PERSPECTIVES: BIOENERGETICS

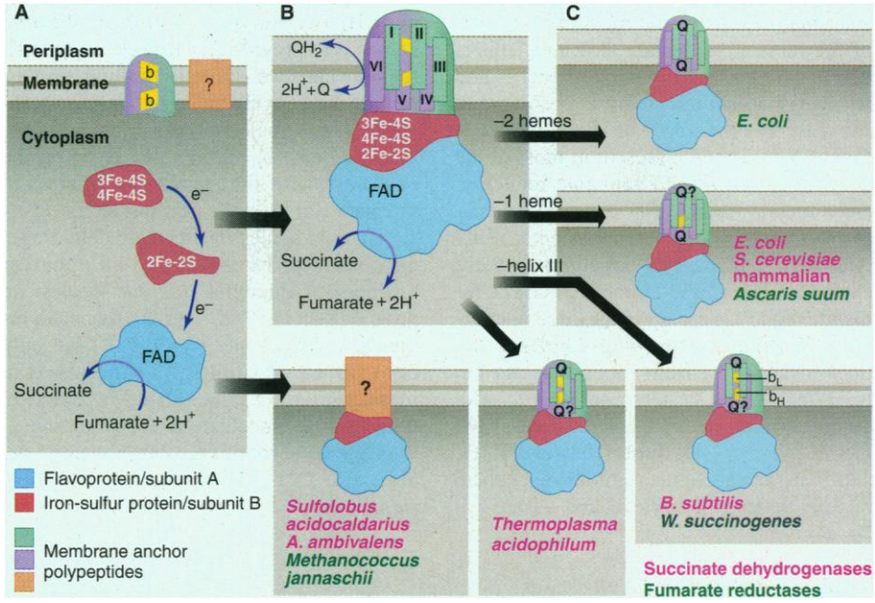
# Respiration Without $O_2$

Lars Hederstedt

Fumarate, a dicarboxylic acid anion, is used for respiration by bacteria and eukaryotic cells that grow under anoxic conditions (*1*). The 3.3 Å x-ray crystal structure of the key enzyme in fumarate respiration, fumarate reductase, is reported by Iverson *et al.* on page 1961 of this issue (*2*). For several reasons, the structure is exciting. It is the first available for a family of membrane-bound enzymes with important function in central carbon metabolism and energy production in cells. They catalyze succinate-fumarate interconversion coupled to the reduction/oxidation of quinone (Q). Succinate dehydrogenase, which is both a citric acid cycle enzyme and respiratory complex II of the mitochondrial respiratory chain, is a member of this family. The fumarate reductase crystal structure can be used as a framework to model succinate dehydrogenase. Such a model, added to the already known complex III, complex IV, and cytochrome c crystal structures, provides a view of the entire mitochondrial succinate oxidase respiratory chain—a site for energy conservation in the cell—in three dimensions at about 3 Å resolution (*3*). In contrast to complexes I, III, and IV, complex II is not considered to be energy coupled. The *Escherichia coli* fumarate reductase structure presented by Iverson *et al.*, and other recent experimental data on members of the same enzyme family, pose new views about the bioenergetic role of complex II–type enzymes.

Facultative bacteria or eukaryotic organisms that live under both oxic and anoxic conditions during their life cycle, such as some parasitic helminths, contain a fumarate reductase and succinate dehydrogenase, but these are not expressed simultaneously (*4*). Although the enzymes in vitro generally can catalyze both Q reduction and quinol ($QH_2$) oxidation, in vivo catalysis only occurs in one direction. Being respiratory enzymes, they are functioning in the cytoplasmic membrane in bacteria and in the mitochondrial inner membrane in eukaryotic cells.

Enzymes of the complex II family are composed of three or four proteins (*5*). Subunit A contains an 8α-*N*(3)-histidyl covalently bound flavin-adenine dinucleotide (FAD) and harbors the dicarboxylate active site. This site is well defined in the x-ray structure of fumarate reductase by one molecule of oxaloacetate, a reversible inhibitor. Subunit B is an iron-sulfur protein with one each of a 2Fe-2S, 3Fe-4S, and 4Fe-4S cluster. Subunits A and B of all enzymes in the complex II family are similar in primary sequence and composition. They form a dimer firmly attached to the membrane by an anchor composed by one larger (subunit C) or two smaller (subunits C and D) membrane-spanning polypeptides and containing two, one, or no protoheme IX molecules, depending on the organism. The AB dimer in the assembled enzyme complex protrudes out from the membrane on the negative side—that is, into the cytoplasm in bacteria and the matrix in mitochondria. There is a minimum of protein mass exposed to the aqueous phase on the positive side of the membrane. The *E. coli* fumarate reductase membrane anchor CD heterodimer does not contain heme, as expected, but surprisingly contains two tightly bound menaquinones. Electron donors to fumarate reductases are generally quinone compounds with a low-midpoint redox potential, such as menaquinone or rhodoquinone (*4, 5*). Flavin, the three iron-sulfur clusters, and the proximal menaquinone ($Q_P$) are arranged in a linear array separated by only 11 or 14 Å, which provides for efficient electron transfer form $QH_2$ in the membrane to fumarate at the dicarboxylate binding site.




**Possible evolution of the complex II enzymes (*5*). (A)** Ancient anaerobic bacterium contained soluble fumarate reductase with noncovalently bound FAD. Electron donor to the enzyme was a 2Fe-2S ferredoxin, which in turn was reduced by a 7Fe-8S ferredoxin that was an electron acceptor for, for example, a soluble cytoplasmic hydrogenase or NADH reductase. The membrane contained small transmembrane proteins, some forming four-helix bundles ligating heme. During evolution, the genes for the 2Fe ferredoxin and the 7Fe ferredoxin were duplicated and fused; the product (the first version of subunit B) became the electron donor to the fumarate reductase domain (subunit A of modern enzymes), forming a complex with it. **(B)** The AB dimer was bound to heterodimeric membrane-bound proteins of two kinds. One kind is a four-helix bundle protein that spans the membrane and ligates two protoheme IX molecules. The other kind is without heme and of unknown general structure but similar to that of heterodisulfide reductase of methanogenic archaea. Formation of the membrane complex was promoted by the bioenergetic advantages that respiration provides. FAD became covalently bound to make the enzyme also functional in succinate oxidation. **(C)** Sites for quinone oxidation and reduction were optimized for electron transfer to and from various types of quinones (menaquinone, caldariella quinone, rhodoquinone, ubiquinones, etc.), leading to diversification. Succinate dehydrogenase of *E. coli* and mammalian cells in this process lost the distal heme group, $b_L$, to primarily function in reduction of ubiquinone; *E. coli* fumarate reductase lost both hemes but retains two menaquinone binding sites. In *W. succinogenes* fumarate reductase and *B. subtilis* succinate dehydrogenase, the two hemes are kept. Succinate dehydrogenase of the cyanobacterium *Synechocystis* and thermoacidophilic archae such as *Sulfolobus acidocaldarius* and *Acidianus ambivalens* contain the "heterodisulfide reductase" type of anchor, which contains two 4Fe clusters (*12*).

The author is in the Department of Microbiology, Lund University, Sölvegatan 12, SE-223 62 Lund, Sweden. E-mail: Lars.Hederstedt@mikrbiol.lu.se

The *E. coli* fumarate reductase structure is clearly modular. Subunit A is in physical contact only with subunit B, which is attached to the membrane anchor. The modular composition indicates that the enzymes of the complex II family have originated from a soluble monomeric flavoprotein, a 2Fe-2S ferredoxin, a 7Fe-8S ferredoxin, and an integral transmembrane protein possibly with two b hemes and functioning in transmembrane electron transfer. Soluble monomeric fumarate reductase with a noncovalently bound FAD is found, for example, in promitochondria of the yeast *Saccharomyces cerevisiae* (*6*), and water-soluble ferredoxins are common, particularly in anaerobic bacteria. The first complex formed has been gradually modified over time to optimize succinate oxidation or fumarate reduction under the metabolic conditions prevailing in different cells.

It is the integral membrane domain, however, that is perhaps the most exciting part of the fumarate reductase structure. This is where quinone oxido-reduction occurs and where remarkable diversity is seen among the different members of the complex II family. Why is heme present in most of the enzymes when *E. coli* fumarate reductase and a mutant variant of *E. coli* succinate dehydrogenase (*7*) are functional without it? Are there undiscovered functions in these enzymes? The C and D polypeptides of fumarate reductase each provide three tilted α-helical membrane-spanning segments: helices I, II, and III from subunit C, and helices IV, V, and VI from subunit D. The amino termini are on the same side of the membrane as the AB dimer. Helices I, II, IV, and V are arranged in a right-handed helical bundle, whereas helices III and VI are peripheral to the bundle. The two bound menaquinone molecules in the fumarate reductase, $Q_P$ and $Q_D$, are located at the respective ends of the helix bundle. $Q_P$ is in a polar pocket ~11 Å from the 3Fe-4S cluster, whereas $Q_D$ is in an apolar environment ~27 Å away from $Q_P$ on the opposite side of the membrane dielectric.

The two low-spin hemes, $b_H$ and $b_L$, in the diheme membrane anchor of, for example, *Bacillus subtilis* succinate dehydrogenase and *Wolinella succinogenes* fumarate reductase are predicted to have bis-histidine axial ligation and to be located within the four-helix bundle (*8, 9*). The two axial ligands for $b_H$ are on helices II and V, whereas those for $b_L$ are on helices I and IV. Applied to the *E. coli* fumarate reductase structure, the two hemes in the diheme anchors would be positioned between $Q_P$ and $Q_D$, filling the ~27 Å gap. This is amazingly similar to the situation in complex III, where the function of the two hemes is to provide efficient electron transfer between two quinone binding sites as part of the Q-cycle energy-coupling mechanism (*3*). A major difference is, however, that the heme $b_H$ in complex III is on the positive side of the membrane, whereas in complex II it is on the negative side.

The two hemes in succinate dehydrogenase seem to be the key to an apparent bioenergetic dilemma. *Bacillus* species can grow on succinate ($E_m'$ for the succinate/fumarate redox couple is +30 mV) but only contain low-potential quinones like menaquinone ($E_m' \approx -80$ mV). Electron transfer from succinate to menaquinone is thus thermodynamically highly unfavorable. The succinate oxidase activity in *B. subtilis* drops drastically when the cells are disrupted (*10, 11*). This phenomenon is also observed if uncouplers are added to intact *B. subtilis* cells, but is not observed with mitochondria or *E. coli* cells. The explanation is most likely that the electrochemical potential across the cytoplasmic membrane, which is lost when the cells are disrupted, drives electron transfer from $b_H$ ($E_m' \approx +16$ mV) to $b_L$ ($E_m' \approx -130$ mV), which then can reduce menaquinone. The energy invested for the reduction of menaquinone is regained, and in excess, by the action of coupled terminal oxidases. This mechanistic model is supported by data from mutant enzymes. For example, a variant of *B. subtilis* succinate dehydrogenase deficient in heme $b_L$ but containing $b_H$ does not support growth on succinate and lacks Q reductase activity (*8*).

The *E. coli* fumarate reductase structure is indeed wonderful. It brings us a big step closer to a full understanding of the aerobic enzyme succinate dehydrogenase but also to respiration without molecular oxygen.

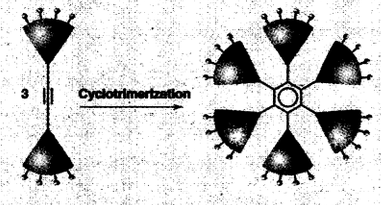

**References**

1. A. Kröger, V. Geisler, E. Lemma, F. Theis, R. Lenger, *Arch. Microbiol.* **158**, 311 (1992).
2. T. M. Iverson, C. Luna-Chavez, G. Cecchini, D. C. Rees, *Science* **284**, 1961 (1999).
3. M. Saraste, *ibid.* **283**, 1488 (1999).
4. A. G. M. Tielens and J. J. V. Hellemond, *Biochim. Biophys. Acta* **1365**, 71 (1998).
5. C. Hägerhäll, *ibid.* **1320**, 107 (1997).
6. H. Muratsubaki and K. Enomoto, *Arch. Biochem. Biophys.* **352**, 175 (1998).
7. K. Nakamura *et al.*, *J. Biol. Chem.* **271**, 521 (1996).
8. L. Hederstedt, *Biochem. Soc. Trans.* **26**, 408 (1998).
9. C. Hägerhäll and L. Hederstedt, *FEBS Lett.* **389**, 25 (1996).
10. E. Lemma, G. Unden, A. Kröger, *Arch. Microbiol.* **155**, 62 (1990).
11. J. Schirawski and G. Unden, *Eur. J. Biochem.* **257**, 210 (1998).
12. C. M. Gomes *et al.*, *Biochim. Biophys. Acta* **1411**, 134 (1999).


**NOTA BENE: CHEMISTRY**

## Completing the Cycle

Dendrimers are treelike molecules that branch out from a central core and subdivide into hierarchical branching units (*1*). They are potentially useful for diagnostic and therapeutic applications, for example, as contrast agents in magnetic resonance imaging (*1*), because of their ability to anchor many substituents on their surface. Dendrimers can be synthesized in two ways. In the divergent method, the dendrimers are built up step by step from the core. With the convergent method, the dendrimer segments are constructed first and then assembled around the central core. Now, Hecht and Fréchet (*2*) report a new approach to convergent dendrimer synthesis. Previous studies reported trimerization via supramolecular interactions, based on the formation of metal complexes, ionic interactions, or hydrogen bonds (*3*). Hecht and Fréchet now achieve covalent cyclotrimerization of dendritic precursors. The alkyne spacers connecting two dendrimer branches (see figure) trimerize to form a benzene core with six dendrimer ligands.




**Clean dendrimer synthesis.** A benzene dendrimer core is formed in situ, catalyzed by a cobalt catalyst, from alkyne spacers between dendrimer units. [Adapted from (*2*)]

This approach is very clean, as no side products or partially reacted products are formed. Precise macromolecular structures can be constructed and easily purified. The method should prove particularly valuable for making hybrid dendrimers from differently substituted precursors. Such clean, versatile syntheses will greatly enhance the potential for successful applications.


**References**

1. M. Fischer and F. Vögtle, *Angew. Chem. Int. Engl.* **38**, 885 (1999).
2. S. Hecht and J. M. J. Fréchet, *J. Am. Chem. Soc.* **121**, 4084 (1999).
3. J. Issberner *et al.*, *Chem. Eur. J.* **3**, 706 (1997); M. Plevoets *et al.*, *New J. Chem.* **63** (1999); M. Kawa and J. M. J. Fréchet, *Chem. Mater.* **10**, 286 (1998); S. C. Zimmermann *et al.*, *Science* **271**, 1095 (1996).


–JULIA UPPENBRINK