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Electron transfer between periplasmic formate dehydrogenase and cytochromes c in *Desulfovibrio desulfuricans* ATCC 27774

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Abstract *Desulfovibrio* spp. are sulfate-reducing organisms characterized by having multiple periplasmic hydrogenases and formate dehydrogenases (FDHs). In contrast to enzymes in most bacteria, these enzymes do not reduce directly the quinone pool, but transfer electrons to soluble cytochromes c. Several studies have investigated electron transfer with hydrogenases, but comparatively less is known about FDHs. In this work we conducted experiments to assess potential electron transfer pathways resulting from formate oxidation in *Desulfovibrio desulfuricans* ATCC 27774. This organism can grow on sulfate and on nitrate, and contains a single soluble periplasmic FDH that includes a cytochrome c_3 like subunit (FdhABC$_3$). It has also a unique cytochrome c composition, including two cytochromes c not yet isolated from other species, the split-Soret and nine-heme cytochromes, besides a tetraheme type I cytochrome c_3 (TpIc_3). The FDH activity and cytochrome composition of cells grown with lactate or formate and nitrate or sulfate were determined, and the electron transfer between FDH and these cytochromes was investigated. We studied also the reduction of the Dsr complex and of the monoheme cytochrome c-553, previously proposed to be the physiological partner of FDH. FdhABC$_3$ was able to reduce the c-553, TpIc_3, and split-Soret cytochromes with a high rate. For comparison, the same experiments were performed with the [NiFe] hydrogenase from the same organism. This study shows that FdhABC$_3$ can directly reduce the periplasmic cytochrome c network, feeding electrons into several alternative metabolic pathways, which explains the advantage of not having an associated membrane subunit.

Keywords Formate dehydrogenase · Cytochrome · Sulfate-reducing bacteria · *Desulfovibrio* · Hydrogenase

Introduction

Sulfate-reducing organisms (SRO) comprise a heterogeneous group of prokaryotes found in a wide variety of anaerobic habitats, where they play a major role in both the carbon and the sulfur cycles. SRO obtain energy by coupling the oxidation of H_2 or organic compounds to the reduction of sulfate [1, 2]. Many redox proteins have been isolated from organisms belonging to *Desulfovibrio* spp., the most studied genus of SRO, but the exact mechanism of how energy conservation is achieved remains to be fully elucidated. There is evidence to suggest that several energy-conserving pathways may contribute to the overall process [3]. Hydrogen and formate are two of the main electron donors in natural habitats, and in this case the energy conservation mechanism is rather straightforward, since their oxidation releases protons in the periplasm, directly contributing to a proton motive force across the membrane. The same does not apply to the oxidation of lactate in the cytoplasm, which has to be associated with chemiosmotic energy transduction in order to provide enough energy gain from sulfate reduction. Hence, cycling of redox intermediates, such as hydrogen, CO, or formate, was proposed as an alternative mechanism for energy conservation in *D. vulgaris*, when growing with lactate as an electron donor [4–6]. Thus, hydrogen or formate may be available exogenously or formed intracellularly in SRO. In addition, a *Desulfovibrio* strain was shown to convert

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formate to hydrogen [7]. In both cases, periplasmic c-type cytochromes are considered to have a crucial role in their periplasmic oxidation. SRO belonging to the *Deltaprote-obacteria* class are characterized by a high content of soluble cytochromes c [2, 3]. In parallel, they have also periplasmic hydrogenases and formate dehydrogenases (FDHs), which in contrast to enzymes in most bacteria lack a membrane subunit for quinone reduction and thus are soluble proteins. The most widespread c-type cytochrome in *Desulfovibrio* spp. is the tetraheme type I cytochrome c_3 (TpIc_3), which is a physiological redox partner of the periplasmic hydrogenases (reviewed in [8, 9]), but which can also receive electrons from periplasmic FDHs [10–12]. The TpIc_3 cytochromes are thought to function as shuttles between the periplasmic dehydrogenases and membrane-bound complexes such as Qrc and Tmc/Hmc, which in turn transfer electrons from the periplasm to the menaquinone pool (Qrc) [13], or directly to the cytoplasm for sulfate reduction (Tmc/Hmc) [9, 14–16]. The cytochrome c_3 family comprises other members that are associated with membrane complexes, such as the type II cytochrome c_3, which is a subunit of the Tmc complex [16], the high molecular weight cytochrome (HmcA) of the Hmc complex [17], and the nine-heme cytochrome (NhcA) of the Nhc complex [18]. In addition, some hydrogenases and FDHs have a dedicated cytochrome c_3 subunit [19–21].

D. desulfuricans is one of the most studied species of *Desulfovibrio*, because it can also reduce nitrate and nitrite, besides sulfate [22]. Interestingly, this organism prefers to reduce sulfate when it is provided with both sulfate and nitrate, even though the reduction of nitrate is energetically more favorable [22, 23]. Recently, it was shown that the operon coding for the Nap nitrate reductase, responsible for reduction of nitrate in the periplasm, is regulated at transcriptional level by nitrate and sulfate [23]. Nap expression is induced by nitrate but is repressed if the preferred electron acceptor, sulfate, is present even in limited amounts [23].

Three multiheme c-type cytochromes have been isolated from *D. desulfuricans* ATCC 27774: TpIc_3, NhcA, and the split-Soret cytochrome (Ssc) [22, 24]. NhcA is a monomeric nine-heme cytochrome c that is part of the Nhc membrane redox complex [18]. NhcA is homologous to the C-terminal domain of HmcA, the 16-heme cytochrome c subunit of the Hmc complex [17, 25]. Owing to the similarity of both proteins, it was suggested that they have an analogous function, which is supported by the fact that the Hmc complex is absent in organisms possessing Nhc [3]. However, the Nhc complex does not have cytoplasmic subunits, so it should only transfer electrons between periplasmic proteins and the quinone pool. In *D. desulfuricans* ATCC 27774, NhcA was reported to receive electrons from hydrogenase via TpIc_3, on the basis of a

biochemical and structural study of these proteins [25]. However, in another strain, *D. desulfuricans* Essex, it was proposed that NhcA receives electrons directly from hydrogenase [26].

The Ssc is a dimer of two identical subunits and derives its name from the unusual split observed in the Soret band (420 nm, with a shoulder at 415 nm) of the ferricytochrome [22, 24, 27]. The function of this cytochrome is unknown, but it is possibly related to nitrate reduction since its expression is higher in growth with nitrate than with sulfate [22, 27]. In addition, *D. desulfuricans* ATCC 27774 also contains the three-heme cytochrome c DsrJ, unrelated to the cytochrome c_3 family, which is a subunit of the strictly conserved Dsr membrane complex [28]. DsrJ is not reduced by hydrogenase and TpIc_3, and is thought to interact with an unknown periplasmic partner [28].

In a previous study we investigated the possible electron transfer pathways involving the periplasmic hydrogenases in a *Desulfovibrio* organism [15], and several other studies have addressed the same issue (reviewed in [9]). By comparison, relatively little is known about the electron pathways involving FDHs, besides their reduction of TpIc_3 and the monoheme cytochrome c-553, proposed to be the physiological electron acceptor [10, 11, 21]. Since formate is an important metabolite in natural anaerobic habitats and an important energy source for SRO [1, 29], it is essential to understand how energy is conserved from formate oxidation. To gain further insight into this subject, we chose to use the organism *D. desulfuricans* ATCC 27774, which has only one soluble periplasmic FDH, FdhABC$_3$. We studied the reduction of TpIc_3, Ssc, NhcA, DsrJ, and the monoheme cytochrome c-553 by FdhABC$_3$. For comparison, the same set of experiments were performed with the *D. desulfuricans* ATCC 27774 [NiFe] hydrogenase.

Materials and methods

Culture medium, growth conditions, and preparation of the soluble fraction

D. desulfuricans ATCC 27774 cells were grown in Postgate medium C containing lactate or formate as electron donors at a concentration of 40 mM, and sulfate or nitrate as electron acceptors at a concentration of 38 and 28 mM, respectively. Cells adapted to either sulfate or nitrate were used as an inoculum in each case. Nickel, selenium, and molybdenum were also present in the medium at a final concentration of 1 μM (Ni and Se) or 0.1 μM (Mo). Growth took place at 37 °C in 100-ml closed flasks containing half the volume of medium and a gas phase of 100 % N_2. The cells were collected after 17 h of growth, and the soluble fraction was obtained with a Bugbuster

Plus Lysonase kit (Merck) following the manufacturer's instructions. The procedure was conducted in anaerobic conditions inside a Coy glove box.

Protein quantification and gel electrophoresis

The protein content of soluble fractions was determined by the Bradford method (Sigma) with bovine γ-globulin as the standard (Bio-Rad). The cytochrome c composition of the soluble fractions was analyzed with a 12 % sodium dodecyl sulfate–polyacrylamide gel stained as described in [30] for visualization of heme-containing proteins.

Protein purification

TpIc_3, NhcA, and Ssc were purified as described in [22], and FdhABC$_3$ and [NiFe] hydrogenase were purified as described in [19, 31]. Dsr was purified as described in [28]. Since cytochrome c-553 could not be isolated from *D. desulfuricans*, we used the protein from *D. vulgaris*, which was purified as described in [32].

Enzymatic measurements

Enzymatic assays with soluble fractions, purified FDH and [NiFe] hydrogenase, were performed in strict anaerobic conditions inside a Coy glove box as described elsewhere [33]. The activity of FDH and [NiFe] hydrogenase was tested with benzyl viologen or methyl viologen prior to their use in the cytochrome reduction experiments. The reduction of cytochromes was measured by following the increase in absorption at 553 nm for cytochrome c-553 ($\varepsilon = 29.1$ mM^{-1} cm^{-1}), 552 nm for TpIc_3 ($\varepsilon = 120$ mM^{-1} cm^{-1}), Ssc ($\varepsilon = 120$ mM^{-1} cm^{-1}), and NhcA ($\varepsilon = 270$ mM^{-1} cm^{-1}), and 555 nm for the Dsr membrane complex ($\varepsilon = 132.4$ mM^{-1} cm^{-1}). When the reductions were performed in the presence of catalytic amounts of TpIc_3 or cytochrome c-553, the enzymes were preincubated with these cytochromes prior to the experiments. All the experiments were repeated at least twice, except with Dsr complex, owing to the limited amount available.

Reductions with *D. desulfuricans* ATCC 27774 FDH

FDH (purified aerobically) was deoxygenated before all experiments, by leaving the sample for about 1 h at 4 °C in the glove box atmosphere, and then diluted to the required concentration in buffer containing 0.2 mM dithiothreitol. The cytochrome solutions were deoxygenated by performing several cycles of argon/vacuum before their insertion in the glove box. To compare the reduction of the different cytochromes, we used the same total heme concentration (15 μM). Thus, the concentrations of each cytochrome used depended on the number of heme groups present. The concentrations were as follows: 0.5 nM FDH, 15 μM cytochrome c-553, 3.75 μM TpIc_3 and Ssc, 1.7 μM NhcA, and 3 μM Dsr.

Reductions with *D. desulfuricans* ATCC 27774 [NiFe] hydrogenase

The [NiFe] hydrogenase was activated before the experiments, by performing several cycles of H$_2$/vacuum followed by incubation under H$_2$ at 30 °C for 1 h. All solutions used in the assays were H$_2$-saturated. The concentrations of each cytochrome and hydrogenase used were as follows: 6 nM [NiFe] hydrogenase, 15 μM cytochrome c-553, 3.75 μM TpIc_3 and Ssc, 1.7 μM NhcA, and 3 μM Dsr.

Results and discussion

### FDH and cytochrome levels in different growth conditions

D. desulfuricans is able to use both sulfate and nitrate as electron acceptors, but the electron transfer pathways involving FDH in each of these growth conditions are not well known. To gain further insight into the relative expression levels of FDH and cytochromes c, we grew cells in parallel with formate or lactate as electron donors, and sulfate or nitrate as electron acceptors. As a measure of growth we used protein quantification to avoid interference in the optical density from metal sulfides present only in the growth with sulfate. In agreement with the findings of a previous report [23], we observed a higher cell yield with nitrate than with sulfate, in the presence of either lactate or formate (Table 1). Both electron donors produced similar cell yields with nitrate, but in contrast the yield of formate/sulfate-grown cells was considerably lower than that of lactate/sulfate-grown cells. In terms of FDH activity, the

Table 1 Total protein and FDH activity of the soluble fraction of *Desulfovibrio desulfuricans* cells grown in different conditions

Growth conditions	Total protein (mg)	Total FDH activity (U)	FDH-specific activity (mU mg^{-1})
L/S	39.9 ± 0.5	3.85 ± 0.16	97 ± 5
L/N	96.2 ± 2.3	1.84 ± 0.42	19 ± 4
F/S	15.8 ± 1.5	0.53 ± 0.03	34 ± 1
F/N	100.8 ± 13.8	14.3 ± 5.9	140 ± 40

The values are means of two independent biological experiments ± the standard deviation (SD)

L/S lactate/sulfate, *L/N* lactate/nitrate, *F/S* formate/sulfate, *F/N* formate/nitrate
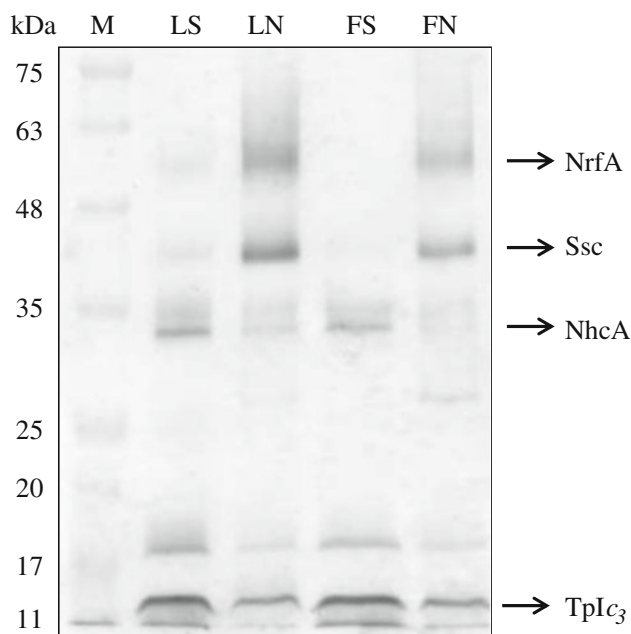
Fig. 1 Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of soluble fractions from *Desulfovibrio desulfuricans* cells grown in different conditions, stained for heme *c* containing proteins. The cytochrome bands were identified by comparison with the purified proteins. *M* prestained molecular mass markers, *LS* lactate/sulfate, *LN* lactate/nitrate, *FS* formate/sulfate, *FN* formate/nitrate, *NrfA* catalytic subunit of nitrite reductase, *Ssc* split-Soret cytochrome, *NhcA* nineheme cytochrome, *TpIc₃* tetraheme type I cytochrome c_3

highest total and specific activities were observed for formate/nitrate-grown cells. In contrast, the FDH activity of formate/sulfate-grown cells was rather low. These results indicate that FDH is more highly expressed in formate/nitrate-grown cells and that formate is a preferred electron donor for growth with nitrate, as observed in *Escherichia coli* [34], but not for growth with sulfate.

The content of cytochromes *c* in soluble fractions of cells grown in these different conditions was analyzed with a sodium dodecyl sulfate–polyacrylamide gel stained for covalently bound hemes (Fig. 1). This revealed significant differences between the use of sulfate or nitrate as an electron acceptor, but not between the use of lactate or formate as an electron donor. NhcA and TpIc₃ are more highly expressed in sulfate-grown cells, whereas Ssc and NrfA (the catalytic subunit of nitrite reductase) are more abundant in cells grown with nitrate, which agrees with the findings of previous reports regarding NhcA and the Ssc [18, 22, 27].

Reduction of cytochromes *c* by FdhABC₃

In *D. desulfuricans* ATCC 27774 a single soluble FDH of the FdhABC₃ type is present, which contains a cytochrome c_3 like subunit, besides the catalytic and electron transfer

subunits [19], and is similar to FdhABC₃ from *D. vulgaris* [21]. In *D. vulgaris*, for which more studies of FDH activity have been conducted, the physiological redox partner of FdhABC₃ is proposed to be the monoheme cytochrome *c*-553 [11, 21]. In several *Desulfovibrio* spp., the gene coding for this cytochrome is found next to the genes for cytochrome *c* oxidases [35], indicating that cytochrome *c*-553 is the electron donor to the oxidases. Also, cytochrome *c*-553:oxidase activity was recently reported in *D. vulgaris* membranes [36, 37]. However, there should also be a pathway to transfer electrons from formate oxidation to sulfate or nitrate reduction, and in this study several cytochromes *c* were tested as possible electron acceptors for FdhABC₃.

FdhABC₃ was purified from nitrate-grown cells and its specific activity (109 U mg⁻¹) was slightly higher than the value previously reported for this enzyme [19]. The rate of reduction of different cytochromes *c* by this enzyme was measured, and we tested also the effect on this rate of adding small, catalytic amounts of TpIc₃ or cytochrome *c*-553 as intermediaries between FdhABC₃ and the final cytochrome. Since cytochrome *c*-553 could not be isolated from *D. desulfuricans* ATCC 27774, we used the corresponding cytochrome from *D. vulgaris* instead.

We observed a very high rate for reduction of cytochrome *c*-553 by FdhABC₃, which was much higher than that observed with the other cytochromes (Table 2). Preincubation with catalytic amounts of either TpIc₃ or cytochrome *c*-553 caused a small increase in this reduction rate, probably owing to increased activation of the enzyme. The lack of a significant effect of TpIc₃ is likely because it is already present as a subunit of FDH. This subunit was shown to be essential for electron transfer between the enzyme and cytochrome *c*-553 [11, 21].

The reduction rate of TpIc₃ by FdhABC₃ is high, and in the same range as the value reported for the [NiFe] hydrogenase and TpIc₃ from *D. vulgaris* [15], but lower than the rate obtained for cytochrome *c*-553 reduction, as previously reported for *D. vulgaris* FdhABC₃ [21]. The addition of catalytic amounts of cytochrome *c*-553 caused a small increase in the reduction rate of TpIc₃.

With Ssc the reduction rate is also quite high (albeit lower than for TpIc₃), suggesting that this may be a physiologically relevant pathway for electrons resulting from formate oxidation. In this case, the presence of either TpIc₃ or cytochrome *c*-553 did not influence the reduction rate. Since the FDH activity and the level of Ssc are both higher with nitrate as an electron acceptor, the electron transfer pathway from FdhABC₃ to nitrate may involve Ssc. In this organism, the dissimilatory nitrate reductase is a periplasmic enzyme [38], whose electron donor is either a membrane-bound NapC cytochrome or a soluble NapM cytochrome [39]. However, neither NapM nor NapC could

Table 2 Cytochrome reduction rates with *D. desulfuricans* FdhABC$_3$, with or without preincubation with catalytic amounts of TpI*c$_3$* (from *D. desulfuricans*) or cytochrome *c*-553 (from *D. vulgaris*) (μmol cytochrome min^{-1} nmol enzyme^{-1}) (mean ± SD, using the same enzyme preparation)

	Cytochrome *c*-553	TpI*c$_3$*	Ssc	NhcA	Dsr
FdhABC$_3$	14.06 ± 0.53	1.27 ± 0.07	0.71 ± 0.07	0.21 ± 0.01	0.13
FdhABC$_3$/cytochrome *c*-553	15.51 ± 0.01	1.95 ± 0.04	0.69 ± 0.09	0.17 ± 0.02	0.12
FdhABC$_3$/TpI*c$_3$*	15.38 ± 0.18	1.16 ± 0.01	0.72 ± 0.08	0.17 ± 0.01	0.09

Ssc split-Soret cytochrome, *NhcA* nine-heme cytochrome, *Dsr* dissimilatory sulfite reductase complex

be isolated by us, so the complete pathway could not be tested.

FdhABC$_3$ is also able to reduce NhcA at a significant rate, although three times lower than that obtained with Ssc. Once more, the presence of catalytic amounts of TpI*c$_3$* or cytochrome *c*-553 does not influence the NhcA reduction rate. As opposed to Ssc, NhcA is present at higher levels when cells grow with sulfate than with nitrate as an electron acceptor.

A low reduction rate is observed for the DsrJ cytochrome as part of the membrane-bound Dsr complex, which is involved in electron transfer to the cytoplasmic dissimilatory sulfite reductase [28, 40]. The [NiFe] hydrogenase, on the other hand, is not able to reduce Dsr, even in the presence of TpI*c$_3$*, as reported previously [28], and confirmed in the present work (see below).

Kinetic parameters of cytochrome *c*-553, TpI*c$_3$*, and Ssc reduction by FdhABC$_3$

To further evaluate the possible role of cytochrome *c*-553, TpI*c$_3$*, and Ssc as electron acceptors for FdhABC$_3$, we determined the kinetic parameters for their reduction by this enzyme (Table 3). The very limited amounts of NhcA and Dsr complex obtained by native purification did not allow similar determinations for these two proteins. The highest turnover number and lowest K_m were obtained for cytochrome *c*-553. The FdhABC$_3$ from *D. desulfuricans* shows a higher turnover number and lower K_m (and thus increased efficiency) with this cytochrome than the enzyme from *D. vulgaris* [11]. The turnover number of Ssc is slightly higher than that of TpI*c$_3$*, whereas the K_m for TpI*c$_3$* is lower than for Ssc. This further supports the reduction of Ssc as a physiologically relevant pathway.

Reduction of cytochromes *c* by [NiFe] hydrogenase

For comparison, the electron transfer between the *D. desulfuricans* [NiFe] hydrogenase and the different cytochromes was also tested. The specific activity of the purified [NiFe] hydrogenase (14 U mg^{-1}) with an artificial electron donor was 65 % below the reported value

Table 3 Kinetic parameters of *D. desulfuricans* FdhABC$_3$ reduction of different cytochromes

|	Turnover no. (s^{-1})	K_m (μM)	k_{cat}/K_m (μM^{-1} s^{-1})
Cytochrome *c*-553[a]	239	2.7	88
TpI*c$_3$*	17.1	3.9	4.4
Ssc	27.0	13.0	2.1

[a] From *D. vulgaris*

(40 U mg^{-1}). All attempts to fully reactivate the enzyme were not successful, so part of the protein remained inactivated. As such, the absolute reduction values observed are lower than the real ones, but it is still possible to compare the relative reduction rates for the several proteins. The monoheme cytochrome *c*-553 is reduced by the hydrogenase only in the presence of TpI*c$_3$* (Table 4). A similar result was obtained previously for the [NiFe] hydrogenase from *D. vulgaris* [15], although the reduction rate obtained in that case was sixfold lower than the one we observed here for the *D. desulfuricans* hydrogenase. As expected from the low activity of the isolated [NiFe] hydrogenase, the value obtained for TpI*c$_3$* reduction was five times lower than that obtained for this reaction in *D. vulgaris* [15, 41]. The reduction rate for Ssc is four times lower than for TpI*c$_3$*, and the presence of TpI*c$_3$* in catalytic amounts does not influence the rate. The lowest reduction rate was obtained for NhcA, confirming the value previously reported [25], although in the present work we could not observe a catalytic effect of TpI*c$_3$*, in agreement with what was previously reported for the [NiFe] hydrogenase and NhcA of another strain, *D. desulfuricans* Essex [26].

The soluble FdhABC$_3$ from *D. desulfuricans* ATCC 27774 includes a cytochrome *c$_3$* like subunit [19]. Thus, the presence of catalytic amounts of TpI*c$_3$* should be redundant, and that explains the fact that very little or no effect is visible for this cytochrome in the reduction experiments with FdhABC$_3$ and the other proteins tested. On the other hand, the [NiFe] hydrogenase does not have a cytochrome *c$_3$* subunit, and TpI*c$_3$*, considered to be its physiological partner, should be necessary to mediate electron transfer from H$_2$ oxidation to other soluble or membrane-bound

Table 4 Cytochrome reduction rates with *D. desulfuricans* [NiFe] hydrogenase, with or without preincubation with catalytic amounts of TpIc_3 (from *D. desulfuricans*) or cytochrome *c*-553 (from *D. vulgaris*) (μmol cytochrome min^{-1} nmol enzyme^{-1}) (mean $\pm$ SD, using the same enzyme preparation)

	Cytochrome *c*-553	TpIc_3	Ssc	NhcA	Dsr
[NiFe] hydrogenase	–	0.32 $\pm$ 0.02	0.08 $\pm$ 0.005	0.02 $\pm$ 0.003	–
[NiFe] hydrogenase/TpIc_3	2.97 $\pm$ 0.11	0.27 $\pm$ 0.001	0.09 $\pm$ 0.002	0.02 $\pm$ 0.003	–

Fig. 2 Periplasmic electron transfer pathways from formate oxidation in *Desulfovibrio* spp. Cytochromes *c* are in *dark gray*. QmoABC is involved in electron transfer to APS reductase, and DsrMKJOP (Dsr complex) is involved in electron transfer to the dissimilatory sulfite reductase. In *D. desulfuricans* ATCC 27774 the pathway for oxygen reduction via cytochrome *c*-553 does not operate. *Nhc* nine-heme cytochrome, *FeS* iron–sulfur centers, *MQ* menaquinone, *MQH2* menaquinol, *Moco* molybdopterin cofactor
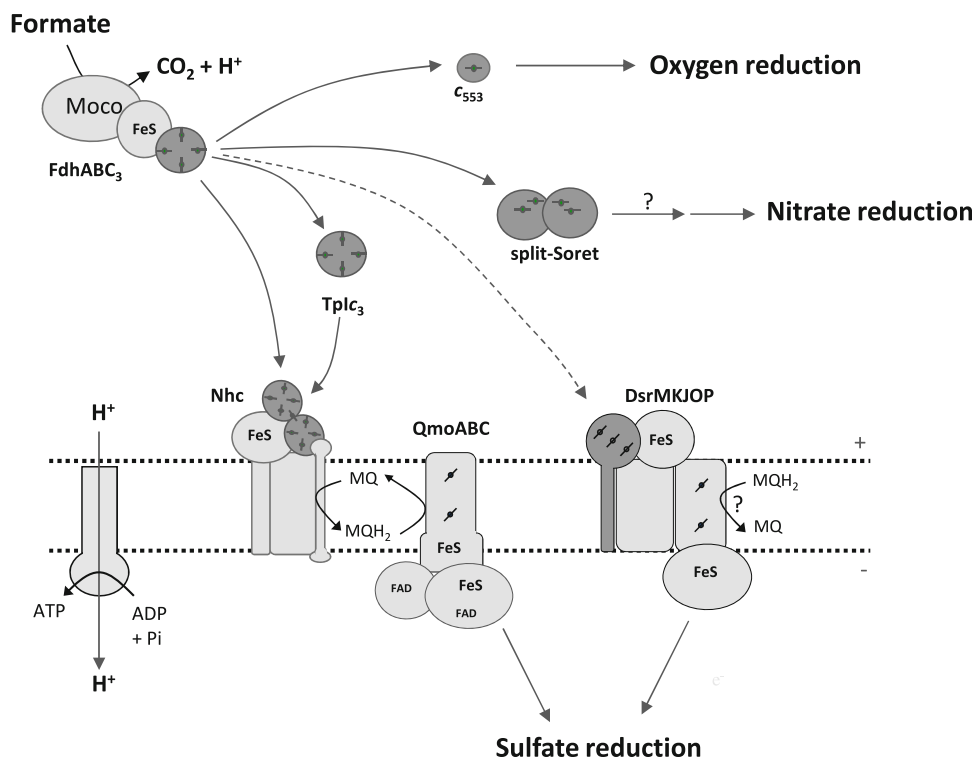
proteins [9]. Nevertheless, Ssc and NhcA are reduced directly by the [NiFe] hydrogenase, indicating that TpIc_3 is not an absolutely essential intermediate. However, given the very high concentration of TpIc_3 in the cell, it seems likely that this always mediates electron transfer from the hydrogenase.

Concluding remarks

Recently, and subsequently to performing these experiments, the genome of *D. desulfuricans* ATCC 27774 was made available (Integrated Microbial Genomes, http://img.jgi.doe.gov/cgi-bin/w/main.cgi). Analysis of this genome shows that neither a cytochrome *c*-553 nor a cytochrome *c* oxidase, the electron acceptor of cytochrome *c*-553, is present in this organism [3]. Thus, in *D. desulfuricans* ATCC 27774, the cytochrome *c*-553 is not a physiological redox partner of FDH. This cytochrome has very high

redox potentials (20–80 mV), in contrast to the low redox potentials of TpIc_3 (-200 to -400 mV). Thus, it is more plausible that TpIc_3 is involved in electron transfer from formate ($E^{0\prime}$ formate/CO$_2$ = -432 mV) to sulfate reduction, rather than cytochrome *c*-553. Nevertheless, the data obtained in the present work show that cytochrome *c*-553 is indeed an efficient electron acceptor for FDH, but most probably for oxygen, and not sulfate, reduction.

Our results show that the FdhABC$_3$ from *D. desulfuricans* ATCC 27774 is able to reduce cytochrome *c*-553, TpIc_3, and Ssc with a high rate. These cytochromes are probable electron input points for reduction of different electron acceptors: oxygen in the case of cytochrome *c*-553 (although this pathway is not operational in the specific case of *D. desulfuricans* ATCC 27774, it occurs in other *Desulfovibrio* spp.), sulfate in the case of TpIc_3, and nitrate in the case of Ssc (Fig. 2). In addition, the membrane-associated NhcA is also reduced with a significant rate, not requiring the involvement of either TpIc_3 or

cytochrome *c*-553. The Nhc complex provides a pathway for reduction of the menaquinone pool from formate oxidation. *D. desulfuricans* ATCC 27774 does not contain the Qrc complex, which in *D. vulgaris* was shown to be responsible for transferring electrons from periplasmic hydrogen or formate oxidation to the menaquinone pool [13]. Thus, in *D. desulfuricans* the Nhc complex most likely replaces the Qrc complex. The direct reduction of the Dsr complex by FdhABC$_3$, although possible, is of doubtful physiological significance owing to its low rate and by analogy to the absence of its reduction by the periplasmic hydrogenases.

This study shows that in *D. desulfuricans* ATCC 27774 the profile for cytochrome *c* reduction by the periplasmic FDH is similar to that of the periplasmic [NiFe] hydrogenase, which further supports the equivalent role of formate and H$_2$ in the metabolism of SRO [29]. These two compounds are provided as substrates for SRO by the metabolism of other organisms, or are formed intracellularly in the cycling of redox intermediates. The fact that both periplasmic dehydrogenases can transfer electrons to different acceptors can explain the advantage of not having an associated membrane subunit, which would necessarily direct the electrons to the quinone pool, and highlights the flexibility of the electron transfer pathways operating in *Desulfovibrio* spp. Nevertheless, further work is required to fully elucidate these pathways, namely, in the case of nitrate, but also sulfate, reduction.

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