Hypothesis

Is a Q-cycle-like mechanism operative in dihaemic succinate:quinone and quinol:fumarate oxidoreductases?

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Abstract Succinate:quinone (SQR) and quinol:fumarate oxidoreductases (QFR) are members of the same enzyme family. These are membrane bound enzymes anchored to the membrane by one or two subunits that may contain two, one or no haems. For the dihaemic enzymes the electron pathway from the flavin at the catalytic centre to the quinones remains to be established. Taking into account that the two haems are located on opposite sites of the membrane, and the possible presence of two quinone binding sites, also located on opposite sides of the membrane, we re-hypothesise the presence of a Q-cycle type mechanism in these enzymes. Such a mechanism can explain an active functional role for two haems and two quinone binding sites, allowing SQR to conserve energy. With this testable hypothesis we intend to challenge the discussion and drive further experimentation to unravel the functional mechanism of SQRs and QFRs.

Key words: Succinate dehadrogenase; Fumarate reductase; Menaquinone

1. Introduction

Succinate:quinone (SQR) and quinol:fumarate oxidoreductases (QFR) are closely related enzymes. Recently, they have been the topic of a special review issue [1] and the crystallographic structures from *Escherichia coli* and *Wolinella succinogenes* QFRs have already been elucidated [2,3]. A flavoprotein, an iron–sulphur protein facing the cytoplasm, and a membrane domain constitute these enzymes (Fig. 1). The flavoprotein contains the FAD site where the catalytic reaction with succinate and fumarate occurs. A $[2Fe–2S]^{2+/1+}$, a $[3Fe–4S]^{1+/0}$ (or a $[4Fe–4S]^{2+/1+}$ in some cases) and a $[4Fe–4S]^{2+/1+}$ cluster are present in the iron–sulphur protein and arranged in a wire-like manner, allowing the electron transfer from the flavin to the membrane domain. This domain can be constituted by one or two subunits composed by transmembrane or possibly monotopic helices [4]. In the former case the transmembrane domain can contain two, one or no haems [5]. The absence of haems is also observed in the enzymes having monotopic helices [4].

2. Presence of two haems

The crystallographic structure of *W. succinogenes* QFR shows the presence of two B type haems, close to the opposite sides of the membrane and with both planes almost perpendicular to the membrane surface [3]. Due to their position in relation to the peripheral domain the haems were named proximal (b_p) and distal (b_d) (Fig. 1). The presence of two B type haems has also been observed in the SQR from *Bacillus subtilis* [6] and *Rhodothermus marinus* [7] and in the QFR from *Desulfovibrio gigas* [8]; on the bases of their amino acid sequences the presence of two haems is anticipated in the SQRs from *Bacillus halodurans*, *Paenibacillus macerans*, *Chlamydia muridarum*, *Chlamydophila pneumoniae*, *Deinococcus radiodurans*, *Mycobacterium tuberculosis*, *Natronomonas pharaonis* and *Halobacterium* sp, and in the QFRs from *Aeropyrum pernix*, *Archaeoglobus fulgidus*, *Campylobacter jejuni*, *Helicobacter pylori*, *Thermoplasma acidophilum* and *Thermoplasma volcanium* [4].

The haems present different absorbance and redox properties [5,7], one having a high reduction potential (b_H) and the other a low reduction potential (b_L). Based on site-directed mutants it was possible to assign b_L as the distal haem and b_H as the proximal haem [9–11].

3. Presence of two quinone binding sites

The number and location of quinone binding sites in SQR/QFR are still under debate. No electron density for a quinone was observed in the crystallographic structure of *W. succinogenes* QFR [3], whereas two menaquinone molecules situated on opposite sides of the membrane were observed in the crystallographic structure of *E. coli* QFR [2]. Similarly to the case of the haems, these quinone binding sites were named proximal (Q_P) and distal (Q_D). Based on kinetics of inhibition by a *sec*-butyl-4,6-dinitrophenol derivative two quinone binding sites had already been proposed for the *E. coli* QFR and for the bovine SQR [12]. A wealth of data using other inhibitors and site-directed mutants have also indicated the presence of two quinone binding sites present in the dihaemic *B. subtilis* SQR [5]. Also using the same approaches a Q_P site is predicted to be present in the monohaemic SQRs from *Bos taurus*, *E. coli*, *Paracoccus denitrificans*, *Ascaris suum* and *Ustilago maydis* [5], and a Q_D site in *W. succinogenes* QFR [13]. Based on random mutagenesis and inhibitor sensitivity, the presence of both Q_P and Q_D binding sites was

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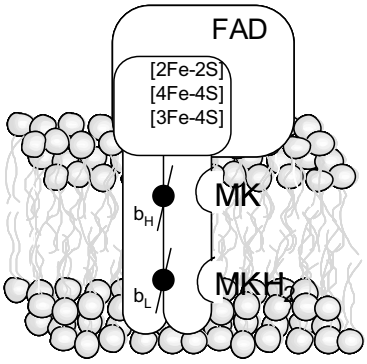

Fig. 1. Schematic representation of a SQR/QFR family member. These enzymes are composed by two peripheral subunits, one containing a flavin and the other containing a $[2Fe–2S]^{2+/1+}$, a $[3Fe–4S]^{1+/0}$ (or a $[4Fe–4S]^{2+/1+}$) and a $[4Fe–4S]^{2+/1+}$ cluster and one transmembrane domain.

proposed for the monohaemic SQR of *Saccharomyces cerevisiae* mitochondria [14].

Fig. 2 shows the amino acid sequence alignment of the membrane subunit(s) of dihaemic SQR/QFRs and their general secondary structure prediction. As mentioned above the presence of a Q_D site in these enzymes is accepted [5,13], while the presence of a Q_P site has been suggested for the *B. subtilis* SQR [5]. The presence of a Q_P site should be near haem b_P and thus it should be situated in a region comprising the end of helix II, loop B and the beginning of helix IV, or in a region including the end of helix V, loop D (Fig. 2) and the beginning of helix VI. An extensive variability can occur in quinone binding sites. However, the amino acids found at these sites are mainly of two types [15]: those able to establish hydrogen bonds with the oxygen atoms of the quinone, and those able to perform ring staking interactions, such as the aromatic amino acid residues. Several motifs for quinone binding sites have been proposed [15]. One such motif is $L(X)_3H(X)_2T$ (being X any amino acid) and indeed a similar motif is present in *W. succinogenes* and *H. pylori* QFRs – $L(X)_2H(X)_2T$ (LeuC117 to ThrC123, *W. succinogenes*, Fig. 2). This motif is present at the end of loop B (Fig. 2), and the beginning of helix IV, which situates it close to haem b_P. In the sequence alignment in Fig. 2, in the same place of that motif in the other enzymes there are several aromatic amino acid residues that could be part of a quinone binding site.

However, as for many other quinone binding proteins, conserved motifs cannot be assigned for other SQRs. Nevertheless, the available data strongly raise the possibility for the presence of two quinone binding sites in dihaemic SQRs/QFRs.

4. The Q-cycle mechanism for SQR/FQR

Based on the previous considerations, besides the flavin and the iron–sulphur clusters, two low-spin haems and two quinone binding sites are assumed to be present in dihaemic SQRs/QFRs. In our perspective the role of all these prosthetic groups has to be functional. A Q-cycle type mechanism [16],

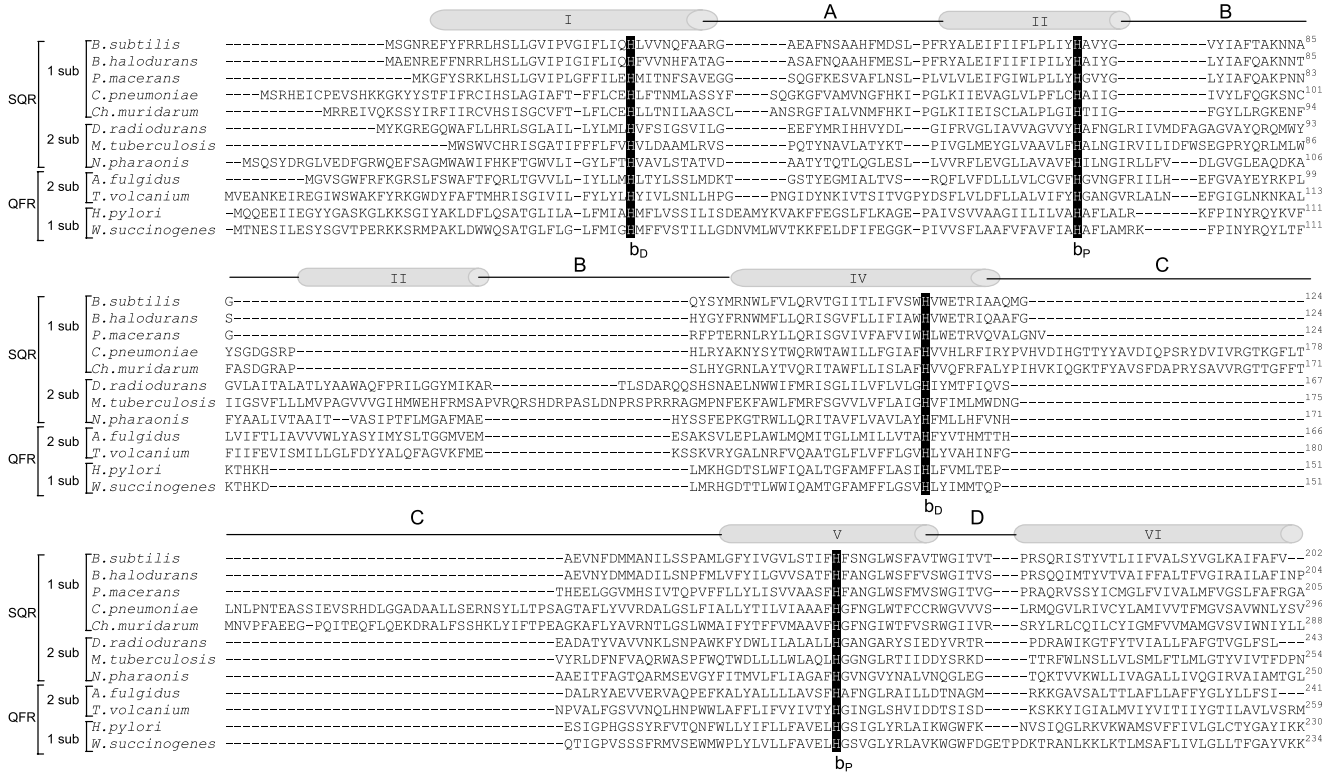

Fig. 2. Amino acid sequence alignment (accession numbers in parentheses) of subunit C and subunits C+D from *B. subtilis* (CAA99546), *B. halodurans* (NP_243959), *P. macerans* (CAA69871), *C. pneumoniae* AR39 (NP_445621), *C. muridarum* (NP_297255), *D. radiodurans* (NP_294678, NP_294677), *M. tuberculosis* (NP_337946, NP_337945), *N. pharaonis* (T44959, T44960), *A. fulgidus* (NP_069518, NP_069517), *T. volcanium* (NP_111264, NP_111263), *H. pylori* (Q9ZMN9), *W. succinogenes* (P17413). Alignments were performed using Clustal W Version 1.6 [24] and manually adjusted.

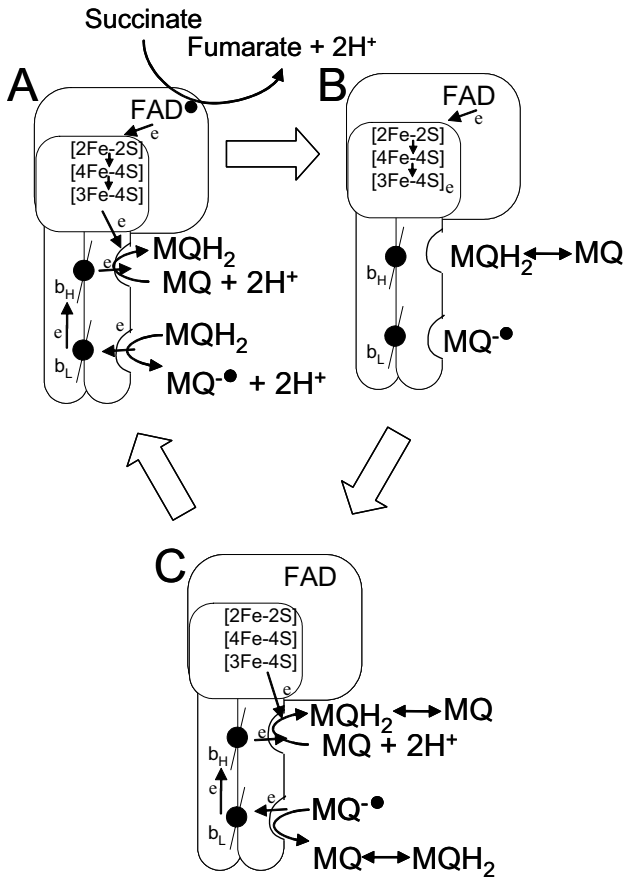

Fig. 3. Schematic representation of the Q-cycle mechanism model for dihaemic SQRs/QFRs. See text for details.

working inversely to that proposed for the bc_1 complexes, is a plausible model for a functional role of all the prosthetic groups present in dihaemic SQR/QFR. Such a model was previously discussed [5]. Here we present a more detailed hypothesis for such a Q-cycle mechanism, using the more extensive experimental data gathered up to date. Fig. 3 shows schematically such a mechanism in dihaemic SQRs. We postulate that the two quinone binding sites have different affinities for menaquinone and menaquinol, similarly to the situation in the bc_1 complex (e.g. [17]). Q_P has preference for menaquinone, while Q_D has a higher affinity for menaquinol, being also able to stabilise the semimenaquinone form. We propose the presence of two electron pathways: one from the FAD through the iron–sulphur clusters to the Q_P site and the other from the Q_D site through the haems b_L and b_H to the Q_P site. At the beginning of a cycle Q_P is occupied with a menaquinone molecule, while in Q_D a menaquinol molecule is present. The presence of succinate starts the reaction by giving two electrons to the flavin and producing fumarate and two H^+. One electron stays in the flavin and the other passes through the iron–sulphur clusters to the menaquinone bound at the Q_P site, which becomes totally reduced by haem b_H (Fig. 3A). By this process a molecule of menaquinol is formed with the concomitant consumption of two H^+. Once haem b_H is oxidised, it is rereduced by haem b_L, which in its turn is rereduced by the menaquinol molecule at Q_D (Fig. 3A). This menaquinol is now in the form of semimenaquinone and two protons were produced; at the same time the menaquinol present in Q_P exchanges with the menaquinone pool and a new menaquinone molecule is now at this site (Fig. 3B). Due to their reduction potentials the second electron that stayed at the flavin should be by now at the [3Fe–4S] centre (Fig. 3B). The cycle is only complete when this electron and another from haem b_H reduce the new menaquinone molecule at Q_P to menaquinol with the consumption of two more protons (Fig. 3C). The rereduction of this haem occurs by the semimenaquinone at Q_D in the same manner as described for the first half cycle, i.e. through haem b_L and at the same time the menaquinol molecule at Q_P exchanges again with the menaquinone pool (Fig. 3C). After the exchange of the menaquinone at Q_D by a menaquinol molecule the enzyme is ready for a new cycle. The mechanism for quinol fumarate oxidoreductases should be exactly the inverse.

To summarise, we propose that in a turnover situation, as the one depicted in Fig. 3, the prosthetic groups are always reduced, being the menaquinone at Q_P almost simultaneously reduced by the [3Fe–4S] centre and haem b_H.

This Q-cycle mechanism where a semimenaquinone is stabilised at Q_D is compatible with the *B. subtilis* SQR behaviour with HQNO, an inhibitor analogous to semimenaquinone. The presence of HQNO in this enzyme affects the spectral and redox properties of haem b_L [18]. Also the possibility that haem b_H is reduced by haem b_L explains the observation that in the isolated *B. subtilis* SQR membrane anchor the haem b_H can be reduced by succinate in the presence of catalytic amounts of purified SQR and DMN (a low reduction potential menaquinone analogue), but not, in the same conditions, in a membrane anchor domain lacking haem b_L [5].

5. Proton translocation balance of the Q-cycle mechanism

Each cycle of the proposed mechanism involves four electrons and eight H^+. Two electrons come from the oxidation of succinate to fumarate and the two others from the oxidation of the menaquinol molecule at the Q_D site to menaquinone. These four electrons reduce two molecules of menaquinone at the Q_P site to menaquinol. From the eight H^+ involved in the reactions two are produced by the oxidation of succinate and two other by the oxidation of the menaquinol molecule at the Q_D site. The other four are consumed by the reduction of the two molecules of menaquinone at the Q_P site. If the protons involved in the reduction and oxidation of the quinones and quinols are taken up and released to the respective sides of the membrane, i.e. if at Q_D protons are released to the periplasm and if at Q_P protons are taken up from the cytoplasm, there would be a net proton translocation by SQRs according to the following equation,

$$\text{succinate} + 2MQ_{in} + 4H^+_{in} + MQH_{2out} \rightleftarrows \text{fumarate} + 2H^+_{in} + 2MQH_{2in} + MQ_{out} + 2H^+_{out}$$

or,

$$\text{succinate} + 2MQ_{in} + 2H^+_{in} + MQH_{2out} \rightleftarrows \text{fumarate} + 2MQH_{2in} + MQ_{out} + 2H^+_{out} \quad (1)$$

In *B. subtilis* it was shown that SQR activity is higher in the presence of a membrane potential, since succinate:oxygen oxidoreductase activity is lower in membranes than in intact cells

[19]. Also Schirawski and Unden [20] have observed in cells from *B. subtilis* and related bacteria that succinate:oxygen oxidoreductase activity is suppressed upon addition of ionophores. This was interpreted as the need of a membrane potential to promote the thermodynamically unfavourable electron transfer from succinate to menaquinone. However, recently Azarkina and Konstantinov [21] observed that in *B. subtilis* the loss of activity by suppression of the membrane potential was not exclusive of succinate:menaquinone oxidoreductase, but rather a general effect of several dehydrogenases dealing with menaquinone. With their results the authors proposed a regulatory common effect of the membrane potential on those enzymes in *B. subtilis*.

In the case of QFRs a decrease of the membrane potential would be observed. Recently it was shown in a reconstituted system that *W. succinogenes* QFR is electrogenically neutral [22]. If in the quinol:fumarate oxidoreduction (inverse to that described above) the protons involved in the oxidation of the menaquinol molecule at the Q_P site were not released to the cytoplasm, but instead could go through some proton channel close to the Q_D site where they could be used in the reduction of the menaquinone molecule at this site, Eq. 1 would be written in the following manner with a $\Delta pH = 0$.

$$\text{succinate} + 2MQ_{in} + 2H^+_{in} + 2H^+_{ch} + MQH_{2out} \rightleftarrows$$
$$\text{fumarate} + 2H^+_{in} + 2MQH_{2in} + MQ_{out} + 2H^+_{ch}$$

or,

$$\text{succinate} + 2MQ_{in} + MQH_{2out} \rightleftarrows$$
$$\text{fumarate} + 2MQH_{2in} + MQ_{out} \quad (2)$$

The existence of such a channel would allow the QFR not to decrease the proton gradient. Recently, the possible existence of a proton channel in the membrane subunit of *W. succinogenes* was proposed, in the context of a completely different hypothesis and based on the crystallographic structure [23]. A glutamate residue (E180 *W. succinogenes* numbering) was proposed to be part of such a channel.

6. Advantages of the Q-cycle

The presence of a Q-cycle type mechanism in dihaemic SQRs provides a functional role to all prosthetic groups of these enzymes and allows the cells to possess another energy conserving respiratory complex. Nature tends to optimisation and thus it would be more advantageous to have an enzyme, involved in the energetic metabolism, with such a functional mechanism that allows energy conservation, than with an energy dissipating mechanism. As discussed previously a Q-cycle type mechanism would result in the net translocation of $4H^+$ in SQRs, while a mechanism involving only one quinone binding site near haem b_L would contribute to the dissipation of the ion motive force by $4H^+$. This hypothesis is testable experimentally and allows designing new experiments to unravel the functional mechanism of these enzymes.

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