Interaction of the *trp* repressor from *Escherichia coli* with a constitutive *trp* operator

Lorna R. CHANDLER and Andrew N. LANE*

National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, U.K.

The interaction of the *trp* repressor from *Escherichia coli* with a 20 bp fragment of DNA (CGTACTGATT·AATCAGTACG) corresponding to a mutant *trp* operator was studied by c.d. in the presence and absence of the co-repressor, L-tryptophan, and as a function of the concentration of K⁺ and Na⁺ ions. The affinity of the repressor for the mutant operator in the presence of tryptophan is about three orders of magnitude lower than the wild-type sequence. Binding in the absence of tryptophan is about 100-fold weaker than to the wild-type. The dependence of the dissociation constant on the concentration of K⁺ or Na⁺ is weak {d(log K_d)/d(log [M⁺]) = 2.5}, and independent of the cation, indicating that electrostatic interactions are not as important for this repressor as for others.

INTRODUCTION

To understand the basis of the tryptophan-dependent sequence specificity of the *trp* repressor, and to determine the dependence of expression of the *trp* operon in response to tryptophan, it is essential to obtain thermodynamic data on the interaction of the repressor with its specific target site and non-specific DNA. The specificity can be further probed by examining the interaction of the repressor with mutant sequences of the operator.

It has been shown that the *trp* repressor has intermediate affinity for its operator ($K_d \approx 1\text{--}5\text{ nM}$) (Platt, 1980; Joachimiak *et al.*, 1983) in the presence of tryptophan, and low affinity for non-specific DNA (Lane *et al.*, 1986, 1987). Further, the dependence of the affinity on the concentration of salt is much less than for other repressor–DNA interaction studies (Lane *et al.*, 1986, 1987), suggesting that electrostatic forces do not dominate the interaction for this repressor.

As part of an investigation of the physical chemistry of the control of expression of the *trp* operon, we are examining the structures and properties of the *trp* operator/promoter. In the present paper we examine the interaction of the *trp* repressor with a mutant sequence of the *trp* operator, which corresponds to an O^c mutation (Bennet & Yanofsky, 1978).

EXPERIMENTAL

trp repressor was purified from an overproducing mutant as described previously (Paluh *et al.*, 1987), and assayed by use of the restriction-endonuclease protection assay (Joachimiak *et al.*, 1983). The 20 bp DNA fragment was synthesized on a Beckman synthesizer, and purified by fast protein liquid chromatography on a Mono Q column (Pharmacia) with a gradient of NH₄HCO₃ (0–1.5 M).

The sequence is:

CGTACTGATT·AATCAGTACG

The point shows the centre of the palindrome, and the underline shows where the sequence differs from the wild-type previously studied (Lane *et al.*, 1986, 1987; Lefevre *et al.*, 1987); these bases are reversed with respect to the wild-type. The duplex was formed by heating the sample at 90 °C for 10 min, and cooling slowly. The sample was dialysed against 10 mM-KCl/10 mM-sodium phosphate buffer, pH 7.5. The integrity of the double-stranded state was checked by electrophoresis on 20 % polyacrylamide gels, and by one- and two-dimensional n.m.r. Concentration was determined by absorbance at 258 nm, by using an absorption coefficient for the oligonucleotide (i.e. double-stranded 20-mer) of 315 mm⁻¹·cm⁻¹ (38 ml/μg).

Binding studies were made at 25 °C by using c.d. (JASCO J-41C spectropolarimeter). Dissociation constants were determined by non-linear regression to the equation:

$$\Delta S = \Delta S^\infty \cdot c \quad (1)$$

where ΔS is the change of the signal intensity and ΔS^∞ is the signal change at saturation; c is the concentration of the complex, and is given by:

$$2c = -(o_t + r_t + K_d) + [(o_t + r_t + K_d)^2 - 4o_t r_t]^{1/2} \quad (2)$$

where o_t is the concentration of duplex, r_t is the concentration of dimeric repressor and K_d is the dissociation constant.

The sensitivity of the dissociation to the concentration of salts was determined by addition of small portions of concentrated buffered salt solutions to preformed complex, and monitoring the change in the c.d. signal over the range 320–250 nm (Kirpichnikov *et al.*, 1985; Lane *et al.*, 1987). The salt-sensitivity was determined by linear regression to eqn. (3) (deHaseth *et al.*, 1977):

$$\log K_d = n\psi \cdot \log [M^+] \quad (3)$$

where $[M^+]$ is the concentration of cation, and $n\psi$ is the salt-sensitivity parameter. Dissociation constants were determined in triplicate with two independent lots of repressor.

* To whom correspondence should be addressed.

RESULTS AND DISCUSSION

Fig. 1 shows near-u.v. c.d. spectra of the DNA fragment alone, of the holo-repressor and of the holo-repressor-DNA complex. The DNA spectrum is typical for the B family of conformations, and n.m.r. spectra

Fig. 1. C.d. spectra of the DNA fragment and the *trp* repressor

The buffer was 10 mM-KCl/20 μ M-sodium phosphate, pH 7.5, at 25 $^{\circ}$ C. Curve 1 (upper), 1.9 μ M-*O^c* DNA + 10.9 μ M-*trp* apo-repressor (dimer concentration) + 45 μ M-L-tryptophan; curve 2 (middle), 1.9 μ M-*O^c* DNA (duplex concentration); curve 3 (lower), 10.9 μ M-repressor + 60 μ M-L-tryptophan.

indicate that the conformation is similar to that of the wild-type sequence (Lefèvre *et al.*, 1987; A. N. Lane, unpublished work). The c.d. of the holo-repressor is relatively weak, as previously observed (Lane, 1986). Mixing the components produces a large increase in ellipticity (about 70%) and a small blue-shift. The increase in ellipticity is similar to that induced in the wild-type sequence by the apo-repressor (Lane *et al.*, 1987). This signal change was then used for titrations in the presence of L-tryptophan. Fig. 2(a) shows the change in ellipticity obtained on adding portions of repressor to the DNA. The best-fit line gives a K_d of $1.7 \pm 0.2 \mu$ M. This value is larger than for the wild-type sequence, for which K_d is less than 0.1 μ M by c.d. (Lane *et al.*, 1987) and 1–5 nM by restriction assay (Joachimiak *et al.*, 1983). At a concentration of 60 μ M-tryptophan, the fractional saturation of the repressor is about 0.79. Calculations on the three-component system DNA, repressor and L-tryptophan (not shown) indicate that the apparent dissociation constant obtained for the holo-repressor is overestimated by about 25% under these conditions. This is similar to the experimental error.

As apo-repressor binding was much weaker than for the holo-repressor, high concentrations of protein were needed to achieve saturation, leading to very high absorbances in the cuvette. The saturation signal was therefore estimated from that of the holo-repressor complex by subtracting the difference c.d. signal between holo-repressor and apo-repressor. The c.d. intensity of the holo-repressor is about twice that of the apo-repressor (Lane, 1986). This leads to an approximate dissociation

Fig. 2. Titration of *O^c* DNA with *trp* holo-repressor

The conditions were as in Fig. 1 legend. (a) Portions of a concentrated solution of repressor were added to 1.0 ml of DNA solution containing 60 μ M-L-tryptophan, and the increase in ellipticity was recorded at 275 nm. The line is the best-fit non-linear-regression line to eqn. (1) and eqn. (2). (b) Dependence of the dissociation constant for the *O^c* DNA-holo-repressor complex on the concentration of salt: ○, NaCl; ●, KCl. The line is a weighted linear-regression fit to eqn. (3).

Table 1. Interaction of the *trp* repressor with 20 bp DNA fragments

$\lambda_{\max}$ is the wavelength at the maximum of the c.d. curve for DNA. f is free, b is bound. $\Delta\theta/\theta_0$ is the change in ellipticity at 275 nm at saturation with repressor. K_d is the dissociation constant at 25 °C at pH 7.5. W indicates the presence of tryptophan. Values in parentheses are for the wild-type operator (Lane *et al.*, 1987). dAdT and O refer to dA₂₀dT₂₀ and wild-type operator respectively (Lane *et al.*, 1986, 1987).

Parameter	Dimension	Value
$\lambda_{\max}$ (f)	nm	278
$\lambda_{\max}$ (b)	nm	274
$\Delta\theta/\theta_0$	—	1.7 (1.5)
K_d (+W)	μM	1.7 ± 0.5 (< 0.1)
K_d (−W)	μM	100 ± 30 (2.0)
K_d (dAdT)	μM	75
$d\{\log K_d(+W)\}$ $d(\log [K^+])$	—	2.6 ± 0.5
$d\{\log [K_d(+W)]\}$ $d(\log [Na^+])$	—	2.3 ± 0.2
$d\{\log [K_d(dAdT)]\}$ $d(\log [Na^+])$	—	2.0
$d\{\log [K_d(O)]\}$ $d(\log [Na^+])$	—	1.0

constant of 100 μM for the apo-repressor. The ellipticity of the wild-type sequence increases 50 % on saturation with the apo-repressor (Lane *et al.*, 1987). If the same is true for the mutant sequence, then the dissociation constant is about 80 μM . Certainly the dissociation constant for the apo-repressor is much higher than for the holo-repressor. The dissociation constant for the apo-repressor–wild-type 20-mer complex was 2 μM (Lane *et al.*, 1987). Hence both the specific and the non-specific binding are greatly decreased by the mutation, as expected for an operator-constitutive mutation (Bennet & Yanofsky, 1978). Binding to poly[d(AT)] was undetectable at the concentrations of repressor used (up to 15 μM), indicating that non-specific binding is weak. This is in agreement with previous experiments with dA₂₀dT₂₀, where the dissociation constant was estimated to be 70 μM (Lane *et al.*, 1986).

The effect of added salt on the dissociation constant is shown in Fig. 2(b) as a log–log plot. For both KCl and NaCl the dissociation constant is relatively weakly dependent on the concentration of added salt and independent of the nature of the univalent cation. The slope of the line is 2.5, compared with 8–12 for other repressor–DNA interactions (Kirpichnikov *et al.*, 1985; Lane *et al.*, 1987). The results are summarized in Table 1, where they are compared with previous results for other sequences of DNA.

The salt-dependence of the affinity of the *trp* repressor for DNA is independent of the base sequence or the total free-energy change, as both specific binding and non-specific binding to dA₂₀dT₂₀ are characterized by slopes of the log–log plots of 1–2 (Table 1). Clearly cation release is not the dominant driving force in *trp* repressor–DNA interactions. The slope of the log–log plot is $n\psi$ (cf. eqn. 3) where ψ is the fraction of

phosphate charges neutralized by univalent cations (Manning, 1969; deHaseth *et al.*, 1977) and n is the apparent number of salt contacts formed in the complex. ψ has the value 0.76 for B DNA in the condensation model (Manning, 1969; deHaseth *et al.*, 1977) and 0.34 for the Poisson–Boltzmann model (Nordenskiöld *et al.*, 1984). Measurements on polydisperse DNA indicate a value of ψ of between 0.34 and 0.6 (Braunlin & Nordenskiöld, 1984). Hence the slope of 2–2.5 (Table 1) indicates that about four or five salt bridges are formed per dimeric repressor (i.e. about two per subunit). This is consistent with the X-ray structure, which shows that there are few lysine and arginine residues in the DNA-binding domain (Kelly & Yanofsky, 1985; Zhang *et al.*, 1987).

The non-specific binding of the *trp* repressor is weak, and K_d would be of the order of several hundred μM at physiological concentrations of K^+ . This is reasonable if the specific binding is of only moderate strength ($K_d \approx 1$ nm), given the high concentration of non-specific sites on the chromosomal DNA (≈ 20 nm). Computer simulations similar to those published for the *lac* repressor (von Hippel *et al.*, 1974) (not shown) indicate that for the *trp* repressor to respond to tryptophan at physiological concentrations (i.e. near 70 μM) the non-specific binding has to be about 10^6 times weaker than the specific binding, assuming a K_d of tryptophan for the holo-repressor of 10–20 μM (Lane, 1986; Marmorstein *et al.*, 1987) and a repressor concentration of 150 dimers per cell (i.e. approx. 0.2 μM) (Kelley & Yanofsky, 1982). The measured K_d for the mutant operator is sufficiently high that it would be unable to compete with the chromosomal DNA for the repressor, therefore leading to constitutive expression of the *trp* operon.

This work was supported by the Medical Research Council. We thank Brian Peck for the synthesis of the oligonucleotide, Professor C. Yanofsky for the *trp*-repressor-overproducing strain CY1050, Dr. S. Martin for assistance with the c.d. measurements and Dr. P. M. Bayley for helpful discussions.

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Received 11 November 1987/17 December 1987; accepted 19 January 1988