Minireview

A structural model for the membrane-integral domain of succinate:quinone oxidoreductases

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Abstract **Many succinate:quinone oxidoreductases in bacteria and mitochondria, i.e. succinate:quinone reductases and fumarate reductases, contain in the membrane anchor a cytochrome *b* whose structure and function is poorly understood. Based on biochemical data and polypeptide sequence information, we show that the anchors in different organisms are related despite an apparent diversity in polypeptide and heme composition. A general structural model for the membrane-integral domain of the anchors is proposed. It is an antiparallel four-helix bundle with a novel arrangement of hexa-coordinated protoheme IX. The structure can be applied to a larger group of membrane-integral cytochromes of *b*-type and has evolutionary and functional implications.**

Key words: Succinate dehydrogenase; Fumarate reductase; Cytochrome *b*; Heme protein; Quinone reductase

1. Introduction

Succinate:quinone reductase (SQR; EC 1.3.5.1) in aerobic cells is a functional part of both the Krebs' cycle and the respiratory chain. This membrane-bound enzyme is also called complex II or succinate dehydrogenase. It catalyses the oxidation of succinate to yield fumarate and reduces quinone. SQR in many organisms contains cytochrome of *b*-type [1,2]. The function and structure of this cytochrome is poorly understood as compared to that of the cytochrome *bc1bf* (complex III, quinol:cytochrome *c* reductase) and cytochrome *c* oxidase (complex IV) [3–5].

Anaerobic cells respiring with fumarate as terminal electron acceptor contain quinol:fumarate reductase (QFR) [6,7]. This enzyme is very similar to SQR in composition, and probably also in structure; in vitro QFRs and SQRs can generally both catalyse succinate oxidation and fumarate reduction, but at different rates. In this paper we collectively refer to SQRs and QFRs as succinate:quinone oxidoreductases (for review see [1,2,8,9]).

Succinate:quinone oxidoreductases consist of a peripheral domain, exposed to the cytoplasm in bacteria and to the matrix in mitochondria, and a membrane-integral domain that spans the membrane (Fig. 1). The peripheral domain, which contains the dicarboxylate binding site and several redox (electron-transfer) components, is very conserved with respect to composition and primary sequence of polypeptides when the enzymes from different organisms are compared. It is composed of a flavoprotein (Fp; 64–79 kDa) subunit, with one covalently bound FAD, and an iron-sulfur protein (Ip; 27–31 kDa) subunit containing one each of a [2Fe-2S], a [3Fe-4S], and a [4Fe-4S] cluster. The membrane-integral domain functions to anchor the Fp and Ip subunits to the membrane and is required for quinone reduction and oxidation. This anchor domain consists of one larger (SdhC/FrdC; 23–30 kDa) or two smaller (C_{II-3}/SdhC/FrdC and C_{II-4}/SdhD/FrdD; 13–18 and 11–16 kDa) hydrophobic polypeptides containing one or two protoheme IX groups with hexa-coordinated iron, or no heme at all. When two hemes are present they have different spectral and thermodynamic properties. In addition to this variability in macromolecular composition the amino acid sequences of the membrane-anchor polypeptides show poor apparent similarity. For example, sequence comparisons using conventional computer programs like TFASTA [10] often do not result in the identification of the anchor polypeptides (cf. [11]).

In this minireview we present an update on the membrane-anchor proteins of succinate:quinone oxidoreductases from different organisms. It is shown that they are related and we propose and discuss a general structural model for the membrane-integral domain of succinate:quinone reductases.

2. Classification of membrane anchors

The membrane-anchor domain of succinate:quinone oxidoreductases can be classified into 4 types, here denoted A–D, based on differences in polypeptide and heme composition (Table 1). Type A, so far only represented by the *Thermoplasma acidophilum* SQR, has two polypeptides and two heme groups. Type B has a single polypeptide and two hemes. Examples of this type are *Bacillus subtilis* SQR and *Wolinella succinognes* QFR. Type C, with two polypeptides and one heme, seems to be the most common type and is found in mammalian mitochondrial and *Escherichia coli* SQR. Type D anchors contain two polypeptides without heme. This latter type is present in *E. coli* QFR, *S. acidocaldarius* (*Sulfolobus* sp. strain 7) SQR and seemingly in *Saccharomyces cerevisiae* SQR.

3. Transmembrane topology of anchor polypeptides

It is known from surface-labelling experiments with mammalian and *B. subtilis* SQR in situ that the anchor domain spans the mitochondrial inner membrane and the cytoplasmic membrane, respectively [1,2]. The two polypeptides in type A, C and D anchors both show a common pattern of 3 stretches

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with hydrophobic amino acids predicted to constitute transmembrane α-helical segments in the membrane-spanning folded proteins. The single polypeptide anchors, i.e. those of type B, contain 5 such predicted transmembrane segments. Experimental data with the *B. subtilis* anchor polypeptide, SdhC, confirm the presence of 5 transmembrane segments in this protein and show that the N-terminal end is exposed to the cytoplasm [12]. The topology of this protein and those predicted for the *T. acidophilum* and *E. coli* SdhC and SdhD polypeptides are illustrated in the top part of Fig. 2.

4. The cytochrome b component

The single heme in mammalian and *E. coli* SQR and the two hemes in *B. subtilis* SQR have bis-histidine axial ligation as demonstrated by EPR spectroscopy combined with low-temperature near-infrared MCD spectroscopy [13–15]. The ligand histidine residues have been identified in the primary sequence of the *B. subtilis* and *E. coli* SQR anchor polypeptides by site directed mutagenesis analysis [12,16,17]. These residues are located in predicted transmembrane segments as shown in Fig. 2 (top panel). In the mono-heme *E. coli* SQR one ligand is provided by SdhC and the other by SdhD, i.e. the heme is ligated by both polypeptides [17]. In the diheme anchors the heme axial ligands are distributed over 4 transmembrane segments.

5. Primary structure comparisons

Fig. 3 is an alignment of the deduced primary sequences of anchor polypeptides from 16 different succinate:quinone oxidoreductases. The alignment is based on the conserved pattern of predicted transmembrane segments and the histidine residues identified as axial ligands in *B. subtilis* and *E. coli* SQR. For the purpose of comparison we have included sequences from three type D anchors, namely those of *S. cerevisiae* SQR (ScS), *E. coli* QFR (EcF) and *Haemophilus influenzae* QFR (HiF). Several conclusive features are apparent from the alignment: (i)Two histidines, one in helix II and one in helix V, known to ligate the high potential heme (b_H) in *B. subtilis* SQR and the single heme in *E. coli* SQR, are present in all the heme-containing anchors, i.e. those of types A–C. The two histidines in helices I and IV that ligate heme b_L in *B. subtilis* SQR, are only present in the type A and B anchors (BsS, WsF and TaS). This confirms the assignment of axial ligands and makes it possible to predict the heme content from primary sequence in those anchors for which no biochemical data is available. (ii) Helix III is absent in the type B anchors (Figs. 2 and 3). (iii) The hydrophilic segments on the negative side of the membrane, i.e. the N-terminal ends and the (inside) loops connecting transmembrane segments, are rich in residues with positively charged side chains that can act as stop-transfer signals when the polypeptide is inserted into the membrane. The eucaryotic anchor polypeptides, i.e. those of *B. taurus* and *S. cerevisiae*, are as deduced from the DNA sequence synthesised with a N-terminal, cleaved, extension of about 30 residues that presumably functions as a mitochondrial inner-membrane targeting sequence (Fig. 3) [18–20]. The N-terminal end of the bacterial polypeptides function as signal peptides [12] but are essentially not processed, i.e. four residues are removed from *E. coli* SdhC [21] and the initiating-methionine in *B. subtilis* SdhC [22] is removed in about 50% of the cases. (iv) Proline and glycine residues are frequent in the hydrophilic segments, supporting the view that these parts are structurally flexible to function as connecting loops. (v) Conserved motifs (marked in Fig. 3) are present and mainly in segments exposed on or close to the negative side of the membrane. This probably reflects the fact that these parts interact with or directly bind to the Fp and Ip subunits. It is known that both polypeptides in type C and D anchors are required for the binding of Fp and Ip [1,17]. A mutation (the conserved $G_{168} \rightarrow D$) in the loop between helices V and VI in *B. subtilis* SdhC blocks binding of Fp and Ip [2,12]. Two carboxyl groups and one histidine residue in the bovine SdhD polypep-

Table 1
Types of membrane anchors in succinate:quinone oxidoreductases

Type of anchor	No. of polypeptides	No. of hemes	Example of enzymes of respective type	Quinone[a]	Properties of cytochrome[b]		References
					E_m (mV)	EPR signal(s) (g_{max})	
A	2	2	*Thermoplasma acidophilum* SQR	CQ	b_H; +75 b_L; −150	3.2	[46,47]
B	1	2	*Bacillus subtilis* SQR	MQ	b_H; +65 b_L; −95	b_H; 3.68 b_L; 3.42	[48]
			Wolinella succinogenes QFR	MQ	b_H; −20 b_L; −200	n.d.	[49]
C	2	1	*Bos taurus* heart SQR	UQ	−185	3.46	[15,50]
			Escherichia coli SQR	UQ	+36	3.63	[14,51]
			Paracoccus denitrificans SQR	UQ	−175[c]	n.d.	[52]
			Adult *Ascaris suum* QFR	RQ	−34	3.6	[7,53]
D	2	0	*Sulfolobulus acidocaldarius* SQR	CQ			[54,55]
			Escherichia coli QFR	MQ			[1,56]
			Saccharomyces cerevisiae SQR[d]	UQ			[57]

[a]CQ, caldariella quinone; MQ, menaquinone; UQ, ubiquinone; RQ, rhodoquinone.
[b]The redox data are for the isolated enzyme or cytochrome at pH 7–7.5. b_H and b_L denote the high- and low-potential cytochrome component, respectively. n.d., no data available.
[c]M. Matsson and L. Hederstedt, unpublished data.
[d]It still needs to be confirmed that yeast SQR lacks heme [19].

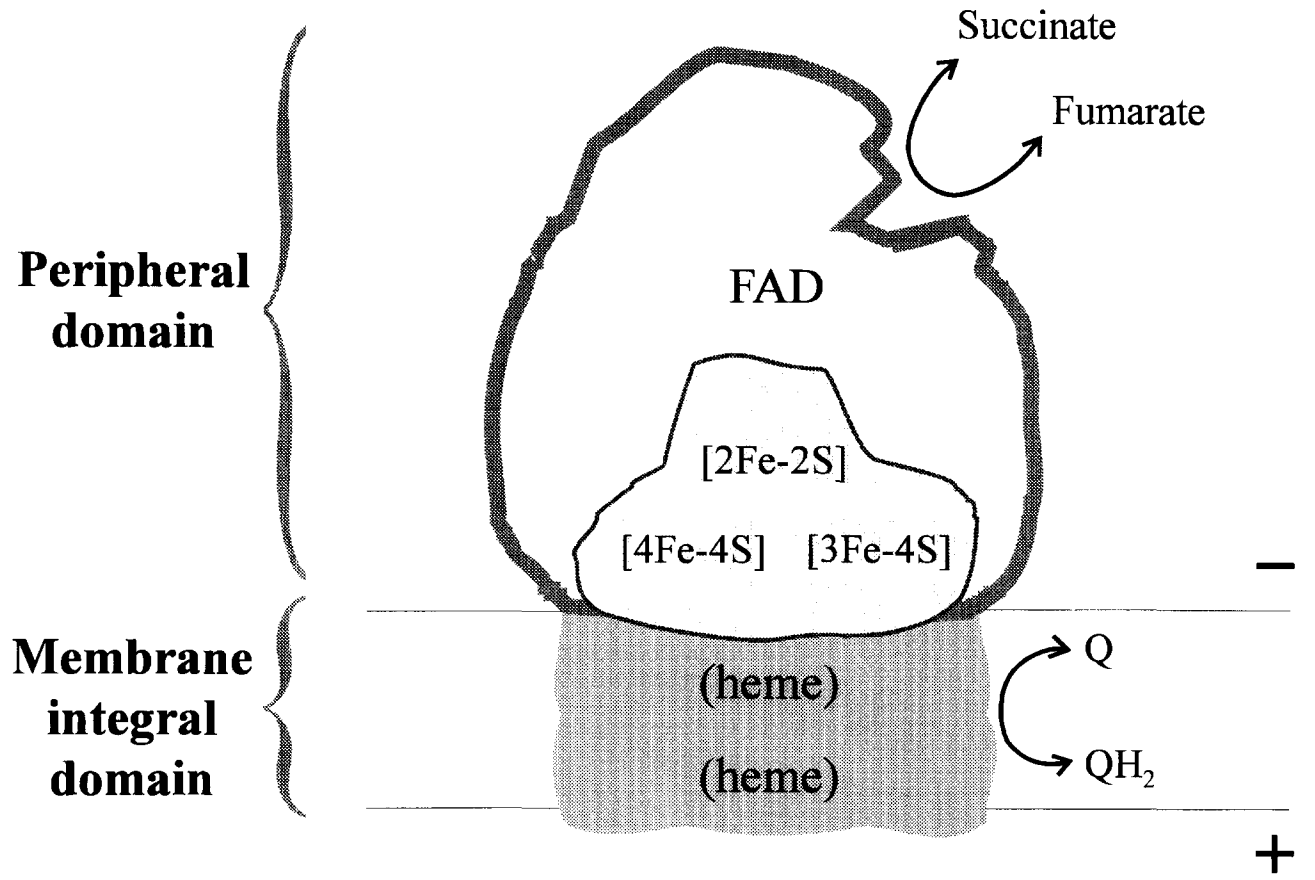

Fig. 1. Composition and organisation of succinate:quinone oxidoreductases.

tide are important for binding the peripheral domain [23,24]. Conserved residues involved in quinone binding are also expected. The loop between helices II and III has been pinpointed in bovine SdhC using photoaffinity labelling of SQR with a quinone analogue [25]. Mutations in the outer loop close to helix V in *E. coli* FrdD ($F_{57} \rightarrow V$, $Q_{59} \rightarrow V$, and $S_{60} \rightarrow A$) affect quinone oxidoreductase activity [26]. Type B anchors, composed of one polypeptide (i.e. BsS and WsF), show common motifs different from those of the two subunit anchors. These type B anchors both interact with menaquinone (Table 1). (vi) SdhC and SdhD polypeptides show sequence similarity. For example, the H/Q R/AxxG/A motif in the beginning of helices I and IV, and the HxxxGxxxxxx D motif at the end of helices II and V. This suggests that the *sdhC* and *sdhD* genes have originated by duplication of a primordial gene. The *sdhC* and *sdhD* genes as well as the *frdC* and *frdD* genes are adjacent in bacteria, and cotranscribed in the direction *C* to *D* [1,2]. In eukaryotic organisms the genes for Fp, Ip and the anchor polypeptides are generally encoded by unlinked nuclear genes (cf. [19,20,27–29]). However, very recently it has been found that the mitochondrial genomes of the photosynthetic red algae *Porphyra purpurea*, *Chondrus crispus* and *Cyanidium caldarium*, the zooflagelate *Rectinomonas americana* and the liverworth *Marchantia polymorpha* carry genes for SQR anchor polypeptides and in some cases also the gene for Ip [30,31]. In *P. purpurea* and *R. americana* the *sdhC* and *sdhD* genes are arranged as in bacteria, whereas in *Ch. crispus* and *M. polymorpha* they are separated. The SdhC sequences deduced from these mitochondrial genes are similar to those of nuclear and bacterial genes. In contrast, the corresponding SdhD (PpS, CrS, MpS) deviate in sequence from other SdhD polypeptides, i.e. the loop between helices IV and V is missing and the sequence at the C-terminus is very polar. The deduced sequences of the mitochondria encoded *sdhC* and *sdhD* genes lack the above-mentioned N-terminal targeting sequence found in the nuclear encoded anchor proteins. It remains to be determined whether the mitochondrial *sdhC* and *sdhD* encode functional proteins or if they are pseudo-genes. (vii) Helices III and VI are overall poorly conserved in sequence, indicating that they do not play central roles in the function of the anchors. *E. coli* QFR anchor polypeptide mutants lacking one or two complete helices at the C-terminal end show substantial quinone reductase activity but are impaired in binding the peripheral domain [32].

6. A 3-dimensional structural model

Predictions of 3-dimensional structure of proteins from primary sequence data are rather ambiguous but much less so for the membrane-integral domain of α-helical polytopic membrane-proteins. This is due to the 2-dimensional restriction in space impeded by the membrane bilayer. If the transmembrane topology of the different α-helical segments is known and the number of helices is small only a few structural variants are possible. This number of variants is further restricted when ligands to prosthetic groups have to be taken into account in the structure. Accordingly, the four heme-ligating transmembrane helices of the diheme *B. subtilis* membrane anchor (type B) can principally be arranged in only three alternative ways. The preferred structural model is

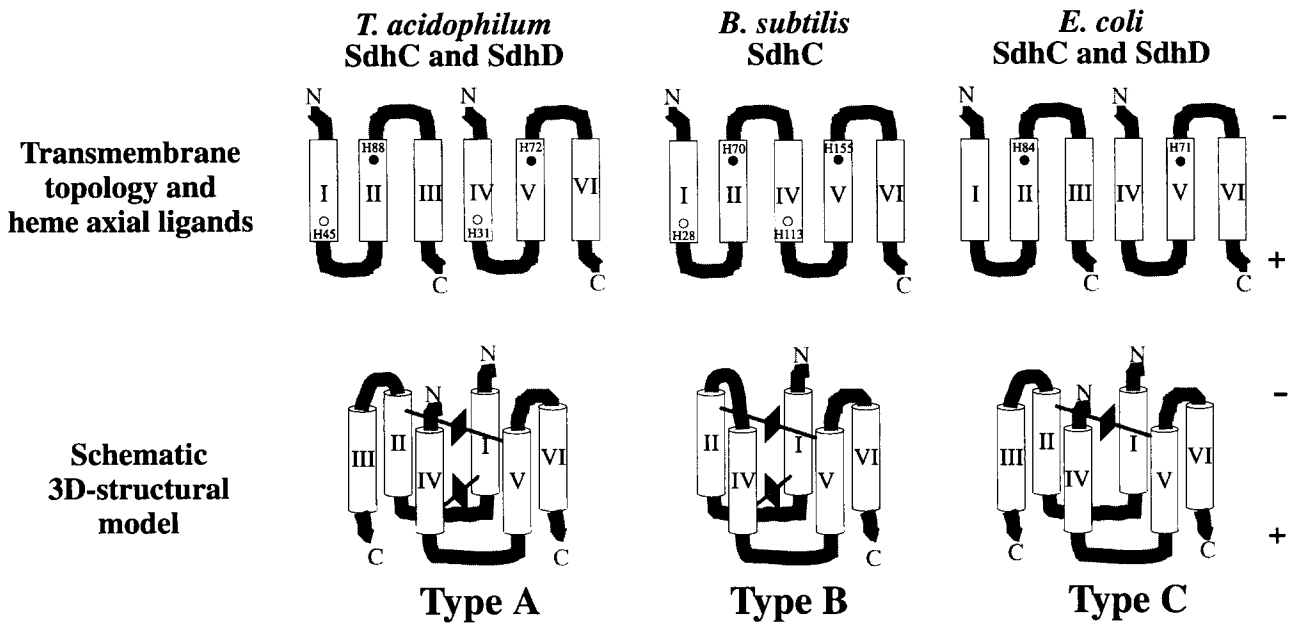

Fig. 2. Proposed structure of the membrane-integral domain. The upper part of the figure shows the topology of *B. subtilis* SdhC in the membrane as verified experimentally [12] and that for the others as predicted (see text for details). The location of histidine residues identified as heme-iron axial ligands in the *B. subtilis* [12] and the *E. coli* [17] proteins are indicated. Those of *T. acidophilum* are deduced from sequence data (see Fig. 3). The ligands to heme b_H and heme b_L in the diheme proteins are indicated ● and ○, respectively.

that which gives a conserved structure for all succinate:quinone oxidoreductases, which does not require complicated folding of hydrophilic loops and which is consistent with accumulated experimental data.

Shown in Fig. 2 (bottom middle) is the 3-dimensional structural model that we find most likely to be correct for the *B. subtilis* membrane anchor (type B). It also applies to type A and C anchors (Fig. 2) and probably those of type D (not shown). Figures of rejected alternative structures are available from the authors upon request; they require that the loops between helices I and II and between IV and V intersect or are difficult to envisage from an evolutionary point of view (discussed below).

The core of the proposed structure is a four helix anti-parallel bundle comprised of helices I, II, IV and V. The heme group(s) is oriented approximately perpendicular to the membrane plane. The two hemes in the type A and B anchors are essentially positioned on the same transmembrane vector but in perpendicular orientation to each other. The large g_{max}-type low-spin EPR signal from heme iron (Table 1) indicates a nearly perpendicular relative orientation of the planes of the two imidazole groups being axial ligands to each heme and a geometrically somewhat 'strained' bond between the tele-nitrogen of histidine and the iron atom [13,33]. The heme molecules in the bundle are most likely oriented such that the propionate side groups are at the membrane surface making electrostatic or hydrogen bonding to residues at the end of transmembrane segments or in loops, as is the case in cytochrome oxidase [3]. We wish to emphasise that the model is only schematically presented in Fig. 2, to illustrate clearly the organisation and topology of the transmembrane segments and the ligation of hemes. In the authentic membrane-bound protein helices II and V can be somewhat tilted relative to both the membrane plane and each other; i.e. the ends of these helices can be closer together at the negative side of the membrane to ligate heme b_H and more distant at the positive side of the membrane to accommodate the heme b_L molecule. Helices I and V would be arranged correspondingly to ligate heme b_L and leaving room for heme b_H. Modelling work with the proposed structure has not provided geometric arguments against the structure. Degli Esposti et al. [34] have previously suggested a four-helix bundle structure for the membrane anchor of *W. succinogenes* QFR with the two hemes at its centre, but that structure is different from the one we propose here since it was based on another transmembrane topology for the anchor polypeptide.

The position of helix VI in the type B anchors, and helices III and VI in that of types A and C, cannot be predicted with any certainty. We have tentatively chosen to put these helices where they could interact with N-terminal ends and act as 'hydrophobic zippers' to stabilise the four helix bundle (Fig. 2). Hexa-coordinated heme probably stabilises the structure by 'cross-linking' the helices in the bundle. The two heme groups in type B anchors may be needed to compensate for the lack of helix III. Helix III might for a similar reason stabilise the type C and D anchors lacking one and both hemes, respectively.

In Fig. 4 the proposed four helix anti-parallel bundle is presented in the form of a helical wheels plot with the type A anchor of *T. acidophilum* SQR as an example. A 2-fold axis of symmetry is apparent in the structure, most clearly seen in the constellation of conserved residues in the top segment around heme (b_H). Statistical calculations using the sequence data for the heme-containing anchors, and that of *S. cerevisiae*, show that hydrophobic residues are predominantly present on the outer surface of the bundle facing lipids (Fig. 4). Several conserved residues are oriented towards the centre of the bundle, close to heme (b_H). The residues in the bottom segment in type C anchors are less conserved and have more bulky side chains as compared to those in types A and B (Figs. 3 and 4). This is consistent with the lack of heme b_L in the type C anchors. It should be noted that the type C anchor sequences dominate in the results of the statistical analysis shown in Fig. 4.

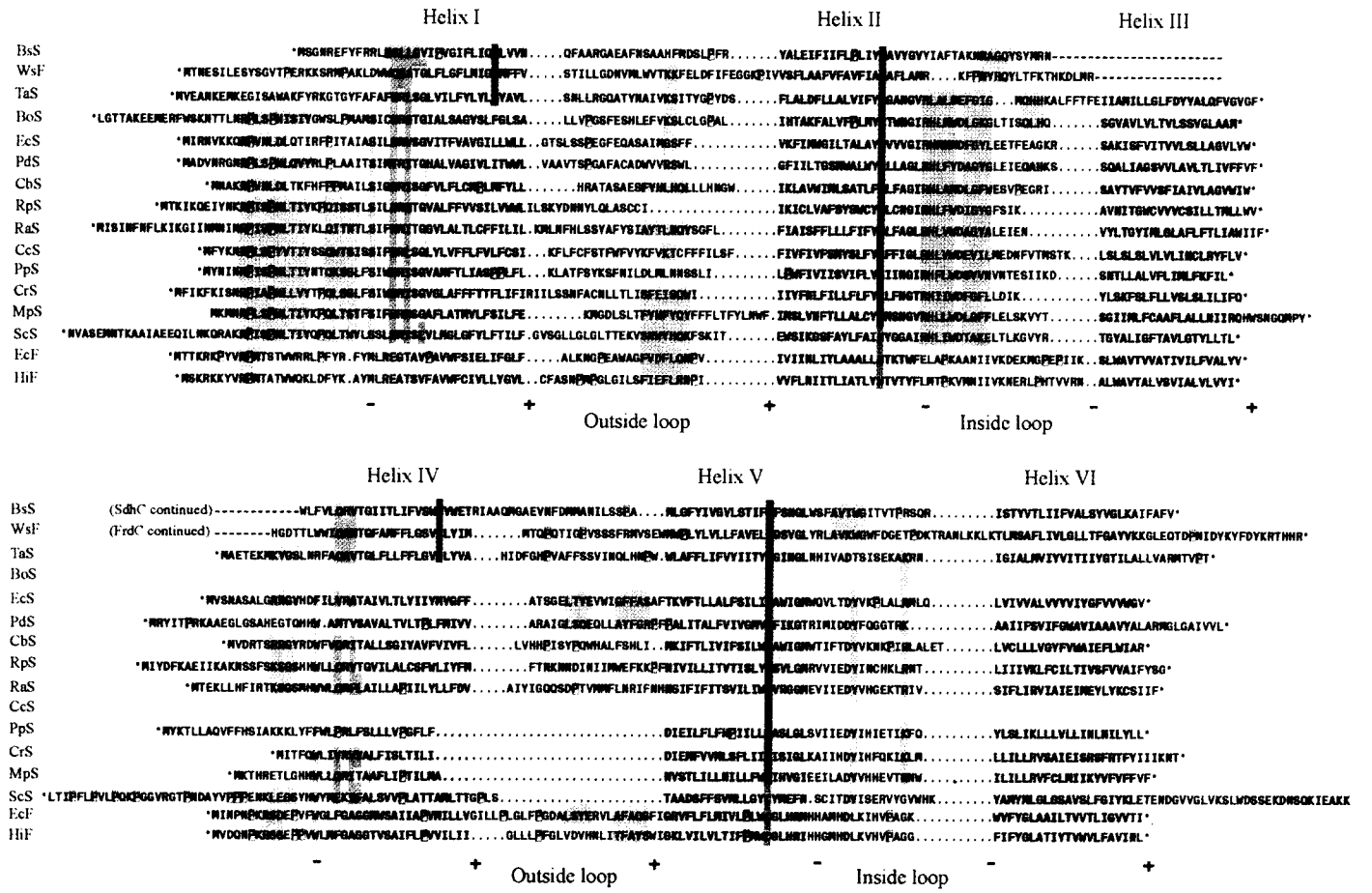

Fig. 3. Sequence alignment of succinate:quinone oxidoreductase anchor polypeptides. Stars indicate the start and end of polypeptides. Amino acid residues are in the one-letter code. The alignment is based on histidines (marked in red) serving as axial ligands to heme iron and located in predicted transmembrane α-helical segments (marked in green). Membrane-spanning segments are for simplicity defined as 20 residues in length, mainly with hydrophobic residues and preferentially with aromatic residues at the ends. Sequences in the loops connecting transmembrane segments are aligned with the bias to show similarities and avoid gaps. Conserved motifs are marked in blue and those present in both anchor polypeptides are indicated in yellow. Inside is the matrix side in mitochondria and the cytoplasm in bacteria and is the side on which the peripheral domain is bound. The organisms, enzyme and accession numbers (Genebank or Swiss Prot) or reference are: BsS, *Bacillus subtilis* SQR (P08064); WsF, *Wolinella succinogenes* QFR (P17413); TaS, *Thermoplasma acidophilum* SQR (X70908); BoS, *Bos taurus* SQR (P35720); EcS, *Escherichia coli* SQR (P10466, P10445); PdS, *Paracoccus dentrificans* SQR (U31902); CbS, *Coxiella burnetii* (SQR (L33409); RpS, *Rickettsia prowazekii* SQR P41085, P41086); RaS, *Rectinomonas americana* SQR [30]; Ccs, *Cyanidum caldarium* SQR (P48935); PpS, *Porphyra purpurea* SQR [30]; CrS, *Chondrus crispus* SQR (P48934); MpS, *Marchantia polymorpha* (P35721, M68929); ScS, *Saccharomyces cerevisiae* SQR (P33421, P37298); EcF, *E. coli* QFR (P03805, P03806); HiF, *Haemophilus influenzae* (QFR (P44892, P44891). DNA sequence information is also available for *Proteus vulgaris frdCD* [58] and for hamster *sdhC* [27]. The deduced polypeptide sequences are very similar to *E. coli* FrdCD and *Bos taurus* SdhC, respectively, and were therefore not included in the alignment. The N-terminus of the BsS [22], BoS [18], and ScS SdhC [20] and SdhD [19] polypeptides as shown in Fig. 3 is that determined by analysis of polypeptide isolated from the respective enzyme.

The model is supported by various experimental data but due to page limitations only some of these can be mentioned here. For example, the $H_{13} \rightarrow Y$ mutation in helix I in the *B. subtilis* anchor strongly affects the properties of heme b_H but not those of heme b_L [12]. The position of H_{13} in the structural model corresponds to I:2 (the second residue in helix I as viewed from the negative side of the membrane) assuming that the proline residue in helix I is an α-helix breaker as suggested from the sequence alignment (Fig. 3). In the helical wheel plots we have otherwise not considered proline residues in predicted transmembrane segments as helix-breakers. The anchor polypeptides of *E. coli* QFR have been subjected to extensive random and site specific mutagenesis [26,32,35,36]. Among residues identified as important for quinone reduction and/or quinol oxidation are E_{29} (I:2), A_{32} (I:5), H_{82} (II:6), W_{86} (II:2), F_{87} (II:1) and H_{80} (V:3). These residues have been proposed to contribute to a 'Q_B site' [26] and are (with the exception of F_{87}) clustered in the structural model. Residues of the postulated 'Q_A site' are the in section **5** mentioned residues in the outside loop between helices IV and V [26]. A few identified functionally important residues like F_{38} (I:11) would be positioned on the outside of helix I in the middle of the membrane and interact with helix VI.

7. Implications from the sequence comparisons and the model

From the data presented in this paper one can speculate on how the membrane-anchor domains of succinate:quinone oxidoreductases have evolved. The ancestral anchor might first have been comprised of a homo-dimer of a polypeptide similar to today's SdhC polypeptides. This protein most likely carried the histidine residue corresponding to that in helix II of present anchors, and could also have contained the one in helix I of present type A and B anchors, and the dimer pos-

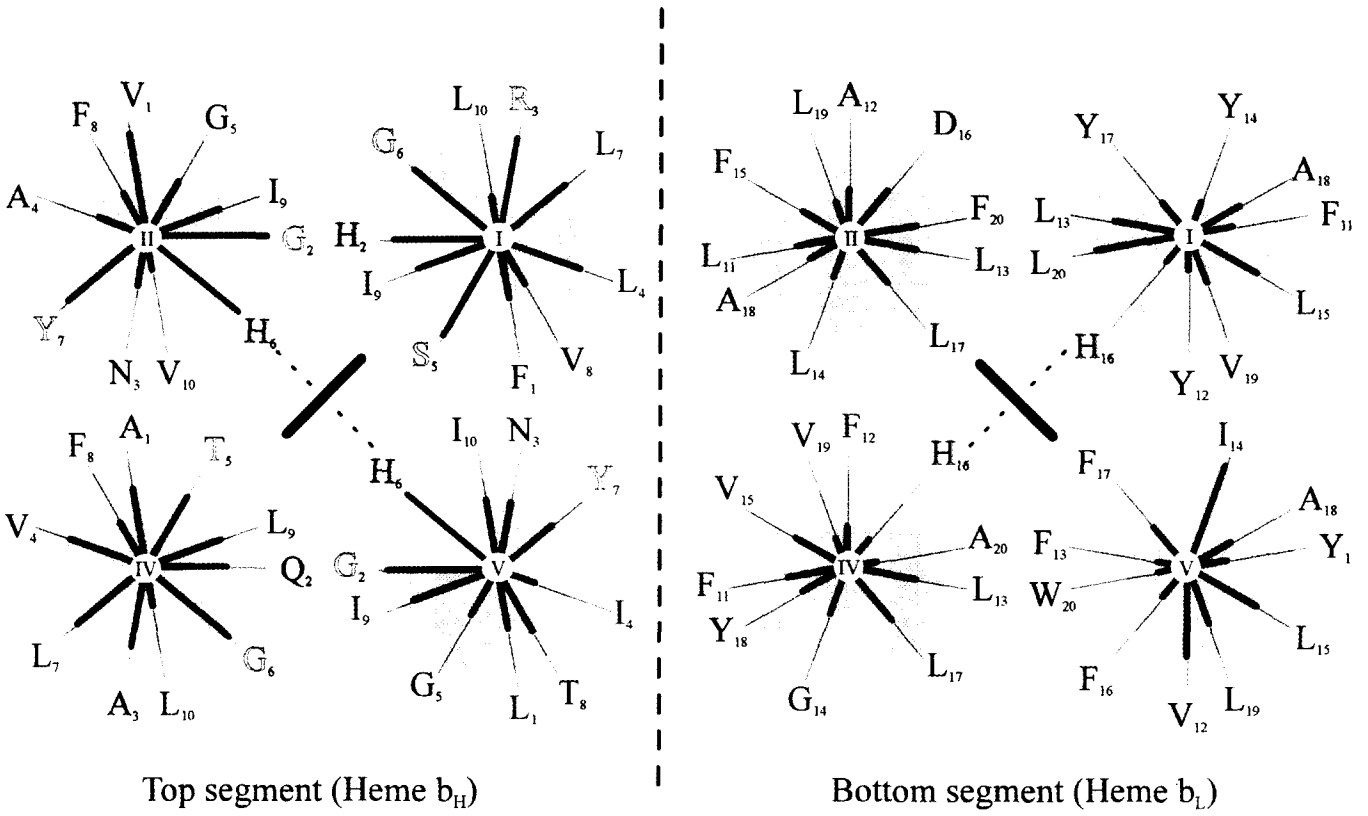

Fig. 4. Helical wheels plot of transmembrane segments I, II, IV and V in the structural model. The sequence of the *T. acidophilum* membrane anchor domain is shown as an example and is combined with statistical data. Each helix comprises 20 residues (those indicated in Fig. 3), is viewed down from the negative side of the membrane, and is presented as two segments; top segment (residues 1–10) and bottom segment (residues 11–20). Amino acids are in the one-letter code and coloured consistent with Fig. 3. The residues in each helix are numbered from the negative side to the positive side. The top segment contains the histidine ligands to heme b_H and the bottom segment those of heme b_L. The bars extending from the centre of each wheel shows the result of a statistical analysis incorporating sequences of all type A–C anchors of Fig. 3 and also that of *S. cerevisiae*, i.e. 14 sequences for helices I and II, and 12 for helices IV and V. Red bars show the frequency of occurrence in percent of the most common amino acid at the respective position (Fig. 3); i.e. the bar for H_6 represents 100%. Blue bars show this frequency when conserved substitutions are taken into account. The calculated hydrophobic moment in each helix is shown in green shading. Conserved substitutions and the general hydrophobicity were calculated as in [59].

sibly contained heme. The gene for this polypeptide was then duplicated and this allowed for divergent evolution of the two polypeptides. This resulted in anchors of the present types A and C. The type B anchor was then derived from a type A anchor by the deletion of helix III and concomitant fusion of the obtained C-terminal to the N-terminal end of helix IV. Such a deletion could easily be accomplished as seen in Fig. 2. Type D anchors can have evolved directly from the ancestral type of polypeptide or from a type C anchor.

The role of heme (cytochrome *b*) in sucinate:quinone oxidoreductases is not understood [37]. Heme is clearly not obligatory for succinate:quinone oxidoreductase activity, as demonstrated by the enzymes with type D anchors and also by e.g. *E. coli* SQR mutants [17], and may thus have other function(s). Electron transfer at significant rates between the peripheral domain and cytochrome *b* has been demonstrated in some cases c.f. [38] but it is not known whether electrons pass via heme upon quinone reduction or oxidation. In vivo experiments with *B. subtilis* and *E. coli* SQR suggest an important role of heme in assembly of the enzyme [17,39]. It is, as mentioned in connection with helix III, plausible that heme functions to stabilise the four helix bundle of the proposed structure. Such a function of heme and metal has been demonstrated with designed water-soluble four helix bundle structures [40,41]. The alignment of the type D anchor polypeptides with those of types A–C is uncertain due to the overall weak sequence similarities (Fig. 3). Notable is that the *S. cerevisiae* (ScS) SdhC polypeptide shows clear sequence similarity to type C anchors, whereas SdhD does not. It cannot be excluded that the sequenced *S. cerevisiae sdhC* [20,42] and *sdhD* [19] genes encode anchor peptides of two different succinate:quinone oxidoreductases; a *sdhD* knockout mutant still contained SQR activity [19]. Generally, however, it seems as if the conserved histidine of helix II (ligand to heme b_H) is present also in type D anchors despite the lack of heme. The histidine in helix V (second ligand to heme b_H) appears to correspond to a cysteine residue in the type D anchors. This raises the question of whether the type D anchors contain some small prosthetic group, e.g. a metal atom such as iron, with the histidine and cysteine residues as ligands. Such a group could, as for heme, stabilise the four helix bundle or play a role in electron transfer to/from quinone. The conserved histidine (H_{82}) in helix II of *E. coli* FrdC has been mutagenised and this affects quinone reductase to various extent depending on the substitution [26,35].

Our proposed model indicates that the cytochromes *b* in diheme succinate:quinone oxidoreductases play some role in transmembrane electron-transfer. The two cytochromes *b* (both with bis-histidine axial ligation) in quinol:cytochrome *c* reductase (complex III) have such a function. The arrange-

ment of the heme groups in the membrane-integral domain of these two enzymes is, however, very different [43]. In quinol:-cytochrome *c* reductase the two hemes are ligated by only two transmembrane helices; each pair of histidines on the two helices are 13–14 residues apart. As a consequence, the two hemes are approximately oriented in the same plane and positioned at a fixed distance, 20 Å [5], from each other. In the diheme succinate:quinone oxidoreductases the relative position of the two hemes can be more flexible. The heme-heme distance might, at least theoretically, be modulated in the enzyme and could be a way to, e.g. regulate electron-transfer rates between the hemes. There are several examples of diheme integral membrane-bound cytochromes *b* with axial heme ligands distributed over more than two transmembrane segments; e.g. cytochrome *b*-561 of chromaffin granule [44] and cytochrome *b* of phagocyte NADPH oxidase [45]. We hope the proposed general structural model of the membrane-integral domain of succinate:quinone oxidoreductases can help in the design of experiments aimed at a better understanding of the structure and function also of these other cytochromes.

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