Biochem. J. (1988) 250, 897–902 (Printed in Great Britain)

Binding of the cyclic AMP receptor protein of *Escherichia coli* to RNA polymerase

Michael PINKNEY* and James G. HOGGETT†
Department of Biology, University of York, York YO1 5DD, U.K.

Fluorescence polarization studies were used to study the interaction of a fluorescein-labelled conjugate of the *Escherichia coli* cyclic AMP receptor protein (F-CRP) and RNA polymerase. Under conditions of physiological ionic strength, F-CRP binds to RNA polymerase holoenzyme in a cyclic AMP-dependent manner; the dissociation constant was about 3 μM in the presence of cyclic AMP and about 100 μM in its absence. Binding to core RNA polymerase under the same conditions was weak ($K_{diss.}$ approx. 80–100 μM) and independent of cyclic AMP. Competition experiments established that native CRP and F-CRP compete for the same binding site on RNA polymerase holoenzyme and that the native protein binds about 3 times more strongly than does F-CRP. Analytical ultracentrifuge studies showed that CRP binds predominantly to the monomeric rather than the dimeric form of RNA polymerase.

INTRODUCTION

The cyclic AMP receptor protein (CRP) of *Escherichia coli* is a pleiotropic gene activator that, when bound to its allosteric effector cAMP, produces an increased rate of initiation of transcription from catabolite-sensitive promoters. Kinetic studies performed with the abortive initiation assay indicate a dual mode of action of the cyclic AMP–CRP complex at the *lac* [1] and *gal* [2] promoters; the complex blocks RNA polymerase binding to weak competing and overlapping promoters, and also directly increases the affinity of RNA polymerase for the more efficient promoters. The kinetic evidence supports the view that the cyclic AMP–CRP complex enhances the stability of the closed complex of RNA polymerase with the promoter but does not influence the isomerization step or steps leading to the open complex. The mechanistic basis of this stabilization of the closed complex is unclear. Binding of the cyclic AMP–CRP complex does not cause destabilization [3] or unwinding [4] of the DNA, but it does produce a significant local bending of the DNA [5]. In addition to changes in the DNA structure, it is also possible that the closed complex is stabilized by direct protein–protein contact between CRP and RNA polymerase; the arguments and indirect evidence supporting this view have been summarized [6].

Early studies [7] with sucrose-density-gradient centrifugation provided no evidence of an interaction between CRP and RNA polymerase (in the absence of DNA), nor did a more recent investigation based on gel chromatography [8]. The first indication of an interaction between the two proteins was reported by Wu and his co-workers [9], who showed that RNA polymerase caused a blue-shift in the emission spectrum of a fluorescently labelled conjugate of CRP. However, the effect was not dependent on cyclic AMP and was too small to allow quantitative analysis. Much clearer evidence of a cyclic AMP-dependent interaction was provided by Blazy *et al.* [10], who demonstrated that the cyclic AMP–CRP complex and RNA polymerase co-sedimented in ultracentrifugation experiments. The importance of the sigma subunit in this interaction was emphasized by a subsequent immunological study [11], in which it was shown that CRP enhanced the complement fixation response both of the RNA polymerase holoenzyme and also of the isolated sigma subunit, implying that CRP can change the conformation of RNA polymerase by binding to this subunit.

Despite this evidence, no satisfactory quantitative information exists on CRP–polymerase interactions. We report here the results of a fluorescence polarization study of the binding of RNA polymerase to a fluorescently labelled conjugate of CRP. The technique exploits the large difference in the M_r values of RNA polymerase (about 450000) and CRP (about 45000). Rotation of the labelled CRP would be expected to cause a greater depolarization of the fluorescence than that of the much larger CRP–polymerase complex. This technique has been used successfully to study quantitatively the binding of protein synthesis initiation factors to ribosomal subunits [12,13].

MATERIALS AND METHODS

Purification of proteins

CRP was prepared from *E. coli* M.R.E. 600 (obtained from the Centre for Applied Microbiological Research, Porton Down, Wilts., U.K.) by a method involving chromatography on phosphocellulose, Sephacryl S-200 and DNA–cellulose. The steps up to and including phosphocellulose chromatography were based on the procedure of Boone & Wilcox [14], and later stages were based on the method of Eilen *et al.* [15]. Samples were judged by SDS/polyacrylamide-gel electrophoresis to be 95% pure, and the specific activity was 5000–7000 units/mg. Stock solutions (3–5 mg/ml) were stored in 50% (v/v) glycerol, pH 7.5 at −20 °C.

RNA polymerase was prepared from *E. coli* M.R.E.

Abbreviations used: CRP, cyclic AMP receptor protein; F-CRP, fluorescein-labelled cyclic AMP receptor protein.

* Present address: Department of Genetics, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, U.K.
† To whom correspondence should be addressed.

600 by the method of Burgess & Jendrisak [16]. Core polymerase was separated from holoenzyme by DNA–agarose chromatography as described by Lowe *et al.* [17]. The content of sigma factor was estimated by SDS/polyacrylamide-gel electrophoresis. Samples (about 5 mg/ml) were stored in 50% (v/v) glycerol, pH 7.9, at −20 °C.

Preparation of fluorescein-labelled CRP

Fluorescein-labelled CRP (F-CRP) was prepared in a heterogeneous reaction with fluorescein isothiocyanate adsorbed on Celite by following the procedure of Rinderknect [18].

Fluorescein isothiocyanate–Celite was prepared by dissolving 40 mg of fluorescein isothiocyanate isomer 1 (Sigma Chemical Co.) in acetone (2 ml), stirring the resulting solution with 160 mg of Celite (Sigma Chemical Co.) and removing the solvent by rotary evaporation and drying in a vacuum desiccator.

Labelling of CRP was carried out at 4 °C. A sample of CRP was dialysed for 16 h against labelling buffer [10 mM-Tris/HCl buffer, pH 7.9, containing 10 mM-$MgCl_2$, 0.1 mM-EDTA, 0.13 M-KCl and 5% (v/v) glycerol]. The concentration of CRP was determined spectrophotometrically (ϵ_{280} 3.98×10^4 $M^{-1} \cdot cm^{-1}$ [19]), and the solution was stirred for 15 min with sufficient fluorescein isothiocyanate–Celite (20%, w/w) to give a molar ratio of dye to protein of 120–130:1. The suspension was centrifuged (8000 ***g*** for 5 min) to remove fluorescein isothiocyanate–Celite, and free dye was removed either by dialysis or gel filtration on Sephadex G-25. The extent of labelling was typically in the range 1.5–2.0 dye residues/CRP dimer. The labelling procedure decreased the cyclic AMP-binding activity in the $(NH_4)_2SO_4$ precipitation assay by about 30%, but did not affect the non-specific binding of the protein to calf thymus DNA (results not shown).

Fluorescence polarization measurements

Measurements were made with a Perkin–Elmer MPF-3 fluorescence spectrophotometer. Protein samples were dialysed against titration buffer before use. Titrations were performed in a 4 mm-pathlength-cuvette by adding portions of a mixture of F-CRP and RNA polymerase to a solution of F-CRP so that the concentration of labelled receptor protein remained constant. In order to correct for any changes in background fluorescence, blank titrations were also performed in which RNA polymerase was added to titration buffer.

Samples were excited at 493 nm with vertically polarized light, and the intensity of the emission at 520 nm was determined with the emission filter in the vertical (V_v) and horizontal (V_h) positions. The excitation filter was rotated through 90° and values for emission in the vertical (H_v) and horizontal (H_h) directions were determined. Corresponding values for the blank were determined similarly, and if significant these were subtracted from the solution values [20]. The fluorescence anisotropy (A) was expressed as:

$$A = \frac{V_v - t \cdot V_h}{V_v + 2t \cdot V_h}$$

in which the correction factor t ($= H_v/H_h$) allows for the selective transmission of light by the emission monochromator [21]. Fluorescence intensities could be determined to a precision of 1%, which typically leads to an error or 3.5% in the derived anisotropies.

Anisotropy is a more convenient quantity to use than polarization for present purposes because the total anisotropy of a mixture of bound and free F-CRP is the weighted sum of the anisotropies of the two individual components. With the assumption that the complex formed is a 1:1 complex, the dependence of anisotropy on the concentration of RNA polymerase is given by the following expression:

$$A = A_f + \Delta A \cdot \frac{[\text{F-CRP}]_{\text{bound}}}{[\text{F-CRP}]_{\text{total}}}$$

in which A_f is the anisotropy of the free F-CRP and ΔA is the change in anisotropy on binding polymerase.

Table 1. Dissociation constants for the binding of F-CRP and CRP to RNA polymerase

Errors for $K_{diss.}$ values are 67% confidence limits (see the text).

Interaction	[KCl] (M)	[Cyclic AMP] (μM)	$K_{diss.}$ (μM)
F-CRP + core	0.05	0	$2.5\binom{+3.0}{-0.5}$
enzyme	0.05	66	$4.5\binom{+10}{-1.5}$
F-CRP + mixed core enzyme and holo-enzyme	0.05	0	$10\binom{+5}{-1}$
	0.05	66	$1.5\binom{+0.3}{-0.3}$
F-CRP + core enzyme	0.2	0	$80\binom{+60}{-30}$
	0.2	66	$80\binom{+60}{-30}$
F-CRP + holo-enzyme	0.2	0	$100\binom{+65}{-30}$
	0.2	66	$3\binom{+2.5}{-0.5}$
CRP + holo-enzyme	0.2	55	$1\binom{+1.5}{-0.5}$

Analytical ultracentrifugation

Sedimentation-velocity studies were performed with a Beckman model E analytical ultracentrifuge equipped with photoelectric scanning absorption optics.

RESULTS

Fluorescence titrations of F-CRP and RNA polymerase

Experiments were carried out at 22 °C with three preparations of RNA polymerase: core enzyme, holoenzyme (with a sigma subunit content of > 90%) and a mixture of core enzyme and holoenzyme in which the sigma subunit content was about 40%. The buffer conditions were 50 mM-Tris/HCl buffer, pH 8.5, 10 mM-$MgCl_2$ and either 50 mM-KCl (low ionic strength)

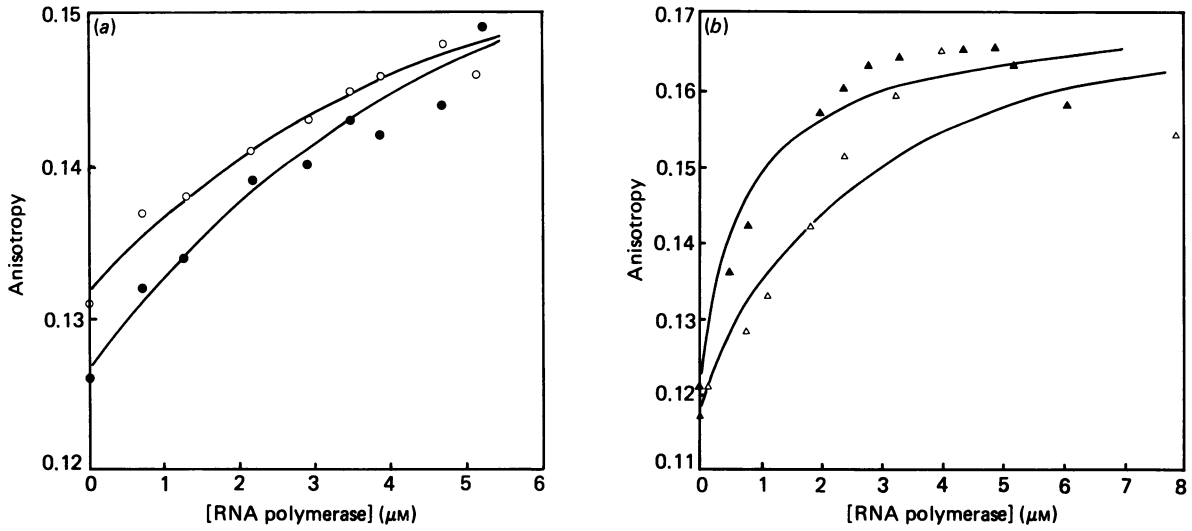

Fig. 1. Binding of F-CRP to (*a*) core RNA polymerase and (*b*) mixed core enzyme and holoenzyme at low ionic strength

The medium contained 50 mM-Tris/HCl buffer, pH 8.0, 10 mM-$MgCl_2$ and 50 mM-KCl. (*a*) [F-CRP] = 0.67 μM: ○, [cyclic AMP] = 0; ●, [cyclic AMP] = 66 μM. (*b*) [F-CRP] = 0.3 μM: △, [cyclic AMP] = 0; ▲, [cyclic AMP] = 66 μM.

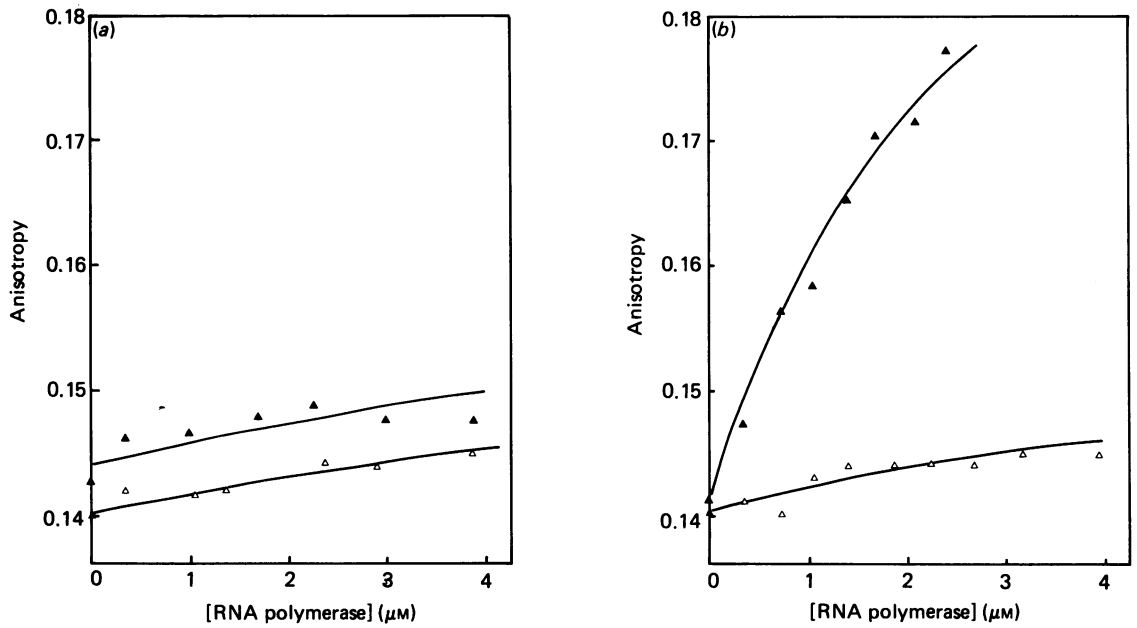

Fig. 2. Binding of F-CRP to (*a*) core RNA polymerase and (*b*) holoenzyme at physiological ionic strength

The medium contained 50 mM-Tris/HCl buffer, pH 8.0, 10 mM-$MgCl_2$ and 200 mM-KCl. (*a*) [F-CRP] = 0.49 μM: △, [cyclic AMP] = 0; ▲, [cyclic AMP] = 66 μM. (*b*) [F-CRP] = 0.49 μM: △, [cyclic AMP] = 0; ▲, [cyclic AMP] = 66 μM.

or 200 mM-KCl (approximately physiological ionic strength). Cyclic AMP, when added, was at a concentration of 66 μM, which is sufficient to saturate the available sites on CRP. Two experimental points that limit the accuracy of the results deserve mention. The first is that, despite attempts to standardize the heterogeneous labelling procedure, different preparations of F-CRP showed somewhat different magnitudes of anisotropy change on binding RNA polymerase, so comparison of absolute values of anisotropies between different preparations is difficult. Secondly, it was not always possible for practical reasons to add enough RNA polymerase to saturate the binding. The data were analysed by a non-linear least-squares procedure on the assumption of a single dissociation constant K for the interaction. In view of the different binding properties of F-CRP to core and holoenzyme, it is clear that this represents only an approximate analysis for the mixed core enzyme and holoenzyme experiments, but the data were not sufficiently accurate to warrant more rigorous analysis.

Confidence limits to the values of K were determined by analysing the sum of residual squares according to the procedure described by Hoare [22]; 67% confidence limits are shown in the results collected in Table 1, the lower limits to the values of K being better defined than

the upper limits. The assumption of 1:1 stoichiometry is supported by the fact that at 200 mM-KCl RNA polymerase exists in the monomeric form throughout the titrations, and, except at the highest polymerase concentrations, this is also the case at low ionic strength. We discuss below the evidence that binding to dimeric polymerase is much weaker than to the monomer.

Fig. 1 illustrates the results of binding experiments in the presence and in the absence of cyclic AMP at low ionic strength for core enzyme (*a*) and mixed core enzyme plus holoenzyme (*b*). The values of dissociation constants are collected in Table 1. Binding to core enzyme at this ionic strength is fairly tight ($K_{diss.}$ approx. 3–5 μM) and within the limits of error is independent of cyclic AMP. The results for the mixed core enzyme plus holoenzyme titration shown in Fig. 1(*b*) do not appear to fit simple binding curves very well. The reason for this is the pronounced decrease in anisotropy that occurs at high (> 5 μM) concentrations of RNA polymerase. Repetition of these experiments showed this phenomenon to be reproducible. The explanation of this in terms of the effect of CRP on the monomer–dimer equilibrium of RNA polymerase is considered in the Discussion section; the values of the relevant dissociation constants shown in Table 1 (1.5 and 10 μM) were obtained by neglecting the data above an RNA polymerase concentration of 5 μM.

Results of experiments with core enzyme and holoenzyme in the presence and in the absence of cyclic AMP at high ionic strength, much closer to the presumed physiological conditions [23], are shown in Fig. 2. These experiments were all conducted with the same batch of F-CRP, and analyses of the weak binding curves were made on the basis that the change in anisotropy at saturation would be the same for all four curves. Under these conditions binding to core enzyme was weak ($K_{diss.}$ approx. 80–100 μM) both in the presence and in the absence of cyclic AMP, as was binding to holoenzyme in the absence of cyclic AMP. However, in the presence of cyclic AMP, binding to holoenzyme was much stronger ($K_{diss.}$ approx. 3–4 μM).

Competition experiments between F-CRP and unlabelled CRP

In order to investigate whether native CRP and F-CRP bind to the same site on RNA polymerase, competition experiments were carried out in which the interaction of F-CRP with holoenzyme has been monitored in the presence and in the absence of CRP. The results are shown in Fig. 3.

In the absence of native CRP, binding of F-CRP to RNA polymerase is described by the following equilibrium condition:

$$K_1 = \frac{[\text{F-CRP}]_{free} \cdot [\text{RNA pol}]_{free}}{[\text{F-CRP–RNA pol}]} \quad (1)$$

In the presence of native CRP there is an additional linked equilibrium that draws on the same pool of free RNA polymerase:

$$K_2 = \frac{[\text{CRP}]_{free} \cdot [\text{RNA pol}]_{free}}{[\text{CRP–RNA pol}]} \quad (2)$$

The equilibrium constant K_1 and the anisotropy parameters were evaluated from the titration carried out in the absence of native CRP, and these quantities were

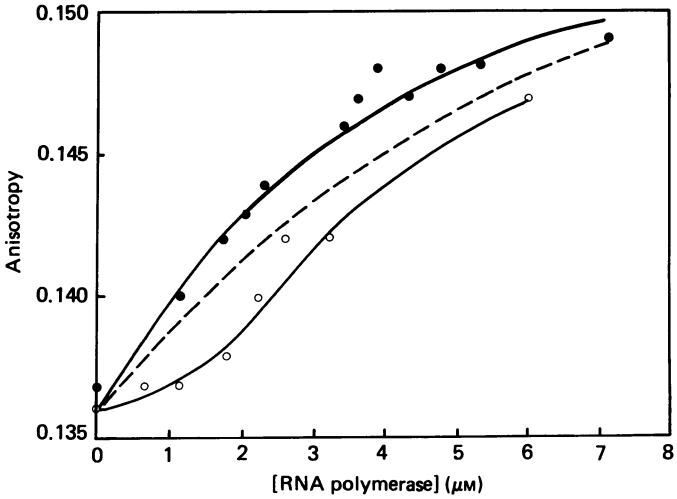

Fig. 3. Binding of F-CRP to RNA polymerase holoenzyme in the presence and in the absence of native CRP

The medium contained 50 mM-Tris/HCl buffer, pH 8.0, 0.1 mM-EDTA, 200 mM-KCl and 66 μM-cyclic AMP. ●, [F-CRP] = 1.1 μM; ○, [F-CRP] = 1.1 μM and [CRP] = 2.3 μM. The broken line indicates the curve that would have been expected if CRP bound with the same affinity as F-CRP (see the text).

used to evaluate the concentration of bound F-CRP corresponding to each of the anisotropy values obtained in the presence of native CRP:

$$[\text{F-CRP}]_{bound} = [\text{F-CRP}]_{total} \cdot \frac{(A - A_f)}{\Delta A}$$

The concentration of free RNA polymerase was then determined by combining eqn. (1) with the conservation equation:

$$[\text{F-CRP}]_{total} = [\text{F-CRP}]_{free} + [\text{F-CRP}]_{bound}$$

to yield the expression:

$$[\text{RNA pol}]_{free} = \frac{K_1 \cdot [\text{F-CRP}]_{bound}}{[\text{F-CRP}]_{total} - [\text{F-CRP}]_{bound}}$$

from which the concentration of bound native CRP can be evaluated from:

$$[\text{CRP}]_{bound} = [\text{RNA pol}]_{total} - ([\text{F-CRP}]_{bound} + [\text{RNA pol}]_{free})$$

The derived values of $[\text{CRP}]_{bound}$ and $[\text{RNA pol}]_{free}$ were fitted by non-linear least-squares analysis to the function:

$$[\text{CRP}]_{bound} = [\text{CRP}]_{total} \cdot \frac{[\text{RNA pol}]_{free}}{K_2 + [\text{RNA pol}]_{free}}$$

to obtain an estimate of K_2, the binding constant of native CRP to RNA polymerase. The value obtained (K_2 = 1 μM) clearly depends on the difference between two binding curves each of which is associated with error. This estimate is therefore itself associated with a considerable error, probably by plus or minus a factor of 2-fold. However, it is clear that native CRP and labelled CRP bind to the same region of RNA polymerase, and that native CRP does so somewhat more tightly than does F-CRP; the broken line in Fig. 3 indicates the curve that would have been expected if CRP were to bind with the same affinity as did F-CRP.

Table 2. Effect of CRP on the monomer–dimer equilibrium of RNA polymerase

The medium contained 50 mM-Tris/HCl buffer, pH 8.0, 10 mM-$MgCl_2$ and added KCl as indicated; the concentration of RNA polymerase was 1.4 μM. The rotor speed was 42680 rev./min.

[KCl] (M)	[CRP] (μM)	$s_{20,w}$ (S)
0.22	0	14.3
0.16	0	22.9
0.05	0	28.3
0.05	6	16.3

Effect of CRP on the RNA polymerase monomer–dimer equilibrium

The influence of native CRP on the monomer–dimer equilibrium of RNA polymerase was investigated by analytical ultracentrifugation. The results are shown in Table 2. Values of $s_{20,w}$ were obtained by using published values for the partial spcific volumes of CRP (0.752; [24]) and RNA polymerase (0.743; [25]), with the assumption that the partial specific volume of the complex was the weight average of the two values and assuming that the two proteins bind in a 1:1 ratio.

The value of $s_{20,w}$ obtained at 0.22 M-KCl was characteristic of monomeric RNA polymerase [25], whereas the value obtained at 0.05 M-KCl in the absence of CRP corresponds to that of the dimer. In the presence of CRP at low salt concentrations, where binding is expected to be tight, a sedimentation coefficient close to that of monomeric RNA polymerase was obtained, indicating that CRP promotes dissociation of dimeric RNA polymerase.

DISCUSSION

The results of the fluorescence polarization titrations provide good evidence for direct protein–protein interactions between CRP and RNA polymerase. The competition experiments show that the native and labelled proteins both bind to the same region of RNA polymerase, and that, although labelling does weaken the interaction somewhat (by a factor of about 3-fold), the two proteins bind RNA polymerase with comparable affinities. The principal findings of relevance to the mechanism of gene activation are that at ionic strength of about 0.2, which corresponds to the effective physiological values [23], binding of CRP to core enzyme is weak both in the presence and in the absence of cyclic AMP, but binding to the holoenzyme, which is the form of the enzyme required for initiation of RNA synthesis, is strongly dependent on cyclic AMP. The observed binding constants in the presence of cyclic AMP lie in the range 1–3 μM, corresponding to a free energy of interaction of 34–32 kJ/mol. The weakness of binding to the core enzyme emphasizes the importance of the sigma subunit and suggests that the strong cyclic AMP-dependent CRP binding to the holoenzyme arises from favourable interactions with that subunit. This suggestion is supported by the finding that CRP influences the complementation fixation response of the isolated sigma subunit as well as that of the holoenzyme [11]. It is, of course, also possible that the effects of the sigma subunit are mediated indirectly; however, our present lack of knowledge of the structure of RNA polymerase makes it difficult to judge the extent to which the conformation of the core enzyme and holoenzyme are significantly different.

An observation that is relevant to the region of RNA polymerase where CRP binding occurs comes from the sedimentation-velocity experiments (Table 2). These show that at low ionic strength (50 mM-KCl), where RNA polymerase normally exists in the dimeric form, addition of an excess of CRP causes dissociation to monomers. This implies that CRP binds more tightly, perhaps exclusively, to the monomeric form. The simplest explanation of this observation is that CRP interacts with a region of RNA polymerase that becomes shielded when monomers associate into dimers. It seems probable that the preferential binding of CRP to RNA polymerase monomers is the reason for the reproducible decrease in anisotropy observed at high polymerase concentrations in titrations at low ionic strength. Under these conditions dimerization becomes progressively more favoured and the concentration of CRP–polymerase complexes falls. It is noteworthy that no corresponding decrease in anisotropy was observed in titrations at higher ionic strength (0.2 M-KCl), where RNA polymerase exists in the monomeric form.

Our results relating to the holoenzyme are in good agreement with those obtained by Blazy *et al.* [10], who estimated binding constants of 3 μM and > 10 μM in the presence and in the absence of cyclic AMP respectively. However, we find binding to the core enzyme to be very much weaker than they did ($K_{diss.}$ approx. 80–100 μM compared with their estimate of 3 μM). Our estimates for the core enzyme are close to those that we obtained for the holoenzyme in the absence of cyclic AMP.

The binding energy for the cyclic AMP-induced interaction of CRP and holoenzyme in the absence of DNA is well into the range required to explain the stabilization of promoter complexes by direct protein contact [26]. These findings provide strong corroborative evidence that protein–protein contact is at least a component of the mechanism of gene activation by CRP.

Protein–protein contact has also been proposed to explain gene activation by both λ and P22 repressors [27]. These repressors closely approach RNA polymerase while activating transcription, and mutant repressors that bind DNA normally but fail to activate transcription show structural alterations in those regions of the repressors that are expected to be in close proximity to the polymerase.

The extent of protein–protein interaction between CRP and RNA polymerase when both are bound to specific promoter sites has not yet been explored. In view of the major changes in DNA structure that are known to occur when the two proteins bind singly, and the possibility of yet further changes when they are in combination, it would be difficult to disentangle the contributions of direct and DNA-mediated interactions from DNA-binding studies with native proteins. We are therefore attempting to obtain CRP mutants that retain normal cyclic AMP- and DNA-binding properties but that are altered in their ability to bind RNA polymerase with a view to characterizing their effects on initiation of transcription.

We acknowledge the award of a research studentship to M. P. from the Science and Engineering Research Council.

REFERENCES

1. Malan, T. P., Kolb, A., Buc, H. & McClure, W. R. (1984) J. Mol. Biol. **180**, 881–909
2. Spassky, A., Busby, S. & Buc, H. (1984) EMBO J. **3**, 43–50
3. Unger, B., Clore, G. M., Gronenborn, A. M. & Hillen, W. (1983) EMBO J. **2**, 289–293
4. Kolb, A. & Buc, H. (1982) Nucleic Acids Res. **10**, 473–485
5. Liu-Johnson, H.-N., Gartenberg, M. R. & Crothers, D. M. (1986) Cell **47**, 995–1005
6. De Crombrugghe, B., Busby, S. & Buc, H. (1984) in Biological Regulation and Development (Yamamoto, K., ed.), pp. 129–167, Plenum Press, New York
7. Nissley, P., Anderson, W. B., Gallo, M., Perlman, R. L. & Pastan, I. (1972) J. Biol. Chem. **247**, 4264–4269
8. Zubay, G. (1980) Methods Enzymol. **77**, 856–877
9. Wu, F. Y.-H., Nath, K. & Wu, C.-W. (1974) Biochemistry **13**, 2567–2572
10. Blazy, B., Takahashi, M. & Baudras, A. (1980) Mol. Biol. Rep. **6**, 39–43
11. Stender, W. (1980) Biochem. Biophys. Res. Commun. **96**, 320–325
12. Weiel, J. & Hershig, J. W. B. (1981) Biochemistry **20**, 5859–5865
13. Weiel, J. & Hershig, J. W. B. (1982) J. Biol. Chem. **257**, 1215–1220
14. Boone, T. & Wilcox, G. (1978) Biochim. Biophys. Acta **541**, 528–534
15. Eilen, E., Pampeno, C. & Krakow, J. S. (1978) Biochemistry **17**, 2469–2473
16. Burgess, R. R. & Jendrisak, J. J. (1975) Biochemistry **14**, 4634–4638
17. Lowe, P. A., Hager, D. A. & Burgess, R. R. (1979) Biochemistry **18**, 1344–1352
18. Rinderknect, H. (1960) Experientia **16**, 430–433
19. Saxe, S. A. & Revzin, A. (1979) Biochemistry **18**, 255–263
20. Dandliker, W. B., Shapiro, H. C., Meduski, J. W., Alonso, R., Reigen, G. A. & Hamrick, J. R. (1964) Immunochemistry **1**, 165–191
21. Azumi, T. & McGlynn, S. P. (1962) J. Chem. Phys. **37**, 2412–2420
22. Hoare, D. G. (1972) Anal. Biochem. **46**, 604–615
23. Kao-Huang, Y., Revsin, A., Butler, A. P., O'Connor, P., Noble, D. W. & van Hippel, P. H. (1977) Proc. Natl. Acad. Sci. U.S.A. **74**, 4228–4232
24. Anderson, W. B., Schneider, A. B., Emmer, M., Perlman, R. L. & Pastan, I. (1971) J. Biol. Chem. **246**, 5929–5937
25. King, A. M. Q. & Nicholson, B. H. (1971) J. Mol. Biol. **62**, 303–319
26. Majors, J. (1975) Nature (London) **256**, 672–674
27. Hochschild, A., Irwin, N. & Ptashne, M. (1983) Cell **32**, 319–325

Received 8 June 1987/21 September 1987; accepted 17 November 1987