Inhibitor-complexed Structures of the Cytochrome $bc_1$ from the Photosynthetic Bacterium *Rhodobacter sphaeroides* $^{*}$

Lothar Esser$^1$, Maria Elberry$^3$, Fei Zhou$^5$, Chang-An Yu$^4$, Linda Yu$^6$, and Di Xia$^{*}\$1

From the $^1$Laboratory of Cell Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892 and the $^2$Department of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, Oklahoma 74078

The cytochrome $bc_1$ complex ($bc_1$) is a major contributor to the proton motive force across the membrane by coupling electron transfer to proton translocation. The crystal structures of wild type and mutant $bc_1$ complexes from the photosynthetic purple bacterium *Rhodobacter sphaeroides* (Rsbc1), stabilized with the quinol oxidation ($Q_\text{F}$) site inhibitor stigmatellin alone or in combination with the quinone reduction ($Q_\text{R}$) site inhibitor antimycin, were determined. The high quality electron density permitted assignments of a new metal-binding site to the cytochrome $c_1$ subunit and a number of lipid and detergent molecules. Structural differences between Rsbc1 and its mitochondrial counterparts are mostly extra membranous and provide a basis for understanding the function of the predominantly longer sequences in the bacterial subunits. Functional implications for the $bc_1$ complex are derived from analyses of 10 independent molecules in various crystal forms and from comparisons with mitochondrial complexes.

A central component of the cellular respiratory chain is the cytochrome $bc_1$ complex (cyt $bc_1$ or $bc_1$)$^{12}$ that catalyzes the electron transfer (ET) from quinol to cytochrome $c$ (cyt $c$) and simultaneously pumps protons across the membrane, contributing to the electrochemical potential that drives ATP synthesis and many other cellular activities (1). In chloroplasts and cyanobacteria a related membrane protein complex, the cytochrome $b_{6f}$ (cyt $b_{6f}$), bridges photosystem I and II, enabling oxygenic photosynthesis and conversion of light energy into a proton gradient for ATP generation (2). For non-oxygenic photosynthetic bacteria, such as *R. sphaeroides* (Rs), which can grow both aerobically and photosynthetically under anaerobic condition, the $bc_1$ complex is involved in both growth modes; however it is essential only under anaerobic conditions (3).

The critical importance of $bc_1$ has made it a target for numerous antibiotics, fungicides, and anti-parasitic agents. As a result, resistance to these agents has been documented in a wide variety of organisms (4–8). Disorders that are related to defects in $bc_1$ complex are manifest clinically as mitochondrial myopathy (9), exercise intolerance (10), and Leber’s optical neuropathy (11). Mounting evidence suggests a correlation between aging and the production of reactive oxygen species from defective $bc_1$ complexes (12, 13). The elucidation of the molecular mechanisms underlying these phenomena requires a combination of experimental approaches and in particular, structural investigations that can provide a molecular framework for further experiments.

Significant advances in elucidating architectural features of this complex have been made by crystal structure determinations of mitochondrial $bc_1$ (14–17) and $b_{6f}$ from a bacterium (18) and an alga (19). In particular, crystal structures of mitochondrial $bc_1$ in complex with various $bc_1$ inhibitors provide important mechanistic insights (20–27), leading to a significant increase in the number of experimental studies and analyses of this enzyme. However, most recent functional investigations have been conducted with bacterial $bc_1$ complexes, especially those of non-oxygenic photosynthetic purple bacteria such as *R. sphaeroides* and *R. capsulatus* (RC). These bacterial systems contain simpler $bc_1$ complexes consisting of either three (RC) or four (Rs) subunits whose sequences have remained close to their mitochondrial counterparts. Chromatophore vesicles are easy to isolate in large quantities. Site-specific mutants can be readily prepared and tested in an optical pulse mode due to its coupling to the photosynthetic reaction center (28). Because of the importance of bacterial $bc_1$ in functional studies, a high-resolution structure has been actively pursued for many years. The crystal structure of the $bc_1$ complex from *R. capsulatus* (Rc$bc_1$) reported at 3.8 Å resolution represented the first step toward this goal (29), though it lacks sufficient resolution of structural details that distinguish the bacterial form from the mitochondrial one. Here, we report the crystal structures of the wild type and mutant $bc_1$ complex from the *R. sphaeroides* (Rs$bc_1$) with bound inhibitors ranging in resolution from 3.1 to 2.4 Å.

---

* This research was supported in part by the Intramural Research Program of the Center for Cancer Research, NCI, National Institutes of Health, and in part from National Institutes of Health Grant GM 30721 (to C. A. Y.). The costs of publication of this article were deferred in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The atomic coordinates and structure factors (code 2QJP, 2QJY, 2QJK) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

1 The on-line version of this article (available at http://www.jbc.org) contains supplemental information and Tables S1–S3.

1 To whom correspondence should be addressed: Laboratory of Cell Biology, Center for Cancer Research, NCI, National Institutes of Health, Tel.: 301-435-6315; Fax: 301-480-2315; E-mail: dixia@helix.nih.gov.

2 The abbreviations used are: cyt, cytochrome; $2Fe2S$, two iron two sulfur cluster of ISP; Pbc1, ubiquinol cytochrome c oxidoreductase; Btbc1, *B. Taurus bc_1*; $b_{6f}$, high potential b heme; $b_{6}$, low potential b heme; EPR, electron paramagnetic resonance; ET, electron transfer; IMS, intermembrane space; ISP, iron-sulfur protein subunit; $Q_{F}$, $Q_{R}$, quinol oxidation; $Q_{O}$, quinone reduction; $QH_{2}$, ubiquinol; r.m.s. deviations, root-mean-square deviations.

---

* This research was supported in part by the Intramural Research Program of the Center for Cancer Research, NCI, National Institutes of Health, and in part from National Institutes of Health Grant GM 30721 (to C. A. Y.). The costs of publication of this article were deferred in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The atomic coordinates and structure factors (code 2QJP, 2QJY, 2QJK) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

1 The on-line version of this article (available at http://www.jbc.org) contains supplemental information and Tables S1–S3.

1 To whom correspondence should be addressed: Laboratory of Cell Biology, Center for Cancer Research, NCI, National Institutes of Health, Tel.: 301-435-6315; Fax: 301-480-2315; E-mail: dixia@helix.nih.gov.

2 The abbreviations used are: cyt, cytochrome; $2Fe2S$, two iron two sulfur cluster of ISP; Pbc1, ubiquinol cytochrome c oxidoreductase; Btbc1, *B. Taurus bc_1*; $b_{6f}$, high potential b heme; $b_{6}$, low potential b heme; EPR, electron paramagnetic resonance; ET, electron transfer; IMS, intermembrane space; ISP, iron-sulfur protein subunit; $Q_{F}$, $Q_{R}$, quinol oxidation; $Q_{O}$, quinone reduction; $QH_{2}$, ubiquinol; r.m.s. deviations, root-mean-square deviations.
**TABLE 1**

Statistics for X-ray diffraction data sets and qualities of Rsbc1 models

|                      | Rsbc1 wild-type<sup>a</sup> | Rsbc1 double mutant<sup>a</sup> | Rsbc1 double mutant<sup>b</sup> |
|----------------------|-----------------------------|---------------------------------|---------------------------------|
|                      | stigmatellin/antimycin      | stigmatellin/quinone            | stigmatellin/antimycin          |
| **Data collection**  |                             |                                 |                                 |
| Cell (Å<sup>2</sup>) | 135.1, 146.5, 141.0         | 147.0, 161.3, 160.8             | 147.4, 160.8, 161.8             |
| α, β, γ (°)          | 90, 110.2, 90               | 104.3, 90, 90                  | 104.1, 90, 90                  |
| **Space group**      | P<sub>2</sub><sub>1</sub>   |                                 |                                 |
| Resolution (Å)       | 50.26 (2.62-2.60)           | 50.2-35 (2.43-2.35)            | 50-3.1 (3.21-3.10)             |
| Wavelength (Å)       | 0.75                        |                                 |                                 |
| Rmerge<sup>d</sup>   | 0.110 (0.67)                | 0.106 (0.56)                   | 0.168 (0.849)                  |
| R<sub>merge</sub>/R<sub>res</sub> | <1;i>|<q;i> | 10.5 (1.4) | 14.5 (1.30) | 9.81 (1.07) |
| Completeness (%)     | 98.8 (94.3)                 | 91.9 (66.0)                    | 99.7 (98.7)                    |
| Average redundancy   | 4.5 (2.8)                   | 4.8 (2.5)                      | 6.0 (4.3)                      |
| No. unique reflections | 154,627                    | 305,667 (293,559)              | 143,931                        |
| No. free reflections  | 3,027                       | 4,927                          | 2,755                          |
| **Model refinement** |                             |                                 |                                 |
| Resolution           | 18-2.60                     | 18-2.40                        | 18-3.10                        |
| No. dimers/ assigned atoms | 2                        | 2                               | 3                               |
| R<sub>merge</sub>/R<sub>free</sub> | 0.24/0.28                  | 0.23/0.25                      | 0.24/0.27                      |
| R<sub>merge</sub>/R<sub>free</sub> (outer shell) | 0.41/0.43 | 0.32/0.35 | 0.41/0.43 |
| No. all atoms        | 28,227                      | 42,656                         | 42,048                         |
| No. unique reflections | 3,452/96.4 (84.7)          | 5,178/96.4 (84.7)              | 5,178/96.4 (84.7)              |
| No. co-factors       | 16                          | 24                             | 24                             |
| No. Stigmatellin at Q<sub>s</sub> site | 4                        | 6                              | 6                              |
| No. Antimycin at Q<sub>s</sub> site | 4                        | 6                              | 6                              |
| No. quinone at Q<sub>s</sub> site | 4                        | 6                              | 6                              |
| No. Lipids/detergent | 4/4                         | 6/6                            | 6/6                            |
| R.m.s.d. bond length (Å) | 0.013                     | 0.014                          | 0.011                          |
| R.m.s.d. bond angle (°) | 1.7                        | 1.7                            | 1.6                            |
| PDB entry            | 2QJP                        | 2QJY                           | 2QJK                           |
| **a** Statistics for the data sets were computed at -1.5, -1, -1e cutoffs for reflection intensities for the three data sets, respectively.
| **b** The Rsbc1 double mutant bears a 5287R mutation in the cyt b subunit and a V135S mutation in the ISP subunit.
| **c** Values in parentheses are for the highest resolution shells.
| **d** R<sub>merge</sub> is defined as ∑|I<sub>h</sub>−|<I<sub>h</sub>|)/|I<sub>h</sub>−|<I<sub>h</sub>|>, where |<I<sub>h</sub>| is the mean intensity for all measured |<I<sub>h</sub>|<sub>s</sub> and |<I<sub>h</I>|<sub>s</sub> is the mean intensity for all measured |<I<sub>h</I>|<sub>s</sub> and Friedel pairs.
| **e** Two crystals were merged.
| **f** Unique reflections to 2.40 Å.
| **g** Number of residues in the model and percentage of completeness. Completeness in parentheses if subunit IV were included.

**EXPERIMENTAL PROCEDURES**

Protein Purification and Crystallization—Cyt bc<sub>1</sub> complexes of both wild-type and double mutant S287R<sup>Cytb</sup>/V135S<sup>ISP</sup> from R. sphaeroides were prepared as described (30). The concentrated protein solution (90 mg/ml) was diluted by a factor of six with a buffer containing 50 mM Tris, pH 7.5, 200 mM NaCl, 10% glycerol, 5 mM NaN<sub>3</sub>, 0.5% β-OG (Anatrace), 200 mM histidine, and 2 mM diheptanoyl phosphatidyl choline (Avanti). The solution was left on ice for 12 h after adding a 5-fold molar excess of stigmatellin (Fluka). A second detergent, sucrose monocaprate (Fluka), was added to a final concentration of 0.12% followed by 10 mM strontium nitrate and 10% PEG400. The resulting solution was incubated overnight at 4 °C. A small amount of precipitation was centrifuged off and the supernatant was used in sitting drop crystallization experiments that yielded small, red, translucent crystals after 2 months of incubation at 15 °C. The reservoir solution was prepared separately and contained 100 mM Tris, pH 8.0, 600 mM NaCl, 20% glycerol, 5 mM NaN<sub>3</sub>, and 26% PEG 400.

Structure Determination—Crystals of Rsbc1 were frozen without additional cryoprotectants but showed decay during the low temperature (100 K) data collection. The crystal structures were measured with the program Denzo and merged and scaled with Scalepack (HKL2000 package) (31). The structures of Rsbc1 inhibitor complexes were solved by molecular replacement (MR) using a dimeric Rsbc1 model based largely on the structure of bovine bc<sub>1</sub> (32) with minor modification as input for the program MolRep (33) of the CCP4 (34) program package. The successful solution consisted of a set of three dimers related by non-crystallographic symmetry (NCS). This model was subjected to thorough rigid body refinement and simulated annealing (35), followed by cycles of standard crystallographic refinement and model building in O (36).

Clear electron density for residues that were missing from the initial model in particular for insertions into cyt b and a rapid drop of R<sub>free</sub> confirmed the correctness of the MR solution. The final model contains continuous polypeptide chains of cyt b, cyt c<sub>1</sub>, and the ISP but none of the supernumerary subunit IV. Difference Fourier maps showed positive density for six lipid molecules, six β-OG molecules, six molecules of the substrate ubiquinone, and nine strontium ions. Throughout the refinement, NCS restraints were maintained except for a few regions at crystal contacts or in places of apparent local disorder. The structure of the wild type complex (P<sub>2</sub><sub>1</sub>) was solved by MR using a refined dimeric polypeptide-only model of the double mutant Rsbc1. A difference Fourier synthesis revealed the position of all prosthetic groups as well as the presence of both inhibitors stigmatellin and antimycin.

**RESULTS AND DISCUSSION**

Structure Determination and Overall Structure of the Rsbc<sub>1</sub>—The presence of the Q<sub>s</sub> site inhibitor stigmatellin, the use of the amino acid histidine and a mixture of β-octyl glucopyranoside (β-OG) and sucrose monopaste are important for obtaining high quality crystals. A batch of Rsbc1 with the double muta-
Crystal Structures of *R. sphaeroides* bc₁

FIGURE 1. Structure of the Rsbc₁ complex. The subunits are colored as follows: green, cyt b; blue, cyt c₁; and yellow, ISP. Insertions and extensions that distinguish Rsbc₁ from Btbc₁ are in red. Heme groups, 2Fe2S₃, stigmatellin, and ubiquinone are shown as stick models with black carbon, red oxygen, blue nitrogen, green sulfur, and brown iron atoms. Ribbon diagrams were produced with the graphics programs Molscript (57), Bobscript (58), Povray, and Gli. A, ribbon representation of the dimeric Rsbc₁, with the bound Q₁ site inhibitor stigmatellin and Q₅ site substrate quinone. The boundary of lipid bilayer is indicated with two parallel lines. The transmembrane domain (TM), the periplasmic (PP), and cytoplasmic (CP) space are labeled. Modeled lipid and detergent molecules are shown as stick models in red. B, ribbon diagram of the cyt b subunit with labeled TM helices and connecting loop. C, structure of cyt c₁ in the ribbon form showing all secondary structure elements. Residues important for Sr²⁺ binding are drawn in stick models with carbon atoms in yellow, oxygen red, and sulfur green. The Sr²⁺ ion is shown as a metallic sphere. D, putative metal-binding site in cyt c₁ subunit. The electron density is shown as a semi-transparent hull contoured at 1σ level. The Sr²⁺ ion is coordinated by three acidic residues and one backbone carbonyl oxygen atom. Residues in the vicinity of the binding site are given in the ball-and-stick form. E, structure of ISP in the ribbon form.

Three C group for growing well-behaved crystals (37). These crystals (space group C2) diffracted x-rays to 2.35 Å resolution (Table 1). Three bc₁ dimers occupy the crystallographic asymmetric unit (ASU). The wild type enzyme crystallizes with two dimers per ASU (space group P2₁) and diffracted x-rays to 2.6 Å resolution (Table 1). Surprisingly, only the three core subunits are present in both wild type and mutant Rsbc₁ crystals; apparently, subunit IV was lost upon crystal formation. The assembly of the three-subunit Rsbc₁ (Fig. 1A) resembles closely that of the corresponding subunits in bovine mitochondrial bc₁ (Bos taurus bc₁ or Btbc₁) (14), and the remarkable conservation in architectural features not only pertains to a single monomer but also to an assembled homodimer. The root-mean-square (r.m.s.) deviation between the cyt b dimers of *R. sphaeroides* and bovine bc₁ is less than 1.1 Å for 726 Ca atoms (Table 2). Consequently, the distances between prosthetic groups are virtually identical, implying functional conservation (Supplemental Table S1). As in mitochondrial bc₁, the extrinsic domain of the iron-sulfur protein subunit in Rsbc₁ crosses over, connecting one molecule of cyt b to the adjacent one. In contrast to the seven or eight supernumerary subunits in mitochondrial enzymes, Rsbc₁ has only one. Thus, it has been speculated that supernumerary subunits represent functional or structural equivalents of the insertions, extensions, and deletions found in the sequences of the catalytic subunits of bacterial bc₁ (38).

Structure of the Cytochrome b Subunit—The cyt b subunit of Rsbc₁ has eight membrane spanning helices named A to H, forming two helical bundles (A–E and F–H) (Fig. 1B). The two heme groups, b₇ and b₈, reside within the first bundle. Extra membranous loops connect pairs of transmembrane (TM) helices and those that are longer than 20 residues are the AB, CD, DE, and EF loop. The quinol oxidation site (QP, positive side, P-side) near the periplasmic side of the membrane and quinone reduction site (Q₅, cytoplasmic or negative side, N-side) on the opposite side can be identified with bound stigmatellin and antimycin, respectively. When compared with structures of Btbc₁, Rs cyt b features two terminal extensions and two major insertions. The N- and C-terminal extensions are 22 and 29 residues long, respectively. Both contain helices named a0 and i, respectively (Figs. 1B and 3A). One insertion (de helix) is in the cytoplasmic DE loop and another (ef1 helix) inserts after the ef helix on the periplasmic side.

Structure of the Cytochrome c₁ Subunit—The cyt c₁ subunit folds in a manner similar to that of its mitochondrial counter-
part, having a C-terminal TM helix (Fig. 1C) and featuring the Cys36-X-X-Cys39-His40 motif characteristic for c-type cytochromes (Fig. 3B) with the heme iron atom being coordinated by the side chains of His40 and Met185 as 5th and 6th ligand, respectively. The heme group is located and positioned identically to that of mitochondrial enzymes. Crystals of Rsbc1 grown in the presence of strontium ions revealed a metal ion-binding site on cyt c1 (Fig. 1, C and D), which is not present in mitochondrial bc1, but appears to be conserved in photosynthetic bacteria (Fig. 3B). The strontium ion, confirmed by the appearance of a strong anomalous signal from the data set collected above the strontium absorption edge, is accessible from the periplasm and coordinated by side chains of Asp8, Glu14, and Glu129 as well as by the backbone carbonyl oxygen atom of residue Val8 in a distorted octahedron. To our knowledge, this metal ion-binding site has not been described previously and its possible physiological role is currently under investigation.

**Structure of the Iron-Sulfur Protein Subunit**—The ISP subunit has a C-terminal periplasmic head domain (extrinsic domain, ISP-ED), which connects through a flexible hinge region to its N-terminal TM helix (Fig. 1E). The ISP-ED is predominantly a β-structure consisting of three β-sheets arranged in three parallel layers with the 2Fe2S cluster located at the apex of the ISP-ED between the 2nd and 3rd β-sheet. The conserved ADV motif in the hinge region (Fig. 3C) adopts an α-helical (HA) conformation, unlike the random coil secondary structure of bovine bc1 in the space group P4221. One insertion with respect to the bovine sequence is located between Thr96 and Ala199, filling a surface depression that would otherwise exist between β-sheets 2 and 3.

**Inhibitor Binding Sites**—Stigmatellin is a potent Qp site inhibitor (39) and has frequently been used to arrest the mobile head domain of the ISP (26). This may be of particular importance in crystallizing the bare-bone bc1 complexes that cannot rely on lattice contacts formed by large hydrophilic core subunits (14, 15), or by antibody fragments (17). In all Rsbc1 structures described in this work, stigmatellin is clearly visible in the difference Fourier maps and oriented in a way that bridges the side chains of the residues Glu295 (cyt b) and His152 (ISP) (Fig. 2A). The hydrophobic tail, known to contribute to the small $K_d$ (0.4–1 nM) of stigmatellin binding (40), is fully visible (Fig. 3A) and makes the same contacts as in complexes from mitochondria (17, 26) or in bof complexes (19, 41).

Antimycin molecules were refined in all four (P21) or six copies (C2) of cyt b in the asymmetric unit. The antimycin complex of bovine cyt b superimposes very well with the corresponding Rsbc1 subunit (Fig. 2B), and the inhibitors fit nicely into the difference density observed in the latter. Antimycin binds strongly to mitochondrial bc1 with a $K_d$ of 32 pm (42), but the $K_d$ for Rsbc1

### TABLE 2

| Subunit | Rsbc1, no. residues | Root mean square deviations (Å) (number of residues superimposed) | Rsbc1-bovine | Rsbc1-yeast | Rsbc1-bf |
|---------|---------------------|-------------------------------------------------|-------------|-------------|--------|
| Cyt b   | 445                 | 0.997 (364) | 0.898 (366) | 1.602 (311) |
| Cyt c1  | 263                 | 1.40 (205) | 1.251 (204) | N/A         |
| ISP     | 187                 | 1.28 (163) | 1.364 (163) | 1.653 (81)  |
| Sub IV  | 124                 | –         | –           | –           |

* Coordinates for the bovine complex are from the PDB: 1SQX.
* Coordinates for the yeast complex are from the PDB: 1EZV.
* Coordinates for the bof complex are from the PDB: 1F90.
* Cyt c cannot be compared with cyt c1.
* Head domain only.

**FIGURE 2. Inhibitor binding sites in cyt b.** A, stereoscopic diagram showing electron density for stigmatellin (white) and its binding environment (blue). Stigmatellin and its surrounding residues are portrayed in the stick model with yellow carbon, red oxygen, and blue nitrogen, green sulfur and brown iron atoms. His41 of ISP is 2.64 Å away from the atom O4 of stigmatellin and its phenolic oxygen atom O6 is 3.01 Å away from OE1 of Glu295. The electron density is contoured at 1.5σ. B, stereoscopic view of the Qp site. The bound antimycin in yellow forms two hydrogen bonds (red dotted lines) with the 3-FASA moiety of the inhibitor. The b1 heme is shown as a stick model in gray. The inhibitor displays anti conformation as in 1PPJ (27).
Crystal Structures of R. sphaeroides bc

A

{image of a figure showing crystal structures and sequence alignments}

FIGURE 3. Structure-based sequence alignment of bc1 subunits. Secondary structure elements are shown as boxes for α-helices and arrows for β-strands, and are illustrated above the sequences of Rsbc1 subunits. Green boxes and blue arrows indicate shared secondary structure elements between bacterial and mitochondrial bc1, whereas those colored in orange are found in Rsbc1. Residues that are ligands to prosthetic groups are red in bold face. Residues that are fully conserved in the alignment are shown in red; those having conserved changes are brown. The sequences are Rs (R. sphaeroides), Bt (B. taurus), Sc (S. cerevisiae), Cr (C. reinhardtii). A, sequence alignment of cyt b subunits. B, sequence alignment of cyt c1 subunits. C, sequence alignment of ISP subunits.

is not known. As prominent contributors to the binding energy, the formyl amide and the phenolic OH group of antimycin are in excellent hydrogen bonding distance to Asp252 (Asp228, Btbc). However, two amino acid changes could strongly influence the binding of antimycin. The substitution of Ser35 in Btbc to Val49 in Rsbc eliminates the H-bond between the hydroxyl group of Ser and the central amide group (N2) of inhibitor’s binding affinity to antimycin by 10–20-fold (43). The impact of the change from Ser35 to Val49 is difficult to judge and may be masked by the addition of the 10–20-fold increase in the binding affinity. Remarkably, the insertions occur only on or near the periplasmic or cytoplasmic side and not within the transmembrane domain. These insertions are not found in the cytochrome bc1 subunits. Cyt b of Rsbc1 is 66 residues longer than cyt b of R. sphaeroides. The insertion occurs only on or near the periplasmic or cytoplasmic side and not within the transmembrane domain. These insertions are not found in the cytochrome bc1 subunits.
structure-based sequence alignment and from its structure (Figs. 1B and 3A), bacterial bc1 has extensions at both termini (α0 and i helices), a helical insertion between the D and E helices (de helix) as well as an insertion between the ef-loop and the F helices (a0 and i helices), a helical insertion between the D and E helices, and a deletion of the last 15 residues does not affect the function of Rsbc1 (38). Except for the ef1 helix, all extensions and insertions are located on the N-side of the membrane (Figs. 1B and 4A), which likely function to maintain the structural integrity of the quinone reduction site by preventing potential electron leakages and by safeguarding channels for proton influx (24). Indeed, without the supernumerary subunits, especially core1 and core2, the heme b_{14} (and with it the QN site) of the mito-
Crystal Structures of R. sphaeroides bc₁

Chloroplastial cyt b is only weakly shielded from the aqueous matrix by a thin layer of protein side chains. In contrast, the Q₅_N site of Rsbc₁ is well protected by an additional layer consisting of the de-helix insertion and the two terminal extensions. The location of the de-helix permits interaction with its own C-terminal extension and with the end of the N terminus from the neighboring cyt b through a network of hydrogen bonds (Fig. 1, A and B, Supplemental Table S2). The a₀-helix reaches to the cyt b of its symmetry mate and forms a pair of salt bridges between Arg²² and Glu₁₂⁶ of the symmetry-related cyt b and a number of hydrogen bonds as well as van der Waals interactions (Fig. 1, A and B). Mutational studies have shown that C-terminal truncations as far as residue 421 lead to increasing detergent sensitivity, loss of ISP and subunit IV during purification and lowering the potentials of both heme groups, leading to eventual inactivation of Rsbc₁ (38). The structure qualitatively explains these observations by demonstrating the interaction of the C terminus of cyt b (via the i helix) with the C terminus of cyt c₁ (indirectly to the N terminus of the ISP) and with the de-helix, which is in close proximity to the b₁₄ heme (38).

On the periplasmic side, there is one large insertion of 18 residues (310–327) between Pro²⁸⁶ and Asn²⁸⁶ (Bt cyt b) containing the e₁-helix (Fig. 4B), which protrudes from cyt b laterally and runs parallel to the membrane surface (Fig. 1B). This insertion occurs only in species that belong to the phylum proteobacteria (Fig. 3A). However, it is functionally important, as the point mutation S322A or deletion of residues 309–326 significantly lowers the enzyme activity (46). The e₁-helix may play an important role in lipid binding, as features of several potential lipid molecules are visible in the electron density (Figs. 4B and 5C). It also enhances crystal contacts through aromatic stacking interaction between Trp¹¹³ of adjacent cyt b subunits.

Insertions and Deletions in Cyt c₁—The structure-based sequence alignment (Fig. 3B) shows that cyt c₁ of Rsbc₁ has undergone both insertions and deletions relative to mitochondrial complexes. Apart from the two small insertions in the Rs cyt c₁ after Glu⁵² (4 residues) and Ala¹⁴⁶ (3 residues), there is one large insertion between Gly¹⁰⁹ and Gly¹²⁷. It features a short helix (H1d) that protrudes from cyt c₁ into the lipid bilayer sealing off a compartment between cyt c₁ and cyt b (Fig. 1, A and C). In Btbc₁, the absence of this insertion creates a niche at the interface between the end of the Helix E of cyt b and cyt c₁. A possible function of this insertion may relate to lipid binding (next section). The only insertion in cyt c₁ that may replace the function of a supernumerary subunit is the 18-residue insertion starting at position 162, which is spatially close to the head domain of ISP (Fig. 4C). Containing a short helix H2a, this region is characterized by an increased disorder (high B-factor) but features a stabilizing disulfide bridge (Cys₁⁴⁵–Cys¹⁶⁸), whose existence is in agreement with recently published data (37). Approaching the ISP-ED within ~8 Å (Cα distance from cyt c₁ Asn¹⁷³ to ISP Asp¹⁴₃), this insertion presumably functions as an extended arm to limit the motion of the ISP-ED (Fig. 4C). However, the intrinsic flexibility and extent of solvent exposure renders it susceptible to proteolytic attack and, conceivably, places it in an evolutionarily disadvantageous position, possibly leading to the replacement of its function by the supernumerary subunit VIII in mitochondrial enzymes (Supplemental Fig. S1).

Compared with mitochondrial cyt c₁, two large deletions, near residues Thr⁷⁷ and Ser⁸₂, respectively (Fig. 3B), result in the loss of bridging interactions between the two cyt c₁ subunits within the dimer (Fig. 4C). The absence of these contacts in Rsbc₁ creates a large continuous groove (13 Å wide) on the P-side surface. Beyond possible functional implications, the closure of the gap improves stability around the heme group in mitochondrial cyt c₁.

Insertions in the ISP—Structure-based sequence alignment shows one insertion in the sequences of Rs ISP (Fig. 3C). This insertion (residues 97–108) is located on the surface of ISP-ED distal to cyt c₁ and stays 20–25 Å away from the 2Fe2S cluster as predicted (47); it forms a globular structure containing three β-turns and one inverse γ-turn (Fig. 4D). There is an intricate network of interactions employing both main chain and side chain atoms, suggesting a stabilizing role for this insertion (Supplemental Table S3). Disruption of this network of interactions by more than one point mutation led to the loss of the ISP subunit in the complex (47). From a morphological point of view, the insertion 97–108 in Rsbc₁ and 97–107 in Rsbc₁ help maintain the globular shape of the ISP-ED as compared with its mitochondrial homologues (Fig. 4D).

Structures of Bound Lipid and Detergent Molecules—Membrane proteins depend on the presence of lipids to maintain their functional and/or structural integrity. Bovine and yeast mitochondrial bc₁ are inactivated through delipidation (21, 48), a process that can be reversed by restoring specific lipids (49). In crystals of Rsbc₁, characteristic features of several lipid and detergent molecules appeared in difference Fourier maps. Ordered lipid molecules are often found between symmetry-related dimers, at the dimer or subunit interfaces, and in surface depressions. One lipid molecule was positively identified on the N-side of the membrane and included in the model. However, additional lipids that are only partially recognizable at both sides of the membrane were excluded. The lipid molecule bound at the cytoplasmic surface of cyt b is modeled as a lauryl oleoyl phosphatidyl ethanolamine (PE); its head group aligns with the surface plane of the cytoplasmic leaflet of the membrane and its fatty acid chains flank the TM helices B and G of cyt b (Fig. 5A). The exact identities of the fatty acids are unknown but the assignment as PE is supported by comparing it to the lipids present in bovine and yeast (21) mitochondrial bc₁. The phosphate group is hydrogen bonded to two highly conserved consecutive tyrosine residues (Tyr¹¹⁷ and Tyr¹¹⁸), and the lipid head group is further stabilized by the side chain of Arg⁵⁵⁸ by forming an ion pair with the lipid phosphate. Common to all structures is the lining of the groove between TM helices B and G (cyt b) with one of the fatty acid chains. In contrast to the lipid in mitochondrial bc₁, whose terminal n-alkyl (n~6~9) moieties of both chains reach into the groove, the bulky side chain of Phe¹¹³ prevents this interaction in Rs cyt b.

On the periplasmic side, a few clusters of residues are involved in lipid binding. The 17-residue insertion, including the H1d helix, in the cyt c₁ subunit (110–126, Figs. 1C and 3B) is positioned parallel to the plane of lipid head groups of the membrane and protrudes laterally into the membrane bilayer,
creating a cavity bounded additionally by the TM helices E and G and the ef-loop (including PFWY) of cyt b. The C-terminal end of this helical insertion is \( \sim 5 \) Å away from the cyt \( c_1 \) metal binding site and \( \sim 5 \) Å away from the sugar ring of a detergent molecule (β-OG). There is weak electron density in the void, which resembles the head group of another lipid molecule with fatty acid hydrocarbon chains extending toward the cytoplasmic side (Fig. 5B). Weak density is present in all six copies of the \( bc_1 \) monomers but is insufficient to build and refine a lipid molecule with confidence.

At the N-terminal end of the ef1 insertion in cyt b, the side chain of Trp313 forms a stacked pair with its symmetry-mate from a neighboring dimer at a distance of 3.8 Å. This pair is symmetrically flanked by at least six pieces of extra electron density, most likely stemming from bound lipid or detergent molecules (Fig. 5C). A strontium ion, clearly confirmed by its anomalous signal, sits right above the indole rings of the tryptophan pair. Its exact coordination environment cannot be resolved, but might involve the head groups of two pairs of putative lipid molecules. We observed the tryptophan pair formation in all crystal forms, and the presence of strontium ions seems to strengthen the interaction but is not required (Fig. 5C).

**Fate of the Subunit IV**—Purified \( Rsb_{c1} \), both wild type and mutant, contains one additional 14.4-kDa subunit (subunit IV), which has been shown to enhance the activity of the core subunits by 68% (50) but is not essential for the function of the complex or the survival of the organism. The same observations have been made about the non-essential 6-kDa subunit in \( Rhodovulum sulfidophilum \) \( bc_1 \) (51). In fact, many of the known bacterial forms of \( bc_1 \), including \( Rhodobacter capsulatus \) and \( Paracoccus denitrificans \), contain only the required three core subunits, cyt \( b \), cyt \( c_1 \) and the ISP. In the crystal structure,
Crystal Structures of R. sphaeroides bc$_1$

however, subunit IV is missing from the complex, indicating that the crystallization medium (including PEG400, detergents, etc.) must have caused the detachment of subunit IV. A SDS-PAGE gel revealed the presence of subunit IV in solution, but showed no detectable amount in crystals (data not shown). It is not uncommon to lose a supernumerary subunit during crystallization of mitochondrial bc$_1$ complexes (17, 27). To test whether subunit IV is indirectly required for the crystallization of Rsbc$_1$, we purified the ∆-subIV mutant (32) and subjected it to the same crystallization conditions. Triclinic crystals grew readily, displaying the same plate-like morphology as the monoclinic ones, and diffracted x-rays to 3.1-Å resolution. The structure could be readily solved and refined (Data not shown), demonstrating that the subunit IV is not required for crystallization.

Functional Implications of the Rsbc$_1$ Structure—Despite lacking supernumerary subunits, bacterial bc$_1$ operates in exactly the same way as mitochondrial enzymes and has therefore been widely used as a model system for mechanistic studies. The modified Q cycle mechanism for bc$_1$ function has received most experimental support (1); it defines separate Q$_p$ and Q$_N$ sites and requires an obligatory bifurcated ET pathway at the Q$_p$ site. Previous crystallographic studies on mitochondrial bc$_1$ complexes revealed the physical locations for the Q$_p$ and Q$_N$ site, respectively (14), demonstrated the importance of the ISP-ED mobility in the electron bifurcation at the Q$_p$ site (15), and outlined a possible mechanism for the ISP-ED conformation switch (46). The structures of Rsbc$_1$ in this work contain additional dynamic structural information encoded in multiple copies of the complexes that promises further insight into its mechanism. For example, the crystal with C$_2$ symmetry consists of six copies of Rsbc$_1$ monomers in a crystallographic asymmetric unit, and crystals of space group $P_2_1$ have four copies. By properly superimposing various parts of the structure, dynamic information with respect to substrate binding and subunit motion is revealed.

Multiple Binding Positions of Quinone at the Q$_N$ Site—Toward the end of the refinement of the Rsbc$_1$ structure in the absence of the Q$_N$ site inhibitor antimycin, non-crystallographic symmetry (NCS) restraints for side chains of the quinone interacting residues (His$^{217}$, Asp$^{252}$, and Asn$^{221}$) as well as for the bound substrate ubiquinone (UQ) were released. This permitted a more realistic estimate of ~70% occupancy of the bound natural substrate UQ based on the comparison of average B factor of UQ to side chain atoms of those of surrounding, interacting residues. The long isoprenoid tail of UQ falls rapidly into discontinuous electron density and was therefore modeled with only two isoprenoid repeats. The quinone molecules are roughly perpendicular to the parallel planes of Phe$^{216}$ and heme b$_{14}$ on one side and parallel to the plane of Phe$^{244}$ on the other side (Fig. 6A). The side chains of three polar residues, His$^{217}$, Asp$^{252}$, and Asn$^{221}$, are within contact distances to the bound UQ.

When the six independent cyt $b$ monomers were superimposed in pairs, the average r.m.s. deviation of the superposition is 0.14 Å for 428 residues. Except for the ef1 helix, the rest of the main chain atoms align almost perfectly. Additionally, the chromone rings of stigmatellin molecules at the Q$_p$ pockets superimpose well. At the Q$_N$ pocket, hydrophobic residues lining the wall and the b$_{14}$ heme groups are also well aligned, whereas the positions and orientations of bound quinone substrates and side chains of the three interacting residues, His$^{217}$, Asp$^{252}$, and Asn$^{221}$, were spread over a small range (Fig. 6A). The largest positional and rotational displacements among the six bound UQ molecules are 1.3 Å and 38°, respectively. The UQ molecules form H-bonds with His$^{217}$ (His$^{201}$, bovine) with the O$_2$-NE2 distances in the range of 2.2–2.4 Å, indicating that the imidazole ring of His$^{217}$ follows the motion of UQ, consistent with its observed conformational flexibility (24). Unlike the Btbc$_1$ (1NTZ, Ref. 24) and the yeast structure (1KB9, Ref. 23) structure, no bridging water molecule was identified. Residue His$^{217}$ is well-conserved and has been identified by spin resonance techniques as the residue that binds directly to the UQ radical (52). Asn$^{221}$ and Asp$^{252}$ (Ser$^{205}$ and Asp$^{228}$ in bovine) were observed to H-bond to UQ in Btbc$_1$ (24) but the bonding distance in the independent Rsbc$_1$ monomers varied from 3.6 to 4.5 Å for Asn$^{221}$ and from 3.8 to 5.5 Å for Asp$^{252}$. The motions exhibited by bound UQ demonstrate a weak binding of the Q$_N$ pocket for the substrate UQ; such a variation was not observed for the binding of the Q$_N$ site-specific inhibitor antimycin. Furthermore, this observation provides a direct structural support for the notion of low-binding affinity of the substrate during catalysis (24) or functional conformation (53). In one of the six monomers, Asp$^{252}$ H-bonds to a well-ordered water molecule. The conserved Lys$^{251}$, proposed previously to be important for proton uptake, displays considerable conformation variations.

Positional Anisotropy in the Subunits of Rsbc$_1$—The presence of six independent copies of each subunit (C$_2$ form) permits an analysis of the degree to which cyt $b$, cyt $c$, and the ISP show flexibility when they assemble into the complex. Of particular interest is the question of the position of the ISP-ED, which is known to undergo large scale conformational changes as part of the mechanism of bc$_1$ function (15, 46). However, in this inhibited complex the ISP-ED is firmly locked down in the Q$_p$ site by stigmatellin. By superimposing only cyt $b$ subunits and calculating the r.m.s. deviations between the position of pairs of cyt

![FIGURE 4. Structure of insertions and deletions in Rsbc$_1$. A, stereo pair displaying the N-side structure of a monomeric Rsbc$_1$. Emphasized in red are the N- and C-terminal extensions and one insertion (de-helix) of cyt b. Yellow and blue CO$_2$ traces represent N-terminal and C-terminal helices of ISP and cyt $c$, respectively. B, stereo diagram of the structure and environment of the P-side insertion (ef1-helix) in cyt b. The CO$_2$ trace of Rsbc$_1$, is yellow and that of Btbc$_1$, is black. At the N-terminal side of the ef1-helix is a large piece of electron density shown in green. Labels in italics refer to the bovine cyt $b$ sequence. C, stereo view of a superposition of CO$_2$ traces of Rs (green) and bovine (brown) cyt $c$, looking down the molecular 2-fold axis from the periplasmic side into membrane bilayer. Also shown is the rib ISP (gray) in the hypothetical $c$ position obtained from the position of ISP found in the 1BE3 structure. Mitochondrial cyt $c$, extends the hairpin structure around residues 73–79 (bovine numbering) to reach out to the helix around residues 92–108. The contact area between the two elements that reach across the dimer is shown in transparent cyan and beige surfaces. In contrast, equivalent residues (77, 95) in Rsbc$_1$, are more than 13 Å apart. D, stereo view of the ISP insertion. The insertion of residues 97–108 (in stick model) is shown under a transparent surface. The locations of the 2Fe2S cluster with its ligands (His$^{134}$, His$^{132}$, Cys$^{129}$, Cys$^{149}$), the C terminus Gly$^{187}$ and N-terminal Ala$^{146}$ that connects to the TM helix are marked on the surface in green, cyan, and yellow, respectively.](2854)
c_{1} and pairs of ISP Ca positions, the following picture emerges: The cyt b subunits themselves are very rigid and superimpose with r.m.s. deviations between 0.05 to 0.15 Å, providing baseline r.m.s. deviation values. The Ca positions of cyt c_{1} display r.m.s. deviations in the range of 0.22–0.54 Å, which are 2.8–5.4 times larger than the baseline. Even more so, the r.m.s. deviation values (0.36–1.28 Å) of the ISP subunits are 4.5–8.5 times above the background.

While the r.m.s. deviations hint at the increased propensities of cyt c_{1} and the ISP to adopt different positions, these values alone do not reveal the nature of the underlying distributions. To visualize the character of the distributions, each sextet of Ca positions was subjected to a trivariate Gaussian analysis (See Supplemental information) (Fig. 6B). The surface of the ellipsoids is drawn at a constant probability density encompassing a volume representing 90% of the total probability and shows clearly anisotropic spread of the ISP-ED Ca positions that has a pivotal region around the 2Fe2S cluster, which grows larger with distance. We interpret the anisotropic shape and size of the probability ellipsoids of the ISP-ED not as an actual movement of the ISP, as each molecule is locked into the Q_{P} position and restrained by crystal packing forces, but as a qualitative measure for the ability of the ISP-ED to undergo directional movements when unrestrained.

Although the mobility of ISP-ED has been established by a number of experimental approaches (15, 20, 26, 54–56), the ability of the ISP-ED to undergo positional adjustments qualitatively consistent with its function to move toward cyt c_{1} has never been shown in a single structure before.

The extrinsic domain of cyt c_{1} (cyt c_{1}-ED) also showed considerable “motion”. Unlike ISP-ED, the displacements of cyt c_{1}-ED are largely isotropic (Fig. 6B). The increased mobility that arises from the absence of inter cyt c_{1} contacts...
due to the two deletions in the Rsbc1 dimer (Fig. 4c) might result in unsuccessful docking attempts of ISP-ED to cyt c1, leading to reduced efficiency in ET.

Atomic coordinates of the refined inhibitor-bound Rsbc1 structures have been deposited in the Protein Data Bank with accession codes: 2QIP (wild type, stigmatellin and antimycin), 2QJY (double mutant, stigmatellin), 2QJK (double mutant, stigmatellin and antimycin).

Acknowledgments—We thank Dr. X. Wen for his contribution to this work. We thank the staff of the SER-CAT beamline at APS, ANL, for their assistance in data collection.

REFERENCES

1. Brandt, U., and Trumpower, B. (1994) Crit. Rev. Biochem. Mol. Biol. 29, 165–197
2. Hope, A. B. (1993) Biochim. Biophys. Acta 1143, 1–22
3. Trumpower, B. L. (1990) Microbiological Rev. 54, 101–129
4. Trumpower, B. L., and Haggerty, J. G. (1980) J. Bioenerget. Biomembr. 12, 151–164
5. von Jagow, G., Gribble, G. W., and Trumpower, B. L. (1986) Biochemistry 25, 775–780
6. Sauter, H., Steglich, W., and Anke, T. (1999) Angew. Chem. Int. Ed. 38, 1328–1349
7. Jordan, D. B., Livingston, R. S., Bisaha, J. J., Duncan, K. E., Pember, S. O., Picolletti, M. A., Schwartz, R. S., Sternberg, J. A., and Tang, X. S. (1999) Pesticide Science 55, 105–118
8. Kessl, J. J., Lange, B. B., Bermbitz-Zahradnik, T., Zwicker, K., Hill, P., Meunier, B., Palsdottir, H., Hunte, C., Meshnick, S., and Trumpower, B. L. (2003) J. Biol. Chem. 278, 31312–31318
9. Dilmauro, S., and Schon, E. A. (2003) N. Engl. J. Med. 348, 2656–2668
10. Andreu, A. L., Hanna, M. G., Reichmann, H., Bruno, C., Penn, A. S., Tanji, K., Pallotti, F., Iwata, S., Bonilla, E., Lach, B., Morgan-Hughes, J., and DiMauro, S. (1999) N. Engl. J. Med. 341, 1037–1044
11. Brown, M. D., Voljavec, A. S., Lott, M. T., Torroni, A., Yang, C. C., and Wallace, D. C. (1992) Genetics 130, 163–173
12. Turrens, J. F., Alexandre, A., and Lehninger, A. L. (1985) Arch. Biochem. Biophys. 237, 408–414
13. Stariiek, K., Gille, L., Kozlov, A. V., and Nohl, H. (2002) Free Radic. Res. 36, 381–387
14. Xia, D., Yu, C. A., Kim, H., Xia, J. Z., Kachurin, A. M., Zhang, L., Yu, L., and Deisenhofer, J. (1997) Science 277, 60–66
15. Zhang, Z., Huang, L., Shulmeister, V. M., Chi, Y. I., Kim, K. K., Hung, L. W., Crofts, A. R., Berry, E. A., and Kim, S. H. (1998) Nature 392, 677–684
16. Iwata, S., Lee, J. W., Okada, K., Lee, J. K., Iwata, M., Rasmussen, B., Link, T. A., Ramaswamy, S., and Jap, B. K. (1998) Science 281, 64–71
17. Hunte, C., Koepke, J., Lange, C., Rossmanith, T., and Michel, H. (2000) Structure 15, 669–684
18. Kurisu, G., Zhang, H., Smith, J. L., and Cramer, W. A. (2003) Science 302, 1009–1014
19. Stroebel, D., Choquet, Y., Popot, J. L., and Picot, D. (2003) Nature 426, 413–418
20. Kim, H., Xia, D., Yu, C. A., Xia, J. Z., Kachurin, A. M., Zhang, L., Yu, L., and Deisenhofer, J. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 8026–8033
21. Lange, C., Nett, J. H., Trumpower, B. L., and Hunte, C. (2001) EMBO J. 20, 6591–6600
22. Gao, X., Wen, X., Yu, C., Essef, L., Tsao, S., Quinn, B., Zhang, L., Yu, L., and Xia, D. (2002) Biochemistry 41, 11692–11702
23. Lange, C., and Hunte, C. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 1800–2805
24. Gao, X., Wen, X., Essef, L., Yu, L., Yu, C. A., and Xia, D. (2003) Biochemistry 42, 9067–9080
25. Hunte, C., Palsdottir, H., and Trumpower, B. L. (2003) FEBS Lett. 545, 39–46
26. Esser, L., Quinn, B., Li, Y., Zhang, M., Elberry, M., Yu, L., Yu, C. A., and Xia, D. (2004) J. Mol. Biol. 341, 281–302
27. Huang, L., Cobessi, D., Tung, E. Y., and Berry, E. A. (2005) J. Mol. Biol. 35, 573–597
28. Gennis, R. B., Barquera, B., Hacker, B., Van Doren, S. R., Arnaud, S., Crofts, A. R., Davidson, E., Gray, K. A., and Daldal, F. (2001) J. Bioenerget. Biomembr. 25, 195–202
29. Berry, E. A., Huang, L., Saechao, L. K., Pon, N. G., Valkova-Valchanova, M., and Daldal, F. (2004) Photosynthesis Res. 81, 251–275
30. Elberry, M., Xiao, K., Essef, L., Xia, D., Yu, L., and Yu, C. A. (2006) Biochim. Biophys. Acta 1757, 835–840
31. Onobrakwe, Z., and Most, W. (1997) Methods Enzymol. 276, 307–326
32. Yu, L., Tso, S. C., Shenoy, S. K., Quinn, B. N., and Xia, D. (1999) J. Bioenerget. Biomembr. 31, 251–257
33. Vagin, A., and Teplyakov, A. (2000) Acta Crystallogr. Sect. B 56, 1622–1624
34. CCP4. (1994) Acta Crystallogr. Sect. D 50, 760–763
35. Brumberger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, I., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. D., Rice, L. M., Simonson, T., and Warren, G. L. (1998) Acta Crystallogr. Sect. D 54, 905–921
36. Jones, T. A., Zou, J. Y., and Cowan, S. W. (1991) Acta Crystallogr. Sect. A 47, 110–119
37. Elberry, M., Yu, L., and Yu, C. A. (2006) Biochemistry 45, 4991–4997
38. Liu, X., Yu, C. A., and Yu, L. (2004) J. Biol. Chem. 279, 47363–47371
39. Ohnishi, T., Brandt, U., and von Jagow, G. (1988) Eur. J. Biochem. 176, 385–389
40. Covian, R., Pardo, J. P., and Moreno-Sanchez, R. (2002) J. Biol. Chem. 277, 48449–48455
41. Yamashita, E., Zhang, H., and Cramer, W. A. (2007) J. Mol. Biol. 370, 39–52
42. von Jagow, G., and Link, T. A. (1986) Methods Enzymol. 126, 253–271
43. Schnaufer, A., Sbicego, S., and Blum, B. (2000) Curr. Genet. 37, 234–241
44. Miyoshi, H., Tokutake, N., Imaeda, Y., Akagi, T., and Iwamura, H. (1995) Biochim. Biophys. Acta 1229, 149–154
45. Kim, H., Esser, L., Hossain, M. B., Xia, D., Yu, C. A., Rizo, J., van der Helm, D., and Deisenhofer, I. (1999) J. Am. Chem. Soc. 121, 4902–4903
46. Esser, L., Gong, X., Yang, S., Yu, L., Yu, C. A., and Xia, D. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 13045–13050
47. Xiao, K., Liu, X., Yu, C. A., and Yu, L. (2004) Biochemistry 43, 1488–1495
48. Gomez, B., Jr., and Robinson, N. C. (1999) Biochemistry 38, 9031–9038
49. Hayer-Hartl, M., Schagger, H., von Jagow, G., and Beyer, K. (1992) Eur. J. Biochem. 209, 423–430
50. Chen, Y. R., Yu, C. A., and Yu, L. (1996) J. Biol. Chem. 271, 2057–2062
51. Rodgers, S., Moser, C., Martinez-Julvez, M., and Sinning, I. (2000) Eur. J. Biochem. 267, 3753–3761
52. Kolling, D. R. J., Samoilova, R. I., Holland, J. T., Berry, E. A., Dikanov, S. A., and Crofts, A. R. (2003) J. Biol. Chem. 278, 39747–39754
53. Crofts, A. R. (2004) Annu. Rev. Physiol. 66, 28.21–28.45
54. Nett, J. H., Hunte, C., and Trumpower, B. L. (2000) Eur. J. Biochem. 267, 5777–5782
55. Tian, H., Yu, L., Mather, M. W., and Yu, C. A. (1998) J. Biol. Chem. 273, 27953–27959
56. Darrouzet, E., Valkova-Valchanova, M., and Daldal, F. (2000) Biochemistry 39, 15475–15483
57. Kraulis, P. J. (1991) J. Appl. Crystallogr. 24, 946–950
58. Esnouf, R. M. (1997) J. Mol. Graphics 15, 133–138