Serine to Cysteine Mutations in Trp Repressor Protein Alter Tryptophan and Operator Binding*

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The tryptophan repressor regulates expression of the aroH, trpEDCBA, and trpR operons in Escherichia coli. The protein contains no cysteine residues, and the presence of this reactive side chain would allow introduction of spectral probes to monitor binding reactions. Three mutant trp aporepressors, each with a point mutation from serine to cysteine, were produced at positions 67, 86, and 88 by oligonucleotide-directed site-specific mutagenesis. This single conservative substitution affected both tryptophan and operator DNA affinities in all three purified proteins. Cysteine substitution for serine at position 67 decreased tryptophan binding by -6-fold and the operator DNA affinity by -50-fold. The proximity of this amino acid to Gln-68 which is involved in binding to operator DNA (Otwinowski, Z., Schevitz, R. W., Zhang, R.-G., Lawson, C. L., Joachimiak, A., Marmorstein, R. Q., Luisi, B. F., and Sigler, P. B. (1988) Nature 335, 321–329) may account for this effect. Substitution at position 86 diminished tryptophan binding by -4-fold and operator DNA binding by -130-fold. The participation of Ser-88 in the hydrogen bond network required for operator binding to the repressor contains the indole moiety (Lane 1986; Otwinowski, Z., Schevitz, R. W., Zhang, R.-G., Lawson, C. L., Joachimiak, A., Marmorstein, R. Q., Luisi, B. F., and Sigler, P. B. (1988) Nature 335, 321–329) presumably accounts for the DNA binding effects. The diminished corepressor activity in these two mutants may derive from distortions of the binding region, as the tryptophan and DNA binding sites are intimately related. The mutation at position 88 altered tryptophan binding the most of the three mutants (-18-fold and operator binding level -12-fold). Ser-88 forms a hydrogen bond with the amino group of bound tryptophan (Schevitz, R. W., Otwinowski, Z., Joachimiak, A., Lawson, C. L., and Sigler, P. B. (1985) Nature 317, 782–786), and alteration of the geometry of the side chain would be anticipated to perturb the topology of the binding site. The diminished operator affinity may derive from improper alignment of the tryptophan ligand, crucial for high affinity operator binding (Otwinowski, Z., Schevitz, R. W., Zhang, R.-G., Lawson, C. L., Joachimiak, A., Marmorstein, R. Q., Luisi, B. F., and Sigler, P. B. (1988) Nature 335, 321–329). Despite the conservative nature of these substitutions, it is apparent that relatively minor changes in the chemical nature of the substituent and in the geometry of the side chain have profound effects on the functional properties of the altered protein.

The trp repressor protein in Escherichia coli regulates expression of three operons: trpEDCBA, aroH, and trpR (Bennett et al., 1976; Brown, 1968; Grove and Gunsalus, 1987; Gunsalus et al., 1986). The trpEDCBA and aroH operons code for aromatic amino acid biosynthetic enzymes (Brown, 1968; Yanofsky et al., 1981), whereas the trpR operon contains the coding region of the trp repressor so that the protein is autoregulatory (Gunsalus and Yanofsky, 1980; Kelley and Yanofsky, 1982; Bogosian et al., 1984). The binding of this protein to its cognate operator DNA sequences is modulated by the interaction of the protein with the corepressor tryptophan. Trp aporepressor is a small dimeric protein of 25 kDa which binds two molecules of tryptophan to form the operator binding conformation (Gunsalus and Yanofsky, 1980; Schevitz et al., 1985; Arvidson et al., 1986; Lane, 1986). The protein has been examined in detail by crystallographic methods, and the structures of the aporepressor, holorepressor, and holo-repressor-operator DNA complex have been determined at high resolution (Joachimiak et al., 1987; Schevitz et al., 1985; Zhang et al., 1987; Otwinowski et al., 1988; Lawson et al., 1988; Lawson and Sigler, 1988). In addition, genetic studies have identified residues which are essential for the binding activity of the protein and which participate in corepressor binding (Kelley and Yanofsky, 1985; Bass et al., 1987, 1988; Kliger and Yanofsky, 1988). The structural changes which result in an activated protein in response to corepressor binding have been elucidated, and the participation of the corepressor ligand in DNA binding has been demonstrated (Zhang et al., 1987; Otwinowski et al., 1988; Marmorstein and Sigler, 1989).

The trp repressor contains 2 intrinsic tryptophan residues/subunit (Gunsalus and Yanofsky, 1980; Singleton et al., 1980); however, examination of their fluorescence properties in response to ligand binding is complicated by the extrinsic fluorescence of the tryptophan indole, and all analogues which bind to the repressor contain the indole moiety (Lane 1986; Marmorstein et al., 1987). Furthermore, operator binding requires the presence of tryptophan so that monitoring DNA binding is also precluded using spectroscopic methods. The trp aporepressor contains no cysteine (Gunsalus and Yanofsky, 1980), and introduction of the reactive sulfhydryl moiety was undertaken as a means of providing a site for selective modification with fluorescent probes. The sites selected for the conservative change of serine to cysteine were positions near the binding site for corepressor and DNA (Fig. 1). These substitutions resulted in marked effects on both tryptophan and operator DNA binding and illustrate the complexity of

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the ANS fluorometric method (Chou et al., 1989) was employed. ANS and tryptophan compete for binding to the repressor, and the displacement of ANS by tryptophan can be monitored fluorometrically. The mutant proteins showed a similar ANS fluorescence decrease at 480 nm in the presence of tryptophan and similar affinities for ANS (Table I). The data obtained for ANS displacement by tryptophan were plotted according to Horovitz and Levitzi (1987), and the dissociation constant for tryptophan was determined (Fig. 2). The results for several measurements are summarized in Table I. Equilibrium dialysis confirmed that the tryptophan binding constants for all three mutants were $>1 \times 10^{-4}$ M. The insensitivity of ANS affinity to the mutations is in contrast to effects on tryptophan binding. All three mutants have decreased affinity for tryptophan at 25°C. The S67C mutation has the least effect (~6-fold), whereas S88C is greatest (~18-fold). The Horovitz-Levitzi plot for S68C is curved, indicating more than one class of sites which may derive from slight perturbations in the conformation of the binding region. The $K_d$ reported is that for the highest affinity binding indicated by brackets in Fig. 2B; the slope of the curve corresponding to lowest affinity binding yields a $K_d$ similar to S88C.

The sulfhydryl group of the single cysteine allows facile introduction of fluorescent labels. All three mutants were reacted with N-iodoacetyl)-N′-(5-sulfophenyl)ethylenediamine (IAEDANS). IAEDANS-labeled Ser→Cys mutant aheaprepressors were examined for effects of tryptophan binding on fluorescence. Both IAEDANS-labeled S67C and S88C exhibited a decrease of fluorescence at 480 nm in the presence of tryptophan (Fig. 3). However, S67C modified with IAEDANS did not show any tryptophan-dependent fluorescence change. Titration of IAEDANS-labeled S68C and S88C with tryptophan was performed by exciting at 395 nm and measuring the fluorescence intensity at 480 nm (Fig. 4). The dissociation constants are summarized in Table I. Introduction of the bulky fluorophore at Cys-86 did not significantly affect tryptophan binding, whereas the presence of this group at position 88 decreased binding almost 3-fold.

Operator Binding—The operator binding affinity of the Ser→Cys mutants was examined by the gel retardation assay using the 90-bp operator-containing DNA used previously by Carey (1988). In addition, a 40-bp synthetic operator, which contains trpEDCBA sequences from −29 to +10 on the top strand and from −28 to +11 on the bottom strand, was utilized. These sequences encompass those indicated by DNase protection and methylation perturbation to be in-
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Fig. 2. Horovitz-Levitzki plot of tryptophan/ANS displacement curve. Mutant trp aporepressors (1 × 10⁻⁶ M as dimer) and ANS (1 × 10⁻⁴ M) in 10 mM potassium phosphate, pH 7.5, 0.1 mM EDTA were titrated with tryptophan. The samples were excited at 395 nm and the fluorescence intensity monitored at 480 nm at 25 °C. x is ([(ANS)T/([Trp]R)T - Y)/Y and y is [(Trp)I+[(ANS)T/([Trp]R)T - Y)/[(1 - Y)/([Trp]R)T/KANS)] ([(ANS)T([Trp]R)T - Y) - Y] (Chou et al., 1989). A, S67C; B, S86C; C, S88C. The slope yields the equilibrium dissociation constant for tryptophan.

decreased intensity of the band for the operator-repressor complex indicated that the mutant repressor-operator complexes dissociated more rapidly than the wild type (Figs. 5 and 6). The equilibrium dissociation constants estimated for the mutants by this method are summarized in Table I. Measurement of t₁/₂ was not possible, as this reaction occurred more rapidly than detection allowed (t₁/₂ < 10 s). For the S88C mutant, an additional band appeared on the gel when mutant repressor concentration exceeded 10 nM (Figs. 7); this band was more prominent for S88C. Sephardex G-75 chromatography of wild-type and S88C mutant repressors with 1 mM DTT gave a single peak eluting at ~38 kDa. In contrast, in the absence of DTT, a small peak corresponding to ~44 kDa and a larger peak at

FIG. 3. The fluorescence emission spectra of IAEDANS-labeled mutant trp aporepressors. IAEDANS-labeled mutant aporepressors (1 × 10⁻⁶ M as dimer) in 10 mM potassium phosphate, pH 7.5, 0.1 mM EDTA were titrated at wavelength 390 nm and emission scanned from 410 to 550 nm. —, without tryptophan; --, in the presence of 1 × 10⁻⁶ M tryptophan. A, S67C; B, S86C; C, S88C.

FIG. 4. Titration curves for IAEDANS-labeled S86C and S88C mutant aporepressors with tryptophan. IAEDANS-labeled mutant trp aporepressors (1 × 10⁻⁶ M as dimer) in 10 mM potassium phosphate, pH 7.5, 0.1 mM EDTA were titrated with tryptophan. The samples were excited at 395 nm and the fluorescence intensity monitored at 480 nm. , S86C-IAEDANS; ■, S88C-IAEDANS. R is the fractional change in fluorescence.

FIG. 5. Gel retardation assay of wild-type and mutant trp repressor-DNA binding. A 90-bp operator-containing DNA fragment was used (Carey, 1988). Reactions were performed under the standard conditions described by Carey (1988).

additional band was observed (Fig. 7); this band was more prominent for S88C. Sephardex G-75 chromatography of wild-type and S88C mutant repressor with 1 mM DTT gave a single peak eluting at ~38 kDa. In contrast, in the absence of DTT, a small peak corresponding to ~44 kDa and a larger peak at
on x-ray crystallographic data: the serine residues located in or near the ligand binding sites were changed to cysteine. There are 6 serine residues in trp aporepressor (Gunsalus and Yanofsky, 1980). Three of these serines are located in the termini of the subunit (at 5, 8, and 107) which are distal to both tryptophan and operator recognition sites (Fig. 1; Schevitz et al., 1985; Zhang et al., 1987; Otwinowski et al., 1988). A fluorescent probe attached at those positions might not monitor any conformational effects upon ligand binding because of the rigidity of the quaternary structure of the aporepressor (Zhang et al., 1987). Ser-88 is hydrogen-bonded with the tryptophan so that this mutant would be expected to affect corepressor binding. Ser-67 and -86 were selected because their side chains are not directly involved in tryptophan binding but are close to the ligand recognition sites. The O<sub>γ</sub> of Ser-86 has recently been shown to be involved in operator binding (Otwinowski et al., 1988).

All three serine to cysteine mutants showed decreased affinities for both tryptophan and operator DNA. The mutation of Ser to Cys at 67 gives a small change (~6-fold) in tryptophan binding. These data are in agreement with the x-ray crystallographic information which indicates that residue 67 is removed from the tryptophan binding site (Schevitz et al., 1985). In addition, no fluorescence change of IAEDANS-labeled S67C mutant upon tryptophan binding was observed, consistent with these results. The thiol group at position 67 may result in a subtle change in conformation of trp aporepressor. Such a change may derive from the differences in the geometry of serine (C–O bond distance = 1.43 Å, O–H = 0.88 Å, C–O–H = 107°; Kistenmacher et al., 1974) and cysteine (C–S bond distance = 1.8 Å, S–H = 1.4 Å, C–S–H = 93°; Kerr et al., 1975). This type of structural shift may contribute to the decreased affinity of the S67C mutant for operator DNA by 50-fold. In particular, the hydrogen bond from N<sub习近</sub> to operator DNA found in the crystal structure of the complex (Otwinowski et al., 1988) may be influenced by the cysteine substitution at 67.

The mutant S86C showed a ~4-fold increase in K<sub>d</sub> for tryptophan binding and a 130-fold increase in K<sub>d</sub> for operator DNA binding. The change in dissociation constant for operator DNA binding represents a loss of about 3 kcal/mol in free energy of binding at 25 °C. Since Ser-86 in the repressor makes a hydrogen bond directly with operator DNA (Otwinowski et al., 1988), the changed geometry of cysteine appears to interfere with this contact, and the increase in free energy change may correlate to the change for hydroxyl to sulfhydryl. The curvature of a Horovitz-Levitzki plot of tryptophan binding to this mutant implies a conformational distortion in the tryptophan binding region for this protein. The absence of an effect of IAEDANS modification on corepressor binding for this mutant would be anticipated from its position based on the crystal structure analysis (Schevitz et al., 1985; Zhang et al., 1987).

The mutant S88C showed the least change in operator binding and the most change in tryptophan binding. As shown by x-ray crystallographic data (Schevitz et al., 1985; Zhang et al., 1987), Ser-88 forms a hydrogen bond with the α-amino group of bound tryptophan. Thus, the change in the mutant from OH ⋯ N to SH ⋯ N appears to yield an increase in the free energy change on complex formation by 1.7 kcal/mol at 25 °C. This major effect on tryptophan affinity does not appear to alter the ability of ligand binding to generate the DNA binding conformation of the repressor. However, the somewhat decreased operator affinity may be ascribed to the effects on tryptophan binding and improper alignment of the corepressor which in turn affects the configuration required for functional binding.

**DISCUSSION**

Oligonucleotide-directed site-specific mutagenesis was used to construct three serine to cysteine trp aporepressor mutants. The rationale for changing serine to cysteine residues in the aporepressor derives from the similarity between sulfhydryl and hydroxyl moieties and the reactivity of the sulfhydryl group. Following chemical modification to introduce a fluorescent probe at this single site in the structure, the reacted protein could be used to study the interactions of the aporepressor with its ligands. The positions chosen for mutation were based upon information from x-ray crystallographic analysis of both the free and bound forms of the repressor. The positions chosen were based on the positions of hydroxyl and tryptophan residues of the structure, the rigidity of the quaternary structure, and the accessibility of the location of the mutation.

**FIG. 6. DTT effects on the gel retardation assay patterns for S88C mutant.** Concentrations of both wild-type and S88C are shown. A, no DTT. The arrow indicates the extra operator complex species of S88C. B, with 1 mM DTT in running buffer.

**FIG. 7. Electrophoresis of mutant repressors.** Proteins (20 μg) were electrophoresed on a 20% polyacrylamide gel in the presence of 0.1% sodium dodecyl sulfate. No sulfhydryl reagent was present in the running buffer nor in lanes 1, 3, 5, and 8. β-Mercaptoethanol (5 μl) was added to samples in lanes 2, 4, 6, and 9; lanes 1 and 2, wild-type trp repressor; lanes 3 and 4, S67C mutant; lanes 5 and 6, S86C mutant; lane 7, molecular weight standards; and lanes 8 and 9, S88C mutant.

~29 kDa were observed for mutant protein (data not shown). The formation of a disulfide bond may trap the higher molecular weight (possibly tetrameric) form of the repressor and allow it to be detected as a separate species.
for operator binding. In addition, the sulfhydryl may influence the backbone conformation and distort Ser-86 and/or Asn-87 contacts with the operator DNA (Otwonowski et al., 1988). The magnitude of the decrease in tryptophan binding observed upon IAEDANS modification presumably derives from a significant perturbation of the binding site as well as interruption of the hydrogen bond with the α-amino group of the ligand.

The effects of these three mutations on ANS binding affinity are minimal (Table 1), in contrast to the marked alterations in tryptophan binding. Since ANS and tryptophan compete for the same site on the protein (Chou et al., 1989), it is noteworthy that the effects on their respective affinities differ considerably. This difference may arise from the discriminating contacts required for tryptophan binding, specifically the indole ring and α-carboxylate (Marmorstein et al., 1987). In contrast, ANS binds to hydrophobic pockets on many proteins and in a relatively nonspecific fashion (Slavik, 1982). The sulfonate moiety may contribute to the binding in a mode analogous to the carboxylate, but the geometric requirements appear to be relaxed for ANS relative to tryptophan. Similar results have been observed with mutant trp repressors having altered amino acid residues in the tryptophan binding site: the ANS binding is diminished less significantly than tryptophan binding.

The dissociation rates for all three mutants from operator DNA are faster than 0.06 s⁻¹ which is the upper limit of resolution for the gel retardation method. These fast dissociation rates may account for the observed increases in the values of equilibrium dissociation constants for operator binding. Hydroxyl radical footprinting using these proteins did not result in significant protection of the DNA backbone. The basis for the inability to obtain a hydroxyl radical footprint may derive from loss of sequence-specific contacts, especially for the S67C and S86C mutants.

From x-ray crystallographic studies, Zhang et al. (1987) indicate that the trp repressor can be divided into three domains: central core, DNA-reading head, and hydrophobic brace. The central core consists of residues in the aminoterminal half of the molecule and residues 90–104 in both subunits. This central structure is not perturbed by the binding of L-tryptophan. The DNA-reading head is comprised of the helix-turn-helix motif which involves sequence positions 66–86 in the carboxy-terminal region. This domain exhibits considerable plasticity within the operator with other amino acids, such as Gln-68, Arg-69, Lys-72, Ser-86, Asn-87, and Lys-90 (Otwinowski et al., 1988). These changes in hydrogen bonding may yield structural shifts which result in increased equilibrium dissociation constants and rates of dissociation of mutant proteins from operator DNA.

All three mutants showed a single protein band, corresponding to 12.5 kDa, on sodium dodecyl sulfate-polyacrylamide gels in the presence of 2-mercaptoethanol. However, in the absence of 2-mercaptoethanol, all three mutants showed two protein bands, corresponding to 12.5 and 25 kDa, respectively. These data imply that the serine to cysteine mutation enables disulfide bond formation to occur to some extent between two monomers. This reaction is most prominent in the S86C mutant protein. Whether this disulfide linkage occurs within the dimer or derives from intermolecular interactions between dimers is not clear. However, the gel filtration results suggest the possibility of tetramer in rapid equilibrium with dimer. Data from differential scanning calorimetry and fluorescence polarization measurements are consistent with this possibility (Bae et al., 1988). Further experiments are in progress to examine this oligomer formation.

In summary, the substitution of cysteine for serine at three different sites within the trp repressor results in alterations in both corepressor and operator affinity. The changes observed can be rationalized in terms of the known three-dimensional crystal structure of the protein, but the effects illustrate clearly the complexities involved in even a relatively conservative substitution. The formation of disulfide linkage between monomers and behavior of these mutant and wild-type proteins on gel filtration suggests the possibility of tetramer formation.

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REFERENCES

Aridvson, D. N., Bruce, C., and Gunsalus, R. P. (1986) J. Biol. Chem. 261, 236–243

Bae, S.-J., Chou, W.-Y., Matthews, K. S., and Sturtevant, J. M. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 6731–6732

Bass, S., Sugino, P., Arvidson, D. N., Gunsalus, R. P., and Youderian, R. (1987) Genes & Dev. 1, 565–572

Bass, S., Sorrells, V., and Youderian, R. (1986) Science 242, 240–245

Benett, G. N., Schweingruber, M. E., Brown, K. D., Squires, C., and Yanofsky, C. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 2361–2355

Bogosian, G., Somerville, R. L., Nishi, K., Kano, Y., and Imamoto, F. (1984) Mol. & Gen. Genet. 193, 244–250

Böhme, H. J., Koepfchlagler, G., and Schulz, J. (1972) J. Chromatogr. 69, 209–214

Bourgeois, S. (1971) Methods Enzymol. 21, 491–500

Bradford, M. M. (1976) Anal. Biochem. 72, 245–254

Brown, K. D. (1988) Genetics 60, 31–46

Carey, J. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 975–979

Carey, J. (1989) J. Biol. Chem. 264, 1941–1945

Chen, E. Y., and Seeberg, P. H. (1986) DNA 4, 165–170

Chou, W.-Y., Bieber, C., and Matthews, K. S. (1989) J. Biol. Chem. 264, 18309–18313

Clewell, D. B., and Helinski, D. R. (1970) Biochemistry 9, 4428–4440

Ellman, G. L. (1969) Arch. Biochem. Biophys. 82, 70–77

Grove, C. L., and Gunsalus, R. P. (1987) J. Bacteriol. 169, 2158–2164

Gunsalus, R. P., and Yanofsky, C. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 7117–7121

Gunsalus, R. P., Miguel, A. G., and Gunsalus, G. L. (1986) J. Bacteriol. 167, 272–278

Hanahan, D. (1985) in DNA Cloning: A Practical Approach (Glover, D. M., ed) Vol. 1, pp. 109–135, IRL Press, Oxford

Horovitz, A., and Levittzki, A. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 6654–6658

Hudson, E. N., and Weber, G. (1973) Biochemistry 12, 4154–4161

Itakura, K., Rossi, J. J., and Wallace, R. B. (1984) Annu. Rev. Biochem. 53, 323–356

Joachimiak, A., Kelley, R. L., Gunsalus, R. P., Yanofsky, C., and Sigler, P. B. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 665–672

Joachimiak, A., Marmorstein, R. Q., Schevitz, R. W., Mandecki, W., Fox, J. L., and Sigler, P. B. (1987) J. Biol. Chem. 262, 4917–4921

Kelley, R. L., and Yanofsky, C. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 3120–3124

Kelley, R. L., and Yanofsky, C. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 483–487

2 J.-J. He and K. S. Matthews, manuscript in preparation.

4 W.-Y. Chou et al., manuscript in preparation.
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Kerr, K. A., Ashmore, J. P., and Koetzle, T. F. (1975) Acta Crystallogr. B31, 2022-2026
Kistenerman, J. T., Rand, G. A., and Marsh, R. E. (1974) Acta Crystallogr. B30, 2573-2578
Klig, L. S., and Yanofsky, C. (1988) J. Biol. Chem. 263, 243-246
Kumamoto, A. A., Miller, W. G., and Gunnsdale, R. P. (1987) Genes Dev. 1, 56-64
Kunkel, T. A. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 488-492
Lane, A. N. (1986) Eur. J. Biochem. 157, 405-413
Lawson, C. L., and Sigler, P. B. (1984) Nature 313, 659-671
Lawson, C. L., Zhang, R. G., Schvitz, R. W., Otwinowski, Z., Joachimiak, A., and Sigler, P. B. (1986) Proteins 3, 18-31
Manatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
Marmorstein, R. Q., and Sigler, P. B. (1989) J. Biol. Chem. 264, 3143-3150
Marmorstein, R. Q., Joachimiak, A., Sprinzl, M., and Sigler, P. B. (1987) J. Biol. Chem. 262, 4922-4927
Messing, J. (1983) Methods Enzymol. 101, 20-78

Otowinski, Z., Schvitz, R. W., Lawson, C. L., Joachimiak, A., Marmorstein, R. Q., Luisi, B. F., and Sigler, P. B. (1988) Nature 335, 321-329
Path, J., and Yanofsky, C. (1986) Nucleic Acids Res. 14, 7851-7860
Sanger, F., Coulson, A. R., Barrett, B. G., Smith, A. J., and Roe, B. A. (1980) J. Mol. Biol. 143, 161-178
Schvitz, R. W., Otwinowski, Z., Joachimiak, A., Lawson, C. L., and Sigler, P. B. (1985) Nature 317, 782-786
Singleton, C. R., Roeder, W. D., Bogosan, G., Somerville, R. L., and Wetl, H. L. (1980) Nucleic Acids Res. 8, 1551-1560
Slavik, J. (1982) Biochem. Biophys. Acta 689, 1-25
Tullius, T. D., and Dobrosbki, B. A. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 5469-5473
Yanofsky, C., Platt, T., Crawford, I. P., Nichols, B. P., Christie, G. E., Horowitz, H., VanCleemput, M., and Wu, A. M. (1981) Nucleic Acids Res. 9, 6647-6668
Zhang, R. G., Joachimiak, A., Lawson, C. L., Schvitz, R. W., Otwinowski, Z., and Sigler, P. B. (1987) Nature 327, 591-597
Zoller, M. J., and Smith, M. (1982) Nucleic Acids Res. 10, 6487-6500