Specific DNA binding of the cyclic AMP receptor protein to a synthetic oligodeoxyribonucleotide

A circular dichroism study

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The interaction of the cAMP receptor protein (CRP) of *Escherichia coli* with a synthetic DNA undecamer (11mer) comprising a portion of the specific target site in the *gal* operon and containing 8 basepairs out of the 10 basepair concensus making up specific CRP sites, has been studied by circular dichroism spectroscopy. The binding constants for the interaction of CRP with the 11mer in the presence and absence of cAMP have been determined, and it is shown that CRP, both in the presence and absence of cAMP, induces a B–C transition in the conformation of the 11mer.

CRP *Specific DNA binding* *Synthetic oligonucleotide* *Circular dichroism*

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 [1–3]. The cAMP–CRP complex binds to specific DNA target sites near each gene it regulates. In some cases this interaction leads to an increase in transcription as in the case of the *lac* [4] and *ara* [5] operons and for transcription from the P_1 promotor of the *gal* operon [6]; in other cases it leads to an inhibition of transcription as in the case of its own structural gene [3] and the *ompA* gene [7] and for transcription from the P_2 promotor of the *gal* operon [6]. The mechanisms whereby CRP achieves these effects are unknown although numerous hypotheses have been put forward [8–12]. To date, no structural data exist on a complex of CRP with one of its specific DNA target sites.

Circular dichroism (CD) spectroscopy has proved a sensitive diagnostic tool in the investigation of DNA conformations in solution as it can easily distinguish between the various forms of DNA [13,14]. Here, we have therefore investigated the interaction of CRP with the synthetic DNA undecamer (11mer):

5′ AAGTGTGACAT
TTCACACTGTA 5′

by CD spectroscopy. This 11mer comprises a portion of the specific target site in the *gal* operon [15] and contains 8 basepairs out of the 10 basepair concensus, 5′ AA-TGTGA--T----CA, making up specific CRP sites [2]. We demonstrate that CRP, both in the presence and absence of cAMP, induces a conformational change in the 11mer characteristic of a B–C transition.

2. EXPERIMENTAL

CRP was purified to homogeneity (>99% pure as judged by sodium dodecylsulphate–polyacryl-

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amide gel electrophoresis) from an overproducing *E. coli* strain harbouring the plasmid pBScrp 2 [16] as in [17].

The two strands of the 11mer were synthesised using the phosphotriester method and purified by ion exchange high pressure liquid chromatography (HPLC) as in [18].

cAMP was purchased from Sigma, and used without further purification. All other chemicals were of the highest purity commercially available.

The standard buffer used in recording the CD spectra was 100 mM KCl, 5 mM potassium phosphate (pH 6.6) and 0.01 mM EDTA.

CD spectra were recorded digitally from 340–235 nm using a Jasco J41-C spectropolarimeter equipped with a J-DPY data processor, with a sensitivity of 0.5 millidegrees/cm and with an instrumental time constant of 4 s. Semi-micro cells of pathlength 10 mm were used. Reported spectra were recorded at 10°C and represent the average of at least two scans; they are presented as plots of circular dichroic absorbance, ΔA, against wavelength.

Data processing involved base-line subtraction and smoothing over a running interval of 40 points using the quadratic–cubic function in [19]. Difference spectra were obtained by the appropriate numerical subtraction.

3. RESULTS

3.1. *Titration of 11mer with CRP*

CD spectra were recorded for the titration of 4.5 μM 11mer with aliquots of concentrated (0.5 mM) CRP in standard buffer (100 mM KCl, 5 mM potassium phosphate, pH 6.6, 0.01 mM EDTA) at 10°C.

Fig.1a shows the CD spectrum over the 235–340 nm range of 4.5 μM 11mer and the difference spectrum, (CRP + 11mer) − (CRP), generated from data for 4.5 μM 11mer and ~20 mM CRP. These spectra clearly show that the CD spectrum of the complex differs from the sum of the individual protein and 11mer spectra, indicating that the formation of the complex involves a conformational change either in CRP or the 11mer, or both. As the CD of CRP is very weak in this wavelength range, it seems likely that it is the 11mer that undergoes the conformational change, detectable by CD spectroscopy, on formation of the complex.

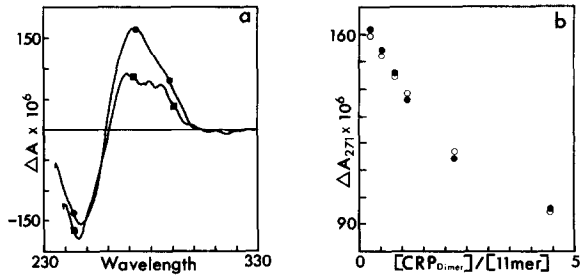

Fig.1. Interaction of CRP with the 11mer: (a) CD spectrum of 4.5 μM 11mer (●) and the CD difference spectrum, (CRP + 11mer) − (CRP), generated from the data for 4.5 μM 11mer and 20 μM CRP (■); (b) titration of the 11mer (4.5 μM) with CRP, monitored by ΔA_{271}, for the family of difference spectra, (CRP + 11mer) − (CRP) (●); (○) computed best fit for a single DNA binding site per CRP dimer model with K = 7.9 (± 2.5) × 10^4 M^{-1}. See text for full details.

Assuming that the CD of CRP is indeed unperturbed by formation of the binary complex, the difference spectrum in fig.1a represents the spectrum of the 11mer in the complex. Compared with the uncomplexed 11mer, this spectrum shows reduced intensity in the 270–290 nm region, even though the intensity of the negative band at ~245 nm is essentially unchanged. Change of this type are characteristic of those seen on inducing the B–C transition [13].

Fig.1b shows a plot of ΔA_{271} as a function of the molar ratio [CRP_{dimer}]/[11mer] for the family of difference spectra, (CRP + 11mer) − (CRP), generated from the titration data. This plot monitors the B–C conversion and thus the binding of CRP to the 11mer. A non-linear least squares fit to the data of a simple model comprising a single DNA binding site per CRP dimer yields a binding constant of 7.9 (± 2.5) × 10^4 M^{-1}.

3.2. *Titration of cAMP with CRP*

The titration of cAMP (50 μM) with CRP (aliquots of 0.5 mM stock) was studied by CD in standard buffer at 10°C. Fig.2a shows the spectrum of 50 μM cAMP and the difference spectrum, (CRP + cAMP) − (CRP) − (cAMP), from data for 50 μM cAMP and 10 μM CRP. Inspection of fig.2a clearly suggests that the difference spectrum

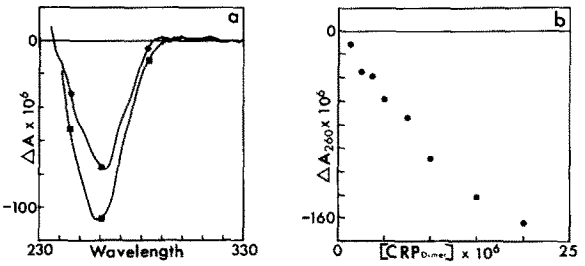

Fig.2. Interaction of CRP with cAMP: (a) CD spectrum of 50 μM cAMP (●) and the CD difference spectrum, (CRP + cAMP) – (CRP) – (cAMP), generated for 50 μM cAMP and 10 μM CRP (■); (b) titration of cAMP (50 μM) with CRP, monitored by ΔA_{260}, for the family of difference spectra, (CRP + cAMP) – (CRP) – (cAMP). See text for full details.

can be accounted for by assuming that the spectrum of bound cAMP is well represented by a simple intensification of the spectrum for free cAMP. Such intensification is presumably due to the immobilization of the bound nucleotide, and these results do not provide evidence for a nucleotide-induced conformational change in CRP, although such a change is known to occur from NMR studies [20].

Fig.2b shows a plot of ΔA_{260} as a function of CRP concentration for the family of difference spectra, (CRP + cAMP) – (CRP) – (cAMP), generated from the titration data. The linear dependence of the ΔA_{260} clearly indicates that under the conditions employed the two cAMP binding sites on the CRP are >90% saturated.

3.3. *Titration of 11mer plus cAMP with CRP*

CD spectra were recorded for the titration of 4.5 μM 11mer and 50 μM cAMP with aliquots of concentrated (0.5 mM) CRP at 10°C in standard buffer.

Fig.3a compared the difference spectrum for the ternary system (CRP + 11mer + cAMP) – (CRP) – (11mer) – (cAMP), with that of the binary system (CRP + 11mer) – (CRP) – (11mer). These difference spectra were calculated from data for 4.5 μM 11mer, 50 μM cAMP and 20 μM CRP. The difference spectrum for the ternary system is clearly more intense than that of the binary system, but interpretation of these changes is complicated by the fact that the binding of CRP to cAMP also generates a difference spectrum (see fig.2a). We have therefore compared the difference spectrum for the ternary system to the difference spectrum sum, [(CRP + cAMP) – (cAMP) – (CRP)] + [(CRP + 11mer) – (11mer) – (CRP)]. This comparison appears in fig.3b and clearly shows that the ternary system difference spectrum is well ac-

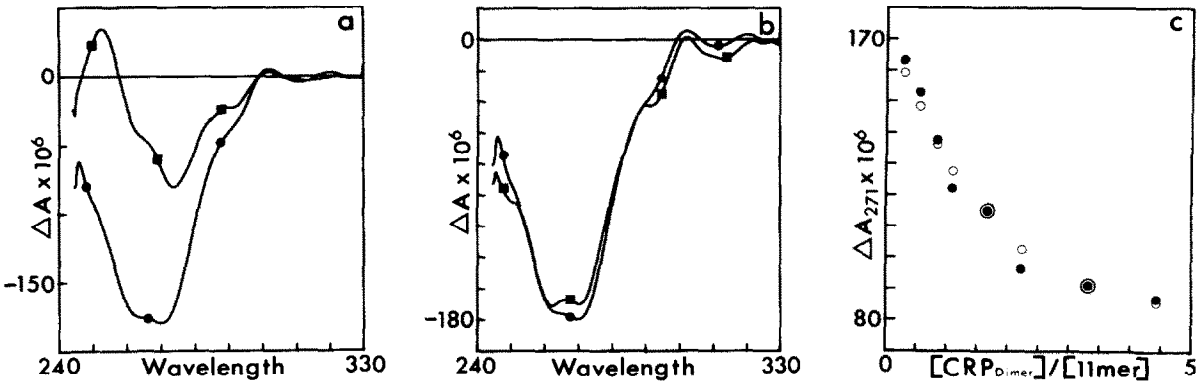

Fig.3. Interaction of CRP with the 11mer in the presence of cAMP. (a) CD difference spectra: (●) (CRP + 11mer + cAMP) – (CRP) – (11mer) – (cAMP); (■) (CRP + 11mer) – (CRP) – (11mer), generated from original data for 50 μM cAMP, 20 μM CRP and 4.5 μM 11mer. (b) CD difference spectra: (●) (CRP + 11mer + cAMP) – (CRP) – (11mer) – (cAMP); (■) [(CRP + cAMP) – (cAMP) – (CRP)] + [(CRP + 11mer) – (11mer) – (CRP)], generated from original data for 50 μM cAMP, 20 μM CRP and 4.5 μM 11mer. (c) Titration of cAMP (50 μM) plus 11mer (4.5 μM) with CRP, monitored by ΔA_{271}, for the family of difference spectra (11mer + cAMP + CRP) – (CRP + cAMP) (●); (○) computed best fit for a single DNA binding site per CRP dimer model with $K = 1.6\ (\pm 0.7) \times 10^5\ M^{-1}$. See text for full details.

counted for by summing up the difference spectra for the two appropriate binary systems.

If, as seems likely, the CD spectrum of CRP is not grossly perturbed by inclusion in the ternary complex, we may assume tha⁺ the effects of CRP on cAMP and of CRP on the 11mer are not altered by formation of the ternary complex; i.e., the effects are simply additive. Providing that this assumption is correct we may generate the spectrum of the 11mer in the ternary complex by the difference spectrum (11mer + cAMP + CRP) − (CRP + cAMP). This was done for the titration data and fig.3c shows a plot of ΔA_{271} as a function of the molar ratio [CRP_{dimer}]/[11mer]. As in the case of fig.1b, this allows us to monitor the B–C transition as a function of CRP concentration. The binding constant of $1.6 (\pm 0.7) \times 10^5$ M^{-1}, obtained from a non-linear least squares fit of this data to a single DNA binding site per CRP dimer model, is a factor of ~2 higher than that obtained in the absence of cAMP.

4. DISCUSSION

Using CD spectroscopy, we have studied the interaction of CRP with a synthetic DNA 11mer comprising a portion of the specific target site in the *gal* operon [15] and containing 8 basepairs out of the 10 basepair concensus sequence making up specific CRP sites [2]. At an ionic strength of 100 mM KCl, the binding of CRP to this 11mer is somewhat tighter in the presence of cAMP (K + $1.6 (\pm 0.7) \times 10^5$ M^{-1}) than in its absence ($K = 7.9 (\pm 2.5) \times 10^4$ M^{-1}). These binding constants are about two orders of magnitude larger than the corresponding binding constants for the binding of CRP to a single non-specific site at the same ionic strength [21]. The change in the CD spectrum of the 11mer induced by the binding of CRP, both in the presence and absence of cAMP, is characteristic of an alteration in the conformation of the 11mer from the B to the C form. That is to say, the binding of CRP to the 11mer increases the helical twist and reduces the helical rise of the 11mer, whilst preserving the handedness of the helix, namely right-handed. These findings are entirely consistent with those in [22] which demonstrated that specific DNA-binding of CRP within the *lac* operon stabilizes double-stranded DNA. Moreover, our results indicate unambiguously that, in the case of the 11mer at least, specific DNA binding does not induce the conversion of right- to left-handed B DNA as proposed in [10] based on speculative modelling of the interaction of CRP with DNA using the 2.9 Å crystal structure of the cAMP–CRP complex.

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