# DNA BINDING OF cAMP RECEPTOR PROTEIN AND ITS N-TERMINAL CORE STABILIZES THE DOUBLE HELIX AND IS MODULATED BY THE ALLOSTERIC EFFECTOR cAMP

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## 1. Introduction

3′,5′-Cyclic AMP (cAMP) receptor protein (CRP) binds sequence specifically to a site at or near promotors of catabolite-sensitive operons in *Escherichia coli*, stimulating the initiation of mRNA synthesis in the presence of cAMP [1–7]. The smaller carboxy-terminal domain of both subunits of CRP may bind to a left-handed helix, resulting in destabilisation of the helix and facilitation of binding and of initiation of mRNA synthesis by RNA polymerase [8]. This model was suggested on the basis of computer modelling on the interactions between DNA and the polypeptide α-carbon backbone of CRP, based on the 2.9 Å resolution crystal structure of the cAMP · CRP complex [8]. If this hypothesis is correct, the N-terminal core of CRP, αCRP, produced by subtilisin digestion of the cAMP · CRP complex, in which the small carboxy-terminal domain has been removed [9], should not bind to DNA. Electron microscopy has shown that the binding of CRP non-specifically to DNA results in the formation of a complex with regular striations along the DNA whose length is ~4-times shorter than that of free DNA [10]. This observation suggests that the binding of CRP alone might result in stabilisation of double stranded DNA. Here, we present a thermal denaturation study on double stranded poly[d(AT)] complexed with CRP and αCRP in the presence and absence of cAMP. We demonstrate that both CRP and αCRP stabilise the double stranded structure of poly[d(AT)], and that this effect is modulated by cAMP.

## 2. Experimental

CRP was prepared by the method of B. B., M. T. and A. B. (unpublished) used in [11,12] αCRP was prepared by subtilisin digestion of the cAMP · CRP complex by a modification of the procedure in [9]. Both CRP and αCRP were >99% pure as judged by SDS–polyacrylamide gel electrophoresis (with app. $M_r$ on the gels of 22 500 ± 500 and 12 500 ± 500 for the constituent polypeptide chains of CRP and αCRP, respectively). Poly[d(AT)] was purchased from PL Biochemicals and had $M_r \sim 1.5 \times 10^6$. cAMP was purchased from Schwarz/Mann. Both poly[d(AT)] and cAMP were used without further purification. All other chemicals were of the highest purity commercially available.

The experimental conditions used for the thermal denaturation measurements were: 20 μM in base pairs poly[d(AT)], 3 mM sodium phosphate pH 7.0 (at 25°C), 1.2 mM NaCl and 0.1 mM sodium EDTA (total ionic strength = 4.3 mM). The concentrations of CRP and αCRP used were between 0.6 μM and 3 μM; the concentration of CRP and αCRP were determined from $\epsilon_{278} = 4.1 \times 10^4\ M^{-1} \cdot cm^{-1}$ and $2.6 \times 10^4\ M^{-1} \cdot cm^{-1}$, respectively (per dimer; [12], B. B., M. T., A. B., unpublished).

In the experiments with cAMP, the concentration of cAMP was 100 μM which is sufficient under these conditions of pH and ionic strength to saturate ≳99% of all cAMP binding sites on CRP and αCRP ([12], M. T., B. B., A. B., unpublished). It should be noted that all samples were initially prepared at an ionic strength of 0.1 M and then dialysed to the final experimental salt conditions given above.

At each temperature spectra from 230–350 nm

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were recorded using a Cary 219 spectrometer with a 1 cm pathlength cuvette. Melting curves were plotted by measuring the increase in absorbance at 260 nm; the total melting of poly[d(AT)] produced a 40% increase in the $A_{260}$ with respect to that of intact double-stranded poly[d(AT)].

The contribution to the absorbance change from the linear temperature dependence of the absorbance of cAMP (0.085 $mM^{-1} . cm^{-1}$ per °C at 260 nm) was eliminated by using cAMP in the reference cuvette at the same concentration (100 μM) and temperature as in the sample cuvette. In contrast, any contribution of this type arising from the protein was negligible, accounting for <0.5% of the total absorbance change. At each temperature sufficient time was allowed for thermal equilibrium to be established, and the rate of increase in temperature in going from one temperature value to the next was maintained at 0.1°C/min.

## 3. Results and discussion

The melting curves for poly[d(AT)] alone and in the presence of CRP, the cAMP · CRP complex, αCRP and the cAMP · αCRP complex at an ionic strength of 4.3 mM are shown in fig.1. This very low ionic strength

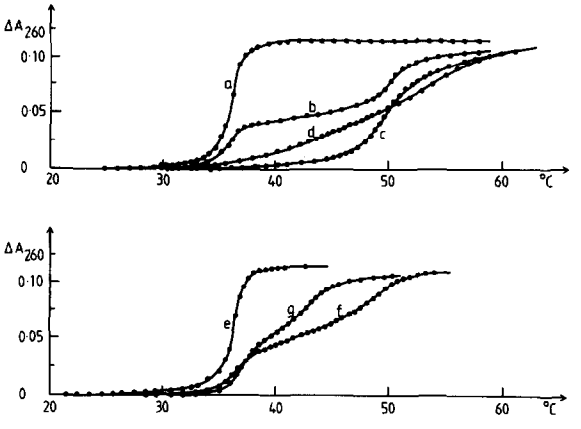


Fig.1. Thermal denaturation curves for the melting of poly[d(AT)] at an ionic strength of 4.3 mM: (a) poly[d(AT)] alone; (b) poly[d(AT)] ~50% saturated with CRP; (c) poly[d(AT)] ≳95% saturated with CRP; (d) poly[d(AT)] ≳95% saturated with the cAMP · CRP complex; (e) poly[d(AT)] in the presence of cAMP; (f) poly[d(AT)] ~60% saturated with αCRP; (g) poly[d(AT)] ~60% saturated with the cAMP · αCRP complex. CRP in (b), (c) and (d) was 0.67 μM, 1.3 μM and 1.3 μM, respectively. αCRP in (f) and (g) was 2.7 μM and 1.3 μM, respectively. All other experimental conditions were as in section 2.

was employed in order to ensure a low value for the mid-point $T_m$ of the melting transition of poly[d(AT)] alone and to circumvent the problem of protein denaturation at high temperatures. Under these conditions CRP and αCRP (both in the presence and absence of cAMP) remain bound to poly[d(AT)] and are not visibly denatured at the highest temperature used here, namely 60°C. In contrast, CRP and αCRP free in solution (both in the presence and absence of cAMP) begin to denature around 50°C, as evidenced by the development of turbidity and consequent increase in light scattering. Thus, it was necessary to use conditions in which the concentration of free protein was minimal so as to avoid problems due to light scattering at high temperatures. When the concentration of protein is sufficient to saturate ≳95% of the poly[d(AT)] lattice, a monophasic melting curve is seen. However, when the concentration of protein is only sufficient to partially saturate the poly[d(AT)] lattice, a biphasic melting curve is seen, the first portion of the curve corresponding to the melting of poly[d(AT)] alone, and the second to the melting of the protein · poly[d(AT)] complex [13].

Table 1

Values of $T_m$ and the melting cooperativity parameter $\sigma_{1/2}$ for poly[d(AT)] alone and in the presence of cAMP, CRP, the cAMP · CRP complex, αCRP and the cAMP · αCRP complex at an ionic strength of 4.3 mM

($\sigma_{1/2}$ is defined as the width corresponding to 50% of the total melting transition centred around the transition mid point)

| Sample | $T_m$(°C) | $\sigma_{1/2}$(°C) |
|---|---|---|
| poly[d(AT)] ± cAMP[a] | 36.0 ± 0.1 | 1.3 ± 0.2 |
| poly[d(AT)] + CRP[a] | 50.2 ± 0.1 | 5.7 ± 0.2 |
| poly[d(AT)] + cAMP · CRP[a] complex | 50.1 ± 0.1 | 11.7 ± 0.2 |
| poly[d(AT)] + αCRP[b] | 47.5 ± 0.3 | 4.5 ± 0.6 |
| poly[d(AT)] + cAMP · αCRP[b] complex | 42.5 ± 0.3 | 3.0 ± 0.6 |

[a] Values of $T_m$ and $\sigma_{1/2}$ are obtained from monophasic melting curves: In the case of the $T_m$ and $\sigma_{1/2}$ values for poly[d(AT)] + CRP, the values obtained from the biphasic melting curve (b) in fig.1 at ~60% saturation of the poly[d(AT)] lattice are identical within experimental error to those obtained from the monophasic melting curve in fig.1 at ≳95% saturation of the poly[d(AT)] lattice

[b] Values of $T_m$ and $\sigma_{1/2}$ are obtained from biphasic melting curves at ~60% saturation of the poly[d(AT)] lattice: The $T_m$ of the first portion of these curves, corresponding to the melting of poly[d(AT)] alone is 36.2 ± 0.3°C

The $T_m$-values and melting cooperativity parameter, $\sigma_{1/2}$ (defined as the width corresponding to 50% of the total melting transition centred about the mid-point) are summarised in table 1. In the case of CRP (both in the presence and absence of cAMP) conditions could be obtained under which $\gtrsim$95% of the poly-[d(AT)] lattice was saturated and the concentration of free CRP minimal. However, in the case of αCRP (both in the presence and absence of cAMP), the maximal saturation of the poly[d(AT)] lattice that could be achieved with a negligible concentration of free αCRP was ~60%. Consequently, the value of $T_m$ and $\sigma_{1/2}$ for poly[d(AT)] in the presence of αCRP and the cAMP. αCRP complex are less precise than those measured in the presence of CRP and the cAMP · CRP complex, as the former could only be obtained from biphasic melting curves while the latter were obtained from monophasic ones.

From the data in fig.1 and table 1, it can be seen that both CRP and αCRP bind to poly[d(AT)] stabilising its double-stranded structure by increasing the $T_m$ and reducing the melting cooperativity (i.e., by increasing the value of $\sigma_{1/2}$). Moreover, the magnitude of the effects of CRP and αCRP in the absence of cAMP are very similar: the $T_m$ is increased by 14.2°C and 11.5°C, respectively, and the $\sigma_{1/2}$ by 4.4°C and 3.2°C, respectively, relative to the respective values for poly[d(AT)] alone ($T_m$ = 36°C, $\sigma_{1/2}$ = 1.3°C). These effects are modulated by cAMP. In the case of CRP, cAMP reduces the melting cooperativity still further, increasing the $\sigma_{1/2}$ from 5.7°C in the absence of cAMP to 11.7°C in the presence of cAMP, but leaves the $T_m$ unaltered at ~50°C. In contrast, in the case of αCRP, cAMP increases the melting cooperativity slightly, reducing $\sigma_{1/2}$ from 4.5°C in the absence of cAMP to 3.0°C in the presence of cAMP, and reduces the $T_m$ from 47.5°C in the absence of cAMP to 42.5°C in the presence of cAMP.

On the basis of these results, we therefore conclude that, with respect to non-specific DNA binding:

(1) CRP and αCRP both in the presence and absence of cAMP are DNA stabilising proteins contrary to what was generally supposed [3,14,15];

(2) The model in [8] for the location of the DNA binding site of CRP solely in the small carboxy-terminal domain is probably incorrect, clearly illustrating the dangers of model building when only the α-carbon polypeptide backbone is taken into account.

Further, it also seems unlikely that binding of CRP to DNA involves the conversion of a right- to a left-handed helix as proposed [8], since one would expect this to result in a destabilisation rather than a stabilisation of double-stranded DNA. Although these experiments were carried out at a very low ionic strength (4.3 mM) in order to depress the $T_m$ of poly[d(AT)] to a reasonably low value, these conclusions will also be valid at physiological ionic strengths as it has been shown that the affinity of CRP and the cAMP · CRP complex is much weaker for single stranded than double stranded DNA [16].

However, these conclusions might not necessarily be true for the interactions involving the specific stie and at present no direct quantitative experimental data on a CRP · specific site complex are available since such studies will have to await the availability of large quantities of the DNA sequence comprising the specific site.

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