

Interaction of the Operator of the Tryptophan Operon with Repressor

(repressor-operator interactions/*trp* repressor/*in vitro* transcription/*Escherichia coli*)

JOHN K. ROSE AND CHARLES YANOFSKY

Department of Biological Sciences, Stanford University, Stanford, California 94305

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ABSTRACT Transcription studies *in vitro* on repression of the tryptophan operon of *Escherichia coli* show that partially purified *trp* repressor binds specifically to DNA containing the *trp* operator with a repressor-operator dissociation constant of about 0.2 nM in 0.12 M salt at 37°, a value consistent with the extent of *trp* operon regulation *in vivo*. The half-life of the *trp* repressor-*trp* operator complex is less than 2 min *in vitro* in 0.12 M salt.

Partially purified *trp* aporepressor (1) represses transcription *in vitro* of the tryptophan (*trp*) operon of *Escherichia coli* in the presence of L-tryptophan or appropriate tryptophan analogues (2-4). Neither tryptophanyl-tRNA synthetase (3, 5) nor tryptophanyl-tRNA (5-7) appears to participate in repression. Repression *in vitro* involves interaction between the *trp* repressor and the *trp* operator, since an operator constitutive mutation eliminates repression (2). On the basis of these findings it may be tentatively concluded that the protein product of *trpR*, the aporepressor, when complexed with the corepressor, L-tryptophan, acts at the *trp* operator to present transcription of the operon.

Attempts to assay *trp* repressor-*trp* operator interaction by conventional DNA-protein binding assays (8) have been unsuccessful. Therefore, we have used the *in vitro* transcription system to study this interaction.

MATERIALS AND METHODS

Bacteriophage Isolation. The transducing phages $\phi 80$ *trpO*⁺ Δ *trpED102 trpCB1* and λ *trpCB1* have been described (2). Phage $\phi 80$ *trpO*⁺ *trpE7* carries *trpE* and the operator proximal portion of *trpD* and was isolated after induction of an *E. coli* $\phi 80$ h⁻ lysogen carrying the deletion *tonB trpAD32*. This deletion removes the operator distal genes *trpA*, *trpB*, and *trpC*, and about 80% of *trpD*. Phage $\phi 80$ *trpO*⁺ *trpE7* carries the *trp* operator and promoter, as shown by enzyme formation analyses in infection experiments similar to those described by Franklin (9). The gene order in the operon is *trp* operator (O)-E-D-C-B-A.

Reaction Conditions *In Vitro* and Repressor Preparation. *In vitro* reactions (2) were for 15 min at 37°. The final volume of each reaction mixture was 30 μ l, containing 0.3 mM L-tryptophan, 12 mM Tris·HCl (pH 7.9), 1.3 mM MgCl₂, 97 mM KCl, 0.13 mM EDTA, 0.1 mM dithiothreitol, 0.09 mM ATP, CTP, and GTP, 0.017 mM [³H]UTP (10 Ci/mmmole), 10 μ g/ml (0.33 nM) to 150 μ g/ml (5 nM) of DNA and 10 μ g/ml to 153 μ g/ml of RNA polymerase holoenzyme (EC 2.7.7.6; nucleosidetriphosphate:RNA nucleotidyltransferase). Except

Abbreviations: *trp*, tryptophan gene or operon; *c* strand and *i* strand, correct and incorrect strand, respectively, of λ *trpCB1* DNA.

where stated otherwise, the ratio of polymerase to DNA was maintained at 1:1 (w/w). Each reaction mixture also contained 6 μ l of buffer IV (1) with the indicated amounts of partially purified repressor. Buffer IV added final concentrations of 2 mM Tris-OAc (pH 8.0), 12 mM KOAc, 2.5 mM Mg(OAc)₂, 0.14 mM dithiothreitol, and 1% glycerol. RNA synthesis, RNA purification, and DNA template preparation were as described (2). The initial steps of aporepressor purification were as described (1), and then the aporepressor was taken through an additional DEAE-cellulose column step which gave 10-fold further purification (5). The final preparation of aporepressor was estimated to be 550-fold purified over that in crude extracts. This preparation contains about 1 nM aporepressor at a protein concentration of 5.2 μ g/ml (Fig. 2). Assuming a molecular weight of 58,000 (1, 5), the active aporepressor would constitute about 1.0% of the protein in the preparation.

RESULTS

The template DNA used in most of the transcription experiments *in vitro* described here was isolated from a specialized transducing phage, $\phi 80$ *trpO*⁺ Δ *trpED102 trpCB1*, which carries the *trp* operator, *trpC*, *trpB*, and a portion of *trpA*, and has a particular deletion of *trpE* and *D*. This deletion results in relatively high rates of *trpCB* mRNA synthesis *in vivo* and *in vitro* (10, 2). *trpCB* mRNA is detected by hybridization of synthesized labeled mRNA to the separated strands of the DNA of λ *trpCB1*. In agreement with previous results (2), we find that approximately 8% of the RNA transcribed from the $\phi 80$ *trpO*⁺ Δ *trpED102 trpCB1* template by purified RNA polymerase hybridizes to the correct or *c* strand of λ *trpCB1* DNA, and less than 1% hybridizes to the incorrect or *i* strand of the latter DNA. A blank value of 0.3-0.4% hybridization to λ DNA has been subtracted from these percentages. In all experiments described below, tryptophan was present in excess at a concentration of 0.3 mM, 30-fold above that required for half-maximal repression (2).

Determination of the dissociation constant

Fig. 1 shows the percent repression of correct strand *trpCB* mRNA synthesis at various repressor concentrations. Each curve presents results obtained at a different template DNA concentration with the ratio of polymerase to DNA held constant. Note that as the DNA concentration is increased (at repressor preparation levels of less than 8 μ g/ml), there is a decrease in percent repression of *trp* operon transcription. Note also that at low DNA concentrations a change in DNA concentration has only a relatively small effect on repression, while at higher DNA concentrations (>1 nM) the effect is large. For example, a repressor concentration that is just

sufficient to give 50% repression with 0.33 nM DNA shows only a 1.3-fold reduction in percent repression (to 38%) when the DNA concentration is increased 3-fold to 1 nM. A much larger (3.8-fold) reduction in repression (to 10%) is seen when the DNA concentration is increased another 5-fold to 5 nM. These results suggest that at the low DNA concentrations tested there are significant levels of free repressor and free operator. Thus, if we assume that percent repression indicates the percent of operators present that are bound to repressor, 10% repression of 5 nM DNA would be equivalent to a repressor concentration of *at least* 0.5 nM. This same repressor concentration, however, gives only 50% repression of 0.33 nM DNA, although the repressor concentration must be higher than the DNA concentration. Therefore, in this case, we conclude that more than half of the repressor is not bound to *trp* operators. Similar reasoning leads to the conclusion that the majority of the *trp* repressor molecules present are bound to *trp* operators at the higher DNA concentrations used in these experiments. The above observations suggest an equilibrium between *trp* repressor, *trp* operator, and the *trp* repressor-*trp* operator complex, with the value for the dissociation constant of the complex near the concentrations of repressor and operator.

We shall show below that these data are consistent with a simple equilibrium situation described as follows:*



The dissociation constant at equilibrium for this reaction is

$$K = \frac{[R - RO][O - RO]}{[RO]} \quad [2]$$

where R = total repressor, O = total operator, [R - RO] = free repressor, [O - RO] = free operator, and [RO] = repressor-operator complex. Equation [2] can be expressed as

$$K = \frac{1 - X}{X} [O] - (1 - X) [R] \quad [3]$$

where [O] = the operator concentration that saturates any given fraction X of repressor [$X = (RO/R)$]. For example, when the operator concentration gives one-half saturation of R, i.e., when $RO = \frac{1}{2}R$ and $X = \frac{1}{2}$, equation [3] becomes

$$K = [O]_{1/2} - \frac{1}{2}[R] \quad [4]$$

To determine if the *trp* repressor-*trp* operator interaction conforms to the equilibrium described above we must know the following values: (i) the total operator concentration, (ii) the concentration of repressor-operator complex, and (iii) the total repressor concentration. The operator concentration is known accurately from the A_{260} of the template DNA, and from its molecular weight (3×10^7), assuming one repressor binding site (*trp* operator) per DNA molecule. The percent repression measured in transcription experiments *in vitro* is

* Actually, however, since the aporepressor (AR) presumably must complex with the corepressor, tryptophan (T), to bind to and remain attached to the operator (O), the following equilibria obtain:



We assume here that in the presence of excess tryptophan, only the dissociation constant for the second reaction is being measured.

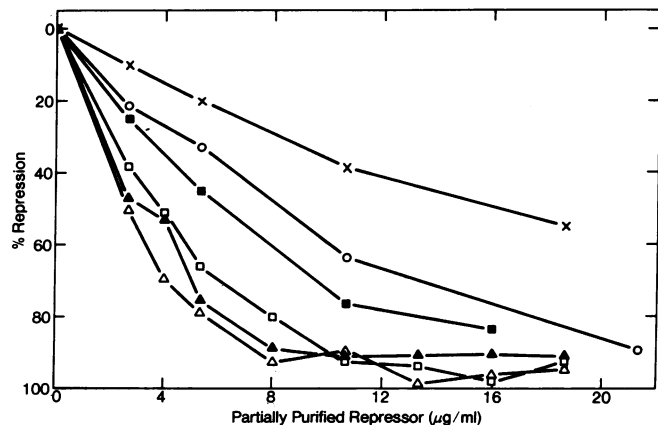


FIG. 1. Repression of *trp* operon transcription as a function of DNA and repressor concentrations. Reaction conditions *in vitro* and partially purified repressor were as described in *Materials and Methods*. The indicated concentrations of partially purified repressor and 0.3 mM L-tryptophan were preincubated with template DNA for 2 min at 0° in the standard reaction mixture. RNA polymerase was added and incubation was continued for an additional 2 min at 0°. Reactions were initiated by addition of nucleoside triphosphates and were run for 15 min at 37°. Template ($\phi 80$ *trpO*⁺ Δ *trpED102* *trpCB1* DNA) concentrations were: Δ , 0.33 nM; \blacktriangle , 0.5 nM; \square , 1 nM; \blacksquare , 2 nM; \circ , 2.5 nM; \times , 5 nM. The RNA polymerase:DNA ratio was constant at each DNA concentration. Total 3 H cpm in RNA ranged from 18,651 (0.33 nM DNA) to 285,033 (5 nM DNA). At each DNA concentration, *trp CB c* strand mRNA (measured by hybridization to λ *trpCB1 c* strand DNA) was 6-8% of the total RNA synthesized. The repressor fraction used has no activity without tryptophan and does not affect either phage transcription or *i* strand *trp* transcription. Longer preincubations of repressor with DNA (37°) at the lowest repressor and DNA concentrations do not enhance repression, indicating that repressor-operator association reaches equilibrium within 2 min at 0°.

assumed to indicate the percent of total operators bound to repressor molecules. The total repressor concentration is determined from the plateau values in experiments in which the operator concentration is increased until the concentration of repressed operators reaches a plateau. If we make the assumptions stated, the results in Fig. 1 can be expressed in the form of repressor saturation curves (Fig. 2). Here the concentration of repressed operators (i.e., the percent repression multiplied by the DNA concentration) is plotted against the DNA concentration. The two sets of data are from experiments performed with 2.6 and 5.2 μ g/ml of the partially purified repressor preparation. The plateau values reached show maximal repression of 0.5 and 1 nM operator at the respective repressor concentrations. That the plateau values are proportional to the amount of repressor added supports the assumption that the plateau values measure the total repressor concentration. Using the repressor concentrations determined in this manner, 0.5 nM and 1 nM, we have constructed theoretical repressor saturation curves using Eq. 3 to determine if the data are consistent with the equilibrium described by Eqs. 1-4. Because theoretical curves (Fig. 2) do follow the data closely, we conclude that an equilibrium is being observed. To determine K , we constructed theoretical curves, setting K at various values. The solid lines (Fig. 2) are the theoretical

TABLE 1. Effect of polymerase concentration on *trp* repression

| RNA polymerase $\mu\text{g/ml}$ | Repressor preparation $\mu\text{g/ml}$ | % [^3H]RNA hybridized to <i>trpCB</i> | | % Repression of <i>trpCB</i> c strand |
|------------------------------------|---|--|----------|---------------------------------------|
| | | c strand | i strand | |
| 20 | 0 | 7.2 | 0.56 | 0 |
| | 2 | 5.4 | 0.54 | 25 |
| | 4 | 3.0 | 0.80 | 58 |
| | 8 | 1.6 | 0.66 | 77 |
| 40 | 0 | 6.7 | 0.81 | 0 |
| | 2 | 4.7 | 0.79 | 30 |
| | 4 | 2.6 | 1.04 | 61 |
| | 8 | 1.5 | 1.31 | 77 |
| 60 | 0 | 7.0 | 1.21 | 0 |
| | 2 | 5.2 | 1.05 | 27 |
| | 4 | 2.7 | 0.74 | 62 |
| | 8 | 2.1 | 1.09 | 70 |

In vitro reactions were done as described in *Materials and Methods* except that the template ($\phi 80$ *trpO*⁺ Δ *trpED102 trpCB1* DNA) concentration was held constant at 30 $\mu\text{g/ml}$ (1 nM). Total incorporation ranged from 14,000 cpm (20 μg of RNA polymerase/ml) to 46,000 cpm (60 μg of RNA polymerase/ml) and was reduced less than ten percent by addition of repressor.

curves constructed with $K = 0.2$ nM and $R = 0.5$ nM or 1 nM. The data are in best agreement with these theoretical curves and fall between the dashed curves calculated for $K = 0.1$ nM and $K = 0.4$ nM. The data are thus consistent with a *trp* repressor-*trp* operator dissociation constant within this range and probably near 0.2 nM in 0.12 M salt at 37°. The upper dotted curve is included for comparison and is calculated for $K = 0.001$ nM, the dissociation constant for lactose (*lac*) repressor-*lac* operator (8) at the ionic strength (0.12 M) used in our transcription experiments *in vitro*.

Following the example of Riggs *et al.* (8), we have not presented the data as double-reciprocal plots because a direct

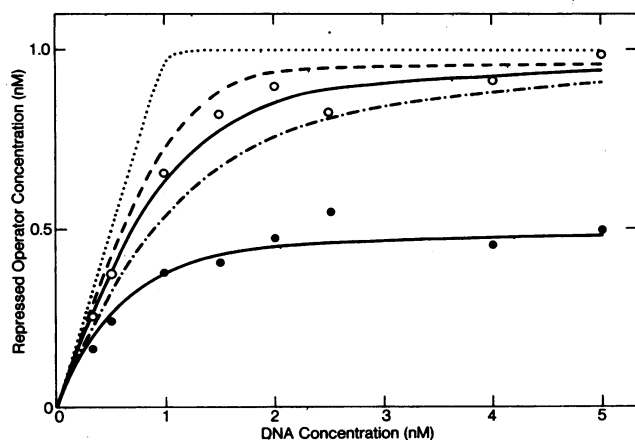


FIG. 2. The concentration of repressed *trp*-operators as a function of DNA concentration. Points are calculated as described in the text from the data of Fig. 1 and additional experiments. The partially purified repressor protein concentrations were 5.2 $\mu\text{g/ml}$ (O) and 2.6 $\mu\text{g/ml}$ (●). All curves shown are theoretical, calculated from Eq. 3 assuming $R = 0.5$ nM, $K = 0.2$ nM (lower solid line); $R = 1$ nM, $K = 0.2$ nM (upper solid line); $R = 1$ nM, $K = 0.4$ nM (dashed and dotted line); $R = 1$ nM, $K = 0.1$ nM (dashed line); $R = 1$ nM, $K = 0.001$ nM (dotted line).

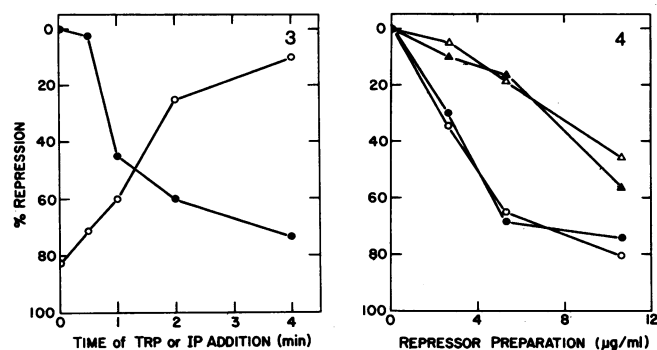


FIG. 3. (left). Effects of time of addition of L-tryptophan or indolyl-3-propionic acid on repression. Reaction conditions were as described in *Materials and Methods* except for the L-tryptophan or indolyl-3-propionic acid (IP) addition. Partially purified repressor (8 μg of protein/ml) was present in all reactions. The percent repression of *trpCB* c strand transcription is plotted against time of addition of 0.1 mM L-tryptophan, O; or against time of addition of 1 mM indolyl-3-propionic acid to a reaction mixture containing 0.1 mM L-tryptophan, ●. Zero time is the time the reaction was started by addition of nucleoside triphosphate. Reactions were run for 15 min at 37°.

FIG. 4. (right). Competition for repressor by *trp* operators. Standard reactions (*Materials and Methods*) contained 1 nM $\phi 80$ *trpO*⁺ Δ *trpED102 trpCB1* DNA, 0.3 mM L-tryptophan, and either 4 nM $\phi 80$ DNA or 4 nM $\phi 80$ *trpO*⁺ *trpE7* DNA were added at the times indicated below. The indicated concentration of partially purified repressor was incubated with the $\phi 80$ *trpO*⁺ Δ *trpED102 trpCB1* DNA (\pm additional DNA) for 2 min at 37° in a reaction mixture containing all components except RNA polymerase and nucleoside triphosphates. The reaction mixtures were then cooled to 0°, and $\phi 80$ DNA or $\phi 80$ *trpO*⁺ *trpE7* DNA were added to reactions that had not been preincubated with either DNA. RNA polymerase was added, and the mixture was incubated for 2 min at 0°. Nucleoside triphosphates were added and reactions were run for 15 min at 37°. Percent repression of *trpCB* c strand transcription is plotted. Repressor preincubated with $\phi 80$ *trpO*⁺ Δ *trpED102 trpCB1* DNA and $\phi 80$ DNA (O) or with $\phi 80$ *trpO*⁺ *trpE7* DNA (Δ). Repressor preincubated with $\phi 80$ *trpO*⁺ Δ *trpED102 trpCB1* DNA; $\phi 80$ DNA (●) or $\phi 80$ *trpO*⁺ *trpE7* DNA (\blacktriangle) added after preincubation. The addition of the 4-fold excess of $\phi 80$ DNA or $\phi 80$ *trpO*⁺ *trpE7* DNA reduced *trpCB* mRNA synthesis to about 1.2% of the total RNA synthesized. To reduce the error due to the hybridization blank (0.4% to λ DNA), all RNA samples were prehybridized to 5 μg of membrane-bound $\phi 80$ DNA for 16 hr at 65°. The supernatant solutions were then hybridized to the separated strands of Δ *trpCB1* DNA and to λ DNA as described (2). Results are expressed as percent of prehybridization to $\phi 80$ DNA (about 3×10^6 cpm), before calculation of percent repression. Hybridization to λ DNA was about 0.05%.

comparison of the data with several theoretical saturation curves calculated for the specific repressor concentration permits an accurate determination of K as well as the range of error. Note that K is not equivalent to the concentration of operator that saturates 50% of the repressor because the total repressor concentration is large compared to the concentration of operator (see Eq. 3).

Since we show below that the *trp* repressor-*trp* operator complex has a short half-life (<2 min) and that RNA polymerase molecules initiate transcription over the initial 4 min of the *in vitro* reaction, we performed the following experiments to examine the possibility that polymerase molecules might prevent rebinding of dissociated repressor and thus

interfere with the estimation of K . The percent repression at several repressor levels was independent of polymerase concentration within the range tested, 20–60 μg of polymerase/ml, at 1 nM DNA (Table 1). Total transcription and *trp* operon transcription increased in proportion to the polymerase concentration, yet a given level of repressor always inhibited the same fraction of *trp* operon transcription, indicating that polymerase does not interfere with repressor-operator binding under our conditions. Recent studies (Catherine Squires and Frank Lee, unpublished) have confirmed this observation and have shown, in addition, that preincubation of RNA polymerase with template DNA in the absence of nucleoside triphosphates at 37° reduces repression by repressor added subsequently. However, RNA polymerase does not interfere with repression in the experiments reported here, presumably because repressor is bound to operator before polymerase is added and because polymerase does not remain near the *trp* operator sufficiently long enough to block re-association of any repressor that dissociates during the initial stages of the *in vitro* reaction.

The data presented here are consistent with a dissociation constant considerably larger than the value determined for other repressor-operator complexes (see *Discussion*). A larger dissociation constant could result from slower association and/or faster dissociation of the repressor-operator complex. Association of repressor and operator occurs so rapidly as to be immeasurable at the concentrations of repressor and operator used in the *in vitro* transcription assay (Fig. 1). However, experiments described below do suggest a rate of repressor-operator dissociation faster than that observed for *lac* repressor-operator complexes. Since interpretation of these experiments requires knowledge of the time during which repressor acts in the *in vitro* system, we performed the following experiments to examine this parameter.

Fig. 3 shows that repression decreases progressively as tryptophan is added at later times during the first 4 min of the standard 15-min transcription reaction period. Conversely, repression increases progressively when indolyl-3-propionic acid (a tryptophan analog that reverses tryptophan-mediated repression, ref. 2), is added at later times to a reaction mixture containing repressor and tryptophan. Thus, the majority of the polymerase molecules in the reaction mixture are no longer subject to repressor action four minutes after addition of triphosphates—probably because they are bound to the template and engaged in transcription.

Specific binding and rapid repressor exchange

Fig. 4 illustrates the results of experiments in which the repressor concentration was varied in transcription reaction mixtures containing template DNA from phages $\phi 80$ *trpO*⁺ Δ *trpED102 trpCB1* and $\phi 80$ *trpO*⁺ *trpE7*, both of which carry the *trp* operator. Synthesis of mRNA hybridizable to λ *trpCB* c strand DNA was measured. Since $\phi 80$ *trpO*⁺ *trpE7* DNA does not contain any region of the *trp* operon homologous to λ *trpCB*, only *trp* mRNA synthesized on the $\phi 80$ *trpO*⁺ Δ *trpED102 trpCB1* template is detected. The results show that the presence of a 4-fold excess of competing $\phi 80$ *trpO*⁺ *trpE7* DNA causes significant derepression of *trpCB* mRNA synthesis whereas, in the control experiment in which a 4-fold excess of $\phi 80$ DNA was added, no derepression was observed. More importantly, competition for repressor is also observed (Fig. 4) when repressor is preincubated with $\phi 80$ *trpO*⁺ Δ *trpED102 trpCB1* DNA before the addition of the competing operator

DNA. This result suggests that the repressor exchanges rapidly among *trp* operators.

Unfortunately, two limitations of the *in vitro* transcription assay procedure preclude an accurate determination of the repressor-operator half-life. First, after addition of the competing *trp* operator DNA to DNA that was preincubated with repressor, a 2 min period is used for the addition of RNA polymerase and nucleoside triphosphates before the reaction is started. Repressor might exchange among operators during this period even though the temperature is held at 0°. [The half-life of the *lac* repressor-operator complex is constant between 0° and 30° (11).] Second, the data in Fig. 3 show that repressor must act throughout the first 4 min of the reaction to prevent all *trp* operon transcription. For example, addition of indolyl propionic acid as late as 1–2 min after initiation of incubation (indolyl propionic acid presumably replaces tryptophan on the repressor and inactivates it immediately) relieves repression significantly, presumably because polymerases are available in the system for initiating *trp* transcription. Thus, in the repressor exchange experiments there is an initial period of 1–2 min at the start of the reaction during which repressor exchange could occur and result in derepression of the template to which repressor was prebound. Beyond this initial period, repressor exchange would not result in derepression because polymerases are no longer available to initiate *trp* transcription. Therefore, the derepression observed must result from repressor exchange before 1–2 min.

The results of the repressor exchange experiments imply that repressor does equilibrate among *trp* operator sites, but considering the above two limitations on determining the time required for this equilibration, we can conclude only that complete equilibration of *trp* repressor among *trp* operators occurs in less than 3–4 min. The apparently complete equilibration (Fig. 4) in less than 4 min implies a half-life of the *trp* repressor-*trp* operator complex of less than 2 min.

DISCUSSION

In estimating the *trp* repressor-*trp* operator dissociation constant we assume that foreign proteins in the impure repressor preparation do not interfere with binding of the repressor to the operator. This appears to be true for *lac* repressor-*lac* operator interaction, since identical values for the dissociation constant are obtained in experiments with either pure repressor or crude cell extracts (12). Since binding of *trp* repressor to the *trp* operator is tight and specific, it seems likely that this is also true of the *trp* repressor and *trp* operator.

We have made the additional assumption that repression results from interaction of repressor with a single *trp* operator site on the DNA. The normal Michaelian hyperbolas obtained in these experiments suggest a lack of cooperative effects of repressor binding, which might be expected if more than one molecule of repressor were binding the operator. However, a more precise assay for repressor-operator binding at low operator concentrations is required for a thorough examination of sigmoidicity in the repressor saturation curves.

The presence of inducer is known to reduce the half-life of preformed *lac* repressor-operator complexes (13). Tryptophan presumably acts to increase the affinity of the repressor for the *trp* operator and/or increase the half-life of the *trp* repressor-operator complex. We cannot decide between these alternatives since our competition assay for binding is indirect and depends on measurements of the amount of repressor available for repression in the presence of excess tryptophan.

Zubay *et al.* (1), using a cell-free coupled system, have calculated a *trp* repressor concentration of 10 molecules per cell from the estimated molecular weight of the repressor and the amount of S-30 extract required for 50% repression of 0.78 nM *trp* operator. In making this calculation, it was assumed that the *trp* repressor concentration that would give 50% repression was half of the DNA concentration. Our results show that the dissociation constant of the repressor-operator complex is relatively large and cannot be ignored in making such a calculation. At 50% repression of 0.78 nM operator ($K = 0.2$ nM), Eq. 4 predicts a repressor concentration of 1.15 nM, 3-fold higher than that estimated by Zubay *et al.* (1). In agreement with our results, their data suggest a relatively large dissociation constant, since a repressor concentration giving an intermediate level of repression is shown to repress increasing concentrations of operator as the DNA concentration is increased from 0.78 nM to 3.12 nM (Fig. 3 of ref. 1). Thus we feel that their estimate should be revised to about 30 molecules of repressor per cell.

Using this estimate and the information that the derepression ratio for the *trp* operon (rate of enzyme synthesis in fully derepressed cultures divided by the rate in fully repressed cells) is about 75 (10), we can calculate a value for the repressor-operator dissociation constant. This calculation is based on the assumption that the repressed level of enzyme synthesis *in vivo* results *only* from a low level of repressor-operator dissociation. Thus, in order to obtain a basal level (fully repressed) of *trp* operon expression equal to $1/75$ th of the fully derepressed value, we assume that one in 75 operators in a repressed culture is not associated with repressor at any given time ($[RO] = 75 [O-RO]$). Thirty molecules of *trp* repressor per cell and 1.7 *trp* operators per cell (assuming 1.7 genomes per cell) are equivalent to intracellular concentrations of about 30 nM repressor and 1.7 nM operator (14). Substituting the above values into Eq. 2, we can calculate $K = 0.38$ nM.† This value is close to our experimentally determined value of about 0.2 nM.

Similar calculations made for the *lac* operon (14), where the induction ratio is 1000, suggested $K \leq 0.01$ nM. At a salt concentration of 0.2 M, close to that believed to exist *in vivo* (15), K for *lac* repressor-operator dissociation is about 0.01 nM (8). *In vitro* *trp* operon transcription is inhibited 6-fold in 0.2 M salt, making determination of K at this ionic strength difficult.

The *E. coli* galactose (*gal*) operon repressor has been shown to bind the *gal* operator with very high affinity ($K = 0.001$ nM in 0.05 M salt, ref. 16). Information on the binding at

higher salt concentrations is not available. *Trp* repressor-operator binding appears to be significantly weaker than that observed for the *lac* repressor and operator at the same salt concentration, and is probably weaker than *gal* repressor-operator binding. Also, the half-life of the *trp* repressor-operator complex is less than 2 min in our *in vitro* transcription buffer (0.12 M salt), shorter than the value of 10 min estimated for the *lac* repressor-operator complex in 0.12 M salt (11).

The relatively large dissociation constant for *trp* repressor-operator interaction could explain the inability to detect binding of *trp* repressor to operator in conventional assays. With more highly purified repressor it may be possible to increase the repressor concentration sufficiently to detect this binding, making possible a detailed kinetic analysis of *trp* repressor-operator interaction.

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† $[RO] = 75 [O-RO]$, $[R] = 30$ nM, $[RO] = 1.7$ nM. Substituting the above values in Eq 2, we have $K = \frac{30 \text{ nM} - 1.7 \text{ nM}}{75} = 0.38$ nM.