Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 668–672, February 1983
Biochemistry

Purification and characterization of *trp* aporepressor

(*trp* repressor/*trpR*/repressor–operator binding)

ANDRZEJ JOACHIMIAK*, RICHARD L. KELLEY†, ROBERT P. GUNSALUS†‡, CHARLES YANOFSKY†, AND PAUL B. SIGLER*§

*Department of Biophysics and Theoretical Biology, University of Chicago, Chicago, Illinois 60637; and †The Department of Biological Sciences, Stanford University, Stanford, California 94305

Contributed by Charles Yanofsky, October 18, 1982

ABSTRACT **We have isolated homogeneous *trp* aporepressor from an overproducing strain of *Escherichia coli* carrying a plasmid containing *trpR* preceded by tandem *trp* operon promoters. Dye-affinity and ion-exchange chromatography were used in conjunction with a gel electrophoresis assay in which the repressor, when bound to the *trp* operator, protects an *Rsa* I restriction site from endonuclease cleavage. Crystals suitable for x-ray diffraction studies were grown from a variety of concentrated salt solutions. Hydrodynamic properties and electrophoretic analysis of unmodified and covalently crosslinked aporepressor show that the free aporepressor has an isoelectric point of 5.9 and is a dimer containing two identical 12.5-kilodalton subunits in the presence or absence of L-tryptophan. The repressor·operator complex binds poorly to nitrocellulose filters, but restriction-site protection studies indicate that, in the presence of tryptophan, one dimer is bound to the operator site with an apparent dissociation constant less than 2×10^{-9} M. Preliminary equilibrium dialysis experiments suggest that tryptophan binds to the aporepressor with a dissociation constant of 1.6×10^{-5} M.**

The *trp* aporepressor,¶ the polypeptide product of *trpR*, regulates transcription of at least three operons in *Escherichia coli* and certain related bacteria: *trpEDCBA*, the biosynthetic operon for tryptophan; *aroH*, responsible for the first step in aromatic amino acid biosynthesis; and *trpR*, the structural gene for the *trp* aporepressor (1–3). There are several features of this protein that make it attractive for studying the molecular details of transcriptional control *in vitro*. It is a small, stable protein of 108 amino acid residues whose sequence has been deduced from the sequence of its gene (1). The operators to which the activated aporepressor binds have been cloned and their sequences have been determined (1, 2, 4–6). Methylation protection experiments and mutational studies have pinpointed critical repressor–DNA contacts sites in the operator of the *trpEDCBA* operon (7). The physiological characteristics of the regulatory systems in which the *trp* repressor is involved have been the subjects of continuing genetic and biochemical studies (1–8). The salient feature of this repressor–operator system that makes it particularly interesting is the fact that the activity of the *trp* aporepressor is modulated by the cellular level of L-tryptophan. When tryptophan binds to the *trp* aporepressor to form the *trp* repressor, the protein increases its affinity for the operator (9). Although the affinity of the aporepressor for the operator and tryptophan have been inferred from biochemical studies of partially purified cell extracts (10, 11), complete characterization of the molecular properties of the *trp* aporepressor has been hampered by inadequate quantities of pure protein. An understanding of the molecular mechanism by which tryptophan stabilizes the repressor–operator interaction will ultimately require detailed structural analysis of pure crystalline protein and its complexes with DNA and tryptophan. Towards this end we describe the preparation of milligram quantities of pure crystalline *trp* aporepressor, the physical characteristics of the aporepressor, and a preliminary analysis of the interaction of *trp* aporepressor with operator DNA and tryptophan.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Reagents and Chromatographic Materials. Sephadex G-75 superfine (Pharmacia), phosphocellulose (P-11, Whatman), and Red A matrix gel (Amicon) were prepared according to the manufacturer's instructions. L-[^{3}H]Tryptophan (10.8 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) and [α-^{32}P]dCTP (400 Ci/mmol) were purchased from Amersham. Restriction endonucleases *Eco*RI and *Rsa* I were from New England BioLabs, and *E. coli* DNA polymerase I was from Bethesda Research Laboratories; these enzymes were used without further purification. Glutaraldehyde (8% aqueous solution) was spectral grade from Merck and dimethylsuberimidate was from Pierce.

Bacterial Strains and Plasmids. The strain used to produce *trp* aporepressor was W3110 *trpL75 leu* carrying pRLK18, a pBR322 derivative with the *trpR* coding region downstream from the tandem *trp* promoters of *Serratia marcescens* and *Salmonella typhimurium*. The plasmid used to assay *trp* repressor activity, pRK9, also is a pBR322 derivative. It contains a single copy of the *Serratia marcescens trp* promoter/operator. A similar plasmid containing the *E. coli trp* promoter/operator, pBN60, was also employed.

Assays for *trp* Repressor Activity. Plasmid pRK9 contains three *Rsa* I sites, one of which is in the *trp* operator. Digestion of pRK9 in the presence of *trp* aporepressor and tryptophan yields only two DNA fragments because the protected operator site fuses two smaller pieces into one large fragment. The extent of binding can be quantitated by the disappearance of the 560- and 1,927-base-pair (bp) fragments and the simultaneous emergence of the 2,487-bp fragment. All of these are normalized to the continuous presence of the 1,598-bp fragment, as visualized after agarose gel electrophoresis, by the fluorescence of bound ethidium bromide, ^{32}P autoradiography, or scintillation counting of excised radioactive bands.

Two microliters of the protein solution to be assayed was added to 18 μl of a standard incubation buffer composed of 6 mM Tris·HCl at pH 7.5, 6 mM 2-mercaptoethanol, 6 mM $MgCl_2$, 50 mM NaCl, and 1 mM L-tryptophan and containing 1.8 μg of bovine serum albumin and 0.68 μg of pRK9 plasmid.

Abbreviations: bp, base pair(s); kDal, kilodalton(s).

‡ Present address: Institute of Molecular Biology, Dept. of Microbiology, University of California, Los Angeles, CA 90024.

§ To whom reprint request should be addressed.

¶ The term "aporepressor" refers to the protein product of the *trpR* gene that upon interacting with tryptophan forms a "repressor" with increased affinity for its operators.

After incubation for 2 min at room temperature, 0.5 unit of *Rsa* I was added and the mixture was incubated for 30 min at 37°C to allow complete cleavage of the unprotected restriction sites. After the digestion was completed 2 μl of 0.1% bromophenol blue in 50% (vol/vol) glycerol was added and the entire incubation mixture was subjected to electrophoresis in Tris/EDTA/acetic acid buffer (12) on a horizontal 1.2% agarose gel containing ethidium bromide at 5 μg/ml. For rapid, semiquantitative assays, the DNA content of the bands was estimated visually from the fluorescence intensity. For quantitative work, nick-translated ^{32}P-labeled plasmid was prepared with [α-^{32}P]dCTP and DNA polymerase I (13). Restriction fragments were located by autoradiography and their DNA content was quantitated by scintillation counting of the excised bands in Aquasol (New England Nuclear). Complete protection indicates at least 10 units of repressor binding activity; when the three variable bands (560, 1,927, and 2,487 bp) were present in equimolar amounts, the plasmid was considered to be half protected and assigned 5 units of repressor activity. Assay aliquots were diluted to the point at which 50% protection was observed.

Aporepressor Production. W3110 *trpL75 leu*(pRLK18) was grown at 37°C in minimal medium (14) supplemented with 0.4% glucose, 0.05% casein hydrolysate, L-tryptophan at 2 μg/ml, and ampicillin at 75 μg/ml. Ten liters in a 14-liter fermenter was inoculated with a fresh overnight culture (1:100) and grown to a turbidity of 40 Klett units (no. 66 filter), at which time an ethanolic solution of indole-3-acrylic acid was added to a final concentration of 25 μg/ml. Cells were harvested the following day after reaching a turbidity of 200 Klett units. A yield of 35 g of wet cell paste per 10 liters of culture was typical.

Aporepressor Purification. Forty-five grams of cells was suspended in 225 ml of 0.1 M Tris·HCl, pH 7.6, and disrupted by sonic oscillation. The extract was centrifuged for 20 min at 29,700 × *g* at 4°C. The supernatant was removed and streptomycin sulfate (20%) was added to 1% with stirring. The supernatant was stirred for 30 min and the mixture was brought to 62°C by shaking in a flask in a 66°C water bath (≈5 min) and then shaken an additional 5 min. The mixture was chilled on ice and the precipitate was removed by centrifugation for 10 min at 36,400 × *g*. Solid ammonium sulfate was added to the supernatant to 45% saturation, the mixture was stirred in an ice bath for 45 min and, after centrifugation, the supernatants were collected. Ammonium sulfate was added to 70% saturation and the mixture was stirred for 1 hr. The precipitate was collected by centrifugation and dissolved in 20 ml of 0.1 M Tris·HCl, pH 7.5/1 mM EDTA and dialyzed vs. 20 mM Tris·HCl, pH 7.5/1 mM EDTA overnight, with one change of buffer. The sample was applied at 30 ml/hr to a 100-ml column of Red A matrix gel. After the sample was applied, 45 min was allowed for binding and the column was washed with 150 ml of 20 mM Tris·HCl, pH 7.5/1 mM EDTA. The aporepressor was eluted with a 600-ml linear NaCl gradient (0.05–2 M) in the same Tris buffer. Fractions were assayed for aporepressor and the active fractions were pooled. The aporepressor generally eluted near the middle of the gradient.

A 1.5 × 21 cm column of Whatman P-11 phosphocellulose was equilibrated with the following buffer: 10 mM $NaPO_4$, pH 7.6/0.1 mM EDTA/0.1 M NaCl/24 μM L-tryptophan/15% (vol/vol) glycerol. The pooled peak fractions from the Red A column were dialyzed against P-11 column buffer. A sample (56 ml, total protein 59 mg) was loaded on the column and the column was washed with 20 ml of P-11 buffer. Aporepressor was eluted with a 150-ml linear gradient of 0.1–0.5 M NaCl in the same buffer. The aporepressor activity elutes late in the gradient (≈0.3 M).

Protein Concentration. All references to protein concentrations are based on an extinction coefficient of 1.2 cm^{-1} mg^{-1} ml for pure aporepressor (see *Results*), or are based on the colorimetric method of Bradford (15) calibrated against photometrically defined concentrations of pure *trp* aporepressor for preparations rich in repressor activity, or against bovine serum albumin for crude protein mixtures.

RESULTS AND DISCUSSION

Aporepressor Production and Purification. Aporepressor was purified from strain W3110 *trpL75 leu* containing the overproducing plasmid pRLK18. This plasmid has the *Salmonella typhimurium* and *Serratia marcescens trp* promoters in tandem fused to the *trpR* structural gene. Growth of this plasmid-containing strain in the presence of the inducer indole-3-acrylic acid yields cells that contain 2–8% of their soluble protein as *trp* aporepressor. The aporepressor was purified to homogeneity (Fig. 1) in good yield in relatively few steps (Table 1). In our procedure we exploited the finding that aporepressor withstands heating to 90°C. In other purification protocols we found that hydroxyapatite chromatography also was effective in aporepressor purification.

Crystallization. Microcrystals can be obtained from a wide variety of precipitants, either in the presence or absence of tryptophan and its analogues. Fig. 2 shows examples of crystals grown by the vapor diffusion technique described by Johnson *et al.* (18). The crystals shown in Fig. 2*b* diffract to atomic resolution and are suitable for single-crystal x-ray diffraction studies. The presence of L-tryptophan and analogues, such as indole-3-propionic acid, that competitively inhibit L-tryptophan's corepressor activity significantly alters the stability of various crystal forms and the rate of crystal growth. A full description of crystalline polymorphism in the repressor/aporepressor sys-

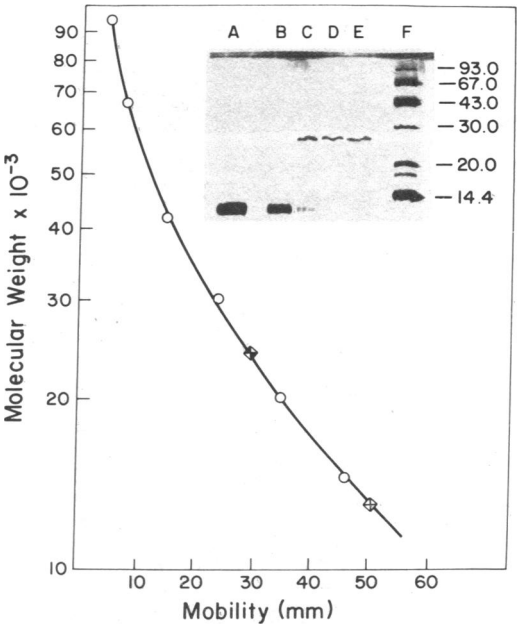

FIG. 1. Gel electrophoresis in 15% polyacrylamide/0.1% Na-DodSO$_4$ (16). Lane A, 1.5 μg of pure *trp* aporepressor. Lanes B–E, *trp* aporepressor (0.5 μg) after treatment with 0.004% glutaraldehyde in 50 mM potassium phosphate, pH 6.8, for 0, 1, 2, or 24 hr, respectively. Gel was stained with silver (17). Lane F, standards, in order of decreasing molecular weight: phosphorylase b, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, ribonuclease A. ⊠, Mobility of *trp* aporepressor; ■, *trp* aporepressor cross-linked with glutaraldehyde.

Table 1. Purification of the *trp* aporepressor

Sample	Volume, ml	Total protein, BU	Repressor specific activity, units/BU	Yield, %	Purification, fold
Crude	210	2,814	34	100	1
Heated, streptomycin	200	850	83	74	2.5
Dialyzed $(NH_4)_2SO_4$ fraction	31	264	206	58	6.1
Pooled Red A	56	59	570	35	18
Pooled phosphocellulose	33	16.5	2,000	31	60

One Bradford protein unit (BU) is equivalent to 1.0 mg of bovine serum albumin or 3.3 mg of *trp* aporepressor.

tem and a discussion of its biochemical significance will be published elsewhere.

Molecular Weight. The mobilities on polyacrylamide gel electrophoresis in $NaDodSO_4$ of the pure *trp* aporepressor and glutaraldehyde-crosslinked derivatives (Fig. 1) indicate that the promoter of the *trp* aporepressor molecule is a 12.5 kilodalton (kDal) polypeptide. This figure agrees with a molecular mass of 12.356 kDal calculated from the *trpR* nucleotide sequence (1).

We determined the diffusion coefficient of *trp* aporepressor under nondenaturing conditions in the absence and in the presence of tryptophan by gel permeation chromatography (Fig. 3*a*).

We also determined the sedimentation coefficient of *trp* aporepressor under nondenaturing conditions by ultracentrifugation on 5–20% sucrose gradients (Fig. 3*b*). The results are summarized in Table 2. The molecular weights calculated from

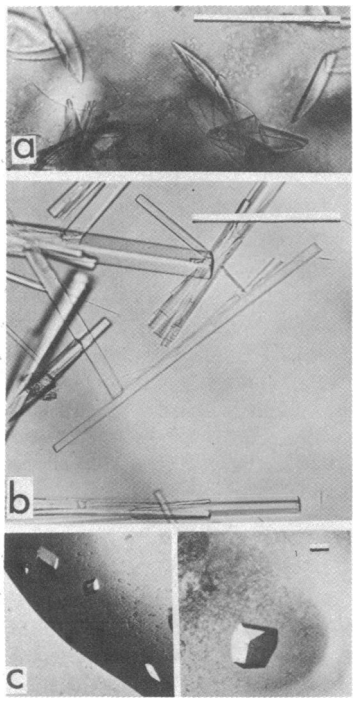

FIG. 2. Crystals of *trp* aporepressor. Conditions of crystallization were as follows: Protein concentration in each case is approximately 15 mg/ml. Precipitants were 1.16 M potassium citrate/50 mM potassium sodium phosphate, pH 7.6 (*a*), 0.95 M ammonium sulfate/50 mM potassium sodium phosphate, pH 7.0/4 mM tryptophan (*b*), 2.8 M potassium sodium phosphate, pH 5.2/4 mM tryptophan (*c*). Bars in upper right represent 0.2 mm.

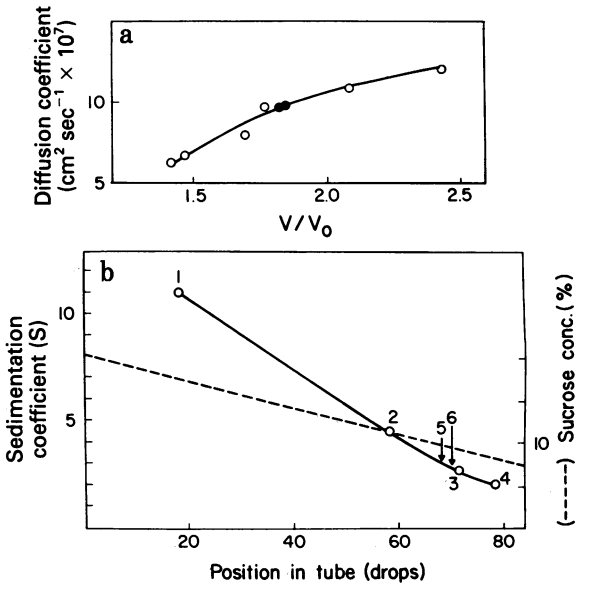

FIG. 3. (*a*) Diffusion of *trp* aporepressor and standard proteins having known diffusion coefficients (ref. 19, pp. C-10–C-25) as measured by gel permeation chromatography on a Sephadex G-75 superfine column (0.7 × 27 cm, 0.1 M KPO_4, pH 6.8). V/V_0 is the elution volume relative to the excluded volume. (*b*) Sedimentation velocity on 5–20% sucrose gradients at 4°C of *trp* aporepressor and standard proteins having known sedimentation coefficients (ref. 19, pp. C-10–C-25): 1, catalase; 2, bovine serum albumin; 3, chymotrypsinogen; 4, lysozyme; 5, *trp* aporepressor plus 0.1 mM tryptophan; 6, *trp* aporepressor.

the diffusion and sedimentation coefficients are 2.8×10^4 for the *trp* aporepressor and 2.7×10^4 for its tryptophan complex. The differences observed between the *trp* aporepressor and the putative tryptophan complex are too marginal to draw any conclusions about functional implications.

In view of the fact that the observed molecular weight of the native protein was slightly greater than an integral multiple of the promoter molecular weight, supplementary evidence for the degree of aggregation was obtained. We allowed *trp* aporepressor to react with the bifunctional crosslinking agents glutaraldehyde or dimethylsuberimidate and analyzed the products by polyacrylamide gel electrophoresis under denaturing conditions. The only high molecular weight band produced by covalent crosslinking had a mobility corresponding to a 24.0-kDal dimer. Even after extending the crosslinking reaction to 24 hr and converting all the monomers to higher molecular weight forms, we observed no product with a molecular weight higher than 24×10^3 (Fig. 1). Crosslinking *trp* aporepressor with dimethylsuberimidate yielded the same dimeric product but with a lower percentage of conversion, perhaps due to abortive monofunctional reactions resulting from rapid hydrolysis of one imidoester function. In contrast to previous suggestions (5, 9, 11), the free *trp* aporepressor in the presence or absence of tryptophan is a dimer of two identical 12.5-kDal subunits. Moreover, the stoichiometry of binding also indicates a functional dimer because two promoters bind to each operator (see below).

Extinction Coefficient. The extinction coefficient at 280 nm was calculated to be 1.2 (±0.07) cm^{-1} mg^{-1} ml from the contributions to the absorbance at that wavelength of the known proportions of tryptophan, tyrosine, and phenylalanine, using the mean of the published values for the absorbance of their *N*-acetyl methyl esters at 280 nm in water and ethanol (ref. 19, p. B-74). The assumption underlying this calculation is that the combined dielectric effects of the protein and its solvent en-

Correction - Proc. Natl. Acad. Sci. USA - V. 80 (1983)

Table 2. Hydrodynamic properties of *trp* aporepressor

Conditions	D_{20}, $cm^2\ sec^{-1} \times 10^7$	s_{20}, S	Molecular weight $\times 10^{-4}$	Effective radius,† Å	f_{eff}/f_0‡
trp aporepressor alone	9.7 ± 0.1	3.1 ± 0.3	2.8 ± 0.2	22.1	1.16
trp aporepressor with 0.1 mM L-tryptophan	9.5 ± 0.1	2.9 ± 0.3	2.7 ± 0.2	22.6	1.19

*Molecular weight calculated from the equation $M = [RTs/D(1 - \bar{v}\rho)]$, in which ρ is density of solvent taken as 1.0 g cm^{-3}, $\bar{v}$ is the partial specific volume taken as 0.725 $cm^3\ g^{-1}$ (20), R is the gas constant, and T is absolute temperature.

†Effective radius calculated from $r_{eff} = RT/6\pi D\eta N_A$, in which η is the viscosity of the solvent and N_A is Avogadro's number.

‡Frictional coefficients were calculated by using $f = 6\pi\eta r$. f_0 is the frictional coefficient of a hypothetical sphere having a specified molecular weight and partial specific volume.

vironment lie between those of pure ethanol ($\varepsilon_{280}^{calc} = 1.27\ cm^{-1}\ mg^{-1}\ ml$) and pure water ($\varepsilon_{280}^{calc} = 1.14\ cm^{-1}\ mg^{-1}\ ml$). The value $1.0\ cm^{-1}\ mg^{-1}\ ml$ was determined experimentally by preparing solutions containing accurately weighed (2–3 mg) samples of protein that had been lyophilized from NH_4HCO_3 solutions; the volatile salt being necessitated by the fact that *trp* aporepressor is insoluble at very low ionic strength. Because slight contamination with salt would cause an underestimate of the specific absorbance, the calculated extinction coefficient of 1.2 ± 0.07 $cm^{-1}\ mg^{-1}\ ml$ was considered more reliable and used in these studies.

Isoelectric Point. The isoelectric point of pure *trp* aporepressor is 5.9 as determined by isoelectric focusing in 5% acrylamide gels with an ampholyte pH range of 5–7. This is consistent with the amino acid composition (1): acidic residues exceed basic residues.

Amino-Terminal Heterogeneity. It has been reported that 90% of the amino-terminal methionine of the original *trpR* translation product had been removed, leaving an amino-terminal alanine in the mature *trp* aporepressor (1). By applying thin-layer chromatographic analysis to the hydrolyzed dansylation products of various pure *trp* aporepressor preparations, we have determined that the fraction of amino-terminal methionine varied from undetectable levels to as much as 25%. We noted no correlation of the amino-terminal composition with repressor activity.

Stoichiometry and Stability of the *trp* Repressor·Operator Complex. We failed to demonstrate repressor-specific binding of labeled operator to nitrocellulose filters under a variety of conditions *in vitro*, even when we used materials that proved to be satisfactory for quantitating protein–DNA interaction in other systems. This was true for both the supercoiled form and the linear form of plasmids pRK9 and pBN60. Nevertheless, under these same solvent conditions tryptophan-specific binding of *trp* repressor to operator can be estimated by the extent to which the *Rsa* I site in the operator is protected (1).

Fig. 4 shows the tryptophan-specific protection of the *Rsa* I site as one saturates the operator with increasing concentrations of repressor. It is clear that complete protection is afforded by the binding of one dimer. More specifically, the binding curve cannot be explained by the binding of three or more 12.5-kDal promoters, which supports the assertion made from hydrodynamic and crosslinking studies that the functional repressor is a dimer of two 12.5-kDal subunits.

An accurate dissociation constant for the repressor·operator complex is difficult to determine by using our restriction-site protection assay. In addition to the nonspecific binding of repressor to nonoperator DNA observed here and with other DNA-binding regulatory proteins (20, 21), one must take into account the competitive and destructive effects of the *Rsa* I endonuclease. These effects lead to an underestimate of the affinity of repressor for its cognate operator. If it were necessary to correct for the artifacts of the restriction-site protection assay, the improved curve in Fig. 4 would have an extended linear (or near-linear) portion and a slight increase in slope. This would reduce rather than increase the apparent number of dimers bound per operator from the experimented value of 1.15 shown in Fig. 4. Therefore we observe no basis for considering more than one dimer bound per operator.

A double-reciprocal plot of the data shown in Fig. 4 is linear with a slope corresponding to an apparent dissociation constant of 2×10^{-9} M (22). For the reasons discussed above this value is likely to be an underestimate of the affinity of the repressor for operator, and it suggests that stability of the *trp* repressor·operator complex is of about the same order as that seen in the *cro*/*cI* system of bacteriophage λ (23, 24).

Although the exact strength of the repressor·operator complex is still uncertain, we consider the current finding of two promoters (i.e., one dimeric repressor) bound to one operator to be more reliable than the ratio of four promoters to one operator previously inferred from biochemical results with a partially purified system (8, 9). Moreover, the stoichiometry of two 12.5-kDal promoters per operator is consistent with recent structural analyses of other DNA regulatory proteins. Comparisons of the

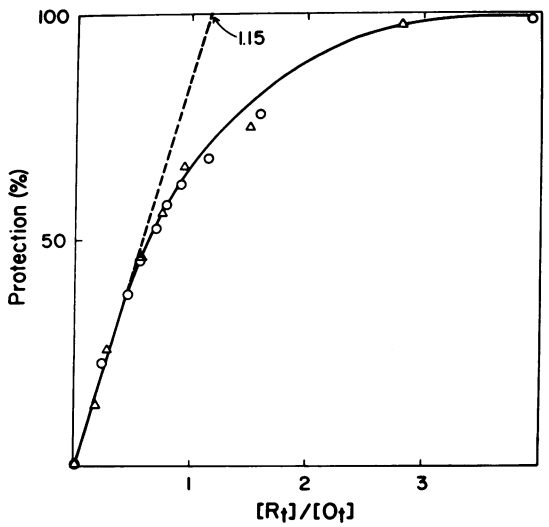

FIG. 4. Saturation of *trp* operator by *trp* repressor measured by using the *Rsa* I protection assay. ^{32}P-Labeled pRK9 plasmid (nick-translated), 9 nM (△) or 15.3 nM (○), was incubated 5 min at 20°C with increasing amounts of *trp* aporepressor and then incubated 8 min at 37°C with *Rsa* I (1 unit/0.5 μg of pRK9). R_t, total concentration (free and bound) of *trp* aporepressor as a dimer, and O_t, concentration of pRK9 plasmid, were determined photometrically at 280 and 260 nm, respectively.

crystallographically determined three-dimensional structures of catabolite gene activator protein (25), cro protein (26), and the amino-terminal fragment of bacteriophage λ repressor (27) suggest a common DNA-binding motif in each of two identical dyad-related promoters. The common substructure in each promoter consists of two seven- to nine-membered α-helices joined by a short and sharply angulated spacer of three amino acids (28–30). It has been proposed (28–30) that this structural motif is arranged such that when the dyad axis of the regulatory protein corresponds to the pseudo-dyad of the binding site on the DNA, the α-helices of each promoter interact with each of the two consecutive deep grooves presented by one side of the operator. Sequence comparisons of a wide range of regulatory proteins that interact with duplex DNA show regions of strong homology with this DNA-binding motif, suggesting that this characteristic substructure may be quite general (31, 32). Ohlendorf *et al.* (32) have pointed out that the carboxyl half of *trp* aporepressor (residues 66–87) shares strong homology with these DNA-binding α-helices.

Binding of Tryptophan to *trp* Aporepressor. *trp* aporepressor is activated by L-tryptophan (8, 9, 11), presumably by enhancing the stability of the repressor·operator complex. Fig. 5 shows a preliminary equilibrium dialysis study of the binding of tryptophan to pure aporepressor in the presence and absence of DNA. The expression $1 + (1/K_d)R_t$ describes the asymmetry of tryptophan concentrations caused by the presence of *trp* aporepressor in only one chamber at a concentration much greater than that of tryptophan. Under the conditions of the restriction site protection assay, tryptophan binds to free aporepressor molecules with a dissociation constant of 1.6×10^{-5} M. Preliminary experiments indicated that the presence of DNA increases the affinity of the aporepressor for tryptophan; however, it is not yet clear to what extent this represents specific ternary complexes formed between tryptophan, aporepressor, and DNA. Further studies using pure operator DNA and pure nonoperator DNA are necessary to clarify these issues.

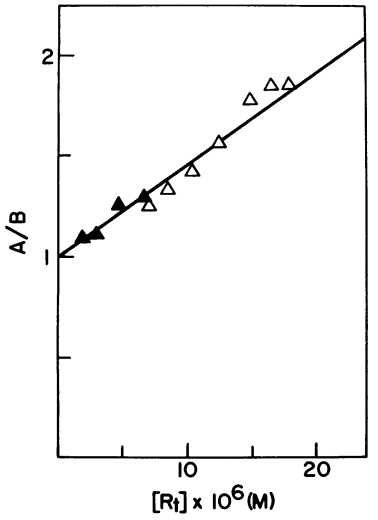

FIG. 5. Equilibrium dialysis binding of L-[^{3}H]tryptophan to *trp* aporepressor under conditions of *Rsa* I protection assay. *A* is the radioactivity at equilibrium (24 hr at 25°C) of 75-μl aliquots taken from the chamber containing a 100-μl sample of aporepressor whose concentration is R_t (as dimer). *B* is the radioactivity of a 75-μl aliquot taken from the opposing chamber containing the same starting components except no protein. Starting tryptophan concentration was 0.123 μM (▲) or 0.495 μM (△). *trp* repressor recovered from this experiment is fully active in the restriction site protection assay.

We thank Drs. K. Agarwal and M. Casadaban for restriction endonucleases, Dr. N. Cozzarelli and his colleagues for help with the nitrocellulose filter assay, Dr. John Westley for helpful discussions, and Drs. Brian Matthews and Carl Pabo for sending us useful information prior to publication. The work at Chicago was supported by a grant from the U.S. Public Health Service (GM15225) and the work at Stanford by grants from the National Science Foundation (PCM77-24333) and the U.S. Public Health Service (GM09738). R.L.K. is a Predoctoral Trainee of the U.S. Public Health Service, and C.Y. is a Career Investigator of The American Heart Association.

1. Gunsalus, R. P. & Yanofsky, C. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 7117–7121.
2. Zurawski, G., Gunsalus, R. P., Brown, K. D. & Yanofsky, C. (1981) *J. Mol. Biol.* **145**, 47–73.
3. Kelley, R. L. & Yanofsky, C. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 3120–3124.
4. Bennett, G. N. & Yanofsky, C. (1978) *J. Mol. Biol.* **121**, 179–192.
5. Gunsalus, R. R., Zurawski, G. & Yanofsky, C. (1979) *J. Bacteriol.* **140**, 106–113.
6. Roeder, W. & Somerville, R. L. (1979) *Mol. Gen. Genet.* **176**, 361–368.
7. Oppenheim, P. S., Bennett, G. N. & Yanofsky, C. (1980) *J. Mol. Biol.* **144**, 133–142.
8. Yanofsky, C. (1971) *J. Am. Med. Assoc.* **218**, 1026–1035.
9. Rose, J. K., Squires, C. L., Yanofsky, C., Yang, H. & Zubay, G. (1973) *Nature (London) New Biol.* **245**, 133–137.
10. Zubay, G., Morse, D. E., Schrenk, W. J. & Miller, J. H. M. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1100–1103.
11. Rose, J. K. & Yanofsky, C. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 3134–3138.
12. Helling, R. B., Goodman, N. M. & Boyer, H. W. (1974) *J. Virol.* **14**, 1235–1244.
13. Kelly, R. B., Cozzarelli, N. R., Deutscher, M. P., Lehman, I. R. & Kornberg, A. (1970) *J. Biol. Chem.* **245**, 39–45.
14. Vogel, H. J. & Bonner, D. M. (1956) *J. Biol. Chem.* **218**, 97–106.
15. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–251.
16. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
17. Merril, C. R., Switzer, R. C. & Van Keuren, M. L. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4335–4339.
18. Johnson, C. D., Adolph, K., Rosa, J. J., Hall, M. D. & Sigler, P. B. (1970) *Nature (London)* **226**, 1246–1247.
19. Sober, H. A., ed. (1968) *Handbook of Biochemistry* (Chemical Rubber, Cleveland, OH), 2nd Ed.
20. Berg, O. G., Winter, R. B. & Von Hippel, P. H. (1981) *Biochemistry* **20**, 6929–6948.
21. Winter, R. B., Berg, O. G. & Von Hippel, P. H. (1981) *Biochemistry* **20**, 6961–6977.
22. Klotz, I. M. & Hunston, D. L. (1971) *Biochemistry* **10**, 3065–3069.
23. Takeda, Y., Folkmanis, A. & Echols, H. (1977) *J. Biol. Chem.* **252**, 6177–6183.
24. Johnson, A. D., Poteete, A. R., Lauer, G., Sauer, R. T., Ackers, G. K. & Ptashne, M. (1981) *Nature (London)* **294**, 217–223.
25. McKay, D. B. & Steitz, T. A. (1981) *Nature (London)* **290**, 744–749.
26. Anderson, W. F., Ohlendorf, D. H., Takeda, Y. & Matthews, B. W. (1981) *Nature (London)* **290**, 754–758.
27. Pabo, C. & Lewis, M. (1982) *Nature (London)* **298**, 443–447.
28. Steitz, T. A., Ohlendorf, D. H., McKay, D. B., Anderson, W. F. & Matthews, B. W. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 3097–3100.
29. Pabo, C. O. & Lewis, M. (1982) *Nature (London)* **298**, 443–447.
30. Ohlendorf, D. H., Anderson, W. F., Fisher, R. G., Takeda, Y. & Matthews, B. W. (1982) *Nature (London)* **298**, 718–723.
31. Sauer, R. T., Yocum, R. R., Doolittle, R. F., Lewis, M. & Pabo, C. O. (1982) *Nature (London)* **298**, 447–451.
32. Ohlendorf, D. H., Anderson, W. F. & Matthews, B. W. (1983) *J. Mol. Evol.*, in press.

*Correction - Proc. Natl. Acad. Sci. USA - 80 (1983)