The Structural Basis for the Interaction between L-Tryptophan and the Escherichia coli trp Aporepressor*

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We have employed equilibrium dialysis to help study the mechanism by which the unliganded Escherichia coli trp aporepressor is activated by L-tryptophan to the liganded trp repressor. By measuring the relative affinity of L-tryptophan and various tryptophan analogues for the co-repressor's binding site, we have estimated the extent to which each of the functional groups of L-tryptophan contributes to the liganding process and discuss their role in the context of the crystal structures of the trp repressor and aporepressor. We have found that the indole ring and α carboxyl group of L-tryptophan are mainly responsible for its affinity to the aporepressor. The α amino group, however, has a small negative contribution to the affinity of L-tryptophan for the aporepressor which may be associated with its essential role in operator-specific binding.

The regulation of tryptophan biosynthesis in Escherichia coli and certain related bacteria has been studied extensively by both genetic and biochemical techniques (1-3). Three mechanisms control the synthesis of tryptophan in E. coli: (i) feedback inhibition of enzyme activity, (ii) attenuation of transcription of the trpEDCBA operon, and (iii) repression of transcription of two operons by the trp repressor (1-3). The two operons are trpEDCBA, which controls the transcription of the genes encoding the enzymes committed to tryptophan biosynthesis, and aroH, which encodes one of the three isozymes responsible for the first step in aromatic amino acid biosynthesis. In addition trp repressor regulates the transcription of its own structural gene, trpR. Repression of these operons is modulated by a feedback loop; as the concentration of L-tryptophan increases, two molecules of L-tryptophan bind noncooperatively (4-6) to the inactive dimeric trp aporepressor forming the active trp repressor which binds specifically and firmly to the appropriate operators (7-9).

The trp repressor is a particularly attractive protein for studying the chemistry of regulated operator-specific binding for two important reasons. (i) The unliganded trp aporepressor is a symmetrical dimer that binds DNA weakly and without sequence specificity. Upon binding two molecules of L-tryptophan the protein is activated to the liganded repressor that binds its cognate operators. The crystal structures of both the trp repressor and aporepressor have been solved to high resolution (10), and the stereochemical changes of the L-tryptophan-induced transition can be visualized directly. The structural changes reflect the effect of bound L-tryptophan and imply the critical elements in sequence-specific DNA interactions. (ii) It has been shown that certain desaminooxy analogues of L-tryptophan can competitively displace L-tryptophan (11), resulting in the formation of trp pseudorepressors. These inactive adducts crystallize in a form that is nearly isomorphous with the active repressor (11), suggesting that these competing analogues also induce most of the structural transition. When the crystal structures of the repressor and pseudorepressor are compared, they should reveal why L-tryptophan, rather than these analogues (some of which bind more strongly), is required for repressor function.

In order to obtain a stereochemical picture of the activation mechanism, we report here our equilibrium binding studies on the affinity of L-tryptophan and 23 of its analogues for the aporepressor. From an analysis of these analogues we infer the role of various functional groups in the binding of L-tryptophan to trp aporepressor and explain these observations qualitatively in terms of the crystal structures of the trp repressor and aporepressor.

MATERIALS AND METHODS

trp Aporrepressor—trp aporepressor was overproduced in E. coli strain W3110 trpL75 leu carrying plasmid pRLK8 and purified as described (12). The protein was homogeneous as determined by electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate and stained with silver. Protein concentrations were determined by absorbance at 280 nm (ε280 = 1.48 × 10^4 M^-1 cm^-1) (4).

L-Tryptophan and Tryptophan Analogues—L-[14C]Tryptophan (53.5 mCi/mmol) was purchased from Amersham Corp. and more than 97% of the radioactivity comigrated with nonlabeled L-tryptophan on reverse phase HPLC (C-8 Aqapore 300, Brownlee Labs, Santa Clara, CA) and thin layer chromatography. L-Tryptophan and its analogues (except for 6-nitro-L-tryptophan) were purchased from Sigma and Aldrich and used without further purification. 6-Nitro-L-tryptophan was a generous gift from Dr. Robert

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L-Tryptophan or tryptophan analogue dilutions were prepared from stock solutions whose concentrations were determined photometrically under the solvent conditions used to establish their extinction coefficients. Except for indole, L-phenylalanine, L-tyrosine, and 6-nitro-L-tryptophan, whose extinction coefficients were taken from the literature (13-15), the extinction coefficient of each ligand was determined under defined solvent conditions (Table I) from solutions prepared gravimetrically as follows. Compounds were desiccated under vacuum over P₂O₅ and samples of 20-200 mg (±0.01 mg) were dissolved in an appropriate solvent such as 0.1 N HCl, 0.1 N NaOH, or 0.5 N HCl. Nonpolar compounds were initially dissolved in ethanol and then diluted greater than 1000-fold with 0.1 N NaOH. UV absorbance spectra were recorded on a Beckman DU-40 spectrophotometer, and extinction coefficients were determined at the wavelength of maximum absorbance.

Equilibrium Dialysis Experiments—The strategy of these experiments was to first establish the affinity of radioactive L-tryptophan for the aporepressor. The affinity of competing tryptophan analogues (or L-tryptophan) used for the L-tryptophan binding site was then determined by the degree to which radioactive L-tryptophan was displaced from its binding site. This approach obviates the need for radioactive analogues and measures the affinity of the analogue for the L-tryptophan binding site only.

Experiments were carried out in 10 mM Tris/HCl, 250 mM KCl, pH 8.1. Polycarbonate equilibrium dialysis chambers (100 ± 10 µl) were separated by Spectrapor 2 membranes (molecular weight cut off 6000-8000) which had been pre-treated at 60 °C for 1 h in 50 mM EDTA. Equilibrium dialysis vessels contained 80.0 µl of solution on each side of the dialysis membrane. Tryptophan aporepressor at a concentration of 34.9 µM was placed on one side of the membrane. On the other side L-[methylem-14C]tryptophan was placed at a concentration of 2.60 µM together with various concentrations of nonlabeled L-tryptophan or one of its analogues. The chamber contents were allowed to equilibrate for 16 h (equilibration was found to be complete after 10 h) at 4 °C after which 65-µl aliquots were removed from each chamber for scintillation counting.

Analytical Treatment—Based on the results of Joachimiak et al. (4), Arvidson et al. (6), and Lane (7) and the binding saturation, Scatchard, and double reciprocal plots shown in Fig. 1, we will assume that the aporepressor dimer binds L-tryptophan and tryptophan analogues noncooperatively. The dissociation constants for L-tryptophan and tryptophan analogues can then be expressed as follows:

\[ K_a = [A]/[W]/[A\cdot W] \]

(1)

\[ K_p = [A]/[I]/[A\cdot I] \]

(2)

where \([A]\), \([W]\), and \([I]\) are the concentrations of unliganded aporepressor subunit, L-tryptophan, and tryptophan analogue, respectively. \([A\cdot W]\) and \([A\cdot I]\) are the concentrations per subunit of bound L-tryptophan and analogue, respectively, irrespective of the status of the aporepressor's dimer-related binding site.

Since the volumes are the same on both sides of the dialysis membrane, the conservation equations can be written in terms of concentrations:

\[ [A_o] = [A\cdot W] + [A\cdot I] + [A] \]

(3)

\[ [W_o] = [A\cdot W] + 2[W] \]

(4)

\[ [I_o] = [A\cdot I] + 2[I] \]

(5)

where \([A_o], [W_o],\) and \([I_o]\) are the starting concentrations of aporepressor, L-tryptophan, and tryptophan analogue, respectively. Since the concentration of free L-[14C]tryptophan is equal in both chambers, the concentration of bound L-[14C]tryptophan can be calculated from its specific activity and the difference in radioactivity in the opposing chambers.⁴

\[ K_w = [A\cdot W]/[2K_c/([A_o] - [A\cdot W]) + 1] \]

(6)

\[ K_c, \text{ the dissociation constant for the complex between L-tryptophan and the aporepressor subunit, was adjusted to optimize the fit of the experimental points to Equation 6 employing sequentially the Simplex (16) and Gauss-Newton algorithms (17).} \]

\[ K_I \text{ Determination—By combining Equations 1, 2, and 3 one can derive Equation 7.} \]

\[ [A_o]/[W] = \frac{K_c}{K_c + [I]K_e} + 1 \]

(7)

Similarly, Equation 8 can be obtained by combining Equations 1, 2, and 5.

\[ [I] = \frac{[I_o]}{K_c[A\cdot W]/K_e[I]W] + 2} \]

(8)

Equation 9 relates the concentration of bound L-tryptophan, \([A\cdot W]\), to the initial concentration of competing analogue, \([I_o]\). The equation is obtained by combining Equations 4, 7, and 8 to eliminate \([I]\) and \([W]\). In effect, it expresses the ability of the analogue to displace L-tryptophan in terms of the two relevant dissociation constants \(K_c\) (for L-tryptophan) and \(K_I\) for the competing analogue. Having already determined the value of \(K_c\), the only unknown parameter is \(K_I\), which was adjusted to optimize the fit of the experimental points to Equation 9 by applying the same least squares approach used to obtain \(K_c\).

RESULTS AND DISCUSSION

L-Tryptophan Binds without Cooperativity—Fig. 1a shows the concentration of bound L-tryptophan, \([A\cdot W]\), as a function of the starting L-tryptophan concentration, \([W_o]\). This binding saturation curve was generated using the nonlinear least squares approach described under "Materials and Methods" and yielded a dissociation constant for L-tryptophan from the aporepressor of 14.6 ± 0.5 µM. In addition, Scatchard and double reciprocal plots (Fig. 1, b and c) indicate no cooperativity and a stoichiometry of 1.0 ± 0.03 L-tryptophan molecules bound per subunit with dissociation constants of 14.6 and 14.8 µM, respectively. The 1:1 stoichiometry and the noncooperativity of binding was also found by several others (4-5). The dissociation constant is in fair agreement with the results of Joachimiak et al. (4) \((K_w = 16 \mu M at 25^\circ C)\) and Lane (6) \((K_w = 18 \mu M at 25^\circ C)\), but is about half of the 28 µM value reported by Arvidson et al. (5) under similar conditions.

The absence of cooperativity in L-tryptophan binding can be explained by the unique architecture of the dimeric protein. Whereas individual subunits of most oligomeric proteins have a globular shape, with the interface between subunits representing a small fraction of the molecule's solvent excluded surface, the crystal structure of the repressor indicates that the individual subunits do not have the usual globular shape. Instead, the two subunits symmetrically interlock, particularly with their first three helices (10), to collectively form a globular shape.

The "starting" concentration of L-tryptophan, \([W_o]\), is determined experimentally at equilibrium from the total amount of [14C]-L-tryptophan in solution (about 5% adventitious loss) after correcting for the radioactivity of L-tryptophan determined by HPLC (about 97.5%).

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⁵The Simplex algorithm is applied first because it has a larger sphere of convergence. The Gauss-Newton method converges more definitively and provides an estimate of the standard deviation.
globular fold which has a large and convoluted interface. Schevitz et al.\textsuperscript{7} in a more quantitative analysis of this unusual interface, point out that in a subunit of trp repressor (taken as a monomer) a strikingly small 4% of the residues can be considered to be buried compared with 20% and greater for typical proteins and their subunits. It is only in the context of the full dimer that a more normal 18% are seen to be buried. The net result is that in the region surrounding the dyad between the tryptophan binding sites the chains of the two subunits are folded into a single globular structure representing a solid "central core" that is not amenable to quaternary shifts on ligand binding. The absence of even tertiary structural changes in the central core in response to the binding of L-tryptophan has been established by the recently solved crystal structure of trp aporepressor\textsuperscript{1} which shows essentially no changes in the backbone conformation of this region (residues 16-64) when compared to the repressor. This stability is in striking contrast to the substantial shifts that L-tryptophan imposes on the DNA interactive segments of the aporepressor (residues 66-86). Thus it would appear that it is part of the molecular design of the trp repressor/aporepressor dimer to use this central core as a rigid scaffold on which to support the peripheral domains that undergo the structural changes responsible for recognizing and adhering to operator DNA.

\textbf{Analogues of Tryptophan Compete for the L-Tryptophan Binding Site—}After thoroughly characterizing the binding of L-tryptophan to trp aporepressor, competition experiments with various tryptophan analogues were conducted under the same conditions. The displacement of L-[\textsuperscript{14}C]tryptophan by competing unlabeled analogues can be readily seen by plotting the concentration of remaining radioactively labeled binding sites, [\textit{A} - \textit{W}], as a function of the initial concentration of competing analogue \([\textit{I}_0]\). Such curves for indole-3-propionic acid, a relatively strong competitor, and tryptamine, a relatively weak competitor, are shown in Fig. 2. Also shown is the curve for nonlabeled L-tryptophan when it is used as a competing analogue. These plots along with those for the 21 other tryptophan analogues were generated by adjusting \(K_t\) to optimize the fit of the experimental points to equation 9 by applying the same least squares approach used to obtain \(K_w\) (Table I).

\textbf{The Indole Group Is Necessary for Binding—}An examination of Table I suggests that the indole ring is the most important moiety in the binding of L-tryptophan to its binding site. Indeed, indole alone binds to the aporepressor more effectively than other aromatic amino acids such as L-phenylalanine or L-tyrosine\textsuperscript{6} which have a phenyl or p-hydroxy phenyl function instead of an indole moiety in their side chain.

The affinity of the indole ring for the aporepressor is sensitive to seemingly small perturbations in electronic structure and/or conformation. For example, indole, which has a saturated 5-membered heterocyclic ring, binds very poorly, as does 7-aza-tryptophan in which a pyridine ring replaces the benzene ring of indole. The apparent rejection of the more polar heterocyclic nitrogen at position 7 of 7-aza-tryptophan is similar to the situation at positions 5 and 6 where the addition of a hydroxyl or nitro group, respectively, also disturbs binding. On the other hand, a nonpolar methyl substituent at position 5 strongly enhances binding, indicating that

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{binding.png}
\caption{Binding of L-[\textsuperscript{14}C]tryptophan to trp aporepressor as measured by equilibrium dialysis. The experimental conditions and mathematical treatment are described under "Materials and Methods." a, binding saturation curve (generated by nonlinear regression to Equation 6); b, Scatchard plot; c, double reciprocal plot. [\textit{A} - \textit{W}] designates the concentration of protein-bound L-tryptophan; [\textit{A}_i], total aporepressor concentration; [\textit{W}_0], total L-[\textsuperscript{14}C]tryptophan concentration, and [\textit{W}], free L-[\textsuperscript{14}C]tryptophan concentration.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{displacement.png}
\caption{Displacement of L-[\textsuperscript{14}C]tryptophan bound trp aporepressor by competing nonradiolabeled analogues. The experimental conditions and mathematical treatment are described under "Materials and Methods." L-[\textsuperscript{14}C]tryptophan bound to trp aporepressor was displaced by indole-3-propionic acid (\textbullet), tryptamine (\textblacktriangleleft), or nonradiolabeled L-tryptophan (\textblacktriangle). The curves were generated by nonlinear regression to Equation 9. [\textit{A} - \textit{W}] designates the concentration of L-[\textsuperscript{14}C]tryptophan bound to trp aporepressor; \([\textit{I}_0]\) is the total concentration of the competing analogue.}
\end{figure}

\textsuperscript{6}The trp aporepressor is clearly designed to ignore L-phenylalanine and L-tyrosine since it must respond over a wide concentration range only to the levels of L-tryptophan in its regulatory function.

\textsuperscript{7}R. W. Schevitz and P. B. Sigler, manuscript in preparation.
**Binding of L-Tryptophan and Analogues to trp Aporepressor**

### Table I

*Extinction coefficients for L-tryptophan and some of its analogues and their dissociation constants in the binding to trp aporepressor*

| Compound                        | Structure | $K_D$ (M$^{-1}$) | $e^*$ (M$^{-1}$ cm$^2$) |
|---------------------------------|-----------|------------------|-------------------------|
| 3-β-Indole-acrylic acid (trans) |           | 0.516            | 12.0 (274)$^a$          |
| S-Methyl-L-tryptophan$^+$       |           | 3.25             | 5.26 (276)$^c$          |
| Indole-3-butyric acid           |           | 5.51             | 5.15 (281)$^a$          |
| Indole-3-propionic acid         |           | 9.74             | 5.23 (280)$^a$          |
| L-tryptophan                    |           | 14.6             | 5.47 (278)$^b$          |
| Tryptophol                      |           | 25.3             | 5.51 (280)$^a$          |
| Indole                          |           | 25.1             | 5.67 (278)$^d$          |
| 4-Methyl-L-tryptophan$^+$       |           | 26.4             | 5.47 (286)$^b$          |
| L-Tryptophanamide               |           | 27.7             | 5.63 (278)$^b$          |
| S-Methyl-tryptamine             |           | 28.8             | 5.59 (287)$^b$          |
| S-Methoxy-L-tryptophan$^+$      |           | 30.7             | 5.69 (274)$^b$          |
| L-Abrine                        |           | 31.1             | 5.57 (278)$^b$          |
| L-Indole-3-lactic acid$^+$      |           | 35.2             | 5.39 (280)$^a$          |
| L-Tryptophan methyl ester       |           | 37.1             | 5.68 (278)$^b$          |
| Tryptamine                      |           | 41.1             | 5.20 (279)$^b$          |
| Indole-3-acetic acid            |           | 72.9             | 5.42 (280)$^a$          |
| 6-Nitro-L-tryptophan            |           | 75.6             | 7.08 (326)$^d$          |
| N-Formyl-L-tryptophan$^*$       |           | 117              | 5.30 (280)$^a$          |
| Indoline                        |           | 174              | 2.66 (289)$^a$          |
| D-Tryptophan                    |           | 343              | 5.47 (278)$^b$          |
| 7-Aza-L-tryptophan              |           | 466              | 8.80 (292)$^b$          |
| S-Hydroxy-L-tryptophan          |           | 2840             | 5.60 (274)$^b$          |
| L-Phenylalanine                 |           | >5000            | 2.06 (258)$^a$          |
| L-Tyrosine                      |           | >5000            | 2.53 (274)$^b$          |

* Racemic mixtures were used in the binding studies, but the dissociation constant ($K_D$) is calculated for the L-isomer only, i.e. it is assumed that binding of the D-isomer is much weaker than the L-isomer and can therefore be neglected.

* Extinction coefficients are given at the wavelength (nm) of maximum absorbance in the following solvents: 0.1 M NaOH; 0.1 M HCl; 0.5 M HCl; 50% aqueous acetic acid; and ethanol.

$^b$ Dissociation constants are all accurate to within 5% as calculated in the Gauss-Newton algorithm (17).

$^a$ Literature value.
the binding pocket does not reject 5-hydroxytryptophan on steric grounds but prefers a hydrophobic moiety in this region. Fig. 3 shows schematically that the indole ring is bound in a well-tailored hydrophobic pocket. The space for this pocket is provided by the strategic placement of Gly-85 in the second helix of the bilential DNA-binding motif. The side chain of any residue other than glycine at this position would seriously impinge on the indole pocket, which explains why mutations that alter Gly-85 fail to repress (18). The α carbon of Gly-85 together with the methylene groups of Arg-84 complete the lid of the indole pocket. The floor of the pocket is provided by the methylene groups of Arg-54, causing the indole ring to be sandwiched between the hydrophobic portions of these two arginine side chains. The methyl group of Thr-81 is very close to the edge of the five-membered ring and Val-58 approaches the opposing edge of the six-membered ring (10).

A detailed comparison of the crystal structures of the aporepressor and the repressor reveals why occupation of the indole pocket is the crucial stereoechemical event responsible for the major structural transition that converts the unliganded inactive aporepressor to the liganded active repressor. The net effect of the indole ligand is to complete a hydrophobic “brace” that maintains the two helices of the bilential motif in their correct relative orientation to another and to the rest of the structure. This transition is necessary but not sufficient for firm operator binding since the tightly bound ligands, trans-3-β-indole acrylic acid, indole-3-propionic acid, and indole-3-butyric acid, in which the 3-indole substituent does not bear an amino group do not bind operator (19–21) even though their crystals are nearly isomorphous to trp repressor (11). Something more than this structural rearrangement is required for operator recognition.

The Carboxyl Group Enhances Ligand Affinity—Replacement of L-tryptophan’s α carboxyl group with a hydrogen atom (tryptamine), a methyl ester (L-tryptophan methyl ester), or a primary amide (L-tryptophanamide) diminishes the affinity of the analogue for the tryptophan binding site 2- to 3-fold. An even more marked decrease in affinity occurs when a hydrogen replaces the carboxyl of 5-methyltryptophan.

The crystal structure of trp repressor (10) shows that the carboxyl group helps L-tryptophan adhere to its binding site (Fig. 3). The guanidino group of Arg-84 (whose methylene groups cover the indole ring) forms a hydrogen bond with one oxygen of the carboxylate, and the hydroxyl group of Thr-445 hydrogen bonds to the other. Clearly, both interactions are lost when the carboxylate is replaced by a hydrogen atom.

The amide and ester modifications bind more weakly even though they can be sterically accommodated because they have lost either an oxygen (atom) entirely or its capacity to form a hydrogen bond, respectively, as well as the presumed stabilizing effect of an ion pair.

Preliminary evidence shows that tryptamine, L-tryptophan methyl ester, and L-tryptophanamide are effective corepressors10; i.e., once the ligand is bound, the resulting complex functions as a repressor. This suggests that although the carboxylate of L-tryptophan enhances the ability of the corepressor to bind to the aporepressor, the unique and immediate structural consequences of the carboxylate’s interactions are not essential for selective affinity for operator DNA.

The Amino Group Helps Only to Activate the Aporepressor—Table I shows that three analogues, 3-β-indole acrylic acid, indole-3-propionic acid, and indole-3-butyric acid, in which the 3-indole substituent does not bear an α amino group (or derivative thereof), bind more tightly to the co-repressor binding site than L-tryptophan itself. This indicates that the amino group does not enhance binding of the co-repressor to the aporepressor. The long established observation that trans-3-β-indole acrylic acid, indole-3-propionic acid, and other 3-indole acids inhibit repression (19–21) has been shown in preliminary in vitro studies to result from diminished operator affinity.10 This indicates that the interactions of L-tryptophan’s α amino group contribute mainly to the creation of a high affinity interface between the repressor and its operators.

The structure of the trp repressor (10) shows that the α amino group is fixed at the carboxyl terminus of the B helix and hydrogen bonds to the exposed backbone carbonyls of Leu-41S and Leu-43S (Fig. 3). Schevitz et al. (10) suggested that the function of the amino group might be to neutralize the negative end of the B-helix’s intrinsic dipole, thereby making the repressor’s presumed DNA-binding surface more acceptable for the negatively charged sugar phosphate backbone of DNA. The amino group’s positive charge may also be needed to accommodate tight binding of L-tryptophan to the aporepressor since N-formyltryptophan, which has no positive charge, binds more poorly than abrine, which bears a comparably bulky methyl substituent but a preserved positive charge.

The Indole-carboxylate Linker, Optimal Length, and Conformation Are Important—Two of the three most strongly bound analogues have links between the carbon 3 of indole and the carboxylate function that are stereochemically quite different to that found in L-tryptophan. Surprisingly, the trans-ethyl-
ene linkage causes \( \text{trans-3,3,3'-indole acrylic acid} \) to bind about 20 times more tightly to the tryptophan site than its saturated counterpart, indole-3-propionic acid, and about 30 times more tightly than \( \text{L-tryptophan} \) itself. In addition, indole-3-butyric acid, which bears a longer indole-carboxylic linker than indole-3-propionic acid and \( \text{L-tryptophan} \), has over twice the affinity for the co-repressor site than \( \text{L-tryptophan} \). In contrast, indole-3-acetic acid, which bears a shorter indole-carboxylic linker than indole-3-propionic acid and \( \text{L-tryptophan} \), binds much more poorly to the aporepressor than these compounds. The reason why the length and conformation of the indole-carboxylic linker is an important determinant of ligand binding awaits a high-resolution structure analysis, now underway, of the appropriate pseudorepressors, i.e., inactive adducts formed with competing analogues of \( \text{L-tryptophan} \) crystallizing nearly isomorphously with \( \text{trp repressor} \).

\textit{D-Tryptophan Is Stereochemically Unacceptable—}The above results clearly indicate that the indole ring and \( \alpha \) carboxyl group of the ligand are primarily responsible for the binding of \( \text{L-tryptophan} \) to the aporepressor, while the amino group has been shown to be the ligand’s most passive substituent in terms of affinity for the protein (although critically important in activation). Therefore, to investigate why \( \text{D-tryptophan} \) binds to the aporepressor so poorly (Table I), we have modelled its binding by first fixing the indole ring and carboxylate as they are observed in bound \( \text{L-tryptophan} \). In the resulting configuration the \( \alpha \) amino group clashes with the C and edge of the heterocyclic 5-membered ring caused by the sharp restriction in rotation about the \( \text{C}_{\gamma}-\text{C}_{\delta} \) and \( \text{C}_{\gamma}-\text{C}_{\delta} \) bonds. This unacceptable configuration is in itself the major deterrent to the binding of \( \text{D-tryptophan} \).

Having developed a stereochemical understanding of the role played by the functional groups of \( \text{L-tryptophan} \) in binding to \( \text{trp aporepressor} \), further studies, both biochemical and crystallographic, are in progress to relate the effect of ligand binding to operator affinity.

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