the classical inhibitor malonate as well as by tetrachlorobenzoquinone, a very potent inhibitor of the *S. acidocaldarius* and the *Thermoplasma acidophilum* SDH [12,16]. Furthermore, the *H. salinarum* SDH activity was also diminished by quinolone analogs like 1-dodecylquinolone, which most probably acts at the quinol-binding site of the enzyme.

3.2.2. The SQR from Natronobacterium pharaonis

The aerobic, haloalkaliphilic Euryarchaeon *N. pharaonis* thrives optimally at pH 9.5 and a salt concentration of 4 M NaCl at 37°C [24]. It was shown to possess an SDH consisting of only one major Coomassie staining band with an apparent molecular mass in SDS–PAGE of 94 kDa [25]. It reflects an unusual dissociation and migration behavior of the extreme halophilic complex (see subunit composition, below). The enzyme possesses an FAD molecule as shown by fluorescence spectroscopy and a *b*-type cytochrome as demonstrated by UV/Vis spectroscopy, respectively. Based on molecular masses derived from genetic information, the solubilized SDH protein complex is most probably a trimer built of the FAD-hosting subunit A, the heme-hosting subunit C and subunit D but lacks the iron–sulfur-containing subunit B.

Thus, the purified enzyme complex did not contain any iron–sulfur clusters. However, in *N. pharaonis* membranes, clearly the typical resonances of the SDH iron–sulfur centers could be detected. The enriched complex was catalytically active in the succinate-PMS/DCPIP oxidoreductase assay system and was totally inhibited by malonate. Specified kinetic data have not been reported, however. Reduction potentials of −312 mV and −340 mV were measured

for the *b*-type cytochrome of the SDH in the purified and in the membrane-bound state, respectively

3.2.3. The SQR from Thermoplasma acidophilum

The thermoacidophilic Euryarchaeon *T. acidophilum* is a facultatively anaerobic and heterotrophic organism, devoid of a cell wall or envelope growing optimally at pH 1–2 and at a temperature of 59°C [26]. Its SQR was investigated in the membrane, i.e. in its native environment, by enzymological studies and, for the first time, the iron–sulfur clusters of an archaeal SDH could be studied in situ due to its high abundance in this organism [16]. Highest turnover values of the SDH activity were observed at pH 7.4, which is somewhat above the internal pH value of *T. acidophilum*. The temperature optimum of the reaction was determined as 78°C and the K_M value for succinate with PMS as electron acceptor at 53°C as 0.32 mM. The membrane-bound enzyme was capable of reducing the artificial electron acceptors PMS, *N*,*N*,*N*′,*N*′-tetramethyl-*para*-phenylenediamine (TMPD) and DCPIP. Succinate oxidation was coupled to oxygen consumption in a totally 2*n*-heptyl-4-hydroxyquinoline *N*-oxide (HQNO)-sensitive manner. In the oxidized state, *T. acidophilum* membranes exhibited an almost isotropic EPR spectrum with $g_{z,y,x}$ values at 2.017, 2.000 and 1.968, respectively, which was assigned to a [3Fe–4S] cluster (S3). Succinate-reduced membranes displayed spectra characteristic of [2Fe–2S] clusters (S1), with $g_{z,y,x}$ values at 2.029, 1.935 and 1.915, respectively. In the dithionite-reduced state the linewidth of the resonances of cluster S1 as well as its signal amplitudes increased. Furthermore, additional resonances of an axial component with $g_{\parallel,\perp}$ values at 2.057 and 1.917 could be detected, which were assigned to a $[4Fe–4S]^{2+/1+}$ cluster. The saturation behavior of the S1 cluster was strongly altered in the dithionite-reduced form, thus indicating spin–spin interaction between the S1 and most probably the S2 cluster. In both the succinate- and the dithionite-reduced membranes parallel mode EPR spectra display a resonance at $g = 14$, which may be due to a transition of the $S = 2$ multiplet of the reduced [3Fe–4S] cluster. Spin quantitation yielded a relative stoichiometry of cluster S1 to cluster S3 of 1:1. The results obtained by EPR spectroscopy indicate that the characteristic iron–sulfur cluster S1 [2Fe–2S], S2 [4Fe–4S] and S3 [3Fe–4S] of 'classical' SDHs are also present in this archaeal SDH. EPR redox titrations of *T. acidophilum* membranes at pH 5.5 yielded a reduction potential of $+60 \pm 20$ mV for cluster S3 and of $+68 \pm 20$ mV for cluster S1. The axial $[4Fe–4S]^{2+/1+}$ center had a reduction potential of -210 ± 20 mV.

3.2.4. The SDH from Sulfolobus acidocaldarius

The thermoacidophilic Crenarchaeon *S. acidocaldarius* grows optimally at pH 2–3 and at temperatures between 70 and 80°C [27]. An SDH complex consisting of four different subunits with apparent molecular masses of 66 kDa, 31 kDa, 28 kDa and 12.8 kDa could be purified from plasma membranes [28]. The isolated enzyme hosted 102.4 nmol Fe, 155 nmol acid-labile sulfur and 4.6 nmol covalently bound FAD per mg protein, corresponding to 14.1 mol Fe/mol SDH, 21.4 mol S/mol SDH and 0.63 mol FAD/mol SDH, respectively. The purified enzyme did not contain heme groups. EPR investigations of the purified enzyme revealed the presence of the characteristic S1 and S2 clusters. The ferredoxin-type cluster S1 exhibited a rhombic spectrum with $g_{z,y,x} = 2.025$, 1.935 and 1.904 [13]. Spin quantitation of this resonance yielded a value of about 1 spin/holoenzyme. Evidence for cluster S2 was obtained indirectly by power saturation studies of cluster S1 resulting in an about 10-fold increase in the half-saturation power of the resonance in the dithionite-reduced state as compared to the succinate-reduced state. Surprisingly, a typical S3 cluster was lacking in the archaeal protein. Although the EPR spectrum of the as prepared enzyme displayed a nearly isotropic resonance with $g_{max} = 2.02$, this signal could not be attributed to a [3Fe–4S] center due to the following reasons: first, spin quantitation of this resonance yielded only a value of about 0.13 spin/holoenzyme; second, the signal amplitude did not increase after addition of oxidant, i.e. ferricyanide; and third, the saturation behavior of this resonance was different from the well studied behavior of classical S3 centers in other SQRs. Therefore, the isotropic resonance was finally assigned as due to a preparation artifact arising during the purification protocol. Instead of cluster S3, the EPR spectrum of the dithionite-reduced enzyme gave evidence for the presence of an additional, novel [4Fe–4S] cluster exhibiting an axial EPR spectrum with $g_{\parallel} = 2.020$ and

$g_{\perp} = 1.930$. Spin quantitation of this resonance resulted in 0.22 spin/holoenzyme. Both the biophysical and sequence data discussed below clearly indicate that a novel iron–sulfur cluster composition is present in the SQR of *S. acidocaldarius*, consisting of one [2Fe–2S] and two [4Fe–4S] clusters instead of the canonical clusters S1, S2 and S3. This novel iron–sulfur cluster composition has important consequences for the catalytic mechanism, since in classical SQRs the S3 center is crucial for enzymatic activity and enzyme stability [29].

Maximal succinate-PMS/DCPIP oxidoreductase activity was recorded at pH 6.5, the internal pH of *S. acidocaldarius* [30]. The temperature optimum of the reaction was measured as 81°C, coinciding with the temperature maximum for growth. From an Arrhenius diagram, the activation energy for the reaction in the range of 50–70°C could be determined as 60 kJ/mol. The K_M for succinate in this assay system could be determined as 1.42 mM. Like the mitochondrial SQR, the *S. acidocaldarius* enzyme was capable of reducing artificial electron acceptors like PMS, TMPD and ferricyanide with K_M values of 295 μM, 99 μM and 1.1 mM, respectively. The archaeal SDH could also reduce DCPIP alone, in the absence of PMS, with a K_M of 65.4 μM. The endogenous Q of the *Sulfolobus* membrane, caldariellaquinone, was only poorly reduced by the isolated SDH. This is in contrast to the complex II preparation described for *S. solfataricus* sp. strain 7 (see below) and may be explained by structural distortions induced by the applied detergents, eventually altering the interaction with the Q; this was the ionic detergent CHAPSO in the case of the *S. acidocaldarius* enzyme, whereas for *Sulfolobus* sp. strain 7 a non-ionic detergent (Lubrol) had been used [14]. Another reason might simply be that the isolation procedure was optimized following the specific activity with the PMS/DCPIP assay, but not with Qs as terminal acceptor. The catalytic activity was strongly decreased by the classical inhibitors of SDH, oxaloacetate and malonate. The most potent inhibitor, however, turned out to be tetrachlorobenzoquinone with a K_I of 1.4 μM.

3.2.5. The SQR from Sulfolobus sp. strain 7

The purified SQR of *Sulfolobus* sp. strain 7 consists of four different subunits with apparent molecular masses of 66, 37, 33 and 12 kDa, respectively [14]. The reported cofactor content is 5.6 nmol FAD and 83 nmol Fe per mg enzyme protein, corresponding to 0.83 mol FAD/mol SQR and 12.4 mol Fe/mol SQR, respectively. The isolated enzyme was devoid of any heme. EPR spectra of the purified SQR in the air-oxidized state displayed a nearly isotropic resonance around $g = 2$ with a g_{max} value at 2.02. This signal was tentatively assigned to cluster S3 of SQR. However, no spin quantitation of the resonance was performed, neither relative to cluster S1 nor relative to the SQR protein. The temperature behavior of the resonance tentatively assigned as FeS cluster S3 was not determined. By comparison of the traces in the EPR spectra of the air-oxidized and the succinate-reduced SQR, it is obvious that the relative spin quantitation of the so-called S3 resonance would only yield a value of maximally 5% of the double integral of cluster S1. If this were true, the signal amplitude of the pseudo-S3 resonance should have drastically increased after addition of oxidant, e.g. ferricyanide. After succinate reduction of the purified enzyme the typical resonances of a [2Fe–2S] cluster with $g_{z,y,x} = 2.03$, 1.94 and 1.90 were observed. Although the attribution of these resonances to the plant-type ferredoxin cluster S1 appears plausible, experimental evidence was not shown, since no EPR spectra were performed at about 70 K, the typical temperature for the measurement of plant-type ferredoxins. Quantitation of the S1 cluster relative to the protein was not reported. In the dithionite-reduced state of the SQR, resonances of another $S = 1/2$ system were reported with g values at about 2.08 and 1.88, which were only detected at 10 K, but not at 25 K. These 'wing' resonances were attributed to cluster S2. However, the classical, indirect characterization of the S2 cluster via the increase of the relaxation rate of cluster S1 in the dithionite-reduced state as compared to the succinate-reduced state is missing. The conclusion that the SQR of *Sulfolobus* sp. strain 7 hosts the typical three iron–sulfur clusters S1, S2 and S3 most probably needs revision. Especially with respect to the results obtained for the SQRs of the closely related Crenarchaea *S. acidocaldarius* [13] and *A. ambivalens* [15], where both the spectroscopic and the genetic data clearly indicate the presence of a novel type of SQR with an additional [4Fe–4S] instead of cluster S3. The answer whether or not the SQR from *Sulfolobus* sp. strain

7 belongs to the described novel class can only be given by the analysis of the cysteine clusters of the SdhB subunit, though this is likely on the phylogenetic relations within the order Sulfolobales.

Interestingly, the isolated SQR exhibited a succinate-ubiquinone-1 oxidoreductase activity with K_M values for succinate and ubiquinone of 280 μM and 20 μM, respectively. The purified enzyme was also capable of using DCPIP alone as electron acceptor with a K_M of 89 μM and V_{max} of 13.6 μmol/min mg. The pH optimum for the reaction with either ubiquinone or DCPIP as acceptor was determined in the range from 6.5 to 6.8, close to the internal pH. Finally, the isolated SQR could also use caldariellaquinone, the endogenous Q of *Sulfolobus*, as electron acceptor. However, the measured V_{max} value of about 1.8 μmol/min mg was very low as compared to the values obtained with either DCPIP or ubiquinone.

Nevertheless, the described preparation of SQR from *Sulfolobus* sp. strain 7 could be functionally reconstituted with a partially purified terminal quinol-oxidase fraction from the same organism, yielding a succinate oxidase activity of 130–150 nmol O_2/min/nmol heme A_S with an excess of the SDH fraction. For comparison, the activity in membranes was only measured as 8–12 nmol O_2/min/nmol heme A_S [14]. The exact path of electrons from succinate to oxygen is not clear because the reconstitution was performed completely with detergent-solubilized fractions but not in a particulate system. The experiments demonstrate, however, the absolute requirement for caldariella Q to maintain electron flow to oxygen.

3.2.6. The SQR from Sulfolobus metallicus

The hyperthermoacidophilic Crenarchaeon *S. metallicus* is an obligate chemolithoautotroph growing aerobically on elemental sulfur in a pH range from 1.0 to 4.5 and temperatures up to 75°C under aerobic conditions [31]. Membranes of *S. metallicus* exhibited succinate-induced oxygen consumption with a respiratory rate of 1.25 nmol O_2/min/mg, which could be totally blocked by cyanide [32]. By EPR spectroscopy, clear evidence for the presence of the characteristic clusters S1 [2Fe–2S] in the membrane-bound enzyme could be shown. However, the resonances of the S1 cluster were only poorly resolved giving unequivocal evidence for only the g_y signal of the cluster at 1.93. Furthermore, a nearly isotropic signal was observed in the as prepared membranes which could be simulated with $g_{z,y,x} = 2.028$, 2.015 and 2.01. This resonance was assigned to cluster S3 of the SDH, since also its signal amplitude decreased after incubation of the membranes with succinate. However, no spin quantitation was performed for this signal relative to the S1 center. Since in the closely related Crenarchaea *S. acidocaldarius* [13] and *A. ambivalens* [15] either the spectroscopic or the genetic data clearly indicate the presence of a novel type of SQR with an additional [4Fe–4S] instead of cluster S3 (see below), the interpretation of the EPR data concerning the isotropic resonance seems still preliminary. UV/Vis redox difference spectra of detergent-solubilized membranes clearly displayed a broad band at about 480 nm, characteristic for flavoproteins. The reduction potential of the flavin could be determined as about +120 mV. This reduction potential is much higher than that for free flavin (−219 mV) [33]; but it is clearly in harmony with a free electron flow from the succinate/fumarate couple (+30 mV) to the acceptor coenzyme.

3.2.7. The SQR from Acidianus ambivalens

The thermoacidophilic Crenarchaeon *A. ambivalens* is an obligate chemolithoautotroph, growing optimally at 80°C and pH 2.5. It is a facultative aerobic organism either oxidizing sulfur to sulfuric acid under aerobic conditions or reducing sulfur to hydrogen sulfide under anaerobic conditions [34]. Its SDH complex was investigated in either the membrane-bound or the purified state. Already in membranes, it could be demonstrated that the iron–sulfur cluster composition of the enzyme deviated from the canonical cluster set [17]. The EPR spectroscopic investigations clearly showed that center S3, the succinate-reducible [3Fe–4S] center of the SDH, was absent in membranes from aerobically grown cells. On the other hand, the typical EPR features associated with the remaining clusters, center S1 and S2, could be observed.

The purified complex contained one molecule of covalently bound FAD and 10 Fe atoms [15]. It consisted of four different subunits with apparent molecular masses of 67 kDa, 33 kDa, 28 kDa and 14 kDa. The isolated SDH was shown to contain bound cal-

dariellaquinone. Furthermore, it was capable of reducing decylubiquinone at a rate of 1.2 U/mg at 70°C. The purified enzyme was devoid of heme *b* [15].

By EPR spectroscopy, the results obtained for the membrane-bound enzyme could be confirmed. The purified SDH was shown to possess two [4Fe–4S] clusters and one [2Fe–2S] cluster, but was devoid of a [3Fe–4S] cluster. In contrast to the membrane-bound state, all three *g* values of the rhombic resonance of cluster S1 could be determined as $g_{z,y,x} = 2.034$, 1.937 and 1.911. Furthermore, the reduction potential of the S1 center was measured as −90 mV. The spectroscopic data were confirmed by the analysis of the sdh operon [32].

4. Structural properties of archaeal SQRs

As yet no structural 3D data exist for archaeal SQRs. However, the general polypeptide composition of these complexes suggests that the recently achieved X-ray structures from *Wolinella succinogenes* and *Escherichia coli* QFRs can serve as a very close scaffold [35,36]. Clearly the large subunit A is the central substrate- and primary acceptor-binding polypeptide containing the canonical FAD in all QFR/SQR complexes. Polypeptide B contains three FeS clusters as a link to further electron acceptors, or donors in the case of QFRs. As will be shown below, a reasonable phylogenetic pattern can be derived for subunits A and B. However, essential diversities between the members of the superfamily of SQRs and QFRs include especially polypeptides C and D as well as the cluster ligandation in the B subunit. Irrespective of the presence or absence of *b*-type hemes as additional redox centers hosted by these latter subunits, these have been assigned as the membrane anchors in any of the preceding structural models [4,37]. Recent elucidation of several archaeal complexes is definitely modifying this picture because both polypeptides C and D reveal completely different properties in the case of thermoacidophilic Crenarchaeota and suggest a novel and specific type of SQRs.

4.1. Operon structure

Fig. 2 gives a survey of the operon structure of archaeal SQR/QFR complexes with the respective operons from *E. coli* as a reference. Resembling the data on Table 1 also the archaeal enzymes share the four-subunit structure with similar lengths of the individual genes. A shuffling of the gene order can be recognized for *N. pharaonis* and *H. salinarum* as members of the extremely halophilic Euryarchaeota, and the Crenarchaeon *A. pernix* (not shown). Another diversion are the thiol-FRDs from methanogens where a gene fusion of subunits B+C occurred whereas an equivalent of subunit D is absent. This is in line with the soluble nature of these enzymes lacking a membrane anchor to which subunit D contributes substantially in most other SQR/QFR complexes. Provided the evolutionary occurrence of the four-subunit complex preceded the appearance of the *tfr* operons of methanogens these may have originated from a deletion of D accompanying the B–C gene fusion. Actually, projecting the rRNA-based phylogenetic tree onto a time scale the Crenarchaeota should in fact have preceded the euryarchaeotic branch.

Most interesting, however, is the occurrence of a

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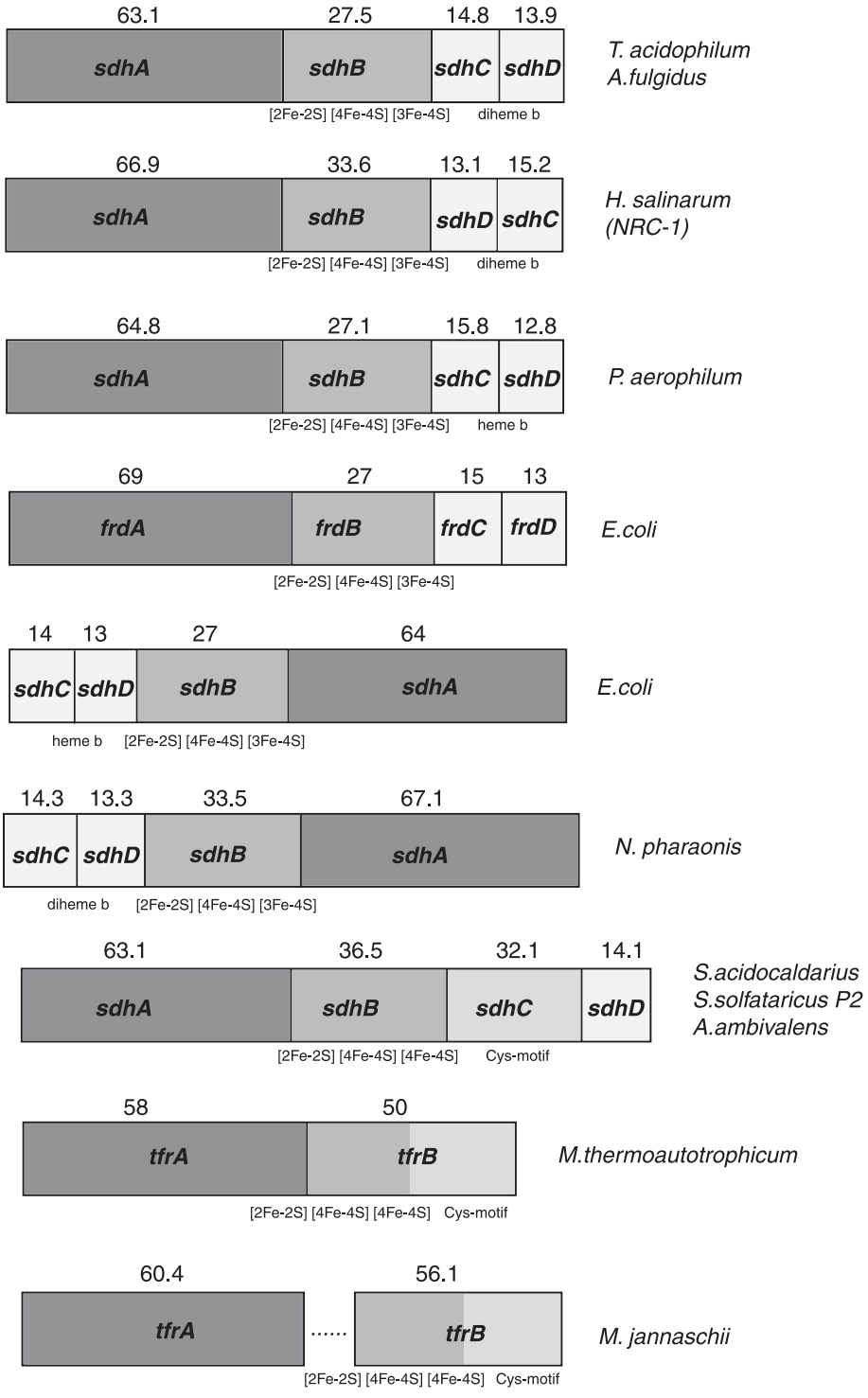
Fig. 2. Organization of archaeal *sdh*/*frd* genes within genomes taking *E. coli sdh* and *frd* operon as reference. Numbers above the genes indicate calculated molecular masses (in kDa) of the corresponding gene products. In all cases genes abbreviated with *A* encode flavoprotein subunits and *sdhB*/*frdB* encode FeS protein subunits. The FeS cluster composition of the B subunits is denoted below the gene symbol. Genes encoding *C* and *D* subunits encode gene products either of the 'classical' type each bearing three transmembrane α-helices and liganding two or one heme molecules (*T. acidophilum, A. fulgidus, H. salinarum* (strain NRC-1), *N. pharaonis, A. pernix* and *P. aerophilum*) or carrying the Cys motif in subunit C in the 'non-classical' type (*S. acidocaldarius, S. solfataricus, A. ambivalens*; for details see text). *TfrB* genes of methanobacteria are apparently fusion products of *B* and 'non-classical' *C* genes. *T. acidophilum, Thermoplasma acidophilum; A. fulgidus, A fulgidus; H. salinarum, Halobacterium salinarum* (strain NRC-1); *P. aerophilum, Pyrobaculum aerophilum; E. coli, Escherichia coli; N. pharaonis, Natronobacterium pharaonis; S. acidocaldarius, Sulfolobus acidocaldarius; S. solfataricus, Sulfolobus solfataricus; A. ambivalens, Acidianus ambivalens; M. thermoautotrophicum, Methanobacterium thermoautotrophicum; M. jannaschii, Methanococcus jannaschii*. *Sdh*, SDH gene; *frd*, FRD gene; *tfr*, thiol:FRD gene. Sequence alignments have been performed using CLUSTAL W 1.7 [46].

special cysteine-rich motif in subunit C shared between these thiol-FRDs and some crenarchaeotic SQR complexes as indicated in the figure. Functional implications of this mutation are discussed below (Section 4.2).

Another general feature relates to the presence or absence of functionally important histidine residues in polypeptides C and D. When present they serve as heme-liganding residues for the *b*-type cytochromes (b_H, b_L) buried between the membrane-traversing



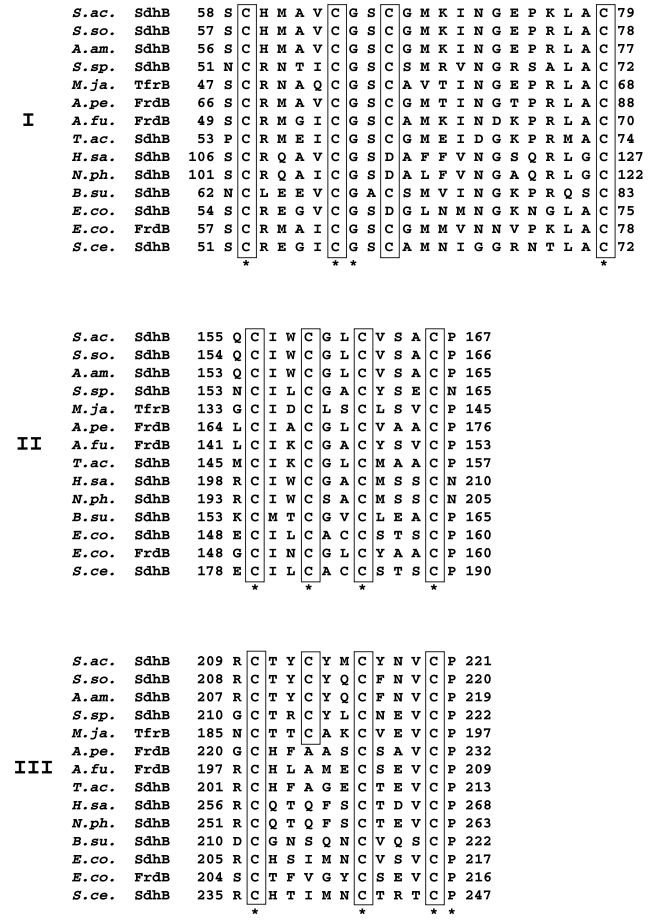


Fig. 3. Sequence alignment of cysteine clusters I, II and III of SdhB and FrdB subunits. Framed cysteines or aspartates are assumed to serve as ligands for the three FeS clusters. Absolutely conserved residues are marked by asterisks. *S.ac., Sulfolobus acidocaldarius; S.so., Sulfolobus solfataricus; A.am., Acidianus ambivalens; S.sp, Synechocystis* sp.; *M.ja., Methanococcus jannaschii; A.pe., Aeropyrum pernix; A.fu., Archaeoglobus fulgidus; T.ac., Thermoplasma acidophilum; H.sa., Halobacterium salinarum; N.ph., Natronobacterium pharaonis; B.su., Bacillus subtilis; E.co., Escherichia coli; S.ce., Saccharomyces cerevisiae.*

α-helices of the anchoring polypeptides. Fig. 2 indicates which complexes provide such heme-binding sites.

4.2. Unusual sequence motifs in archaeal SQRs

As concluded from EPR spectroscopic studies (see Section 4.3) the thermoacidophilic branch of the archaeal order Sulfolobales hosts a second [4Fe–4S] cluster instead of the usual S3 iron–sulfur cluster of the [3Fe–4S] type. This is explained by the presence of an additional cysteine as depicted from the alignment of the iron-binding domains of B subunits compiled in Fig. 3. The altered motif is found also in the thiol:FRDs [Tfr] of *Methanococcus jannaschii* and *Methanobacterium thermoautotrophicum*, as well as in bacterial FrdB subunits of *Aquifex aeolicus*, *Synechocystis* sp., and *Campylobacter jejuni*. Therefore it is not restricted to Archaea. A systematic investigation of the redox potentials of these altered FeS centers as compared to 'typical' S3 centers of SQRs is not available yet.

Even more unusual is the appearance of a 10-cysteine motif in subunit C of the SQRs from thermoacidophilic Sulfolobales. It has no equivalent in any other SQR or QFR but surprisingly has been found in polypeptides B of thiol:fumarate reductase (TFR) complexes from methanogens and also in a small number of completely different and functionally unrelated oxidoreductases included in the alignment of Fig. 4. The motif occurs as a tandem with the general signature -GC-X_{31}-CC(G/P)-X_{35}-CXXC-(repeat I)-X71-(G/P)C-X_{39}-CCG-X_{39}-C-XX-C-(repeat II); the given example refers to the specific case of *S. acidocaldarius*. The second appearance within this tandem contains an invariantly conserved glycine, 12 residues ahead of the CCG motif. Importantly, neither by EPR spectroscopy [13,32] nor by chemical metal analysis [17] could additional iron clusters or other metals be detected in this polypeptide. Therefore, if not involved in disulfide bonds, one or more of these cysteines may be participating in redox reactions, i.e. electron transfer to Qs possibly via thiol radicals. Direct evidence for that in membranes is unavailable at present; however, as mentioned above (cf. Section 3) a reconstituted soluble succinate oxidase system using caldariella Q as intermediate electron carrier has been reported [14], and the isolated SQR from *A. ambivalens* was shown to react with ubiquinone as terminal acceptor [15]. Moreover, and in contrast to the C subunits of SQORs lacking this cysteine-rich motif, these polypeptides are devoid of typical membrane anchors such as for example a membrane-spanning hydrophobic α-helix. For that reason the discovery of an archetypical novel class of SQORs [13] is discussed in more detail in the following.

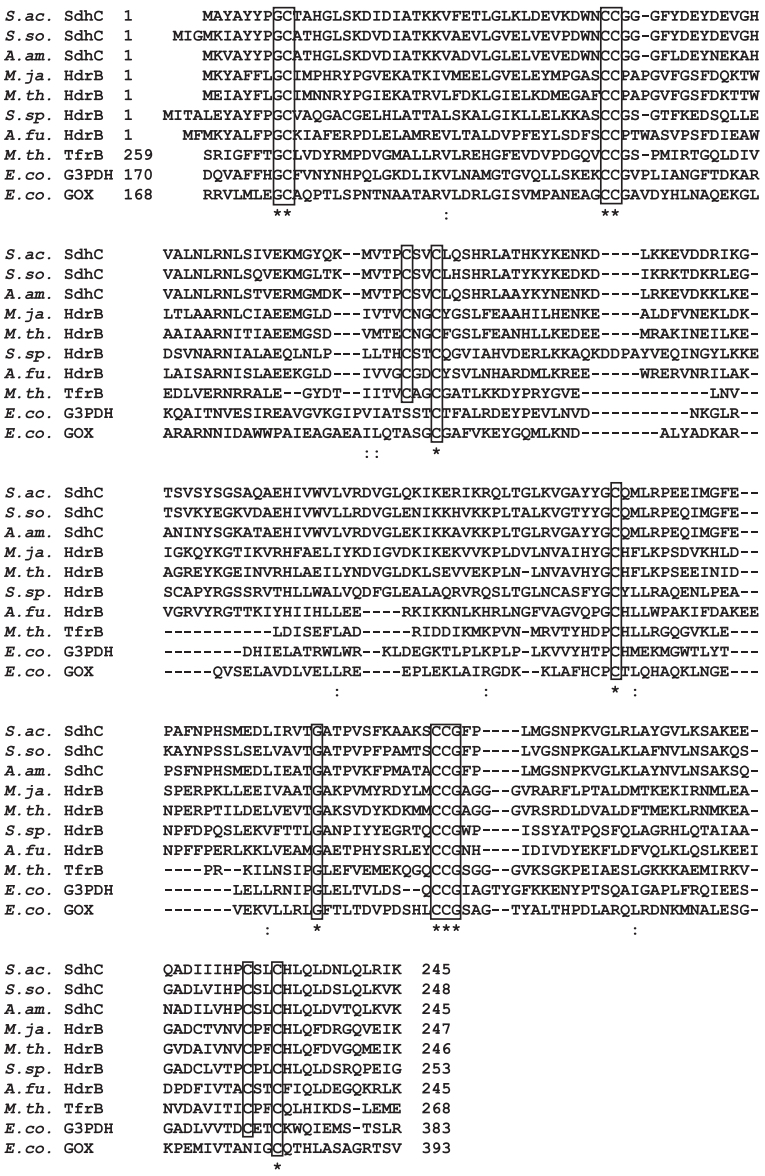


Fig. 4. Sequence alignment of cysteine motif-containing protein subunits. Functionally important residues are framed. Absolutely conserved residues are marked by asterisks, conservative replacements are indicated by dots. Sdh, succinate dehydrogenase; HdrB, heterodisulfide reductase; TfrB, Thiol:FRD; G3PDH, *sn*-glycerol-3-phosphate dehydrogenase; GOX, glycolate oxidase. *M.th., Methanobacterium thermoautotrophicum*, other abbreviations for organism names are depicted in Figs. 2 and 3. Sequence alignment has been performed using CLUSTAL W 1.7 [46].

4.3. *'Classical' versus 'non-classical' archaeal SQOR complexes*

According to the above, SDHs in the archaeal domain of the phylogenetic tree of living organisms can be classified into the so-called 'classical' SQORs and into 'non-classical' or novel SDHs. We use this term in order to distinguish SDH complexes which are similar to known bacterial and eukaryal SQOR complexes from those archaeal SDH complexes displaying a novel structure and unique composition of their FeS clusters. Our knowledge of the classical branch of archaeal SDH has been mostly derived from genomic sequence data, the non-classical branch has

been investigated additionally in biochemical and biophysical terms. Whether sequence-derived data call for SQORs in the classical branch remains an open question until the enzymology of these complexes and the redox potentials of their FeS centers have been further investigated. Today only the physiological situation under which the Archaeon has been isolated may give a functional hint. While classical SQOR sequences have been found in both the euryarchaeal and the crenarchaeal branch of Archaea, the non-classical forms are restricted to date to the order Sulfolobales in the crenarchaeal branch; therefore they constitute a novel class of SDHs.

4.3.1. Classical archaeal SQORs

The euryarchaeal organisms *H.NRC*-1, *N. pharaonis*, *T. acidophilum* and *A. fulgidus*, and the crenarchaeal organisms *Pyrobaculum aerophilum* and *Aeropyrum pernix* clearly belong to the classical branch. Their sdh/frd operons encode four protein subunits: subunit A (flavoprotein subunit), B (iron sulfur protein subunit) and two small subunits C and D (Fig. 2). The gene arrangement is reminiscent of the *E. coli* frd operon in the case of *T. acidophilum, A. fulgidus* and *P. aerophilum*. It differs in the case of *H.NRC*-1 where D precedes C. In *N. pharaonis sdhC* and D precede *sdhB* and *sdhA* analogous to *E. coli* SQR. The arrangement of the four genes also differs in *A. pernix*. Here the operon-like structure is not obvious since an open reading frame is inserted between *sdhA* and *sdhD*. The atypical gene order is *sdhB-sdhC-sdhD-orf-sdhA*.

The iron–sulfur protein (subunit B) contains 11 conserved cysteine ligands distributed among three conserved clusters I, II and III. They may serve as ligands for the binuclear FeS center S1, the bacterial ferredoxin-type tetranuclear center S2 and the trinuclear center S3 (Fig. 3). The conserved cysteine cluster types in the classical archaeal SQORs argue for homologous FeS centers as found in bacterial and eukaryal SQORs. As shown for *T. acidophilum* paramagnetic S1, S2 and S3 centers have been detected in the membrane-bound state of its SQOR. *A. fulgidus* lives under strictly anaerobic conditions; it may possess a QFR instead of SQR activity implying a function in a reductive TCA cycle.

Subunits C and D are likely to fold as transmembranous structures each spanning the plasma membrane three times. They are assumed to serve as membrane anchors for the peripherally associated large subunits. Both small subunits contain conserved histidine residues as ligands for two prosthetic heme molecules except for *P. aerophilum* which only contains His ligands to form a mono-heme cytochrome b_H. At least in the case of *T. acidophilum* SQOR binding of the diheme has been proven spectroscopically [38–40]. According to the classification by Hägerhall [4] the small subunits of classical archaeal SDH complexes belong apparently to the type A membrane anchors whereas *P. aerophilum* SQOR resembles type C.

4.3.2. Non-classical SDH complexes

As a prototype the non-classical SDH complex of Archaea was first described in the aerobically grown hyperthermoacidophilic *S. acidocaldarius* [13]. Later it was shown to occur also in *A. ambivalens* [16] and *S. solfataricus* strain P2 (accession number AL512976) [41]. Remarkably all these organisms are phylogenetically strongly related and belong to the order Sulfolobales. While the sequence of the flavoprotein is essentially conserved among all organisms, substantial differences have been observed in the FeS cluster composition of subunit B (Fig. 3) and the molecular structure of subunits C and D between classical and non-classical forms. It appears justified to assume the same for complex II from the *Sulfolobus* sp. strain 7 [14].

In the novel SQOR complex subunit B contains 12 instead of 11 cysteines and encompasses three iron–sulfur centers. Cluster I contains four cysteine ligands arranged in the characteristic manner of plant-type ferredoxins [2Fe–2S]. Its consensus sequence was found to be Cys-X_4-Cys-X_2-Cys-X_{11}-Cys and agrees with the crenarchaeal sequences reported here. Cysteine ligands of cluster II are conserved in the consensus sequence Cys-X_2-Cys-X_2-Cys-X_3-Cys typical for bacterial type ferredoxins [4Fe–4S]. While cluster I and cluster II are alike to classical archaeal SDH complexes, a remarkable difference appears in the composition of cluster III. This cluster is normally composed of three cysteines liganding the [3Fe–4S] center. Here, the third cluster is liganded by four cysteines and leads to a cluster composition similar to cluster II. The additional cysteine ligand exerts a profound effect on the EPR

resonance of cluster III in the crenarchaeal SDH complexes [16]. The isotropic spectrum indicating a [3Fe–4S] is not detected in the oxidized state. Instead a ferredoxin-like resonance appears in difference spectra indicating the presence of a second [4Fe–4S] center in these complexes.

Subunit C differs totally in terms of molecular mass, amino acid composition and hydrophobicity from the membrane anchor-type subunit. Its molecular mass is in the range of 32–34 kDa as deduced from sequences and thus is much larger than the typical C subunit of the classical complex. Furthermore, its hydrophobicity profile does not suggest membrane-anchoring transmembrane α-helices. A strong hydrophobic sequence stretch can be deduced only at the C-terminal end which might be essential for the attachment of the complex to the membrane. Putative amphipathic α-helices are proposed to contribute to the protein's binding site at the plasma membrane [15]. The major attribute of the non-classical subunit C is the above-mentioned unusual cysteine motif arranged as a tandem. Remarkably, homologs to subunit C have been also shown for other bacterial and archaeal enzymes like heterodisulfide reductases in methanogenic Archaea and some bacterial species (subunits HdrB and HdrD), thiol:FRDs (subunit TfrB) in methanogenic Archaea, anaerobic G3PDHs (subunit GlpC), and glycolate oxidases (subunit GlcC) in bacterial species (Fig. 4). It may be speculated that the cysteine motif containing subunit C or its equivalent in other enzyme complexes is engaged either as a membrane-anchoring protein, and/or as a redox-active subunit supporting electron transport functions in these enzyme complexes. At least in the case of G3PDH this subunit is suggested to interact with Q [42]. The mechanism by which this function is maintained in the conserved cysteine motif is yet unknown. It might be speculated that comparable to thioredoxin function sulfhydryl radicals support redox reactions in this subunit. Further investigations will unravel the role of subunit C in electron transport across the archaeal SDH complex.

Although subunit D of non-classical SDH complexes exhibits a similar molecular mass of 14 kDa as subunit D of the classical ones, the sequences do not reveal any significant homology. While classical subunit D acts as a membrane-anchoring subunit and provides ligands for coordination of the hemes, the non-classical ones display hydrophilic properties and cannot bind hemes. Their function is yet unknown.

4.3.3. FRD encoding genes in methanogenic archaea

Methanogenic archaea like *M. jannaschii* and *M. thermoautotrophicum* possess thiol-driven FRDs (TFR) using reduced thiol-containing coenzymes as electron donors instead of quinols in methanogens [43]. *M. thermoautotrophicum* TFR is a cytosolic enzyme reducing fumarate to succinate by reduced coenzyme M (CoM-SH, 2-mercaptoethanesulfonate) and reduced coenzyme B (CoB-SH, N-7-mercaptoheptanoyl-threoninephosphate) generating a mixed heterodisulfide [44]. Both coenzymes are methanogen-specific compounds acting in the last steps of methanogenesis. The TFR complex consists of two subunits, TFR A and TFR B. TFR A is a flavoprotein and contains the binding site for fumarate while TFR B appears to be the catalytic site for coenzyme oxidation. Interestingly, it contains the three FeS centers of non-classical SDH complexes ([2Fe–2S], 2×[4Fe–4S]) and the cysteine motif of the SDH C subunit as well. Therefore TfrB appears a fusion construct of non-classical SdhB and SdhC.

5. Phylogenetic relations

The large substrate-binding subunit A is present in all known SQRs and QFRs and is a common catalytic element regarding the binding site of FAD and the reaction mechanism. Therefore a rather strong conservation might be expected. Also common to both classes of enzymes is the use of a cascade of three FeS clusters as intermediate electron carriers hosted in subunit B. Because these fundamental elements are also present in soluble enzyme forms such as for example in the thiol FRDs (TFRs) one might speculate that both modules may have a common evolutionary history, whereas the membrane-residing other small subunits may have been adopted from different evolutionary sources.

Based on amino acid sequences an unrooted phylogenetic tree of subunit A is shown in Fig. 5. Despite the expected strong conservation of the functional modules the tree differentiates clearly between

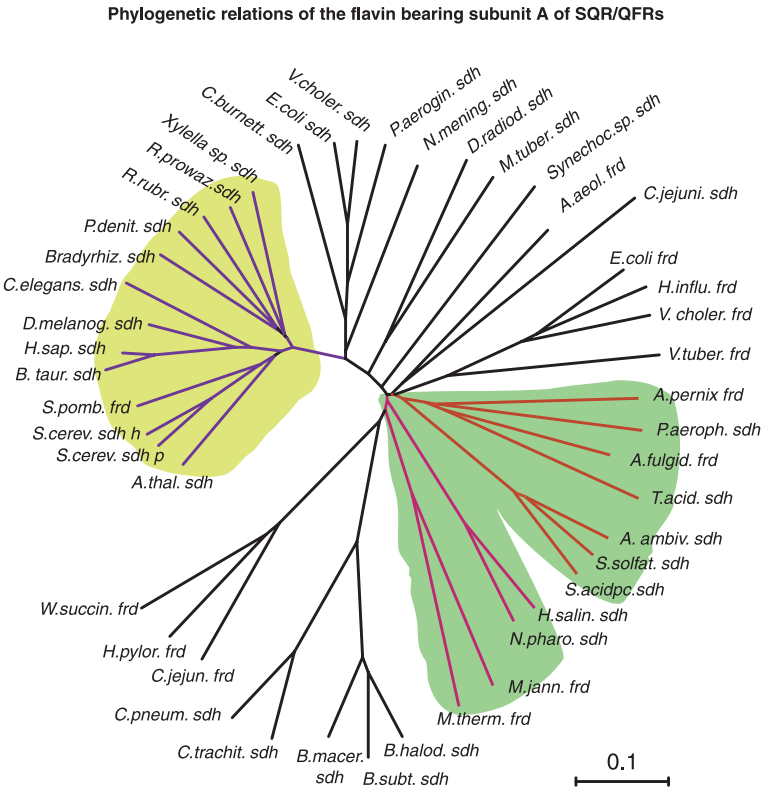


Fig. 5. Unrooted phylogenetic tree of polypeptides A from various archaeal, bacterial, and eukaryal SQRs and QFRS. The archaeal cluster and the cluster comprising proteobacteria and eukaryal mitochondria are emphasized by color. Sequences were extracted form generally accessible published genomes and single entries in data banks. The tree was calculated using CLUSTAL W 1.7 [46]; the unrooted presentation was drawn with 'NJ-Plot' and 'Unroot' [47,48].

the major evolutionary domains. The shaded (colored) areas in Fig. 5 depict the archaeal and the eukaryal kingdoms as far as the organelles of endosymbiontic origin are concerned. Members of the latter originate from a single branch. It splits into the branches of proteobacteria and of mitochondria from plants, yeasts, and animals, each forming a separate cluster. The archaeal domain also originates from a common branching point and the methanogens together with the halophiles correctly cluster within an euryarchaeal domain whereas the Crenarchaea are found in the other major archaeal branch. However, *T. acidophilum* and *A. fulgidus* are incorrectly placed with the Crenarchaeota; according to 16S rRNA phylogeny they belong to the Euryarchaeota. It has to be emphasized, however, that the genome of *Thermoplasma* contains a significant number of genes strongly homologous to Sulfolobales [40]; therefore in protein-based trees it falls frequently into the latter though by 16S rRNA analysis it rather belongs to the Euryarchaeota (note the same situation with subunit B, below). Furthermore, from the bottom of both archaeal branches also two branches of totally unrelated Eubacteria split off. Generally, the distances between bifurcations in this region of the tree are very short though clearly supported by high bootstrap values (not shown). On the one hand, these results definitely underline the close relationship of all SQR polypeptides A, but on the other they suggest a distinct evolutionary history. With only a few exceptions (predominantly in the archaeal domain) SQRs and QFRs are routinely found on separate branches which in most cases show significant phylogenetic distances. For an example the reader is referred to the FRD and SDH clusters containing *E. coli* and *Vibrio cholerae*, or those containing *C. jejuni* SDH and FRD, respectively.

A comparison with a tree calculated for the B subunits from a comparably large collection of SQRs and QFRs is shown in Fig. 6. Actually, the general shape and scaling of the unrooted tree supports the

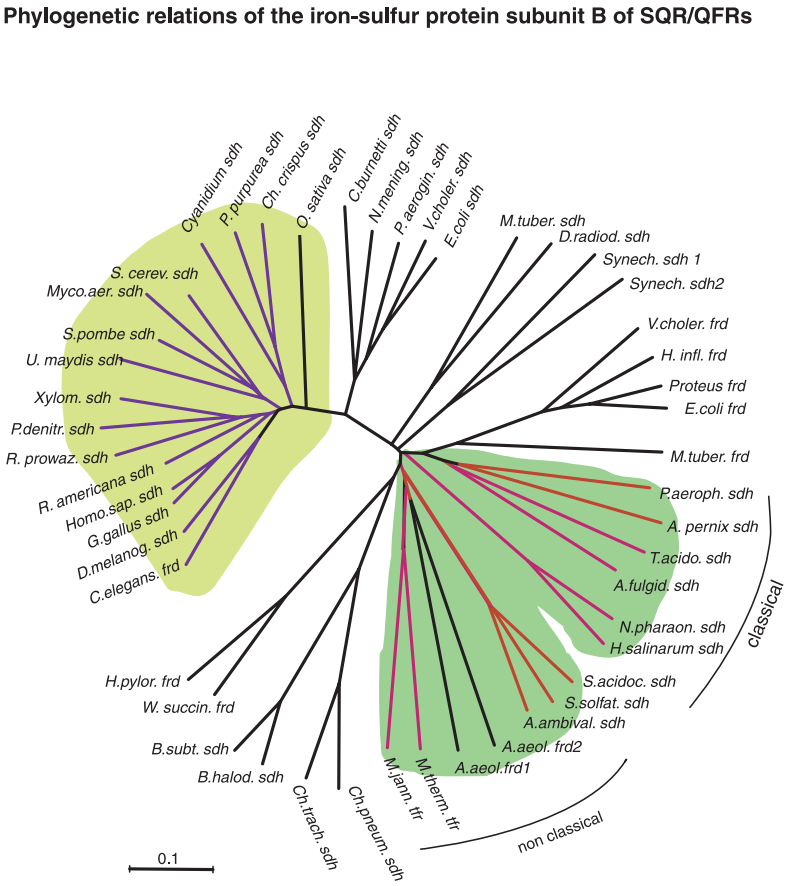


Fig. 6. Unrooted phylogenetic tree of polypeptides B from various archaeal, bacterial, and eukaryal SQRs and QFRs. The archaeal cluster and the cluster comprising proteobacteria and eukaryal mitochondria are emphasized by color. The tree was generated as indicated in Fig. 5. Sequences were derived from generally accessible data banks.

hypothesis of a co-evolution of both components, the flavoprotein (subunit A) and the iron–sulfur protein (subunit B). Also in that tree the proteobacterial and mitochondrial sequences from eukaryotes are located in a common domain, and again the archaeal domain originates from a common branch point splitting into two major subdomains (shaded area). In this case the separation between Crenarchaea and Euryarchaea is less pronounced (several translocations), but the subdomains illustrate clearly the division into the 'classical' complexes containing membrane-anchored subunits C and D all bearing the heme-liganding histidines in their membrane-traversing helices, and the 'non-classical' complexes described above; the latter share the unusual cysteine-rich sequence motif in subunit C as well as the [4Fe–4S] cluster in subunit B. This might indicate hitherto unknown mechanistic differences of electron transport for the complexes from both subdomains which remain to be elucidated, however.

6. Conclusions and open questions

The problem to be addressed was whether or not archaeal complexes II, i.e. the succinate:acceptor oxidoreductases, exhibit unusual or novel features as compared to well-known bacterial and eukaryal enzymes. The answer is yes. As outlined in the above not only the gene order may be different from the classical bacterial one but also novel sequence motifs have been detected. Some of these features have been demonstrated directly with the isolated or membrane-bound enzymes but others have been deduced by analogy with genetic data of organisms from which the actual enzyme has never been accessed

enzymatically. This leaves us with a number of open questions to be investigated in the future. One of these is the electron transport pathway and acceptor specificity of those complexes lacking the typical iron–sulfur cluster S3. The novel [4Fe–4S] cluster may simply replace S3 but might also exhibit an altered redox potential and change the kinetic properties of the enzyme. Not only that has to be verified but also the occurrence of this novel cluster itself in those species where it has only been predicted genetically. Actually, a designed mutant of *E. coli* FRD with the cluster conversion ([3Fe–4S] ⇒ [4Fe–4S]) not only had a midpoint potential lowered by about −220 mV but also lost more than 80% of the wild-type catalytic activity [49]. On the other hand, an analogous mutation in *B. subtilis* did not produce a cluster conversion but rendered the enzyme unstable [29].

A similar question remains open regarding the cysteine-rich tandem motif. Its functional significance is totally open and might be investigated preferentially by directed mutational analysis. The latter, however, is unattainable at present because none of the organisms bearing this motif in complex II can be genetically transformed; neither has the heterologous expression of a whole SDH operon ever been achieved. If this motif is involved in electron transfer employing the sulfhydryl groups directly, a totally new mechanism of electron transport between complex II and the Q pool would have to be expected. Another unresolved aspect is the obvious lack of the membrane-anchoring hydrophobic α-helices in the small subunits of some archaeal SQR complexes. Their deduced largely hydrophilic properties are in contrast to the obvious fact that the intact complex is definitely associated with the plasma membrane. Though some speculations have been made on how by interaction of theoretically predicted amphipathic helices these complexes might be assembled the answer can only be derived from sound structural data; therefore crystallization of an archaeal complex revealing these unusual features is a useful challenge. In view of the vast amount of genetic data which have become available from archaeal organisms in the past 2 years the state of proteomic studies is lagging behind. With respect to archaeal respiratory chains it is notable that in contrast to an energy-transducing, proton-pumping complex I (type-I NADH dehydrogenase) a membrane-bound SDH activity and/or the respective genes for a complex II equivalent have been found ubiquitously in Archaea. Actually, two conclusions we would like to propose are that: (1) these electron-transducing complexes are a very early and essentially largely conserved product of evolution, presumably dating back prior to the split into the three evolutionary domains; and (2) a co-evolution of the core modules of SQRs and QFRs took place as suggested by the persuasive similarity of evolutionary trees emerging for the flavoprotein and the iron–sulfur protein of these complexes.

Acknowledgements

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