

Cooperative non-specific DNA binding of the N-terminal core of the cyclic AMP receptor protein of *Escherichia coli* and its modulation by cyclic AMP

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The non-specific DNA binding of CRP and its N-terminal core, α CRP, to a 298 base pair DNA fragment, in the presence and absence of cAMP, has been studied using the nitrocellulose filter binding technique and analysed quantitatively using the theory of Clore et al. [J. Mol. Biol. (1982) 155, 447–466]. It is shown that both CRP and α CRP bind cooperatively to DNA. At an ionic strength of 100 mM and pH 7.5, the intrinsic equilibrium association constant for the binding of α CRP to DNA is ~ 10 -times smaller than that for CRP, but the cooperativity parameter is ~ 17 -times larger for α CRP than CRP. cAMP exerts its effect solely on the intrinsic equilibrium constant and does not alter the cooperativity. In the case of α CRP, cAMP reduces the intrinsic equilibrium association constant by a factor of 3, in contrast to the case of CRP where cAMP increases it by a factor of 3. The possible location of the DNA binding site present in the N-terminal core of CRP is discussed in the light of crystallographic data on the cAMP·CRP complex [McKay et al. (1982) J. Biol. Chem. 257, 9518–9524].

<i>cAMP receptor protein (CRP)</i>	<i>N-terminal core</i>	<i>cAMP modulation</i>
<i>Cooperative non-specific DNA binding</i>	<i>Nitrocellulose filter binding assay</i>	

1. INTRODUCTION

The cAMP receptor protein (CRP) of *Escherichia coli* regulates the transcription of at least 20 genes, including its own structural gene and all genes subject to carbon catabolite repression, by binding to specific DNA target sites in the presence of cAMP [1–3]. In addition to specific DNA binding, CRP binds DNA non-specifically [4,5]. The function of non-specific DNA binding is probably two-fold: first as a fine control mechanism regula-

ting the amount of CRP bound at specific DNA target sites [6,7], and second as a translocating mechanism to increase the rate of specific site recognition by processes such as one-dimensional diffusion along the DNA [8,9]. At the present time, the molecular mechanisms of both specific and non-specific DNA binding of CRP are unknown, although it has been shown that both types of interaction induce a B to C transition in the DNA structure whilst leaving the handedness of the helix, namely right handed, unchanged [4,10–12]. Most models of the complex of CRP with DNA have assumed that the two F α helices of the C-terminal domain, one from each subunit, interact with the major grooves of B DNA [13–17]. The assumption that the C-terminal domain of

Abbreviations: CRP, cAMP receptor protein of *E. coli*; α CRP, N-terminal core of CRP produced by subtilisin digestion of the cAMP·CRP complex; cAMP, adenosine 3',5'-cyclic phosphate; bp, base pair

CRP contains the entire DNA binding site is based on the observation that the N-terminal core of CRP, α CRP, produced by subtilisin digestion in the presence of cAMP, possesses no cAMP dependent DNA binding capacity at pH 8 [18]. These experiments, however, were only conducted at a single protein concentration so that the conclusions drawn from them must be treated with caution, particularly in view of the fact that α CRP has been shown to stabilize double stranded polyd(AT) [19].

We have characterized here the non-specific DNA binding of α CRP to a small 298 base pair (bp) restriction fragment, derived by *Hinf* I digestion of pBR 322 DNA, by means of the nitrocellulose filter binding technique, and analysed the data quantitatively using the theory of Clore et al. [20]. We demonstrate that α CRP, both in the presence and absence of cAMP, binds non-specifically to DNA in a highly cooperative manner; in addition, non-specific DNA binding of α CRP is modulated by cAMP which reduces the intrinsic DNA binding affinity, in contrast to the case of CRP where cAMP increases it.

2. EXPERIMENTAL

CRP and α CRP were purified as in [21,22] and were greater than 99% pure as judged by sodium dodecyl sulphate-polyacrylamide gel electrophoresis with subunit M_r values of 23 000 and 12 500, respectively.

The pBR 322 DNA [23] was prepared by a modification of the procedure in [24]. DNA fragments were analysed after digestion with the restriction endonuclease *Hinf* I (from New England Biolabs) by agarose gel electrophoresis, and the desired 298-bp fragment was purified by extraction from an 8% thin (0.3 mm) polyacrylamide gel as in [25]. The 298-bp fragment was radioactively labelled by nick translation (using [α - 32 P]dATP, 500 Ci/mmol, from Amersham) as in [25,26].

Equilibrium binding studies of CRP and α CRP to the 298-bp restriction fragment were carried out using the nitrocellulose filter binding technique as in [27,28]. Aliquots (12 ng) of labelled DNA were incubated with various amounts of CRP or α CRP, in the presence or absence of 100 μ M cAMP, in 100 μ l 100 mM Tris-HCl, 100 mM KCl (pH 7.5) at

22°C for 20 min. The concentration of cAMP employed was sufficient to ensure complete saturation of the two cAMP binding sites on CRP and α CRP ([21] and Takahashi, M., personal communication).

3. THEORY AND COMPUTATION

The theory required to analyse equilibrium nitrocellulose filter binding data has been developed by Clore et al. [20] and will only be summarized here. The quantity measured is the fraction θ of DNA to which at least one molecule of protein is bound, and is given by

$$\theta = [\text{DNA}_{\text{bound}}]/[\text{DNA}_{\text{total}}] = (Z - 1)/Z \quad (1)$$

where Z is the binding polynomial. This expression contrasts to that for the parameter which would be measured in a conventional equilibrium binding study, namely the number of moles of protein bound per mole of DNA, which is given by $\partial \ln Z / \partial \ln [L_F]$.

In the case of a DNA lattice, there are $(N - l + 1)$ binding sites available when no protein molecule is bound (where N is the total number of base pairs and l the size of the binding site in base pairs) and the maximum number of protein molecules that can bind is N/l . The expression for Z for the cooperative non-specific DNA binding of a protein is given by

$$Z = 1 + \sum_{i=1}^{N/l} \left(\prod_{j=1}^{i-1} \frac{N - jl + 1 + 2(i - 1)\alpha}{j} \right) K^i L_F^i \quad (2)$$

where L_F is the concentration of free protein, K the non-specific intrinsic equilibrium association constant and α the cooperativity parameter. α may be defined in terms of a local cooperativity parameter β operating over a distance of m base pairs from the occupied site by

$$\beta = \alpha / m + 1 \quad (3)$$

and for $m = l$, the length of a single site, β is equivalent to the cooperativity parameter ω for contiguous binding as defined in [29]. Eq. 2 does not take into account the complication that the average number of binding sites available to bind say $i = n$ protein molecules will be slightly less than

$(N - nl + 1)$, as there will exist states where the binding sites available to n protein molecules is less than $(N - nl + 1)$ if two or more protein molecules are separated by a distance of less than l base pairs. However, in practice, the polynomial eq. 2 only needs to be expanded to degree 5, so that providing $N/l \geq 10$, this complication can be neglected.

The experimental data were fitted using eq. 2 and optimizing the parameters K and α for different values l over the range 10–30. In fact, over this range of l , K and α were found to be independent of l within the accuracy of their determination. Moreover K and α were highly correlated with correlation coefficients, $\rho_{K\alpha}$ in the range -0.98 to 0.99 . Consequently, only values of K and $K\alpha$ are quoted, and the standard deviations of $K\alpha$ calculated from $\text{var}(\ln K\alpha) = \text{var}(\ln K) + \text{var}(\ln \alpha) + 2\rho_{K\alpha}[\text{var}(\ln K) \cdot \text{var}(\ln \alpha)]^{1/2}$.

The data were fitted using the FACSIMILE program [30] which employs Powell's method of non-linear optimization [31] as described in [32].

4. RESULTS

The equilibrium nitrocellulose filter binding curves for the binding of the cAMP·CRP complex, CRP, α CRP and the cAMP· α CRP complex to the 298-bp DNA fragment at an ionic strength of 100 mM and pH 7.5 are shown in fig.1. Although the plasmid pBR 322 has been shown to contain a specific CRP binding site [33] this is not located in the 298-bp *Hinf* I restriction fragment [34] so that the binding curves reflect solely non-specific DNA binding. On purely qualitative considerations, we can deduce the following from the shape of the binding curves:

- (i) CRP and α CRP bind cooperatively to DNA. The intrinsic non-specific equilibrium binding constant is larger for CRP than α CRP as reflected in L_{50} values of 1.8×10^{-7} M and 3.3×10^{-7} M, respectively; the cooperativity factor, however, is greater for α CRP than CRP as reflected in the steeper binding curve for α CRP.
- (ii) The effect of cAMP is solely to shift the mid points of the binding curves without affecting their shape; that is to say cAMP alters the intrinsic non-specific equilibrium association constant, increasing it in the case of CRP ($L_{50} = 6 \times 10^{-8}$ M) and decreasing it for α CRP

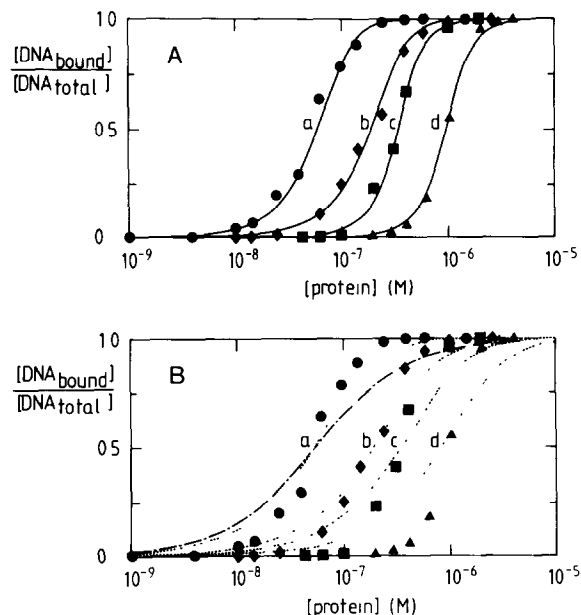


Fig.1. Comparison of the experimental equilibrium nitrocellulose filter binding curves for the non-specific DNA binding of (a) the cAMP·CRP complex (●), (b) CRP (◆), (c) α CRP (■) and (e) the cAMP· α CRP complex (▲) to a 298-bp DNA fragment (derived by *Hinf* I digestion of pBR 322 DNA) with the best fit theoretical curves for (A) cooperative (—) and (B) non-cooperative (---) DNA binding. The theoretical curves were calculated using eqs 1 and 2 and the best fit parameters are given in table 1. A rectangular hyperbola given by $KL_F/(1 + KL_F)$ is shown for comparison in B as an interrupted line (---) with $K = 1.67 \times 10^7 \text{ M}^{-1}$. The ionic strength is 100 mM and the pH 7.5; other experimental conditions are given in section 2. The symbols are the means of the actual data points obtained from two separate experiments.

($L_{50} = 9.6 \times 10^{-7}$ M), without affecting the cooperativity.

These qualitative observations are confirmed by a non-linear least squares fit of the data to eqs 1 and 2. The best fit curves for the cooperative model are shown in fig. 1A and for the non-cooperative model in fig.1B. The S.D. of the fit for the cooperative model is $\sim 3\%$ which is within the estimated standard error of the data ($\sim 5\%$) and the distribution of residuals is random; in contrast the S.D. of the fit for the non-cooperative model is $\sim 9\%$ and there are clear systematic differences between the experimental data and the computed curves. The optimized values of the parameters are given in table 1.

Table 1

Equilibrium binding parameters for the non-specific DNA binding of CRP and α CRP, in the presence and absence of cAMP, to a 298-bp DNA fragment at an ionic strength of 100 mM and pH 7.5^a

	Cooperative binding ($\alpha \neq 0$)		Non-cooperative binding ($\alpha = 0$)
	K (M ⁻¹)	$K\alpha$ (M ⁻¹)	K (M ⁻¹)
cAMP · CRP	$1.2 (\pm 0.2) \times 10^4$	$2.7 (\pm 0.3) \times 10^7$	$4.7 (\pm 0.6) \times 10^4$
CRP	$3.7 (\pm 0.6) \times 10^3$	$8.6 (\pm 1.2) \times 10^6$	$1.3 (\pm 0.2) \times 10^4$
α CRP	$3.2 (\pm 0.9) \times 10^2$	$1.3 (\pm 0.2) \times 10^7$	$7.1 (\pm 1.0) \times 10^3$
cAMP · α CRP	$1.1 (\pm 0.5) \times 10^2$	$4.4 (\pm 0.5) \times 10^6$	$3.0 (\pm 0.4) \times 10^3$
Overall SD of fit (%)	3.2		8.8
Distribution of residuals	random		systematic errors

^aAll the experimental data shown in fig.1 were fitted simultaneously using eqs 1 and 2. In the case of the cooperative model, K and α were initially varied individually for all 4 curves. However, the values of α for the binding of CRP and the cAMP · CRP complex were found to be the same within the relative error (± 30 – 40%) of their determination, and similarly in the case of α CRP and the cAMP · α CRP complex. Consequently, in the final optimization, the values of α for the CRP and cAMP · CRP binding curves were constrained to be equal, and similarly for the values of α for the α CRP and the cAMP · α CRP binding curves. As K and α are highly correlated, the values of $K\alpha$ together with their standard deviations are quoted (see section 3)

5. DISCUSSION

The data presented here demonstrate unambiguously that α CRP binds cooperatively to DNA. At an ionic strength of 100 mM and pH 7.5 the intrinsic non-specific equilibrium association constant for the binding of α CRP to DNA is 10-times smaller than that for CRP (see table 1). The cooperativity parameter α for α CRP, however, is approximately an order of magnitude greater than that for CRP with $\alpha_{\alpha\text{CRP}}/\alpha_{\text{CRP}} = 17 \pm 8$. The cooperativity parameter α is model independent [20]. From α a local cooperativity parameter ω for contiguous binding, as defined in [29], can be calculated using eq. 3 (see section 3); the value of ω is dependent on the site size l , and for $l = 15$, the size of a single non-specific DNA binding site for CRP determined by independent means [4,5], ω has a value of 156 ± 40 and 2590 ± 1200 for CRP and α CRP, respectively. cAMP only exerts its effect on the intrinsic non-specific equilibrium association constant and has no effect on the cooperativity. This effect is equal and opposite in the case of CRP and α CRP with $K_{\text{cAMP} \cdot \alpha\text{CRP}}/K_{\text{CRP}} = 3.2 \pm 0.2$ and $K_{\alpha\text{CRP}}/K_{\text{cAMP} \cdot \alpha\text{CRP}} = 2.9 \pm 0.2$. We note that

in the case of CRP, our findings are in qualitative and approximate quantitative agreement with those of Takahashi et al. [5] who used a conventional equilibrium binding technique.

α CRP is produced by subtilisin cleavage of the cAMP · CRP complex between Leu 116 and Ser 117, and, like CRP, is composed of 2 identical subunits [22]. From the crystal structure of the cAMP · CRP complex [35], it is readily apparent that the whole of the C-terminal domain (comprising α helices D to F and β sheet 9 to 12), together with three quarters of helix C (constituting the C-terminal end of the N-terminal domain) are no longer present in α CRP. The structure of α CRP appears to be identical to that of the corresponding portion of native CRP as judged by ¹H-NMR spectroscopy [36], and is made up of 2 small α helices A and B, a small fragment of α helix C and an 8 strand antiparallel β roll which contains the cAMP binding site [35]. Our results clearly indicate that a site capable of non-specific DNA binding and the factors responsible for cooperative DNA binding are located within this N-terminal core constituting α CRP.

The relevance of the DNA binding site present in

α CRP to either specific or non-specific DNA binding of native CRP cannot of course be ascertained from the present data. Nevertheless, it is still of interest to locate the position of this DNA binding site. In the absence of direct data, we have resorted to model building of the basis of the crystal structure of the cAMP·CRP complex [35] and the amino acid sequence of CRP [37,38]. This reveals that the portion of the β roll comprising the C-terminal ends of β sheets 1, 3, 5 and 7 and the N-terminal ends of β sheets 2, 4, 6 and 8 constitutes an easily accessible positively charged site of the surface of the protein (with 4 lysine residues situated close in space) which could potentially interact with DNA. The spatial arrangement of this site with respect to the geometry of the α CRP dimer is such that only a single subunit at a time could interact with a linear piece of DNA. That is to say the α CRP·DNA complex would be asymmetric with respect to the dyad axis of the α CRP dimer. If this site also constitutes a portion of the complete DNA binding site of native CRP, then the CRP·DNA complex would also be asymmetric with respect to the dyad axis of the CRP dimer, in contrast to the various symmetric DNA binding models [13–17]. In this respect it should be borne in mind that the consensus sequence of the specific DNA target site for CRP [2,39], derived from a large number of specific sites, is also asymmetric, whereas those of other multimeric DNA binding proteins, such as lac repressor, λ repressor and cro protein, exhibit a clear dyad axis [40].

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