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Biochemistry

Size of the amino acid side chain at position 158 of cytochrome *b* is critical for an active cytochrome bc_1 complex and for photosynthetic growth of *Rhodobacter capsulatus*

(electron transport chain/ubiquinol:cytochrome *c* oxidoreductase/site-directed mutagenesis/quinone binding site/membrane protein overproduction)

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ABSTRACT **The nonphotosynthetic mutant R126 of *Rhodobacter capsulatus* has a cytochrome (cyt) bc_1 complex (EC 1.10.2.2) with a defective quinol oxidation $Q_{z(o,p)}$ site but a functional quinone reduction $Q_{c(i,n)}$ site [Robertson, D. E., Davidson, E., Prince, R. C., van der Berg, W., Marrs, B. L. & Dutton, P. L. (1986) *J. Biol. Chem.* 261, 584–591]. Genetic analyses of this mutant have indicated that a single-base-pair change has replaced Gly-158 of cyt *b* with Asp. In this work, Gly-158 was changed by oligonucleotide-mediated mutagenesis to several other amino acids to define its role on quinol oxidation catalyzed by the cyt bc_1 complex. The effects of the mutations were analyzed by measuring the photosynthetic growth rate of mutants and the activity of their cyt bc_1 complexes. The mutants overproduced the cyt bc_1 complex, assembled its subunits, and incorporated its prosthetic groups as shown by immunoblotting and optical difference spectroscopy. Of 14 amino acid residues tested at position 158 of cyt *b* all but alanine and serine resulted in a marked decrease of cyt bc_1 activity and failed to support photosynthetic growth of *R. capsulatus*. The photosynthesis-competent mutants, Gly-158 → Ala and Gly-158 → Ser, had lower cyt bc_1 complex activities that were resistant to myxothiazol, but not to stigmatellin, quinol oxidation inhibitors. These findings indicated that the specific role of Gly-158 of cyt *b* on quinol oxidation and myxothiazol binding may be related to the small size of its side chain and are discussed in terms of the structure and function of the quinol oxidation site of the cyt bc_1 complex.**

The ubiquinol:cytochrome *c* oxidoreductase [cytochrome (cyt) bc_1 complex; EC 1.10.2.2] catalyzes the transfer of electrons from ubiquinol to cyt *c* and translocates protons across the membrane (1–3). It contains a minimum of three redox-active polypeptide subunits: a *c* type cyt of ≈30 kDa, a 2Fe–2S protein of ≈20 kDa, and a polypeptide of ≈50 kDa carrying two distinct *b* type cyts, cyt $b_{L,566}$ and cyt $b_{H,560}$. The primary structure of these subunits has been established by sequencing the corresponding genes from several sources (for an overview, see ref. 3). In the photosynthetic bacterium *Rhodobacter capsulatus*, these genes are clustered as an operon, named *fbc* (4) or *pet* (5) with the 5′ to 3′ order being *fbcF(petA)* (FeS protein), *fbcB(petB)* (cyt *b*), and *fbcC(petC)* (cyt c_1).

The cyt bc_1 complex is thought to contain two distinct quinone binding domains: The quinol oxidation site (called Q_z, Q_o, or Q_p) and the quinone reduction site (called Q_c, Q_i, or Q_n) are on the outer and inner sides of the biomembrane (1–3). Several inhibitors, such as myxothiazol or stigmatellin and antimycin or funiculosin, affect the reactions catalyzed at these sites differently (for review, see ref. 6). To better define the location and structure of these sites, inhibitor-resistant mutants have been isolated, and their molecular basis has been elucidated in mitochondrial (7, 8) and bacterial (9) systems. In *R. capsulatus*, spontaneous Q_z-inhibitor-resistance mutations were confined to two discrete regions of cyt *b*. Five mutational sites were found between amino acids 140 and 163 in the Q_zI region, which links the transmembrane helices III and IV of cyt *b* (9). Two of these sites (positions 144 and 152) have also been observed in yeast cyt *b* (7) and two others (positions 158 and 163) have been observed in mouse mitochondrial cyt *b* (8).

Earlier, a nonphotosynthetic mutant of *R. capsulatus*, R126, has been studied biochemically by Robertson *et al.* (10) and shown to be impaired at the quinol oxidation (Q_z) site of the cyt bc_1 complex while retaining a functionally intact quinone reduction (Q_c) site. This mutant has also been used to isolate by genetic complementation the structural genes of *R. capsulatus* cyt bc_1 complex (11). The mutation in R126 has been identified as a Gly-158 → Asp replacement in cyt *b* (*fbcB*:G158D) (9). In this work, a genetic system for site-directed mutagenesis of the cyt bc_1 complex of *R. capsulatus* was developed and, by using it, the role played by Gly-158 in cyt *b* was probed. Fifteen mutants carrying amino acid substitutions at this position were obtained and the effects of these substitutions on the activity of the cyt bc_1 complex, on inhibitor binding, and on photosynthetic growth of *R. capsulatus* were determined.

MATERIALS AND METHODS

Media, Strains, Plasmids, Growth Conditions, and Recombinant DNA Techniques. *Escherichia coli* and *R. capsulatus* strains were cultured on LB and on MPYE rich or RCV minimal media, respectively, and all recombinant DNA techniques were done as described (9). Plasmids pPET1 and pMT0-404 carrying an expressed copy of the *fbc(pet)* operon and the *R. capsulatus* mutants containing chromosomal deletions eliminating part of it [MT-RBC1 (*Stu* I fragment between residues 540 and 3490) and MT-CB5 (*Eco*RI–*Sma* I fragment between residues 1330 and 1830)] are shown in Figs. 1 and 2 and will be described in detail elsewhere.

Site-Directed Mutagenesis and Chromosomal Allele Replacement. The phage M13mp10-73R2BC$_1$ carrying the 1.8-kilobase-pair *Sma* I–*Sma* I fragment containing the early part of the *fbcB(petB)* gene (9) was used as a template, and the mixed synthetic oligonucleotides petB-G158-1 and petB-G158-2 as mutagenic primers with the Muta-Gene kit of Bio-Rad (Fig. 2). Mutagenized phages were screened by DNA sequencing using Sequenase according to United States

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Abbreviations: cyt, cytochrome; DBH, 2,3-dimethoxy-5-methyl-6-decyl-1,4-benzoquinone; Ps, photosynthetic growth phenotype.
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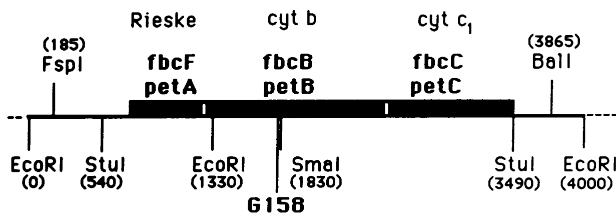

FIG. 1. Restriction map of the *fbcFBC(petABC)* cluster of *R. capsulatus* MT1131. Dotted lines, chromosomal DNA outside the *Eco*RI sites bracketing the *fbcFBC(petABC)* operon; thick line, its coding regions. Position numbers are from ref. 5, and G158 indicates Gly-158 of cyt *b*.

Biochemical. Construction of the merodiploids pBG158X/MT-RBC1 and transfer of the mutations *fbcB*:G158A and *fbcB*:G158S into the chromosome of MT-CB5 are described in Fig. 2.

Biochemical Analyses. Chromatophores were prepared using a French pressure cell as described (13) except that the cell debris was eliminated by centrifugation at 37,000 × *g* for 45 min. This yielded clearer chromatophore preparations that scattered less light. Bacteriochlorophyll content of chromatophores was determined after extraction into acetone/methanol, 7:2 (vol/vol), using an extinction coefficient (ε_{772}) of 75 $mM^{-1} \cdot cm^{-1}$ (14). Protein concentrations were measured according to Lowry *et al.* (15) after extraction of pigments with acetone. Reduced-minus-oxidized optical difference spectra of *c* and *b* type cyts were recorded at room temperature with a Hitachi spectrophotometer U3210. SDS/PAGE and Western blot analyses were performed as described (16) using the monoclonal antibodies D50, D42, and D29 (17) and horseradish peroxidase-conjugated secondary antibodies from Bio-Rad.

Activity of cyt *c* reductase was measured at room temperature as described (18) using chromatophores prepared from chemoheterotrophically grown cells in MPYE medium, 40 μM 2,3-dimethoxy-5-methyl-6-decyl-1,4-benzoquinone (DBH), 50 μM horse heart cyt *c*, and sodium dodecyl maltoside (0.1 mg/ml).

RESULTS

A Genetic System for Site-Directed Mutagenesis of the cyt bc_1 Complex of *R. capsulatus*. Replacement of a particularly interesting amino acid residue of a protein with various residues often contributes to a better understanding of the structural and functional parameters at this site of the protein. Thus, a genetic system was sought to allow the replacement of any amino acid residue of the cyt bc_1 complex by any other residue by using site-directed mutagenesis. A chromosomal deletion eliminating the entire *fbc(pet)* operon, MT-RBC1,

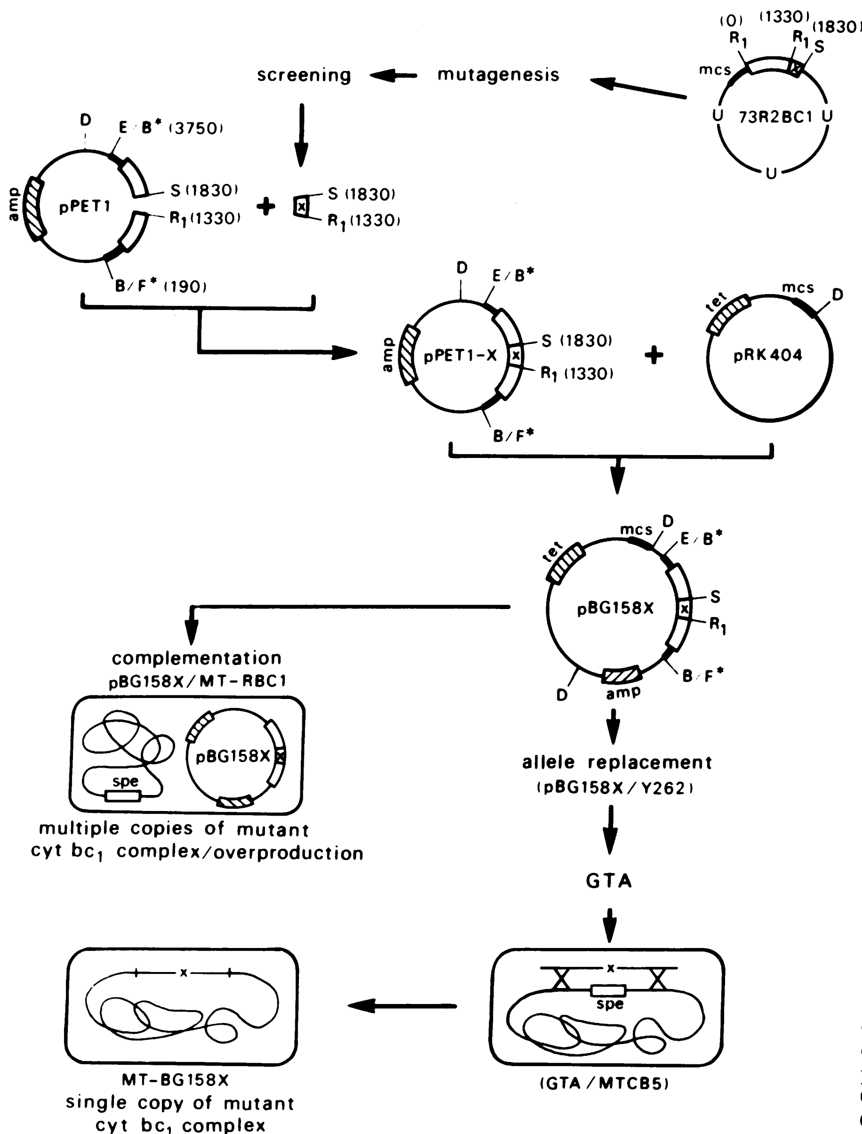

FIG. 2. Genetic system for site-directed mutagenesis of the cyt bc_1 complex of *R. capsulatus*. Phage M13-73R2BC$_1$ was used as a template for mutagenesis and the mutation carried by the 0.5-kilobase-pair *Eco*RI–*Sma* I fragment between residues 1330 and 1830 [R_1(1330)–S(1830)] was shuttled to pPET1 yielding plasmid pPET1-X (X being the substituting amino acid at position 158). This latter plasmid was ligated to the *Hin*dIII site of pRK404 yielding pBG158X, which was conjugated into either the *R. capsulatus* cyt bc_1-minus strain MT-RBC1 for complementation or the gene transfer agent (GTA) overproducer strain Y262 (12) for allele replacement. The GTA obtained from the latter strains were used to introduce the *fbcB*: G158A and -S mutations into the chromosome of MT-CB5 by selecting for Ps^+ and screening for myxothiazol-resistant, spectinomycin-sensitive colonies on MPYE plates. X, U, mcs, amp, tet, and spe indicate the mutated amino acid residue, uracilated template DNA, multiple cloning site, and ampicillin-, tetracycline-, and spectinomycin-resistance genes, respectively. R_1, S, D, E/B* and B/F* correspond to *Eco*RI, *Sma* I, *Hin*dIII, and the hybrid *Eco*RI/*Bal* I and *Bal* I/*Fsp* I restriction enzyme sites, respectively. The nucleotide sequences of the mutagenic primers petB-G158-1 and petB-G158-2 were 5′-CACGGTGGC(G/C)NNC-CAGAACG-3′ and 5′-CACGGTGGC(G/C)(T/C)(C/T/A)CCAGAACG-3′, respectively.

was transcomplemented with the composite plasmid, pMT0-404, carrying a wild-type copy of the same operon (Fig. 2). As expected, the merodiploid pMT0-404/MT-RBC1, but not the haploid strain MT-RBC1, grew photosynthetically on rich or minimal medium. Interestingly, this merodiploid overproduced all three subunits of the cyt bc_1 complex as shown by immunological and spectroscopic analyses. Increased amounts of *c* and *b* type cyts were detected in chromatophores of pMT0-404/MT-RBC1 (Figs. 3 and 4).

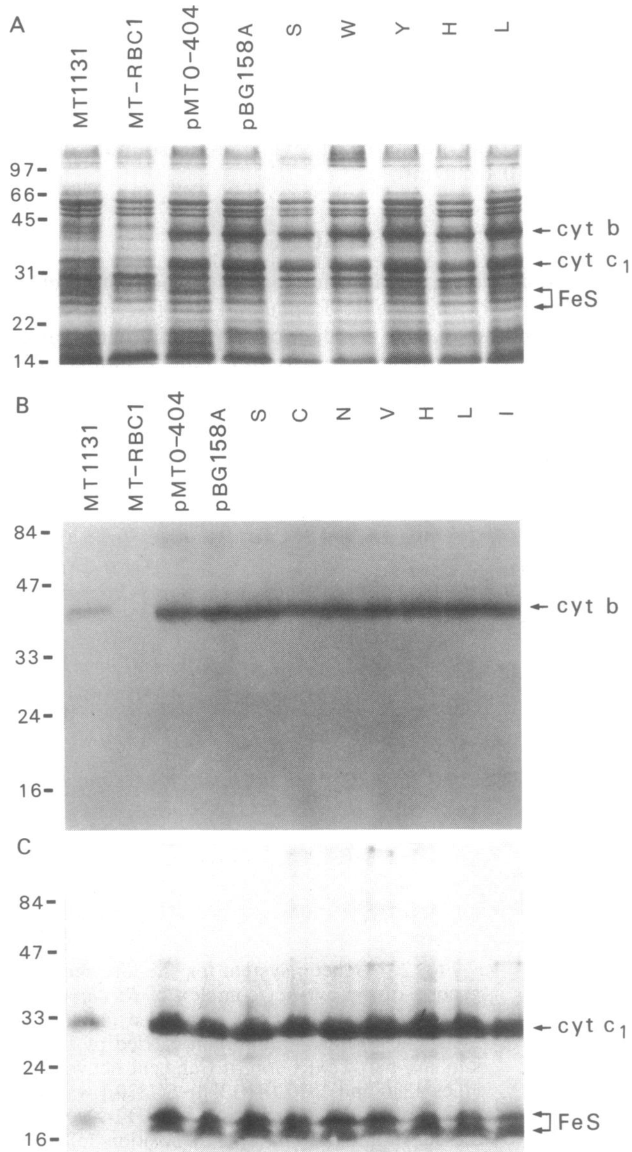

FIG. 3. SDS/PAGE and Western blot analyses of chromatophores of various cyt *b*:G158 substitutions. (*A*) SDS/PAGE of chromatophores of *R. capsulatus* MT1131 (wild-type cyt bc_1 complex, single copy), MT-RBC1 (cyt bc_1 deletion), pMT0-404 (wild-type cyt bc_1 complex, multicopy), pMTG158A, -S, -W, -Y, -H, and -L (cyt bc_1 complexes with cyt *b*:G158A, -S, -W, -Y, -H, and -L substitutions, respectively. Membrane proteins at 25 μg per lane (heated for 10 min at 37°C) were separated on a 15% gel and stained with Coomassie blue G250. Molecular weight markers were from Bio-Rad. (*B* and *C*) Western blot analyses of chromatophores from *R. capsulatus* strains MT1131, MT-RBC1, pMT0-404 (described in *A*), and pMTG158A, -S, -C, -N, -V, -H, -L, and -I strains containing cyt bc_1 complexes with cyt *b*:G158A, -S, -C, -N, -V, -H, -L, and -I substitutions, respectively. Total membrane proteins at 70 μg and 25 μg per lane for *B* and *C*, respectively, were separated by SDS/PAGE on a 15% gel, electroblotted onto a nitrocellulose filter, and probed with monoclonal antibodies D50 (*B*) and D42 and D29 (*C*) recognizing the *R. capsulatus* cyt *b*, cyt c_1, and FeS protein, respectively. Positions of prestained molecular mass markers (Bio-Rad) are indicated in kDa.

Substitutions at Gly-158 of cyt *b* (cyt *b*:G158) Yielding Active cyt bc_1 Complexes. Since two mutations at position 158 of cyt *b* have yielded either nonfunctional (G158D) (9) or myxothiazol-resistant (G142A, homologous to Gly-158 of bacterial cyt *b*) (8) cyt bc_1 complexes, several other amino acid replacements at this position were sought. After mutagenesis and screening, the following 14 amino acid substitutions at position 158 (GGC) were obtained: G158A(GCC), -S(AGC), -C(TGC), -T(ACC), -N(ACC), -D(GAC), -E(GAG), -H(CAC), -R(CGG), -V(GTC), -L(CTC), -I(ATC), -Y(TAC), and -W(TGG) (codons are in parentheses). Of these only the merodiploids pBG158A/MT-RBC1 and pBG158S/MT-RBC1 carrying the Gly → Ala and Gly → Ser substitutions, respectively, supported photosynthetic growth at rates comparable (doubling time of 120 and 140 min on MPYE at 35°C) to that (120 min) of the wild-type merodiploid pMT0-404/MT-RBC1. The mutant pBG158C/MT-RBC1 was unable to grow at temperatures higher than 30°C indicating that the Gly → Cys substitution rendered the photosynthetic growth temperature-sensitive. The remaining mutants (G158T, -D, -N, -E, -H, -R, -L, -V, -I, -Y, and -W) were unable to grow photosynthetically in either rich or minimal medium at any temperature ranging from 25 to 35°C.

Like pMT0-404/MT-RBC1 the mutant merodiploids pBG158A/MT-RBC1 and pBG158S/MT-RBC1 also overproduced the cyt bc_1 complex (Figs. 3 and 4), indicating that the

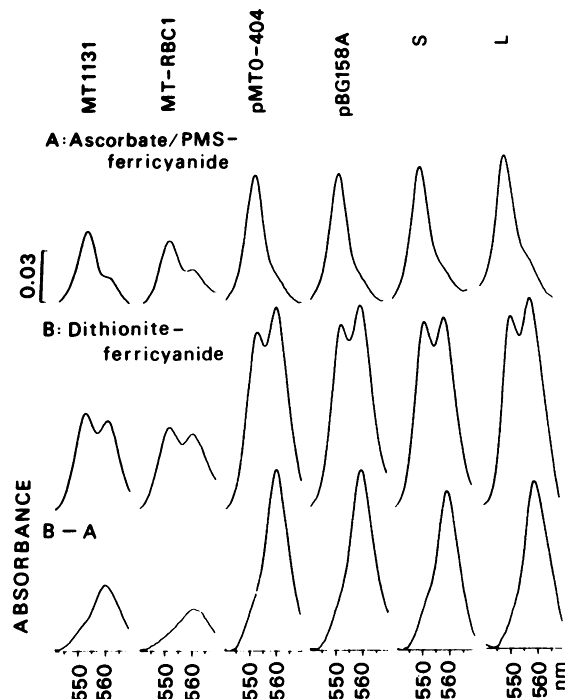

FIG. 4. Absorption spectra of cytochromes in chromatophores of *R. capsulatus* strains carrying the following cyt *b*:G158 substitutions: MT1131 (wild-type, single copy of cyt bc_1 complex), MT-RBC1 (cyt bc_1 deletion), pMT0-404 (wild-type, multiple copies of cyt bc_1 complex), and pBG158A, -S, and -L correspond to the cyt *b*:G158A, -S, and -L substitutions, respectively. Chromatophores (50 μM bacteriochlorophyll) were oxidized with potassium ferricyanide (0.8 mM) and reduced either with sodium ascorbate (1 mM) or sodium dithionite (a few crystals) in the presence of *N*-methylphenazonium methosulfate (PMS) (100 nM) and the difference spectra, indicated as ascorbate/PMS–ferricyanide (spectrum A) and dithionite–ferricyanide (spectrum B) were recorded as in ref. 13. Spectrum B − A was obtained by subtracting spectrum A from spectrum B by using an arithmetric program.

fbcB:G158A and *fbcB*:G158S mutations do not affect the synthesis, assembly, or turnover of the complex. Chromosomal derivatives of these mutations producing a normal amount of mutant complexes MT-BG158A and MT-BG158S were isolated to better define their effect on growth (Fig. 2). MT-BG158A grew photosynthetically at a rate similar to that of MT1131 (doubling time of 120 min on MPYE medium) but MT-BG158S grew markedly slower (doubling time of 190 min), suggesting that this latter substitution affected the cyt bc_1 complex activity more severely. The resistance to Q_z inhibitors was determined for both the haploid and merodiploid strains carrying these mutations. Although the photosynthetic growth of pMT0-404/MT-RBC1 and MT1131 was inhibited completely with 5 μM myxothiazol, that of pG158A/MT-RBC1, MT-BG158A, pG158S/MT-RBC1, and MT-BG158S was resistant to at least 100-fold higher concentrations of this inhibitor. Conversely, these mutations increased the sensitivity of the cyt bc_1 complex to stigmatellin, another cyt bc_1–quinol oxidation inhibitor.

cyt *b*:G158 Substitutions Yielding Nonfunctional cyt bc_1 Complexes. The cyt *b*:G158T, -D, -E, -N, -H, -R, -L, -V, -I, -Y, and -W substitutions, which were unable to support the photosynthetic growth of *R. capsulatus*, were further examined for the presence of nonfunctional cyt bc_1 complexes. SDS/PAGE analysis of chromatophores indicated that the cyt *b*:G158W, -Y, -H, and -L substitutions overproduced the subunits of the cyt bc_1 complex in amounts comparable to that directed by pMT0-404 (Fig. 3*A*). Western blot analysis of membrane proteins of nonfunctional mutants using monoclonal antibodies specific for the cyts *b* and c_1 and the FeS protein of the cyt bc_1 complex are presented in Fig. 3 *B* and *C*. The merodiploids carrying the substitutions G158C, -N, -V, -H, -L, and -I were able to incorporate into chromatophores increased amounts of cyts *b* and c_1 and FeS protein. Similar data were also obtained with the remaining photosynthetic growth-negative (Ps^-) substitutions (data not shown).

Optical difference spectra of chromatophores were taken to determine whether the Ps^- cyt *b*:G158 substitutions affected incorporation of the heme groups of the cyt bc_1 complex (Fig. 4). By using ascorbate-reduced minus ferricyanide-oxidized spectra (spectrum A), dithionite-reduced minus ferricyanide-oxidized spectra (spectrum B), and their arithmetical difference spectra (spectrum B − spectrum A) the total amount of *c* type, (*b* + *c*) types, and *b* type cyts, respectively, present in chromatophores of various mutants was determined. All cyt *b*:G158 substitutions contained *b* and *c* type cyts in amounts similar to those found in pMT0-404/MT-RBC1 (Fig. 4 for alanine, serine, and leucine substitutions; others not shown). Immunological and spectroscopic data in Figs. 3 and 4 indicated that the Ps^- mutants also overproduced, assembled, and incorporated the *b* and *c* heme groups into the cyt bc_1 complex.

DBH/cyt *c* Reductase Activity of cyt *b*:G158 Substitutions. The activity of the cyt bc_1 complex of various mutants are listed in Table 1. Membranes of the wild-type merodiploid pMT0-404/MT-RBC1 showed a cyt *c* reductase activity of 437 nmol of cyt *c* reduced per min per mg of total chromatophore proteins, which was sensitive to 5 μM antimycine or myxothiazol (95% or 97% inhibition, respectively). Membranes of the Ps^+ merodiploids pMTG158A/MT-RBC1 and pMT-G158S/MT-RBC1 had activities equal to 58% and 25%, respectively, of that seen with pMT0-404/MT-RBC1 but, as expected, the activities were resistant to 5 μM myxothiazol. The lower activity observed with the substitution cyt *b*:G158S was in agreement with its slower growth rate in the haploid state. The remaining Ps^- merodiploids exhibited much lower (<5% of wild type) levels of cyt *c* reductase activity (Table 1).

Table 1. DBH/cyt *c* reductase activity of various cyt *b*:G158 substitutions

Mutant	Phenotype	Residue 158	Activity: nmol per min per mg	Activity: % control
pMT0-404/MT-RBC1	Ps^+, Myx^S	Gly (60.1)	437	100
pG158A/MT-RBC1	Ps^+, Myx^R	Ala (88.6)	252	58
pG158S/MT-RBC1	Ps^+, Myx^R	Ser (89.0)	108	25
pG158C/MT-RBC1	Ps^{TS}	Cys (108.5)	12	2.8*
pG158D/MT-RBC1	Ps^-	Asp (111.1)	9	2.1
pG158T/MT-RBC1	Ps^-	Thr (116.1)	5	1.2
pG158N/MT-RBC1	Ps^-	Asn (117.7)	3	0.7
pG158E/MT-RBC1	Ps^-	Glu (138.4)	5	1.2
pG158V/MT-RBC1	Ps^-	Val (140.0)	7	1.6
pG158H/MT-RBC1	Ps^-	His (153.2)	4	1.0
pG158L/MT-RBC1	Ps^-	Leu (166.7)	7	1.6
pG158I/MT-RBC1	Ps^-	Ile (166.7)	8	1.8
pG158R/MT-RBC1	Ps^-	Arg (173.4)	11	2.5
pG158Y/MT-RBC1	Ps^-	Tyr (193.6)	5	1.2
pG158W/MT-RBC1	Ps^-	Trp (227.8)	4	1.0
MT-RBC1	Ps^-	Δcyt bc_1	0	0.0

Volumes in Å^3 (shown in parentheses) of amino acids were taken from ref. 26. Activity is reported as nmol of reduced horse heart cyt *c* per min per mg of membrane proteins, by using an extinction coefficient (ε_{550}) of 20 $mM^{-1} \cdot cm^{-1}$ for cyt *c*, and as percentage of activity found in strain pMT0-404/MT-RBC1 (control). Myx^S and Myx^R, myxothiazol-sensitive and -resistant, respectively.
*Note that this strain is temperature sensitive for growth.

Photosynthetic Growth-Positive (Ps^+) Revertants of *fbcB*:G158 Mutations. Secondary mutations that compensate already existing mutations in a protein are informative for deciphering the effects of the initial mutations. Thus, revertants that grew photosynthetically on MPYE were isolated from pSR7-404/MT-RBC1 carrying the *fbcB*:G158D (GGC → GAC) mutation of R126 (9). Nucleotide sequence analysis of the Q_zI region of two such revertants, pMF-rev1/MT-RBC1 (Ps^+, myxothiazol resistant) and pMF-rev2/MT-RBC1 (Ps^+, myxothiazol sensitive) indicated that G158D(GAC) was changed to G158A(GCC) and to the parental G158(GGC), respectively. These results were consistent with the site-directed mutagenesis studies that demonstrated that all of the other possible substitutions [G158N(AAC), -Y(TAC), -H(CAC), -V(GTC), -D(GAT), and -E(GAG or GAA)] obtainable from D158(GAC) by a single-base-pair change would have yielded Ps^- mutants. Spontaneous Ps^+ revertants were also obtained from the mutants pBG158H/MT-RBC1 [*fbcB*:G158H (CAC)] and pBG158L/MT-RBC1 [*fbcB*:G158L (CTC)]. These strains could not revert by single-base-pair changes at position 158 to glycine, alanine, or serine residues that allow photosynthetic growth. In each case, three Ps^+ isolates were proven to contain a proline (CCC) at position 158 by sequencing of the appropriate *Eco*RI–*Sma* I fragments of cyt *b*. The exchange of these fragments with their counterparts in pPET1 demonstrated that a single G158P substitution could also support the photosynthetic growth of *R. capsulatus* although poorly (doubling time of 250 min on MPYE medium).

DISCUSSION

In this work, a genetic system for site-directed mutagenesis of the cyt bc_1 complex of *R. capsulatus* was developed and used to analyze the structural and functional role of Gly-158 of the Q_zI region of cyt *b* (Fig. 2). The system described here is similar to that reported by Bylina *et al.* (19) for mutagenesis of the photochemical reaction center of *R. capsulatus*. A complete deletion of the *fbc*(*pet*) operon was used to avoid homologous recombination between the chromosomal and

the plasmid-carried copies of these genes. The merodiploids overproduced severalfold the wild-type as well as the mutant cyt bc_1 complexes without any deleterious effect on growth. Although this overproduction facilitated the measurement of low cyt bc_1 complex activities of mutants, it obscured the effect of partially active complexes on growth. Thus, a procedure to incorporate by allele replacement the mutations generated *in vitro* into the chromosomal *fbc(pet)* locus was also developed (Fig. 2). Analysis of the cyt *b*:G158S substitution indicated that the overproduction of a cyt bc_1 complex with decreased activity could also support photosynthetic growth like a wild-type complex.

Site-Directed cyt *b*:G158 Substitutions. Gly-158 is located within a highly conserved sequence of the Q_zI domain (7–9, 20). Out of the 15 substitutions tested at this position glycine, alanine, serine, and to a lesser degree cysteine and proline provided a cyt bc_1 complex active enough to support photosynthetic growth (Ps^+). The remaining 12 substitutions (G158C, -T, -D, -N, -E, -V, -H, -L, -I, -R, -Y, and -W) were Ps^- and had <5% of the wild-type cyt bc_1 complex activity (Table 1). The photosynthetic competence of G158A could be expected based on the conserved occurrence of this residue in chloroplast cyt b_6 (20) and on the mouse mitochondrial mutation (G142/158A) conferring resistance to myxothiazol (8). However, no natural G158S substitution was observed previously in any cyt *b* (20).

The Size of the Amino Acid Side Chain at Position 158 Is Critical for the Activity of the cyt bc_1 Complex. The Ps^- cyt *b*:G158 substitutions indicated that for a functional cyt bc_1 complex the chemical nature of the amino acid side chain at position 158 was not crucial since the presence of acidic (aspartic acid and glutamic acid), basic (arginine and histidine), hydrophobic (valine, leucine, and isoleucine), or aromatic (tyrosine and tryptophan) residues equally resulted in the loss of the activity and the photosynthetic growth. On the other hand, it was noticed that all functional cyt *b*:G158 substitutions (with the exception of proline) had an amino acid side chain smaller than 100 $Å^3$ (Table 1). The effect of the size of the amino acid side chain at position 158 was best illustrated with the serine, cysteine, and threonine substitutions of similar chemical properties that yielded partially active, temperature-sensitive, and highly inactive cyt bc_1 complexes, respectively.

Similarities Between Mutations Affecting the Q_B Domain of the Photochemical Reaction Center and Those Affecting the Q_z Domain of the cyt bc_1 Complex. Herbicide-resistance mutations in the Q_B region of bacterial photochemical reaction centers of known structure (21, 22) were found in an extramembranous region of the complex and affected amino acids with aromatic (Phe-216 and Tyr-222), hydrophobic (Ile-229), and hydroxyl (Ser-223 and Thr-226) side chains or without a side chain (Gly-228) (20, 23, 24). Similarly, mutations in aromatic (Phe-144), hydrophobic (Met-140 and Ile-162), and hydroxyl (Thr-163) side chain-containing residues and side-chain-less residues (Gly-152 and Gly-158) located in the extramembranous Q_zI domain also conferred resistance to quinol oxidation inhibitors (7–9). Further, the Phe-216 and Ile-229 residues of the reaction center are located in close proximity of the conserved residues His-190 and His-230 that make contacts with either quinone or herbicides in the Q_B binding pocket (23, 24). The Met-140 and Phe-144 residues of the Q_zI region of cyt *b* are also in close proximity of the universally conserved heme b_L liganding His-97 and His-198 and may similarly provide direct contacts to quinone/quinol molecules. These observations suggest that the Q_B and Q_z regions may be topologically similar. A detailed analysis of the structural and functional resemblances between the Q_B site of the photochemical reaction center and the Q_z domain of the cyt bc_1 complex is presented elsewhere (ref. 25).

Role of Gly-158 of the Q_zI Region on Quinol Oxidation Catalyzed by the cyt bc_1 Complex. The data obtained in this work indicate that Gly-158 of cyt *b* influences the interactions of the quinone/quinol couple as well as the inhibitors, myxothiazol and stigmatellin, with the Q_z site. Although the presence of a glycine, which has no side chain, appears to allow free access of quinol and myxothiazol to this niche, even a small increase of the size of the side chain, such as the G158A or -S substitutions, restricts their access and yields partially active but myxothiazol-resistant complexes. Side chains of size larger than the methyl (alanine) and hydroxymethyl (serine) groups then further hinder the access of the substrate and lead to assembled but nonfunctional cyt bc_1 complexes. The resolution of the structure of the cyt bc_1 complex is needed to further determine the precise role of Gly-158.

Finally, it is noteworthy that Gly-158 is only one of the five inhibitor-resistant sites located in the Q_zI region of cyt *b* (9). Since the role of the position 158 appears related to the size of the amino acid side chain at this position, the remaining Met-140, Phe-144, Gly-152, and Thr-163 positions should be systematically studied by saturation mutagenesis to better define their contributions to the structure and function of the quinol oxidation site of the cyt bc_1 complex.

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