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# The Structure of the R184A Mutant of the Inositol Monophosphatase Encoded by *suhB* and Implications for Its Functional Interactions in *Escherichia coli*\*

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The *Escherichia coli* product of the *suhB* gene, SuhB, is an inositol monophosphatase (IMPase) that is best known as a suppressor of temperature-sensitive growth phenotypes in *E. coli*. To gain insights into these biological diverse effects, we determined the structure of the SuhB R184A mutant protein. The structure showed a dimer organization similar to other IMPases, but with an altered interface suggesting that the presence of Arg-184 in the wild-type protein could shift the monomer-dimer equilibrium toward monomer. In parallel, a gel shift assay showed that SuhB forms a tight complex with RNA polymerase (RNA pol) that inhibits the IMPase catalytic activity of SuhB. A variety of SuhB mutant proteins designed to stabilize the dimer interface did not show a clear correlation with the ability of a specific mutant protein to complement the  $\Delta$ *suhB* mutation when introduced extragenetically despite being active IMPases. However, the loss of sensitivity to RNA pol binding, *i.e.* in G173V, R184I, and L96F/R184I, did correlate strongly with loss of complementation of  $\Delta$ *suhB*. Because residue 184 forms the core of the SuhB dimer, it is likely that the interaction with RNA polymerase requires monomeric SuhB. The exposure of specific residues facilitates the interaction of SuhB with RNA pol (or another target with a similar binding surface) and it is this heterodimer formation that is critical to the ability of SuhB to rescue temperature-sensitive phenotypes in *E. coli*.

The *suhB* gene in *Escherichia coli* was first identified as a suppressor of temperature-sensitive mutations in protein export (1), the heat shock stress response (2), and DNA repli-

cation (3). Given the diverse mutations it affects, it has been suggested (4) that *suhB* participates in the post-transcriptional control of gene expression. The cold-sensitive lethality of the *suhB* mutant is suppressed by *rnc* mutations that introduce defects in the double-stranded RNA processing enzyme RNase<sup>2</sup> III (4). This particular observation suggested that SuhB could alter mRNA stability by modulating RNase III activity, or binding to the RNA targets and protecting them from degradation by RNase III. However, there is little concrete evidence that defines a normal role for SuhB in *E. coli*.

The protein coded by *suhB* is 29.1 kDa (256 residues) and has significant sequence similarity to human inositol monophosphatase (IMPase). The IMPase activity of SuhB is 2–3-fold lower than most other IMPases, and the enzyme has a slight preference for D-inositol 1-phosphate compared with the L-isomer (5, 6). Like human IMPase, SuhB is strongly inhibited by Li<sup>+</sup> (5, 7). However, *E. coli* SuhB appears to exist in solution as an equilibrium between monomer, dimer, and higher oligomers (5), a difference from other well studied IMPase proteins but similar to IPPases and PAPases belonging to the same structural superfamily.

The relevance of SuhB IMPase activity to its post-translational effects in *E. coli* is unclear. *myo*-Inositol-containing phospholipids and soluble inositol compounds are undetected or represent very minor components in *E. coli*, so that an IMPase activity is not a necessity for these cells (8). The *E. coli* SuhB mutant protein D87N is inactive as an IMPase but fully functional in complementing a defective *suhB* mutant strain (5). Thus, the IMPase is not required for growth at low temperature and is not related to *suhB* suppression effects. This would be consistent with SuhB participating in control of gene expression possibly by interacting with a key enzyme or by interacting directly with mRNA (3).

In this work, we present a structural analysis of the *E. coli* SuhB R184A protein (the wild-type protein failed to crystallize). This mutant protein has high IMPase activity and forms a dimer in the crystal structure that is reminiscent of other IMPases, particularly the human enzyme (9). However, the dimer interface of R184A is somewhat different from other

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

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<sup>2</sup> The abbreviations used are: RNase, ribonuclease; IMPase, inositol monophosphatase; RNA pol, RNA polymerase.

## SuhB Function Involves the Monomer

IMPase proteins with a high cationic character that would tend to destabilize it compared with other IMPases (9–11). The SuhB monomer has a positively charged surface that could bind specifically to other proteins or nucleic acids. One such binding partner, RNA polymerase (RNA pol) can form moderately tight complexes with SuhB as indicated by gel shift assays. This binding partially inhibits the IMPase activity of SuhB. Mutations of residues in the SuhB dimer interface had little effect on IMPase activity, and those constructed to strengthen the dimer interface desensitized the enzyme to inhibition by RNA pol to different degrees. None of the mutations constructed had exclusively monomer or dimer populations, and there was no direct correlation of dimer content with RNA pol inhibition of IMPase suggesting that the loss of RNA pol inhibition reflects removal/alteration of specific binding contacts rather than changes in the monomer/dimer equilibrium of SuhB. Two mutations, G173V and R184I (as well as the related L96F/R184I), showed no inhibition of IMPase activity by RNA pol. These were also the only mutations that rendered extragenic SuhB nonfunctional in recovering growth of the  $\Delta$ suhB strain of *E. coli* at 30 °C. In the structure of R184A, the position of residue 184 is at the core of the dimer interface; Gly-173 runs across the plane of the interface and appears critical for the assembly of the dimer and its twist angle. Because both enzymes are active IMPases, a specific interaction of SuhB with its biological target has been prevented in these two mutant proteins. The accessibility of Arg-184 and regions around this residue (e.g. for binding to RNA pol) would require disruption of the dimer structure. This suggests that the SuhB monomer is the “active” form in *E. coli* and that it may form heterodimers with proteins involved in transcriptional (and possibly post-transcriptional) events or may even form complexes with nucleic acids.

### EXPERIMENTAL PROCEDURES

**Chemicals**—Ammonium molybdate and malachite green oxalate were purchased from Sigma. The Sephadex QFF resin was obtained from Amersham Biosciences. Tryptone and yeast extract were purchased from Difco. SDS-PAGE molecular weight standards were purchased from Bio-Rad. The QuikChange site-directed mutagenesis kit was purchased from Stratagene. The RNA polymerase and core complex were provided by Drs. M. Kashlev and L. Lubkowska of the National Cancer Institute at Frederick, Maryland.

**Protein Expression, Purification, and Mutation**—*E. coli* SuhB was prepared as described previously (5). BL21(DE3) competent cells were transformed with the pET23a(+) plasmid containing the desired *suhB* gene for expression of the protein. SuhB mutant proteins were prepared using the *suhB* gene, QuikChange mutagenesis kit, and appropriate primers; all altered genes were sequenced to confirm the specific mutations. After overexpression of the SuhB protein, the *E. coli* cells were harvested and lysed by sonication. The supernatant, separated by centrifugation, was applied to a Sephadex Q-Sepharose fast flow column (2.5 × 12 cm) and eluted with a linear gradient of 0.05 to 0.5 M KCl in buffer A (400 ml of 50 mM Tris-HCl, pH 8.0, 1 mM EDTA). For nearly all the mutant proteins this resulted in protein that was >90% pure as judged by SDS-PAGE. The fractions of pure protein were dialyzed against

buffer A with 0.2 M KCl and concentrated to 5 ml with a protein concentration of 10 mg/ml. Protein distribution in monomer and dimer forms was analyzed by native gels using polyacrylamide gel electrophoresis without SDS (12). Samples typically contained 60 μg of protein in 40 μl (0.052 μM). Electrophoresis was carried out at 4 °C with a constant voltage of 100 until the migration front reached the bottom of the gels (about 1.5 h). The gels were stained with Coomassie Brilliant Blue.

**Phosphatase Assays**—IMPase activity was measured by colorimetric determination of released inorganic phosphate ( $P_i$ ) (5, 13). Assays for SuhB (500 μl total volume) were carried out in 50 mM Tris-HCl with 8 mM  $MgCl_2$ , pH 8.0, with a range of D-inositol 1-phosphate (0.02 to 1.2 mM) to measure  $K_m$  and  $k_{cat}$ . The amount of enzyme added was adjusted to give a 15–20% conversion of substrate to  $P_i$  during the 1-min incubation at 37 °C. After incubation, 1 ml of ammonium molybdate and malachite green reagent was immediately added to the assay solution. Comparison of the  $A_{660}$  to those for standard  $P_i$  samples was used to calculate the amount of  $P_i$  generated. The effect of RNA polymerase on the SuhB IMPase activity was measured in 20 mM Tris-HCl, pH 7.9, containing 100 mM KCl and 8 mM  $MgCl_2$ , 0.5 mg/ml bovine serum albumin, and 0.5 mM D-inositol 1-phosphate. For these assays, the volume was 15 μl, and 6 μg of SuhB (14 μM) and 15 or 72 μg of RNA polymerase (3 or 14 μM) were preincubated at 25 °C for 15 min. The reaction was carried out at 37 °C for 5 min. A series of other proteins were added 1:1 with SuhB (14 μM) to see if they could also reduce IMPase activity. These included recombinant phosphatidylinositol-specific phospholipase C from *Bacillus thuringiensis*, recombinant *Archaeoglobus fulgidus* inositol-1-phosphate synthase, liver alcohol dehydrogenase (*Saccharomyces cerevisiae*), fructose biphosphatase (*E. coli*), DNase (bovine pancreas), and RNase (bovine pancreas). These assays used 0.2 mM D-inositol 1-phosphate in the assay buffer.

**RNA pol Gel Shift Assay**—RNA pol (0.42 μM) in 20 mM Tris-HCl, 40 mM KCl, 5 mM  $MgCl_2$ , and 3 mM 2-mercaptoethanol, pH 7.9, and SuhB (0 to 4.2 μM) in the same buffer containing 500 mM KCl were mixed at 4 °C. The final KCl concentration varied from 135 to 180 mM. Samples (12 μl) were incubated at 24 °C for 20 min, then 3 μl of native loading buffer (no dye, 50% sucrose and 50 mM EDTA) was added and the sample loaded onto a 6% native gel. The gel was run at 1 watt (constant), 100 volts, and ~15 mA for 2.5 h. The gel was stained with Coomassie Blue (obtained from Sigma), then destained with 7% acetic acid.

**Complementation Experiments**—The  $\Delta$ suhB strain of *E. coli* W3110 was made by recombineering (14), precisely replacing (15, 16) the chromosomal *suhB* *orf* with the *kan* cassette containing *kan* *orf* with its own translation initiation region and *Pkan* promoter exactly as described (17). The *kan* cassette for replacement was made by PCR using the following pair of primers: the forward primer TGCGCCGTTTTCCCGTTCCTTAACA-TCCAGTGAGAGAGACCGTTGCCAGCTGGGGCGCCCTCTGGTAAG and the reverse primer AAGGCGAGGGCGG-GTGAGTGATATCACCCGCCTGAGTCATTATCAGAAG-AACTCGTCAAGAAG. The *suhB*(*kan*) replacement was checked by PCR and transduced using the P1 phage to the W3110 wild-type cells. The resulting *suhB*(*kan*) knock-out cells

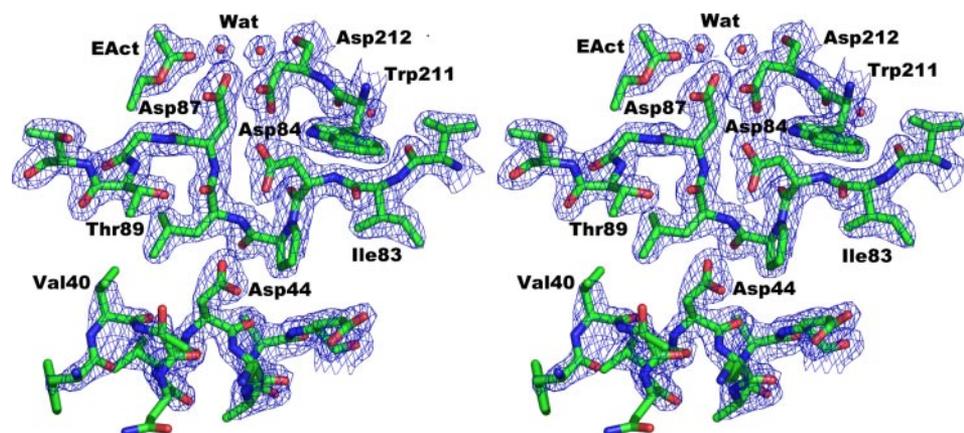
were transformed with plasmids bearing mutant *suhB*, with the plasmid expressing a wild-type *suhB* and with empty pET11 vector, the last two as positive and negative controls, selecting for Ap<sup>R</sup> at 37 °C on LB-Ap agar plates. In these plasmids, *suhB* and its mutant derivatives were cloned in the same orientation as the *bla* (Amp<sup>R</sup>) gene and expressed from the constitutive *Pbla* promoter. The transformants were usually grown at 30, 37, and 42 °C; the  $\Delta$ *suhB* strain could not grow at 30 °C but grew well at 42 °C. Introduction of wild-type *suhB* via plasmid into this mutant strain allowed the cells to grow at 30 °C.

**Crystallography**—Although recombinant SuhB did not crystallize under any conditions examined, the mutant protein R184A, which has equivalent IMPase activity to native SuhB, crystallized under three different conditions: (i) 0.2 M ammonium iodide, 20% PEG 3350, pH 6.2; (ii) 0.2 M potassium acetate, 20% PEG 3350, pH 7.8; and (iii) 0.2 M ammonium acetate, 20% PEG 3350, pH 7.1. Crystal forms for the protein had the lattice organized by C2 symmetry. Diffraction data were collected to 1.9-Å resolution on the RaxisIV++ area detector mounted on the Rigaku rotating anode at the x-ray facility at

**TABLE 1**  
Data collection and refinement summary for SuhB R184A mutant structure (PDB 2QFL)

Space group	C2
$d_{\min}$ (Å)	1.9
Unique reflections	15,113
$I/\sigma(I)^a$	10.5 (4.1)
Completeness (%) <sup>a</sup>	95.3 (39.9)
Average redundancy <sup>a</sup>	3.3 (2.6)
Unit cell (Å)	$a = 87.77, b = 45.45; c = 71.56$
Angles	$\beta = 125.4$
$R_{\text{merge}}^a$	0.050 (0.26)
Working $R$ -factor	0.213 (0.27)
Free $R$ -factor (5%)	0.285 (0.44)
<b>Root mean square deviations</b>	
Bond lengths (Å)	0.021
Bond angles (°)	1.84
<b>Water molecules</b>	90
<b>Ligands</b>	Acetate, ethylacetate
<b>Ramachandran plot (%)</b>	
Most preferred	89
Allowed	10
Generously allowed	1
Disallowed	0

<sup>a</sup> Last shell (1.9–1.95 Å) data in parentheses.



**FIGURE 1.** The stereo diagram of the active site of SuhB R184A mutant protein covered with  $2F_o - F_c$  electron density map contoured at 1.4  $\sigma$ . A single molecule of ethyl acetate (EAct) and two water molecules (Wat) are also visible. This and the following figures were prepared with program PyMOL (26).

the Burnham Institute for Medical Research. HKL2000 was used for integration and scaling (18). The structure was solved by the molecular replacement program MOLREP (19) using the model constructed from the consensus sequences of human, MJ0109 and AF2372 enzymes as a search probe. Data collection and refinement statistics can be found in Table 1.

**Automated Docking Procedure**—The programs AUTODOCK (20), GOLD (Cambridge Crystallographic Data Center) 21, and SURFLEX (22) were used to dock substrate to the active site of the refined structures. The charge on inositol 1-phosphate was set at  $-2$ . For docking with AUTODOCK, the grid box was set at 30 Å<sup>3</sup> centered at the middle of the active site, with a grid spacing of 0.275 Å between grid points. In each case, 50 docking runs were performed using the Genetic Algorithm with a maximum of 500,000 energy evaluations. With GOLD the grid center was defined as the center of gravity of the overlaid inositol 1-phosphate from the human IMPase.

## RESULTS AND DISCUSSION

**General Description of the Structure**—The SuhB R184A protein crystallized in the C2 space group with unit cell dimensions  $a = 87.77$  Å,  $b = 45.45$  Å, and  $c = 71.56$  Å,  $\beta = 125.4^\circ$  (Table 1) with a  $d_{\min}$  of 1.9 Å. The phase problem was solved by molecular replacement and the model refined using REFMAC (23) to an  $R$ -factor of 0.213 and  $R_{\text{free}}$  of 0.285 with good stereochemistry. The backbone stereochemistry as measured by PROCHECK (24) showed no residues in disallowed regions and only 1% in generously allowed region. The structure has very good electron density (an example is shown in Fig. 1) with the exception of the last six residues of the C terminus and a small region of the catalytic loop (residues 29–36).

There is a single monomer in the asymmetric unit. The SuhB dimer is formed by applying the crystallographic symmetry to the monomer present in the asymmetric unit. The overall fold of SuhB is highly conserved as in other members of the entire IMPase superfamily (25). The fold consists of the  $\alpha$  helical layers flanking two perpendicular  $\beta$  sheets in an arrangement of  $\alpha$ - $\beta$ - $\alpha$ - $\beta$ - $\alpha$ . Like human IMPase, this SuhB mutant protein is organized in a crystal as a homodimer. Sequoia alignments with previously solved archaeal IMPase proteins (AF2372 and MJ0109 (10, 11)), and human IMPase (9) indicated that the *E. coli* protein was most similar to the human IMPase. The closer similarity of SuhB R184A to human IMPase as opposed to archaeal IMPase proteins is seen when the dimers are compared (Fig. 2). Superimposing the second subunit of the human enzyme on SuhB after superposition of the first one requires a 12° rotation, whereas superposition of the archaeal *A. fulgidus* IMPase (Protein Data Bank 1LBZ) second subunit on SuhB requires a 38° rotation along the longest axis of the dimer.

## SuhB Function Involves the Monomer

**Active Site of SuhB**—The active site of SuhB, identified by homology to the human enzyme, is well ordered despite the absence of metal cofactors and substrate or substrate analogues (Fig. 1 shows a  $2F_o - F_c$  map of the active site). The molecules of ethyl acetate and acetate refined at the active site of SuhB may serve as an indicator of the binding modes for substrates. However, there are some architectural differences with active sites of other IMPases associated with lack of metal ions and local changes of amino acids, as shown in Fig. 3. There are large differences in the region where protein coordinates the inositol hydroxyl groups (more specifically where human Ser-162 superimposes on SuhB Lys-160). There are also significant differences in side chain positioning when comparing Asp-212 or Glu-67 of SuhB with Asp-220 or Glu-70 of human IMPase (Fig. 3). Another significant difference is the alternative conformation of Asp-44 (SuhB) that breaks the hydrogen bond with Thr-89. Considering the highly conserved active site residues between the human and *E. coli* proteins, these differences most



FIGURE 2. Comparison of the backbone structure of *E. coli* SuhB R184A (dark blue) with the human IMPase (PDB 1AWB) (in cyan and green). The catalytic loops of both enzymes are highlighted in different colors. The loop in human IMPase (residues 27–41) is in red, whereas the loop in SuhB (residues 29 to 41) is in magenta.

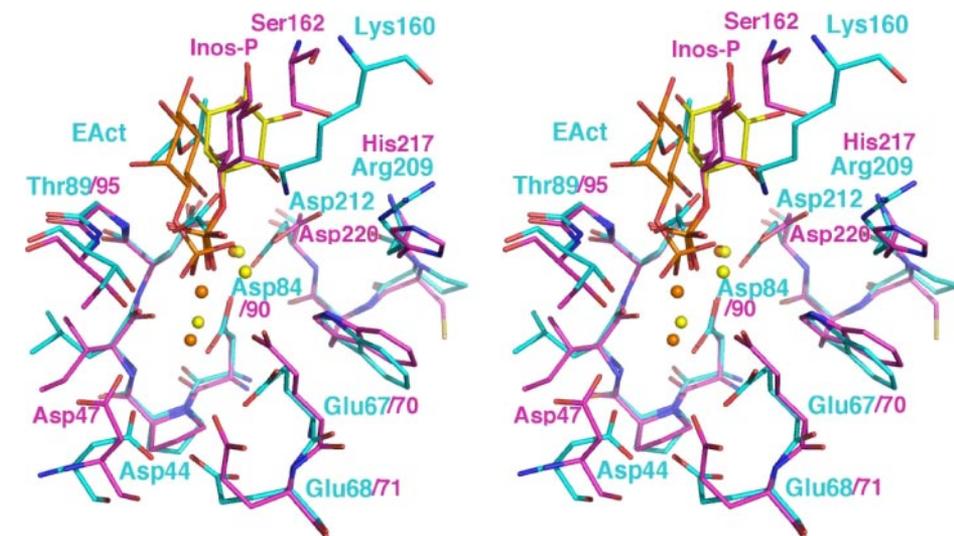


FIGURE 3. The model of the active site region of SuhB R184A (cyan) is overlaid on the active site of the human IMPase (in purple). The labeling colors correspond to the color of the bonds with purple labels for the human enzyme and cyan for SuhB. Two models of D- and L-inositol phosphate as refined in human IMPase are shown (in purple and yellow bonds, respectively) as well as the D-inositol 1-phosphate docked to SuhB (in gold).

likely reflect that the apo form of SuhB adopts a slightly different architecture than the liganded form of the human enzyme.

To further confirm the identification of the active site residues of SuhB, we performed a series of docking experiments with D-inositol 1-phosphate docked into the active site of apo-SuhB with activating metals ( $Mn^{2+}$ ) modeled into the approximate positions of the two calcium ions in the structure of the human enzyme. The third ion was placed into the active site at a position taken from structures of reaction products and  $Mn^{2+}$  (or  $Zn^{2+}$ ). Docking results from three docking programs SURFLEX (22), AUTODOCK (20), and GOLD (21) were compared. The highest scoring result obtained in GOLD is shown in Fig. 3. The position of the inositol ring appears to be different from corresponding positions found in the human enzyme. The close contacts of the Lys-160 side chain with superposed ligands of the human IMPase suggests a different mode of binding. Additionally, the inositol ring superposes well with ethyl acetate, an observation that provides additional support for this mode of binding.

**Monomeric SuhB and the Dimer Interface**—Because biochemical data indicated that the monomer and dimer species of SuhB are in equilibrium in solution (5), we analyzed the dimer interface of SuhB. We calculated the total solvent accessible area of the interface and analyzed its content for contribution of hydrophobic and hydrophilic interactions. We also calculated the volume of the cavities along the surface (data not shown), and identified individual pockets (and residues) along the surface of the SuhB dimer interface. The total surface of the interface as calculated by GRASP was  $\sim 1,450 \text{ \AA}^2$  in which  $\sim 700 \text{ \AA}^2$  were hydrophilic and  $\sim 750 \text{ \AA}^2$  hydrophobic interactions. The interface is almost equally balanced between hydrophobic and hydrophilic interactions (Fig. 4). Two symmetric patches of hydrophobic interactions that make homodimer formation favorable would be counteracted by like-charge repulsive interactions (notably Arg-183 and Arg-184 in the wild-type protein). The mutation of Arg-184 to Ala would be expected to shift the equilibrium toward the dimeric organization and results in

crystal formation, whereas wild type would have a weakened interface (this behavior would explain why we failed to crystallize wild type SuhB).

A particularly important hydrophobic interaction at the interface appears to form an asymmetric crescent arrangement extending from a cluster of two Phe residues (Phe-157, Phe-159) and Pro-158 that interacts with Phe-176 and Phe-182 through the Leu-96 and Pro-97 interacting with Val-200 and Tyr-194 (Fig. 4B). The symmetry related Ile-169 interacts with itself and at the other side of the molecule the same cluster of Phe residues (Phe-157, Phe-159, Phe-176, and Phe-182) completes the crescent. This set of interactions appears to be important for the

homodimer stability. These interactions are supplemented by the charge and polar interactions of Arg-183 and Asp-181 to Asn-91 and Ala-184 in the other subunit. Similarly, Arg-199 and Lys-94 interact with Tyr-23 and Asp-201 of the opposing surface.

All other characterized IMPases are dimers (or a tetramer in the case of the enzyme from *Thermotoga maritima* (27)) and are extremely soluble in low ionic strength buffers and show no tendency to precipitate from solution. *E. coli* SuhB has consid-

erably poorer solubility and is also more hydrophobic, especially along the dimer interface, whereas more solvent accessible areas contain a higher ratio of polar and charged residues. When comparing the number of hydrophilic, polar, and hydrophobic residues of SuhB and other IMPases such as MJ0109 and AF2372 and human IMPase, SuhB has ~53% hydrophobic residues along the dimer interface compared with 29% for MJ0109, 32% for AF2372, and 35% for human IMPase. This balance of hydrophobic and electrostatic interactions in SuhB is likely to control the oligomerization state of the protein as well as interactions with other proteins.

*Dimer Interface Mutations and Aggregation State of SuhB Proteins*—The unusual dimer interface in this IMPase could be responsible for the functional properties of SuhB in *E. coli* (5). To test this hypothesis, we created a number of proteins with single and double mutations of interface residues. Alanine mutations of residues in the SuhB R184A dimer interface (Arg-121, Arg-183, Arg-184, Arg-199, Arg-248, Val-249, and Lys-251) as well as H98F, which occurs in a pocket located at the

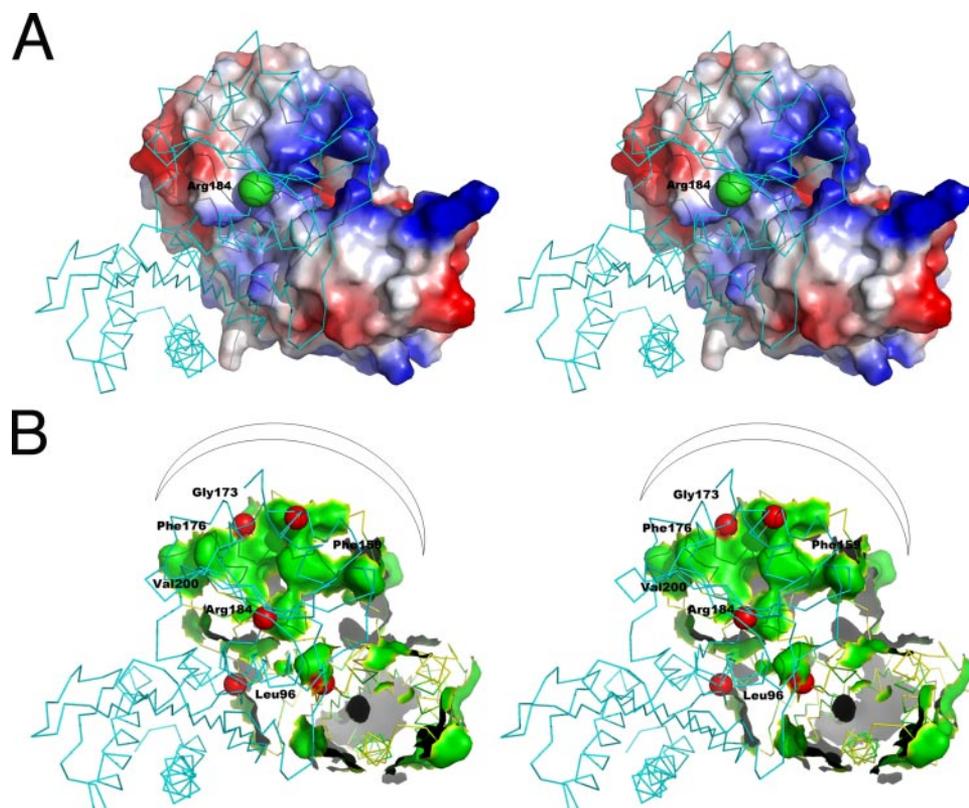


FIGURE 4. The stereo images of the surface representation of one of the SuhB monomers and stick-bond  $\alpha$ -carbon representation for the subunit facing the viewer highlighting the homodimer interface. A, R184A SuhB monomer colored by the electrostatic potential mapped onto the surface. Please note two complementary charged patches at the edges of the molecule, and the location of the Arg-184 at the center of the dimer (green sphere). B, the surface of hydrophobic residues present at the interface (in green). Please note the crescent arrangement of the hydrophobic residues linked to the dimer formation. The red dots depict the location of three residues (Leu-96, Gly-173, and Arg-184) whose mutation most affected the complementation assay (Table 2). The symmetry related Arg-184 location is occluded by the green surface. Some hydrophobic residues present at the interface are also labeled (Phe-159, Phe-176, and Val-200).

TABLE 2

Kinetics parameters, aggregation state, interaction with RNA polymerase and extragenic suppressor analysis of recombinant SuhB and mutants

SuhB	$k_{\text{cat}}$ $s^{-1}$	$K_m$ $mM$	Monomer/dimer <sup>a</sup>	$v_{+RNApol}/v_o^b$	$\Delta\text{suhB}$ growth at 30 °C <sup>c</sup>
WT	0.490 ± 0.024	0.11 ± 0.02	0.40 <sup>d</sup>	0.43	+
H98F	0.504 ± 0.024	0.17 ± 0.04	0.40	0.33	+
R121A	0.369 ± 0.001	0.13 ± 0.02	0.68	0.73	+
G173V	0.295 <sup>e</sup>		1.7	0.97	–
R183A	0.747 ± 0.100	0.16 ± 0.03	1.0	0.71	–/+
R184A	1.183 ± 0.058	0.29 ± 0.03	0.17	0.80	+
R184I	0.357 ± 0.02		0.35	0.98	–
R199A	0.199 ± 0.002		0.81	0.70	+
R248A	0.267 ± 0.014	0.13 ± 0.02		0.75	+
V249A	0.175 ± 0.009	0.06 ± 0.01	0.15	0.64	+
K251A	0.165 ± 0.009		0.88	0.70	+
R184I/L96F	0.324 <sup>e</sup>		0.42	0.98	–

<sup>a</sup> Ratio of monomer SuhB to dimer SuhB as measured from the intensities of each band in native PAGE using the ImageJ program.

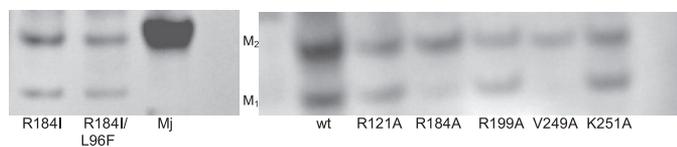
<sup>b</sup> Specific activity of the enzyme towards 0.5 mM D-inositol 1-phosphate in the presence of a 1:1 equivalent of the holo-RNA polymerase compared to the IMPase activity in the absence of the polymerase.

<sup>c</sup> Ability of the extragenic *suhB* mutant to cause the  $\Delta\text{suhB}$  *E. coli* strain to change its growth ability so that it can now grow at 30 °C. An entry of –/+ indicates a small amount of growth at this temperature.

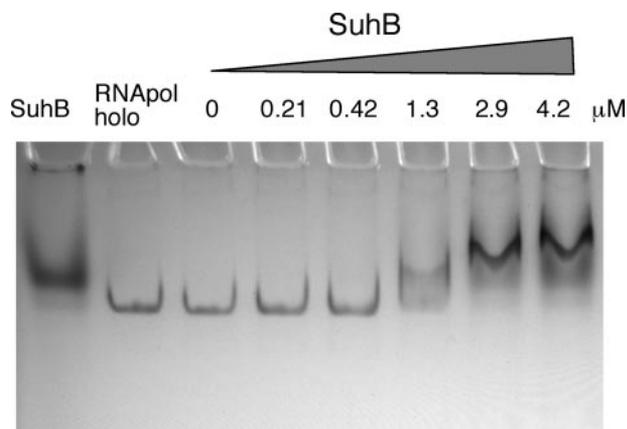
<sup>d</sup> The ratio of monomer to dimer varies considerably, but on average it is usually ~0.4. However, very fresh protein that has been stored in high salt can often show as much as a 2-fold excess of monomer.

<sup>e</sup> Assay done only once at 0.5 mM D-inositol 1-phosphate, a value above the  $K_m$  for SuhB. Under these conditions the observed specific activity should approximate  $V_{\text{max}}$ .

## SuhB Function Involves the Monomer



**FIGURE 5. Native PAGE showing the distribution of monomer and dimer SuhB protein for recombinant wild-type SuhB (wt) and several mutants (indicated on figure).** All the SuhB mutant proteins ( $0.052 \mu\text{M}$  applied to the gels) display some amount of monomer, although this can be a small fraction as in R184A or V249A. For comparison the IMPase from *Methanococcus jannaschii* (labeled Mj), which forms a very tight dimer, is shown.



**FIGURE 6. Native PAGE showing the effect of SuhB on the mobility of holo-RNA polymerase.** The RNA pol holoenzyme ( $0.42 \mu\text{M}$ ) and SuhB ( $0$  to  $4.2 \mu\text{M}$ ) were incubated at  $24^\circ\text{C}$  for 15 min. Samples ( $10 \mu\text{l}$ ) were mixed with  $2.5 \mu\text{l}$  of loading buffer without dye and analyzed in 6% PAGE native gel running it for 80 min only.

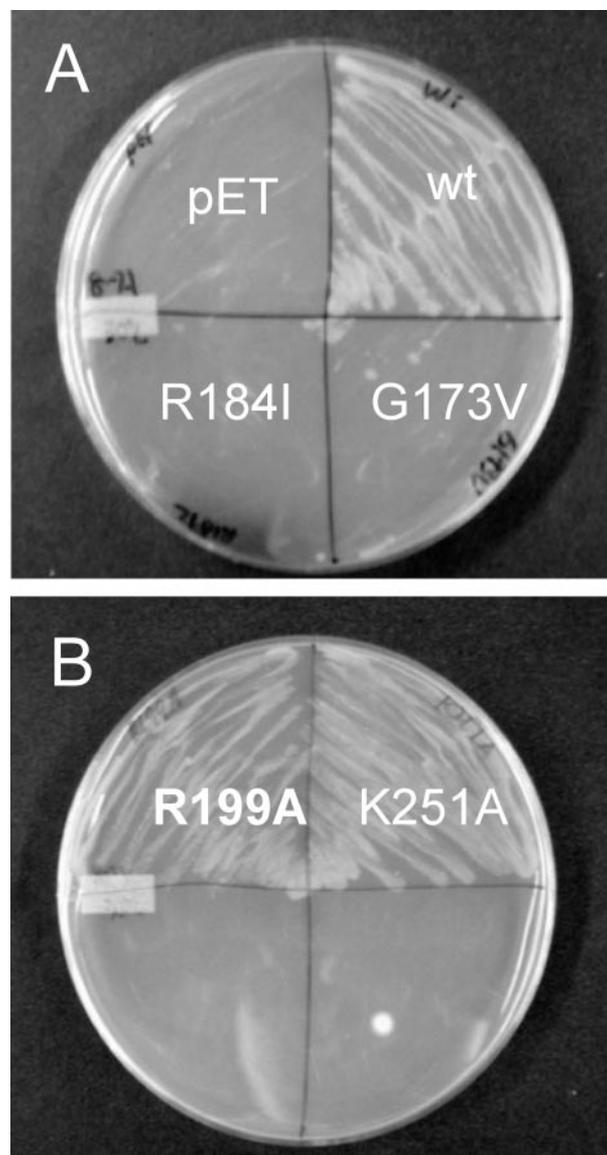
other side of the protein, were constructed. Interestingly, in other IMPase dimers, the residue occupying the position of Arg-184 is a hydrophobic residue, so this residue was also changed to introduce a hydrophobic group, rather than alanine (R184I). Another mutation, G173V, was also constructed as another attempt to enhance subunit interactions at the dimer interface. The double mutant protein L96F/R184I was prepared with the desire to further bias the equilibrium toward dimer formation. All these proteins expressed well and could be purified and studied. These SuhB mutant proteins were active toward D-inositol 1-phosphate, with  $k_{\text{cat}}$  values within a factor of two of recombinant SuhB (Table 2). For several of these  $K_m$  was determined as well, and again values were all similar to that for recombinant SuhB (Table 2). If the aggregation state of the proteins has been altered it is not clearly reflected in the IMPase activity.

Unlike other IMPase proteins, SuhB yields two discrete bands on a native gel (Fig. 5). The slower migrating band corresponds to a dimer, whereas the faster band represents monomeric protein. This behavior allows us to quantify the amount of monomer and dimer at a given concentration of protein (and pH 8.8 of the native gels). All the SuhB mutant proteins still exhibited both monomer and dimer bands, although the ratio of the two bands varied significantly. None had as much monomer as recombinant wild-type SuhB. However, under these conditions there was no simple trend between increase in dimer and change in catalytic activity (Table 2). Clearly, we can modulate dimerization of SuhB by altering residues at the R184A dimer interface. However, what becomes more interesting is whether we can relate these changes to any biological function of SuhB.

**TABLE 3**  
Effect of different proteins on SuhB IMPase activity towards D-inositol 1-phosphate

Added protein	Relative IMPase activity <sup>a</sup>
RNA polymerase ( <i>E. coli</i> , strain K-12)	0.47
Liver alcohol dehydrogenase	0.97
DNase (bovine pancreas)	1.06
RNase (bovine pancreas)	1.02
IPS ( <i>A. fulgidus</i> )	0.98
PI-PLC ( <i>Bacillus thuringiensis</i> )	0.99
Fructose biphosphatase ( <i>E. coli</i> )	1.00

<sup>a</sup> Assay conditions included preincubating  $14 \mu\text{M}$  SuhB with  $14 \mu\text{M}$  of the added protein then measuring hydrolysis of  $0.2 \text{ mM}$  D-inositol 1-phosphate. All assays were done at least in duplicate; standard errors were  $<5\%$ .



**FIGURE 7. Growth of *E. coli*  $\Delta\text{suhB}$  W3110 cells at  $30^\circ\text{C}$  with wild-type or the indicated mutant SuhB introduced via plasmid.** Introduction of the wild-type SuhB, shown in A, allows the *E. coli* strain to grow at this low temperature. The R199A and K251A mutants behave like wild-type (wt). However, two mutants constructed to modify the dimer interface, R184I and G173V, no longer rescue growth at  $30^\circ\text{C}$ . The bottom sectors in B are empty (were not streaked with cells).

**RNA Polymerase Binding and Inhibition of SuhB**—Another way of gaining insight into SuhB behavior is to look for a binding partner and use this complex formation to screen for effects of the dimer interface mutations. Interestingly, we found that

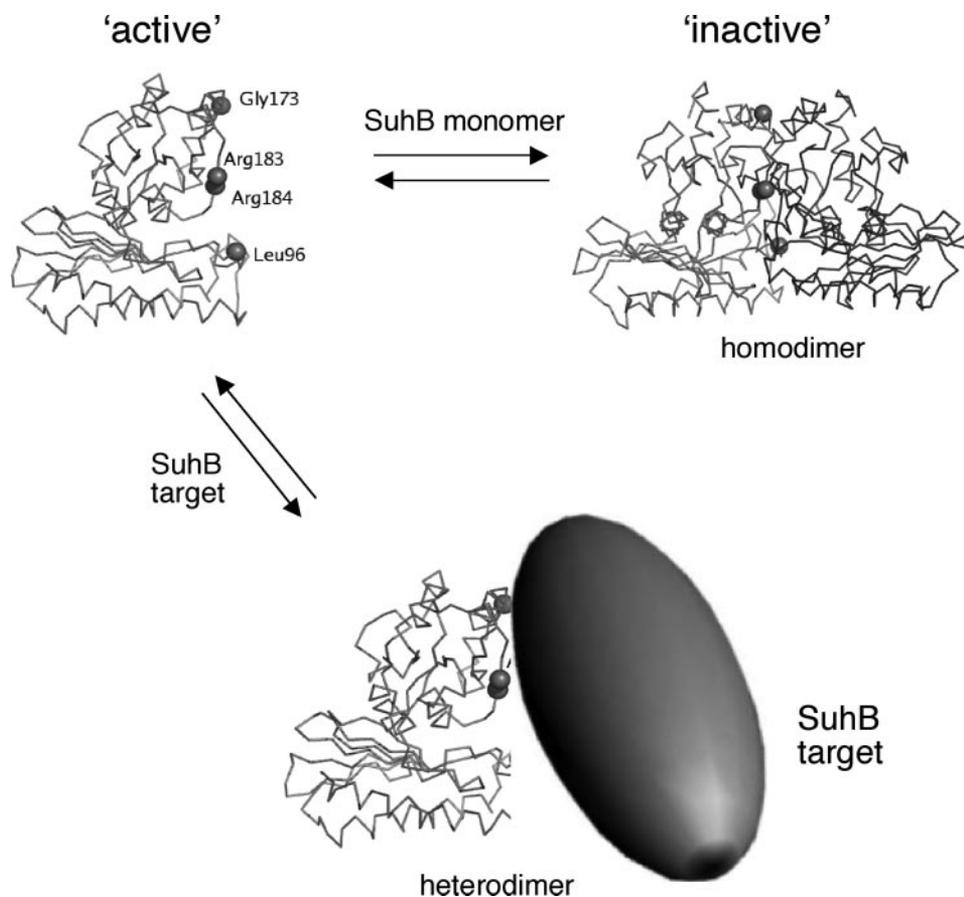


FIGURE 8. **Schematic model for SuhB in *E. coli*.** Dissociation of the SuhB homodimer to monomers presents a binding surface available for binding with target protein(s) (e.g. RNA polymerase or other species) depicted by a large oval. It is the resultant heterodimer of SuhB that is related to its functional behavior in cells. Thus, the SuhB dimer, although an active IMPase, is "inactive" because it cannot form heterodimers, whereas the SuhB monomer is active and the species whose subsequent interactions are critical to altering the growth temperature of  $\Delta$ *suhB* W3110 at 30 °C.

*E. coli* RNA polymerase was one such binding partner. The addition of SuhB to RNA pol (0.33  $\mu$ M in the solution placed in the gel lane) in native gel electrophoresis retards the migration of the RNA pol in a concentration dependent fashion, an indication of binding between the two species (Fig. 6). Under these specific conditions, increasing the amount of SuhB at fixed RNA pol indicated that the optimal SuhB concentration for complex formation was 2.3  $\mu$ M. Formation of this SuhB-RNA pol complex also reduced the SuhB IMPase activity toward D-inositol 1-phosphate. With the ratio of RNA pol to SuhB equal to 1:4.6, corresponding to 3.0  $\mu$ M RNA pol and 14  $\mu$ M SuhB, the specific activity for SuhB decreased to  $69 \pm 10\%$  of wild-type recombinant SuhB. Increasing the RNA pol to 72  $\mu$ g (RNA pol:SuhB = 1:1 with each protein 14  $\mu$ M in the assay) reduced IMPase to  $36 \pm 6\%$  of the original activity. These IMPase assays used a higher concentration or proteins than the gel shift experiments so that there is likely to be more SuhB dimer (whose formation should increase with increasing SuhB) as well as RNA pol-complexed SuhB in solution. Nonetheless, formation of a SuhB-RNA polymerase complex does lead to inhibition of IMPase activity. This is a property that can be used in screening the SuhB mutant proteins. Interestingly, RNA pol inhibition of many of the interface mutant proteins was reduced to different degrees (Table 2). The only mutant protein that was inhibited to the same extent

as recombinant SuhB was H98F, a residue not at the dimer interface. Most of the single alanine mutant proteins showed 65–80% residual IMPase activity (i.e. reduced inhibition by RNA pol binding). However, G173V, R184I, and R184I/L96F showed no inhibition by RNA pol. This suggests that the interaction of SuhB with RNA pol requires residues in the dimer interface of SuhB monomer, and that replacing Arg-184 and Gly-173 with hydrophobic groups impairs this interaction.

To see if RNA pol inhibition of SuhB is nonspecific we screened a diverse group of proteins for their effect on SuhB IMPase activity (Table 3). Two of these were enzymes that bound inositol-containing substrates or products; others interacted with sugar phosphate or nucleotides. Although most are not the *E. coli* proteins, they serve to see if the IMPase inhibition by RNA pol is the result of more generic SuhB/protein hydrophobic or electrostatic binding. At a 1:1 ratio of these added proteins to SuhB, there was no inhibition of IMPase activity. RNA pol was the only additive in this series that was capable of reducing IMPase activity. This suggests that the SuhB interaction with RNA pol is indeed specific.

**Effect of SuhB Interface Mutations on Cell Growth**—Deletion of *suhB* confers a conditionally lethal phenotype to *E. coli* W3110 cells so that they no longer can grow at  $\leq 30$  °C. Reintroduction of wild-type or an active *suhB* mutant restores growth of the  $\Delta$ *suhB* cells at low temperatures providing a functional assay for SuhB constructs with point mutations in the dimer interface (Fig. 7). All expressed SuhB Ala mutant proteins, except for that coding for R183A, behaved the same as wild-type SuhB (Table 2), notably allowing the cells to now grow at 30 °C. Expression of the R183A SuhB mutant protein, which has higher IMPase activity than wild-type enzyme, led to some but significantly less growth of the  $\Delta$ *suhB* cells at that temperature. The G173V and R184I SuhB proteins were unique among the single mutant proteins tested in their inability to allow growth of the  $\Delta$ *suhB* at 30 °C. The related double mutant protein L96F/R184I also did not allow growth at the low temperature. These mutants express well and generate enzymatically active IMPase proteins. SuhB mutant proteins that were no longer able to rescue low temperature growth of  $\Delta$ *suhB* cells were also the ones that were no longer sensitive to RNA pol inhibition of the IMPase activity.

This *in vivo* behavior is consistent with a model where the identity of residues at the SuhB dimer interface is critical to SuhB function, presumably by modulating how SuhB binds

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with a critical partner (Fig. 8). The residue at position 184 can be an Arg or Ala and the extragenic protein can rescue low temperature growth of  $\Delta$ suhB cells, implying that residue charge is not important. However, if a hydrophobic group such as Ile is placed here, the mutant can no longer rescue low temperature growth. Arg-183 may also contribute to the *in vivo* interactions because R183A shows only partial growth at the low temperature. Substitution of Gly-173 with Val also prevents suppressor activity, presumably by blocking specific binding with the larger side chain or disruption of a conformational switch in SuhB. Although there is no correlation between monomer content and suppressor behavior, access of both Arg-183 and Arg-184 would require the dimer to dissociate. This suggests that monomeric SuhB is the active form in cells and participates in forming heterodimers with one or more targets in the cell. Although they were constructed to enhance dimerization, all the interface mutant proteins examined yielded some amount of monomer in solution so that there would likely be enough for heterodimer formation. The extensive hydrophobic patches that become exposed in the monomer are unique to the *E. coli* IMPase and are also likely to be very critical to SuhB function. Whether or not RNA pol is an *in vivo* target is less clear. We tried to identify potential interactions of SuhB with *E. coli* proteins by searching interaction data bases. Some of them (DIP-UCLA) (28) did not show any proteins interacting with SuhB, whereas others (IntAct-EMBL-EBI) (29) showed multiple partners but none of them RNA pol. However, there could certainly be other targets for SuhB heterodimer formation that complement the hydrophobic surface and we would postulate that it is these diverse protein-protein interactions forming a heterodimer that are responsible for the function of SuhB in *E. coli*.

**Summary**—Our crystal structure provides a basis for understanding the catalytic activity of the SuhB as an IMPase. The analyses of electrostatic properties of the protein, its dimer/monomer equilibrium in combination with gel shift assays and *in vivo* extragenic suppressor assays suggest that the direct interaction of SuhB monomer with proteins with a complementary surface (where hydrophobic interactions as well as charges likely participate), RNA polymerase being a potential example, can contribute to the biological role of SuhB in *E. coli*. The results presented in this study cannot exclude participation of other components such as nucleic acids as an additional mechanism for gene suppression. However, they do strongly implicate the SuhB monomer as the active agent in these cells.

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## REFERENCES

1. Shiba, K., Ito, K., and Yura, T. (1984) *J. Bacteriol.* **160**, 696–701
2. Yano, R., Nagai, H., Shiba, K., and Yura, T. (1990) *J. Bacteriol.* **172**, 2124–2130
3. Chang, S. F., Ng, D., Baird, L., and Georgopoulos, C. (1991) *J. Biol. Chem.* **266**, 3654–3660
4. Inada, T., and Nakamura, Y. (1995) *Biochimie (Paris)* **77**, 294–302
5. Chen, L., and Roberts, M. F. (2000) *Biochemistry* **39**, 4145–4153
6. Morgan, A. J., Wang, Y. K., Roberts, M. F., and Miller, S. J. (2004) *J. Am. Chem. Soc.* **126**, 15370–15371
7. Matsuhisa, A., Suzuki, N., Noda, T., and Shiba, K. (1995) *J. Bacteriol.* **177**, 200–205
8. Kozloff, L. M., Turner, M. A., Arellano, F., and Lute, M. (1991) *J. Bacteriol.* **173**, 2053–2060
9. Bone, R., Springer, J. P., and Atack, J. R. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 10031–10035
10. Johnson, K. A., Chen, L., Yang, H., Roberts, M. F., and Stec, B. (2001) *Biochemistry* **40**, 618–630
11. Stieglitz, K. A., Johnson, K. A., Yang, H., Roberts, M. F., Seaton, B. A., Head, J. F., and Stec, B. (2002) *J. Biol. Chem.* **277**, 22863–22874
12. Laemmli, U. K. (1970) *Nature* **227**, 680–685
13. Itaya, K., and Ui, M. (1966) *Clin. Chim. Acta* **14**, 361–366
14. Court, D. L., Sawitzke, J. A., and Thomason, L. C. (2002) *Annu. Rev. Genet.* **36**, 361–388
15. Yu, D., Ellis, H. M., Lee, E. C., Jenkins, N. A., Copeland, N. G., and Court, D. L. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97**, 5978–5983
16. Thomason, L. C., Bubunenko, M., Costantino, N., Wilson, H. R., Openheim, A., Datta, S., and Court, D. L. (2005) in *Current Protocols in Molecular Biology* (Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K., eds) pp. 1.16.11–1.16.21, John Wiley & Sons Inc., New York
17. Bubunenko, M., Baker, T., and Court, D. L. (2007) *J. Bacteriol.* **189**, 2844–2853
18. Otwinowski, Z., and Minor, W. (1997) *Methods Enzymol.* **276**, 307–326
19. Vagin, A., and Teplyakov, A. (1997) *J. Appl. Crystallogr.* **30**, 1022–1025
20. Goodsell, D. S., Morris, G. M., and Olson, A. J. (1996) *J. Mol. Recognit.* **9**, 1–5
21. Jones, G., Willett, P., Glen, R. C., Leach, A. R., and Taylor, R. (1997) *J. Mol. Biol.* **267**, 727–748
22. Jain, A. N. (2003) *J. Med. Chem.* **46**, 499–511
23. Murshudov, G. N., Vagin, A. A., and Dodson, E. J. (1997) *Acta Crystallogr. Sect. D Biol. Crystallogr.* **53**, 240–255
24. Laskowski, R. A., Moss, D. S., and Thornton, J. M. (1993) *J. Mol. Biol.* **231**, 1049–1067
25. York, J. D., Ponder, J. W., and Majerus, P. W. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92**, 5149–5153
26. DeLano, W. L. (2002) *The PyMOL Molecular Graphics System*, DeLano Scientific, San Carlos, CA
27. Chen, L., and Roberts, M. F. (1999) *Appl. Environ. Microbiol.* **65**, 4559–4567
28. Salwinski, L., and Eisenberg, D. (2003) *Curr. Opin. Struct. Biol.* **13**, 377–382
29. Arifuzzaman, M., Maeda, M., Itoh, A., Nishikata, K., Takita, C., Saito, R., Ara, T., Nakahigashi, K., Huang, H. C., Hirai, A., Tsuzuki, K., Nakamura, S., Altaf-Ul-Amin, M., Oshima, T., Baba, T., Yamamoto, N., Kawamura, T., Ioka-Nakamichi, T., Kitagawa, M., Tomita, M., Kanaya, S., Wada, C., and Mori, H. (2006) *Genome Res.* **16**, 686–691

**The Structure of the R184A Mutant of the Inositol Monophosphatase Encoded by *suhB* and Implications for Its Functional Interactions in *Escherichia coli***

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