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CYCLIC ADENOSINE 3':5'-MONOPHOSPHATE RECEPTOR PROTEIN: INTERACTION WITH E. COLI RNA POLYMERASE

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SUMMARY: Antibodies against the σ subunit of E. coli RNA polymerase were used to investigate potential interactions between the cyclic adenosine 3':5'-monophosphate receptor protein and RNA polymerase holo enzyme. Cyclic adenosine 3':5'-monophosphate receptor protein enhances the complement fixation response of RNA polymerase and of the isolated σ subunit in the absence and presence of DNA and cyclic adenosine 3':5'-monophosphate. The results suggest a new quality of the assembly of the σ subunit and RNA polymerase core enzyme.

INTRODUCTION

The cyclic adenosine 3':5'-monophosphate receptor protein in E. coli in the presence of cyclic adenosine 3':5'-monophosphate functions as a positive control element in gene regulation of catabolite sensitive operons(1). The site on the DNA at which the CRP-cAMP complex specifically binds has recently been defined in the lac-operon(2,3). The position of the CRP binding site strongly suggests that stimulation of transcription by CRP-cAMP includes protein-protein interactions between CRP and RNA polymerase(4), and that this is true for other cAMP-dependent promoters as well(5). However, the mechanism by which CRP-cAMP stimulates RNA polymerase, whether directly or mediated by the DNA, is unknown. From the points of contacts between DNA and both proteins, CRP and RNA polymerase could touch each other on the same side of the DNA helix(2), provided DNA being undistorted.

The present investigation deals with the effects of CRP on RNA polymerase holo enzyme as measured by complement fixation with anti σ antibodies. The results show similar effects on holo enzyme and on the isolated σ subunit which may be attributed to specific interactions between CRP and the σ subunit.

Abbreviations: CRP, cyclic adenosine 3':5'-monophosphate receptor protein; cAMP, cyclic adenosine 3':5'-monophosphate; C'fixation, micro complement fixation.

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MATERIALS AND METHODS

CRP. CRP was isolated and purified according to Eilen, Pampeno & Krakow(6) from E. coli MRE 600. The specific activity in the cAMP binding assay was 3600 units/mg.
RNA Polymerase. The enzyme was isolated according to Burgess & Jendrisak (7). The specific activity of the holo enzyme on calf thymus DNA was 4240 units/mg, of core enzyme on poly[d(A-T)] 4500 units/mg. One unit gives the rate of GMP incorporation of 1 nmol/h at 37°. The σ subunit was isolated according to Lowe, Hager & Burgess(8).
Chemicals. Calf thymus DNA and cAMP were purchased from Boehringer(Mannheim). Bacteriophage lambda DNA was from Miles GmbH(Frankfurt/M).
Preparation of Antibodies. The antibodies (IgG-fraction) were raised in rabbits and purified as described(11).
Micro Complement Fixation. The assay was performed according to Levine (9,10) as described(11). No crossreaction between CRP and any of the RNA polymerase subunits was detected by agar gel double diffusion and C'fixation.

RESULTS

The C'fixation of holo enzyme with anti σ (1:2800) is increased to 100 % in the presence of CRP (Fig. 1A). Since the titration of holo enzyme at 10^{-9}M and anti σ (1:2800) with CRP resulted in 100 % C'fixation at the CRP concentrations used (10^{-11}- 10^{-7}M)(data not shown) and for better comparison with the results obtained with the free σ subunit, a titration was performed by using a 1:6000 dilution of anti σ (Fig. 1B)

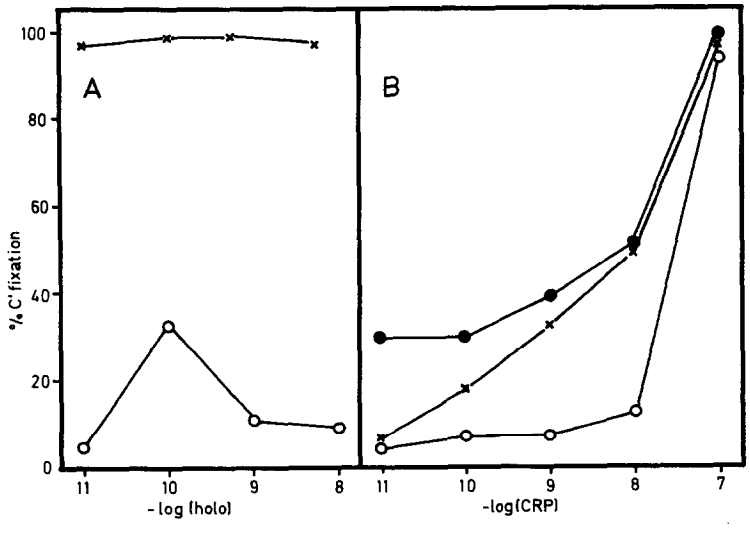

Figure 1: C'fixation of holo enzyme A: with anti σ(1:2800) in the absence (○) and presence (x) of 10^{-7}M CRP. B: Titration of holo enzyme (10^{-9}M) and anti σ (1:6000) with CRP in the absence (○) and presence of 10^{-5}M DNA (x) and 10^{-5}M DNA + 10^{-5}M cAMP (●).

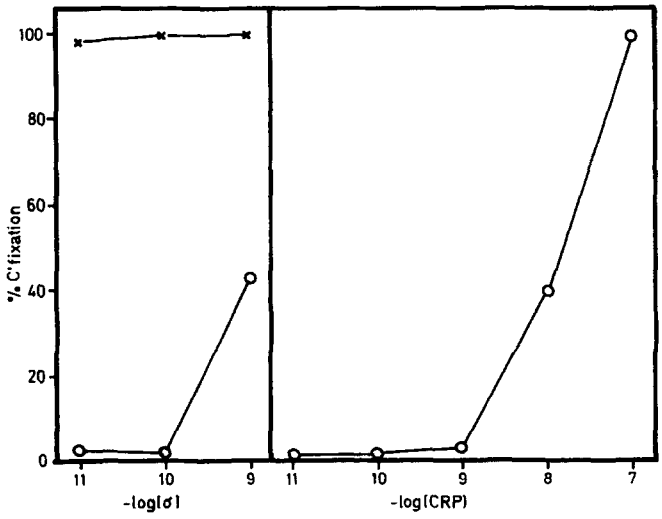

Figure 2: C'fixation of σ subunit with anti σ(1:6000). A: in the absence (○) and presence (x) of 10^{-7}M CRP. B: Titration of σ(10^{-10}M) and anti σ (1:6000) with CRP.

which is proper for free σ (Fig. 2 A) but does not show C' fixation of holo enzyme (data not shown). The titration curve (Fig. 1B) depends on the presence of cAMP and DNA.

The C'fixation of the free σ subunit could not be performed at higher σ concentrations because the high glycerol content of the rather diluted σ solution disturbed the C'fixation assay. However, again the presence of CRP increases the C' fixation with anti σ (1:6000)(Fig. 2A). The titration with increasing amounts of CRP is shown in Fig. 2B and reveals a similar shape than for holo enzyme (Fig. 1B).

RNA synthesis on bacteriophage lambda DNA as template was performed with holo enzyme in the absence and presence of CRP (Fig. 3). As can be seen, the incorporation of radioactivity into acid-precipitable material is reduced to the level of RNA synthesis by core enzyme in the presence of CRP. By adding cAMP however, more RNA is synthesized by holo enzyme in the presence of CRP than by holo enzyme alone.

DISCUSSION

The question of a direct interaction between CRP and RNA polymerase has been examined recently(4). It has been shown that under certain conditions CRP cosediments with RNA polymerase holo or core enzyme, particularly in the presence of

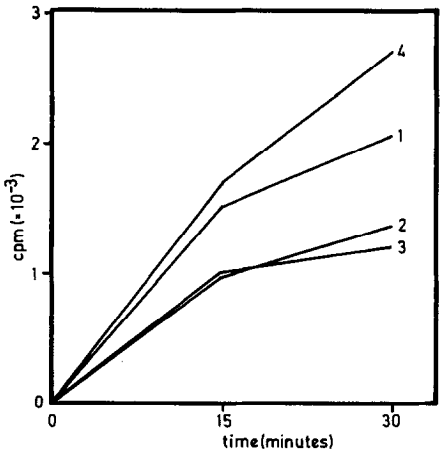

Figure 3: RNA synthesis on lambda DNA with holo enzyme(1), holo enzyme + 10^{-7}M CRP(2), core enzyme(3), and holo enzyme + 10^{-7}M CRP + 10^{-5}M cAMP(4). The assay mixture (100 ul) contained 0.8 pmol RNA polymerase, 0.5 pmol lambda DNA and was 0.7 mmol/l for substrates.

cAMP and calf thymus DNA. To locate subunit specific interactions of CRP on the holo enzyme, I used C'fixation with subunit specific antibodies which has proven successful in studying DNA binding to RNA polymerase and its subunits(11). Antibodies against the subunits α, β, and β' exerted no distinct response (data not shown). However, experiments with antibodies against the σ subunit gave some results which may help to elucidate the role of CRP in stimulating transcription.

The relevant data were obtained at an anti σ dilution of 1:6000. Under these conditions, holo enzyme responded to the presence of CRP in a fashion very similar to the free σ subunit (Fig. 1B and 2B). In addition, the titration of holo enzyme is changed to higher C'fixation in the presence of DNA and even more in the presence of DNA and cAMP. Whatever may be the reason for the increase of C'fixation by CRP, it is obvious that DNA and cAMP which should strengthen the interactions between CRP and RNA polymerase augment the effects of CRP alone (Fig. 1B). Furthermore, holo enzyme in the titration with CRP behaves like the free σ subunit. This strongly suggests that CRP changes the accessibility of anti σ to the σ subunit in the holo enzyme, either by separating σ from the remaining core enzyme or by impairing the contacts between σ and core enzyme. Since a release of σ from the holo enzyme upon addition of CRP has not been found (4) I would favour the

second explanation. Thus, in the presence of CRP, σ could still be loosely associated with core enzyme. This would enable σ to cosediment with the enzyme(4) and would explain the immunological behaviour of σ in the presence of CRP found in this investigation.

It is known that core enzyme transcribes DNA from bacteriophages to a lesser extent than holo enzyme. Thus I compared the RNA synthesis of holo enzyme on bacteriophage lambda DNA in the presence and absence of CRP. The result (Fig. 3) shows that holo enzyme in the presence of CRP behaves like core enzyme, supporting the suggestion of CRP action on holo enzyme made above. However, in the presence of cAMP, the RNA synthesis of holo enzyme is stimulated. Further experiments with isolated promoters will be required in order to show whether CRP and cAMP stimulate RNA synthesis by a premature release of σ subunit from the enzyme compared to the normal case where σ probably dissociates not until after some steps of elongation(12).

If the influence of CRP on σ found in the present study is mediated by a direct contact between these proteins one may ask whether by some distortion of the DNA during the binding of CRP and RNA polymerase, CRP is bound in opposition to RNA polymerase relative to the DNA strand thus allowing the contact between CRP and σ.

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REFERENCES

1. de Crombrugghe, B. & Pastan, I.(1978) in The Operon (Miller,J.A. & Reznikoff,W.S.,Eds.) pp. 303-324, Cold Spring Harbor Laboratory.
2. Simpson, R.B. (1980) Nucleic Acids Res. 8, 759-766.
3. Reznikoff, W.S. & Abelson, J.N.(1978) in The Operon (Miller, J.A. & Reznikoff, W.S.,Eds.) pp. 221-243, Cold Spring Harbor Laboratory.
4. Blazy, B., Takahashi, M. & Baudras, A.(1980) Mol.Biol.Reports 6, 39-44.
5. Taniguchi, T., O'Neill, M. & de Crombrugghe, B. (1979) Proc. Nat. Acad. Sci. U.S.A. 76, 5090-5094.
6. Eilen, E., Pampeno, C. & Krakow, J.S.(1978) Biochemistry 17, 2469-2473.
7. Burgess, R.R. & Jendrisak, J.J.(1975) Biochemistry 14, 4634-4638.

8. Lowe, P.A., Hager, D.A. & Burgess, R.R. (1979) Biochemistry 18, 1344-1352.
9. Levine, L. & VanVunakis, H.(1967) Meth. Enzymol. 11, 928-936.
10. Levine, L. (1978) in Handbook of Experimental Immunology, Vol. I (Immunochemistry), Weir, D.M.,Ed., pp. 5B.1-5B.8, 3rd. Ed., Blackwell Scientific Publishers, Oxford.
11. Stender, W. (1980) Nucleic Acids Res. 8, 1405-1420.
12. Krakow, J.S. & von der Helm, K. (1971) Cold Spring Harbor Symp. Quant. Biol. 35, 73-83.