**Structure of fumarate reductase (FRD)**

The hydrophilic FrdA subunit (analogous to SdhA) contains a covalently bound FAD prosthetic group and the dicarboxylate binding site of the enzyme. FrdA is oriented towards the cytoplasm of *Escherichia coli* and binds the FrdB subunit, which contains a redox active chain of three independent iron-sulfur clusters. The FrdAB subunits are attached to the membrane through interaction with the hydrophobic FrdC and FrdD subunits (Figure s1). The FrdB, FrdC, and FrdD subunits at the interface of the cytoplasmic side of the inner membrane form a quinone-binding site, which can readily accommodate either menaquinone or ubiquinone (Maklashina et al, 2006). The structure of SDH (SdhCDAB) is basically the same as FrdABCD except for the addition of a b heme in the membrane domain ligated between the SdhC and SdhD subunits (Cecchini, 2003; Cecchini et al, 2002). Although FRD is known to function in anaerobic respiration with fumarate as a terminal electron acceptor it is known that its maximal transcription occurs under microaerophilic conditions and that even in highly aerobic environments there is a background level of FRD transcription (Jones & Gunsalus, 1985, 1987; Tseng et al, 1996).

**Supplementary Materials and Methods**

**Chemicals — complete list**

Tris, glycine, 2,6-dichloroindophenol, phenazine methosulphate, succinate (sodium salt), fumarate (sodium salt), polyoxyethylene(9)dodecylether, $[^{14}\text{C}]$fumarate, ampicillin, tetracycline, chloramphenicol, penicillin G (sodium salt), and compounds for SDS-polyacrylamide gel electrophoresis were obtained from Sigma (St. Louis, MO, USA); isopropyl $\beta$-D-thiogalactopyranoside (IPTG) from Fermentas (Burlington, Canada); Bacto Agar, Tryptone Peptone, Antibiotic Medium 3 and yeast extract were from Difco (Franklin Lakes, NJ, USA); benzoate, sodium hydrogen phosphate, sodium dihydrogen phosphate and imidazole were from Merck (San Diego, CA, USA); L-leucine, L-methionine, L-serine, L-threonine, and L-histidine were from Calbiochem (Darmstadt, Germany); Ni-NTA matrices from QIAGENE (Hilden, Germany); sodium boro$[^3\text{H}]$hydride, $[^{14}\text{C}]$leucine, and $[^{3}\text{H}]$leucine were from Amersham (Buckinghamshire, UK). All the compounds were of the highest purity grade available.

**Binding of $[^{14}\text{C}]$fumarate to isolated switch proteins**

**Equilibrium dialysis.** Equilibrium dialysis was carried out as described (Klotz, 1989) in a screw-capped Eppendorf tube. The cap, which holds up to 200 $\mu$l, was used as the compartment to contain the binding protein (FliM, FliN and/or FliG, or, as negative control, BSA). The body of the tube...
contained \(^{14}\text{C}\)fumarate. An 8 kDa cutoff membrane was placed between the cap and body of the tube, and the cap was sealed. The tube was incubated at 4°C overnight with continuous inversion. An aliquot from each compartment was taken to estimate the level of radioactivity by scintillation counting.

**Centrifugal ultrafiltration.** Samples of 2 ml containing \(^{14}\text{C}\)fumarate and FliM, FliN and/or FliG, or, as a negative control, BSA were transferred to Centricon-5 tubes with a 5 kDa cutoff Millipore filter and centrifuged at \(~2500 \times g\) until the volume was reduced to 100 µl. The level of \(^{14}\text{C}\)fumarate was determined both in the concentrate and the filtrate by scintillation counting (Menguy et al, 1998).

**Measurement of FRD binding to FliG by sedimentation velocity**

Sedimentation velocity measurements were performed in a Beckman XLA analytical ultracentrifuge. Samples (0.4 ml) of FRD (2 µM) alone, His-tagged FliG (16 µM) alone, and an equimolar mixture of FRD and His-tagged FliG (2 µM), all in KP, (50 mM, pH 7.4), MgSO\(_4\) (5 mM), NaCl (150 mM) and Thesit (1.3 x 10\(^{-3}\) % v/v), were loaded into standard two-channel analytical ultracentrifuge cells, equilibrated at rest at 4°C for 2 h and spun at 50,000 rpm. Radial sedimentation profiles were recorded by monitoring the absorption at 280 nm. Sedimentation coefficients and molecular weight distributions, \(c(s)\) and \(c(M)\), respectively, were obtained by fitting the raw data to a distribution of the Lamm equation using the software Sedfit (Schuck, 2000) (obtained from http://www.analyticalultracentrifugation.com). Solvent density, viscosity and protein partial specific volumes were estimated from the buffer composition and the amino acid sequence of the proteins, using the program “Sednterp” (Laue et al, 1992). Solvent density and viscosity values of \(\rho_s = 1.01192\) and \(\eta_s = 0.015607\) poise were used, and partial specific volumes of 0.731 and 0.734 ml·g\(^{-1}\) for FRD and His-tagged FliG, respectively. Thesit is a polyethylene glycol 400 dodecyl ether (C\(_{12}\)E\(_9\)). Based on published values of several C\(_{12}\)E\(_n\) detergents (le Maire et al, 2000), we estimated the detergent’s partial specific volume, \(\tilde{\nu}_d\), to be about 0.97. Since the latter is almost neutrally buoyant (\(\tilde{\nu}_d \rho_s = 0.982 \approx 1\)), its contribution to the protein molecular weight estimation was considered negligible.

**Determination of the direction of flagellar rotation by tethering assay**

To determine the direction of flagellar rotation and the chemotactic response, we used the tethering assay (Silverman & Simon, 1974) with intact cells as well as with cell envelopes. With intact bacteria, the cells were tethered through their flagella to glass slides using motility buffer containing
KP$_i$ (10 mM, pH 7.0), EDTA (0.1 mM), and methionine (0.1 mM), in a flow chamber (Berg & Block, 1984), and the rotation of the tethered cells was recorded on videotape. The direction of each spinning cell in the microscope field was assessed visually for 40 s and the data were expressed as the percentage of time spent spinning in the clockwise direction and the frequency of switching both prior to and after addition of stimulus. This analysis was carried out without prior knowledge of the strain being analyzed. Envelopes were similarly tethered, as described earlier (Ravid et al, 1986), in a medium containing Tris-HCl (50 mM, pH 7.9), Tetren (0.1 mM), DL-lactate (2 mM) and fumarate (10 mM). The direction of each spinning envelope in the microscope field was visually assessed for 60 s.
**Figure s1.** Comparison of the SDH and FRD structures. SDH (left side) and FRD (right side) both comprise four polypeptide subunits. The cytoplasmic domain is composed of a flavoprotein subunit (SdhA and FrdA, shown in blue) and an iron-sulfur protein subunit (SdhB and FrdB, shown in orange). The transmembrane domain contains two subunits: SdhC and FrdC (shown in green) and SdhD and FrdD (shown in purple). The SdhAB and FrdAB subunits extend approximately 70 Å above the membrane into the cytoplasm, as noted by the vertical arrow. In the iron-sulfur subunits the FeS clusters are shown as spheres. In the flavoprotein subunits the covalently-bound FAD cofactor is shown in yellow. In the transmembrane subunits the ubiquinone molecule and heme of SDH are shown in yellow. In FRD the menaquinone molecules are shown in yellow. *E. coli* SDH and FRD are encoded by separate operons; however, their respective gene order is different (*i.e.*, *sdhCDAB* and *frdABCD*).
Figure s2. Purities of the isolated intact switch complex and the main studied proteins judged by 12% SDS-polyacrylamide gel electrophoresis. Lane 1, Intact isolated switch complex. The molecular sizes of the marked bands were 61 (FliF), 38 (FliM), 37 (FliG) and 15 (FliN). Lanes 2, 3, Purified FRD and SDH from *E. coli*. Molecular sizes: A subunit, 66 kDa; B subunit, 27 kDa; C subunit, 14–15 kDa; D subunit, 13 kDa. The molecular stoichiometry of the subunits, revealed from the gels, was estimated to be 1:1:1:1. Lane 4, His-tagged FliG, 38 kDa. Lane 5, His-tagged CheY, 15 kDa.
Figure s3. Images of isolated basal bodies lacking the structure of the C-ring following incubation with an anti-FliG antibody and then immunogold-labeled protein A. The figure demonstrates the association of FliG with the M-ring.

Figure s4. Western blots of cell lysates with anti-FliG antibody. Strains RP437, RP437Δfrd and DFB225ΔfliG (Table II) were grown on tryptone broth to mid-logarithmic phase and harvested. The cells were incubated for 10 min at room temperature with CellLytic-B™ (Sigma), followed by 10 min centrifugation at 10,000 × g (4°C). Equal amounts (25 µg) of the supernatants were applied to 12% SDS-PAGE and the gel blotted with an anti-FliG antibody. Lane 1, Strain RP437. Lane 2, Strain RP437Δfrd. Lane 3, Strain DFB225ΔfliG. Lane 4, Purified FliG.
Figure s5. Specificity of anti-FRD and anti-His-tagged-FliG antibodies. A, Western blots with anti-FRD antibody. Lane 1, Purified FRD. Note that the anti-FRD antibody recognized all four subunits of FRD. The three weaker bands in lane 1 between the A and B subunits of FRD are, most likely, proteolytic fragments of FrdA commonly seen in FRD preparations (Luna-Chavez et al, 2000). Lane 2, Membrane fraction of a lysate of the ∆frd strain. The anti-FRD antibody recognized the A and B subunits of SDH, known to be very similar to those of FRD (Figure s1). B, Western blots with anti-His-tagged-FliG antibody. Lane 1, Lysate of strain DFB225∆fliG. Lane 2, Lysate of strain BL-21 containing pEWG1, induced to overproduce His-tagged-FliG. Lane 3, Purified His-tagged FliG.

References


