
Is DNA unwound by the cyclic AMP receptor protein?

Annie Kolb and Henri Buc

Institut Pasteur, Département de Biologie Moléculaire, 25 rue du Dr Roux, 75724 Paris cedex 15, France

Received 20 November 1981; Accepted 14 December 1981

ABSTRACT

Superhelical pBR 322 derivatives have been relaxed by eukaryotic topoisomerase I in the presence or in the absence of *E. coli* cyclic AMP receptor protein (CRP) and of cyclic AMP (cAMP). CRP alone, or cAMP alone do not affect the average linking number of the distribution of the relaxed topoisomers. Hence, they do not unwind the template.

In the presence of cAMP, CRP induces a small unwinding. The extent of this unwinding is barely modified when the relaxation is carried out on a similar vector plasmid where the CRP binding site of the *lac* or of the *gal* operon has been inserted. Under these conditions, we checked that CRP occupies the lactose control site and that upon addition of RNA polymerase, the corresponding promoter is readily activated. These findings are difficult to reconcile with the proposal that activation of these promoters results from the binding of the CRP-cAMP complex to left-handed DNA sequences.

INTRODUCTION

The cyclic AMP receptor protein (CRP) of *E. coli* possesses at least two functions, both of which are mediated by the level of cyclic AMP (cAMP) in the cell. First, it promotes the efficient transcription of several operons, particularly those sensitive to catabolite repression, by increasing the rate of initiation of RNA synthesis (1). Second, it modulates the natural polarity of polycistronic gene units (2). The mechanisms by which the cAMP-CRP complex fulfils these two functions are still unknown.

Two models have been proposed to explain the cAMP-CRP mediated stimulation of the initiation of transcription. The first argues that direct contact between CRP and RNA polymerase provides the energy to increase the binding affinity of RNA polymerase to the promoter (3, 4). The second model assumes that CRP, upon binding to DNA, destabilizes neighbouring regions of the double helix (5) so that RNA polymerase could more easily form the open complex required for efficient initiation of transcription.

The available experimental evidence does not allow us to decide between

one or the other model. On the one hand, direct contact between RNA polymerase and cAMP-CRP may take place since stable complexes have been observed in solution, even in the absence of DNA (6). On the other hand, the position as well as the sequence of several CRP binding sites have been determined by genetic and biochemical means (3, 4, 7, 8, 9, 10). Although all are located upstream of the 5' end of the corresponding message, the distance between the CRP binding site and the start point of transcription varies, ranging from - 35 b.p. for the gal (7) and pBR-P4 (10) promoters to - 90 b.p. for the ara BAD gene (9). This variation makes the direct contact model less plausible, at least in its simplest version suggested by Gilbert (4).

By solving the structure of the CRP-cAMP complex at 2.9 Å resolution, Mc Kay and Steitz have greatly added to our knowledge (11). On the basis of model building studies, they proposed that two α helices of the CRP dimer would precisely fit into two successive major grooves of a left-handed B DNA. They suggested that the transition from a right-handed to a left-handed DNA helices induces "the denaturation of nearby regions of the helix, possibly the region unwound by RNA polymerase itself". Such an hypothesis implies that CRP, in binding to its specific site, would remove negative superhelical turns from a closed circular template ; it might also explain why, in vitro, CRP stimulation of polymerase activity is more effective on negatively supercoiled DNA (12).

This model can be tested by measuring the unwinding angle due to CRP binding to the lac or gal promoters on small, covalently closed DNAs. We show here that the predicted unwinding is not found.

MATERIALS AND METHODS

Proteins

E. coli CRP was generously given to us by B. Blazy and A. Baudras. It is at least 98 % pure as judged by electrophoresis on SDS polyacrylamide gels after staining with silver nitrate (13). It does not contain any endonuclease or topoisomerase activity.

Purified topoisomerase I from thymus DNA was kindly provided by J.C. Wang. We also use a chromatin extract of African green monkey kidney cell VerOP, prepared according to (14). It is further purified by chromatography on phosphocellulose. The column is eluted by a linear gradient of a potassium phosphate buffer pH 7.4 from 0.2 M to 0.6 M, containing 5 % of glycerol. Aliquots of topoisomerase I are made 50 % in glycerol, stored

at - 30°C and suitably diluted before use.

Plasmids

The detailed construction of these plasmids will be reported elsewhere. A 203 b.p. fragment (15), containing the whole promoter-operator site of the lactose operon was inserted in the Eco RI restriction site of pBR 322, either in the same orientation as the tetracycline gene or in the opposite direction. The original plasmid contains a single Pvu II restriction site at position 2068 (16). The fragment has also a single site for this enzyme at 17 b.p. from the i proximal end. Cutting these DNA with Pvu II and religating it in the presence of a Bam HI linker (decanucleotide purchased from BRL) results therefore in two types of plasmids (figure 1).

If the 203 b.p. insert is oriented as the tetracycline gene, the final 2323 b.p. plasmid, pRB 17, contains only a 17 b.p. fragment from the i gene. By contrast, if the 203 b.p. is inserted in the opposite direction, the final 2492 b.p. plasmid, pRB 186, contains the whole promoter-operator region.

This includes the corresponding lac CRP binding site. Several variants of pRB 186 exist, in particular those corresponding to the wild type promoter, to the L8+ and to the L8UV5 mutants.

A similar plasmid was constructed for the galactose control region. pMI 4 plasmid is a shorter derivative (2568 b.p.) of pMI 3 plasmid (17) which contains the 270 b.p. gal operator-promoter region inserted between the Eco RI and Hind III sites of pBR 322. pMI 3 was treated with Pvu II restriction enzyme, which cuts only in the vector and Hind III, which cuts at the frontier of the insert. After ligation, pMI4 is selected among transformants.

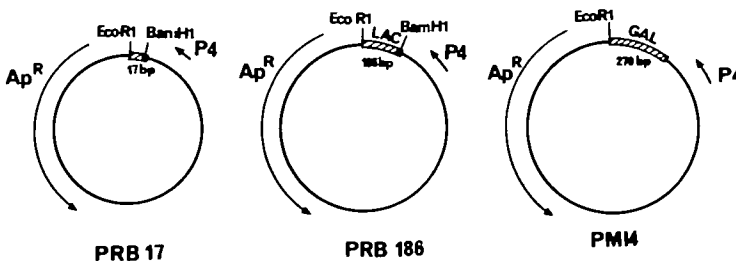


Figure 1 : Maps of pRB 17, pRB 186 and pMI 4 plasmids. The thin line is the remaining part of pBR 322 plasmid from the nucleotide position 2068 (Pvu II site) to Eco RI site at position 4362. The dashed area represents the insert. The arrows point towards the direction of transcription.

Relaxation of closed circular DNA

1.2 μg of DNA are incubated at 35° C in 60 μl of buffer I containing 40 mM Tris-HCl pH 8, 10 mM magnesium chloride, 100 mM potassium chloride, 1 mM DTT and 100 $\mu\text{g/ml}$ bovine serum albumin (Sigma, number A-6003, preheated for 15 minutes at 65° C). When required, CRP is incubated with the DNA for 15 minutes. 10 μl of purified topoisomerase I, diluted in the same buffer (5-50 units) are added and mixed. Incubation is continued either for one hour or overnight, and terminated by addition of SDS (1 % final) and EDTA (final concentration 25 mM) prewarmed at the same temperature.

The mixture is diluted to 220 μl with Tris 100 mM-EDTA 10 mM pH 8.0 and extracted with phenol ; it is made 0.3 M in ammonium acetate, precipitated with ethanol, rinsed and dried. The samples are dissolved in 60 μl of buffer II (10 mM Tris-HCl pH 8, EDTA 1 mM, 6 % Ficoll, 0.025 % bromophenol blue and 0.025 % xylene cyanol blue) before application of 15 μl to the agarose gel.

Electrophoresis

Electrophoresis is carried out at room temperature in a vertical slab gel apparatus using 1.5 % agarose gels (16 cm x 16 cm x 0.3 cm) equilibrated in the electrophoresis buffer III (40 mM Tris-HCl pH 8, 20 mM sodium acetate, 5 mM EDTA, 0.025 $\mu\text{g/ml}$ ethidium bromide). A field of 3 V/cm is applied to the gel until the xylene cyanol blue dye travels 10 cm (approximately 18 hours).

Determination of centers of masses of DNA species by fluorescence photography

After electrophoresis, the gel is stained with ethidium bromide (1.5 $\mu\text{g/ml}$) rinsed and photographed on Polaroid type 55 film ; the negative of the film is traced on a Vernon microdensitometer. As described by Depew and Wang (18), the amount of the respective topoisomers follows a gaussian distribution.

The change in the average linking number between a given sample and the blank, where CRP and cAMP are omitted, is determined as follows :

In the control, one measures the height of the most intense band, A, (corresponding to the i^{th} topoisomer) as well as the heights of the bands immediately below, B, or above, C. B corresponds to $\alpha = i + 1$ and C to $\alpha = i - 1$, α being the linking number. The average linking number associated to the median is $i + x$, with :

$$x = \frac{\log B - \log C}{2 (2 \log A - \log B - \log C)}$$

This operation is repeated for the other lanes. One locates again the most intense band for which $\alpha = j$ (the value $j - i$ is easily obtained by di-

rect inspection of the gel) and the distance x' between the median of the distribution and this band. One deduces the change in linking number with respect to the control : $\Delta x = j - i + x' - x$

Terminal ^{32}P labelling of DNA

The 203 b.p. lac fragment (15) with its Eco RI protruding ends is 5' end-labelled using γ - ^{32}P -ATP (Amersham 3,000 Ci/mole) and T4 polynucleotide kinase (Boehringer) (19). The DNA is digested with Pvu II to give a 186 b.p. fragment uniquely labelled at the Eco RI terminal.

DNAse protection

DNAse protection studies are carried out by the method of Galas and Schmitz (20). We incubate 0.03 μg of the labelled 186 b.p. lac fragment and 0.35 μg of cold relaxed pRB 17 with various amounts of CRP protein with or without 1 mM cAMP in 20 μl of buffer I at 35° C overnight.

In some experiments, buffer I is supplemented with 7 % glycerol. This is the glycerol concentration obtained when undiluted topoisomerase I is added to the mixture ; 5 μl of 0.08 $\mu\text{g}/\text{ml}$ of DNAse I (Worthington) in 10 mM Tris-HCl pH 8, 10 mM magnesium chloride, 10 mM calcium chloride, 0.1 mM DTT, 125 mM potassium chloride are added and the incubation is continued for 15 seconds. The digestion is stopped by addition of 200 μl of ammonium acetate (2.5 M) EDTA (25 mM) and sonicated calf thymus DNA (20 $\mu\text{g}/\text{ml}$). The mixture is extracted with phenol, precipitated and rinsed with ethanol. The DNA is redissolved and loaded on a 10 % urea-polyacrylamide gel as for DNA sequencing (21).

RESULTS

Principle

If a covalently closed circular DNA is fully relaxed by topoisomerase I, a family of topoisomers differing only in their linking number is generated. They can be resolved by electrophoresis. Under appropriate conditions, a set of bands appears on the gel, each DNA band differs from the adjacent one by one unit in the linking number. The relative masses of the different topoisomers conform to a Boltzmann distribution because of thermal fluctuations of the DNA helix at the time of ring closure. The position of the median of the gaussian curve can be precisely determined from intensity of each band, provided they are well resolved by the electrophoresis (22, 23).

When the relaxation of the covalently closed DNA is carried out in the same conditions, but in the presence of a ligand which alters the DNA structure, the relative amount of each topoisomer is modified. After removal of the ligand, the distribution of the species will still conform to a gaussian

curve ; if studied under the same electrophoretic conditions it differs from the preceding one by its median and in some cases by its width.

The change in the position of the median between the two experiments characterizes the change in the linking number at the time of ring closure and therefore the topological winding of the DNA induced by the ligand.

This method has already been used to measure the unwinding angle introduced by *E. coli* RNA polymerase (24), eukaryotic histones (25), the small basic prokaryotic protein HU (26), and *M. luteus* gyrase (27). Here, it is applied to measure the angular alteration induced by CRP on the plasmids described in Materials and Methods and in figure 1.

If the Mc Kay and Steitz model were correct, the cAMP-CRP complex, on binding to 20 b.p. of the lac region in a left-handed DNA form, should have a large effect on the topological structure of DNA. It should unwind two turns of right-handed DNA helix and wind it back two turns in the opposite direction resulting in the removal of at least four negative superhelical turns. After relaxation by topoisomerase I, the set of bands found on the gel will differ from those obtained in the control (- CRP) by at least four units in their linking number ($|\Delta x| \geq 4$). This is the lower expected limit because binding to a left-handed DNA form may also unwind regions adjacent to the fixation site. Additionally other CRP binding sites, located on the plasmid may also contribute to some unwinding such as the cAMP-CRP dependent promoter pBR-P4, which initiates transcription from position 2270 of the pBR 322 vector (10).

Choice of the experimental conditions

cAMP and CRP concentrations are adjusted so that the CRP sites are effectively occupied and so that promoter activation is observed upon addition of RNA polymerase. We have chosen to use a KCl, MgCl₂ buffer (buffer I, see Materials & Methods) since data on the stimulation of transcription initiation with wild-type and mutant lac promoters are available in the same buffer (12). On the wild type promoter, full activation is observed for a CRP to promoter ratio of 2.5 and for a cAMP concentration of 10 μM. Furthermore, the composition and ionic strength of buffer I is very close to the intracellular conditions of the bacteria ; additionally the presence of 100 mM KCl should also minimize binding of CRP to secondary sites of DNA (8).

The CRP concentration to be used is determined by probing the occupancy of the lac CRP site using DNase footprinting on a labelled fragment. During the relaxation experiments, other CRP sites present on the vector plasmid may compete with the lac CRP binding site. To take this fact into account, we add

to the 186 bp, *lac* fragment, labelled on its lower strand, an equimolar amount of cold relaxed plasmid pRB17. Fig.2 shows the pattern of the footprint of the CRP at different concentrations in binding buffer I (lanes 1 to 9) or in the same buffer containing 7% glycerol (lanes 10 to 18, see Materials & Methods). The cAMP-CRP protection against DNase attack is localised in the -60 region from the transcription start. Some modifications induced by the presence of the protein on the DNA (attenuation or enhancement of bands) clearly appear along the gel in the region depicted by the brackets; particularly striking is the disappearance of the band at -66 which maps close to the L8 mutation site; this band completely disappears at CRP concentrations higher than 440 nM. There is no protection in the absence of cAMP (compare lanes 15 to 16) and the pattern is hardly affected by addition of 7% glycerol. However, the pattern of protection we observed is weaker than the one reported by Schmitz (8). This is probably due to a lower glycerol content and to a higher ionic

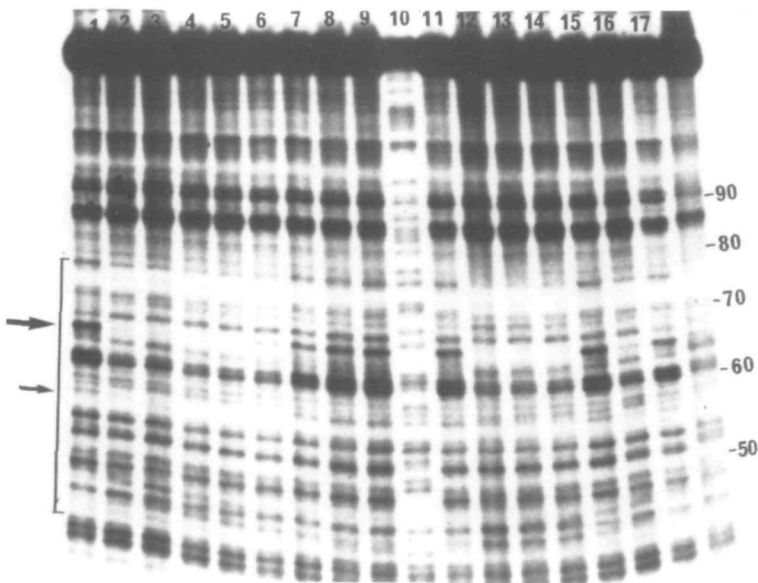


Figure 2 : Footprints of CRP on 186 b.p. *lac* promoter DNA fragment (10 nM) in the presence of relaxed pRB 17 plasmid (10 nM). Lanes 1, 9 and 11 are controls without CRP. Lane 10 contains the sequencing reaction products G + A. Experiments are carried out with 1 mM cAMP except in lanes 1, 8, 15 and 17 where no cAMP is added. In lanes 11-18, 7% glycerol is added in the mixture. Concentrations of CRP are as follows : 1,660 nM (lanes 2, 3, 12) ; 880 nM (lanes 4, 13) ; 440 nM (lanes 5, 14, 15) ; 220 nM (lanes 6, 16, 17) ; 75 nM (lanes 7, 8, 18).

strength in our conditions. We do not detect any change in the footprinting pattern of the CRP interaction site located at -60 upstream to the promoter at CRP concentrations varying from 440/1660 nM. This shows that the functional CRP binding site is already fully occupied by the protein at 440 nM despite the presence of competing plasmid DNA sequences.

Unwinding experiments

Figure 3 illustrates the electrophoretic pattern of the plasmid DNAs after treatment with topoisomerase I in the absence and presence of CRP (1.7 μ M per 10 nM of plasmid DNA) and cAMP (1 mM) in buffer I. The figure shows the distribution of topoisomers on the wild type lac pRB 186 plasmid (lanes 1a to 1d) on the control plasmid pRB 17 (lanes 2a to 2d) and on the gal pMI 4 plasmid (lanes 3a to 3d). 1 mM cAMP has no effect (compare 1a, 2a, 3a to 1b, 2b, 3b), CRP alone has also no effect (lanes 1c, 2c, 3c). In contrast, in the presence of 1mM cAMP, CRP induces a small unwinding in the plasmid DNAs (lanes 1d, 2d, 3d). The scans of the relevant gels are given in fig.

0 1a 1b 1c 1d 2a 2b 2c 2d 3a 3b 3c 3d 4

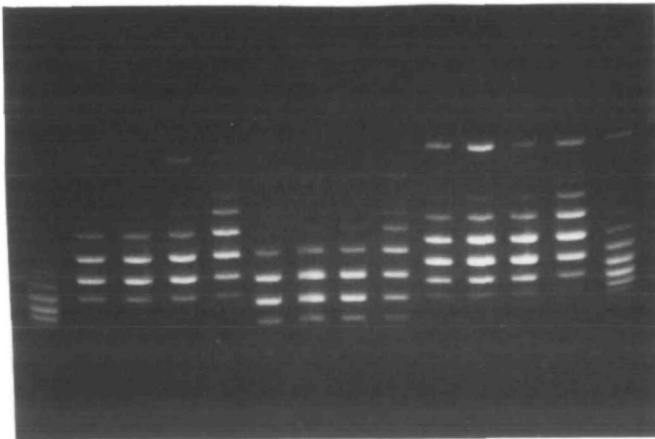


Figure 3 :

Photograph of a 1.5 % agarose gel run with electrophoresis buffer III containing 0.025 μ g/ml ethidium bromide. The purified superhelical plasmid DNAs, pRB 186 and pMI 4 isolated from the bacteria appear on the gel in lanes 0 and 4 as negatively supercoiled species. The plasmids relaxed with topoisomerase I alone are shown in the positive supertwisted domain which offers optimal resolution for the bands. 1a : pRB 186, 2a : pRB 17, 3a : pMI 4. Lanes 1b, 2b, 3b display the same plasmids relaxed in the presence of 1 mM cAMP. In lanes 1c, 2c and 3c, the relaxation is carried out with 1.7 μ M CRP and in lanes 1d, 2d and 3d in the presence of 1.7 μ M CRP and 1 mM cAMP.

4 where the medians of the gaussian curves are indicated by arrows.

Comparison of figures 4A and 4B, which show respectively the pattern of relaxation with CRP in the presence and absence of 1 mM cAMP for the pRB 186 lac plasmid, allows us to determine the unwinding angle induced by the cAMP-CRP complex : the data are summarized in table I. Examination of scans in fig. 4C and 4D, which depict a similar experiment for control plasmid pRB 17, indicates that the presence of lac insert causes no major change in the differential patterns.

Similar experiments can be performed varying the cAMP and the CRP concentrations (table I). The concentration of CRP has been varied from 0 to 3 μ M in the presence or absence of 1 mM cAMP. Again unwinding is only observed when both CRP and cAMP are present. Unwinding increases as the amount of CRP increases (table I) for all the plasmids which have been tested. No significant difference is observed between pRB 17, pMI 4 and the lac pRB 186 plasmid where the L8 mutation has been introduced ; this mutation lowers the affinity of CRP for the lac promoter by a factor of at least 10.

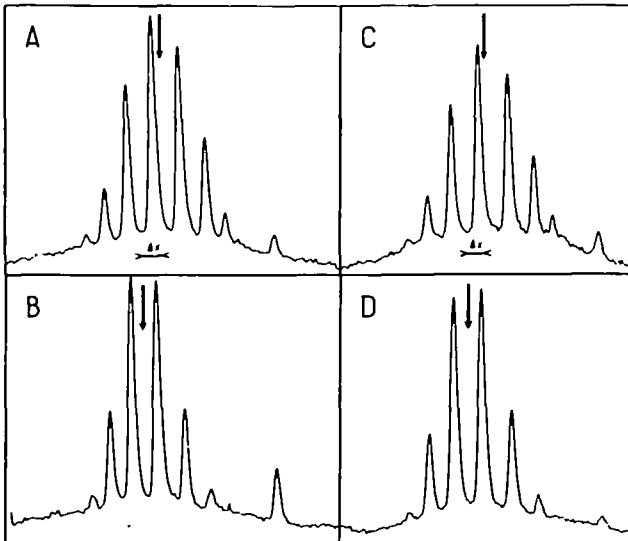


Figure 4 : Representative gel scans from fig. 3. Parts A & B refer to lanes 1d and 1c of fig. 3. Relaxation of 10 nM pRB 186 lac plasmid in the presence of 1.7 μ M CRP with or without 1 mM cAMP. Parts C & D are scans of lanes 2d and 2c which show relaxation of 10 nM pRB 17 control plasmid in the presence of 1.7 μ M CRP with or without 1 mM cAMP. The arrows show the median of the gaussian distribution of topoisomers.

[CRP] _{nM}	75	275	550	1100	1700	3400
pRB 17	0.1(2)	0.2(5)	0.4(6)	0.5(1)	0.6(8)	1.1(1)
pRB 186 <u>lac</u> wild type	0.1(0)	0.3(1)	0.5(3)	0.6(6)	0.7(8)	1.2(2)
pRB 186 L8UV5	0.1(1)		0.4(4)			
pRB 186 L8+			0.4(8)			
pMI 4			0.4 *		0.5 *	1.0
δ (Δx)			- 0.07	- 0.15	- 0.10	- 0.11

TABLE I : Unwinding due to the binding of the cAMP-CRP complex on the plasmid DNAs as a function of CRP concentration. It is expressed in turns and corresponds to $-\Delta x$, the distance which separates the centers of the two gaussian distributions of DNA species relaxed either in the presence of CRP and 1 mM cAMP or in their absence.

In each case, several independant measurements have been done (except for the values labeled with an asterisk). Their average is given in the Table. The absolute errors on each determination is of the order of 0.1 turn, so that the second decimal () is not significant. It is given here only for the sake of the computation of the average change $\delta(\Delta x)$ observed between pRB 186 lac wild type and pRB 17, as well as for the determination of the corresponding standard error (for this computation, CRP concentrations larger than 275 nM have been taken in consideration).

In the case of the wild type lac promoter, for each CRP concentration, the cAMP dependent unwinding which is observed is slightly higher than in the control pRB 17 plasmid (see table I). The average observed change Δx (pRB 186) - Δx (pRB 17) amounts to $- 0.1 \pm 0.06$ turn. Though the trend seems consistent, one has to keep in mind that the error on the determination of the median, in a given experiment is of the order of 0.1 turn. Lowering the cAMP concentration in the assay does not increase the difference between the unwinding of pRB 186 and pRB 17.

The constant increase in unwinding observed for the pRB 17 plasmid, when the CRP concentration is increased, is attributed to non-specific binding. The amount of CRP-cAMP bound in such a way under similar ionic conditions has previously been measured (28). From these data, we estimate that one cAMP-CRP dimer bound non specifically unwinds the DNA by about 0.03 turn.

We repeated the unwinding experiments in the presence of E. coli RNA polymerase in order to check whether the presence of RNA polymerase is required for CRP-cAMP to unwind the DNA. We have compared the unwinding angle observed when RNA polymerase alone is bound to the lactose promoter with the value measured when CRP, cAMP and RNA polymerase are all assembled on the

same control region. The results are complicated as RNA polymerase alone causes a large unwinding of the template plasmid (24). Adding CRP in the presence of cAMP slightly increases this unwinding angle on the pRB 186 lac wild type plasmid. Therefore even in the presence of RNA polymerase, the differential unwinding due to cAMP-CRP binding on the lac insert, is low but seems significant (~ 0.5 turn).

DISCUSSION

From the above results, it appears that the cAMP-CRP complex binds at the lactose and galactose promoters without much unwinding of the template. No specific unwinding can be detected at the galactose control site or at a lactose control site which carries the L8 mutation. We estimate the specific unwinding at the wild type lactose site to be 0.1 turn. The measure of the extent of unwinding is subject to a large margin of error, since our determination is of differential nature, and since the control plasmid shows a significant amount of unwinding. However, it is clear that the specific unwinding at the wild type lactose site, when CRP is bound to it, cannot exceed 0.5 turn.

The presence of RNA polymerase does not modify dramatically this value and we can again ascertain that the differential unwinding due to CRP-cAMP does not exceed 1 turn in this case. Measurements relative to the change of DNA topology are obviously less powerful than structural determinations. However, any structural model depicting the interaction between the cAMP-CRP complex and its cognate site should be compatible with the topological determination. Some models have been proposed (3,4,5,29,30) which do not predict a great unwinding and which therefore cannot be challenged by the present results. But we must conclude that the Mc Kay-Steitz model is either inaccurate or incomplete. Either the CRP molecule does not convert the DNA template from a right-handed to a left-handed helix, or there is a compensatory mechanism taking place in some other part of the DNA-CRP complex which results in a topological winding of the template by c.a. 3.5 turns. Though this latter case cannot be excluded, this seems to be an "ad hoc", unlikely explanation. We would rather suggest that a specific complex between CRP and the lactose promoter can be formed without a considerable structural rearrangement of the DNA. We cannot exclude a minor but significant conformational change as for example a B to A transition.

Our determinations are consistent with measurements of a completely different nature. The abortive initiation assay has been used to character-

size the rate of initiation of transcription *in vitro* at the lactose promoter on plasmids of various supercoiling densities (12). In particular, P. Malan has determined that a linear fragment carrying the wild type lactose control region requires a 40 fold higher concentration of cAMP to be activated than a supercoiled pBR 322 plasmid into which the same DNA had been inserted. From this number one can deduce that during the transition from an inactive to an active promoter, the cAMP-CRP complex contributes to the promoter unwinding for roughly one third of a turn (for the basis of such a computation see 31, 32).

The fact that promoters of catabolite sensitive operons are hypersensitive to inhibitors of DNA gyrase (33) has been interpreted to support the proposal that CRP binds to an unwound form of DNA (5,11). In fact, kinetic analysis of the initiation of transcription at the wild type lac promoter (12) shows that supercoiling does not affect the initial binding of RNA polymerase at the promoter, but rather is necessary for the isomerisation step which leads to transcription initiation. It appears that CRP and supercoiling exert their activating role at two distinct steps of the kinetic pathway which leads to an active RNA polymerase lactose-promoter complex. Therefore it is not necessary to imply direct coupling between the action of CRP and the unwinding of DNA.

ACKNOWLEDGMENTS

We are indebted to B. Blazy and J. Wang respectively for their gifts of CRP protein and topoisomerase I. We thank A. Spassky and S. Busby for the construction of pRB 17, pRB 186 and pMI 4 plasmids. The technical assistance of A. Lechien through the whole course of this work is warmly acknowledged. We are grateful to F. Horodniceanu (Institut Pasteur) for providing us with facilities to grow kidney Vero P. cells in his lab.

This work has been supported by grants from the Délégation Générale de la Recherche Scientifique et Technique (MRM n° 81.E.0419) and from the Fondation pour la Recherche Médicale Française.

REFERENCES

1. de Combrugge, B. and Pastan, I. (1978) in the Operon, Miller, J. and Reznikoff, W. Eds., pp. 303-324, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
2. Ullmann, A., Joseph, E. and Danchin, A. (1979) Proc. Nat. Acad. Sci. USA 76, 3194-3197.
3. Majors, J. (1975) Nature 256, 672-674.

4. Gilbert, W. (1976) in RNA polymerase, Losick, R. and Chamberlin, M. Eds., pp. 193-205, Cold Spring Harbor Laboratory, New York.
5. Dickson, R.C., Abelson, J., Barnes, W.M. and Reznikoff, W.S. (1975) *Science* 187, 27-35.
6. Blazy, B., Takahashi, M. and Baudras, A. (1980) *Molec. Biol. Rep.* 6, 39-43.
7. Taniguchi, T., O'Neill, M. and de Combrughe B. (1979) *Proc. Nat. Acad. Sci. USA* 76, 5090-5094.
8. Schmitz, A. (1981) *Nucl. Acids Res.* 9, 277-292.
9. Lee, N.L., Gielow, W.O. and Wallace R.G. (1981) *Proc. Nat. Acad. Sci. USA*, 78, 752-756.
10. Queen, C. and Rosenberg, M. (1981) *Nucl. Acids Res.* 9, 3365-3377.
11. McKay, D.B. and Steitz, T.A. (1981) *Nature* 290, 744-749.
12. P. Malan's thesis, Harvard University, September 1981 - Malan, P., Kolb A. Buc, H. McClure, R.W. in preparation.
13. Oakley, B.R., Kirsch, D.R. and Morris, N.R. (1981) *Anal. Biochem.* 105, 361-363.
14. Germond, J.E., Hirt, B., Oudet, P., Gross-Bellard, M. and Chambon, P. (1975) *Proc. Nat. Acad. Sci. USA* 72, 1843-1847.
15. Ogata, R. and Gilbert, W. (1977) *Proc. Nat. Sci. USA* 74, 4973-4976.
16. Sutcliffe, J.G. (1978) *Cold Spring Harbor Symp. Quant. Biol.* 43, 77-90.
17. Busby, S.J.W., Irani, M. and de Combrughe, B., *J. Mol. Biol.* submitted for publication.
18. Depew, R.E. and Wang, J.C. (1975) *Proc. Nat. Acad. Sci. USA* 72, 4275-4279.
19. Berkner, K.L. and Folk, W.R. (1977) *J. Biol. Chem.* 252, 3176-3184.
20. Galas, D. and Schmitz, A. (1978) *Nucl. Acids Res.* 5, 3157-3170.
21. Maxam, A. and Gilbert, W. (1980) *Methods in Enzymology* 65, 499-560.
22. Keller, W. (1975) *Proc. Nat. Acad. Sci. USA* 72, 4876-4880.
23. Shure, M., Pulleyblank, D.E. and Vinograd, J. (1977) *Nucl. Acids Res.* 4, 1183-1205.
24. Wang, J.C., Jacobsen, J.H., Saucier, J.-M. (1977) *Nucl. Acids Res.* 4, 1225-1241.
25. Germond, J.-E., Rouvière-Yaniv, J., Yaniv, M. and Brutlag, D. (1979) *Proc. Nat. Acad. Sci. USA* 76, 3779-3783.
26. Rouvière-Yaniv, J., Yaniv M. and Germond, J.E. (1979) *Cell* 17, 265-274.
27. Liu, L.F. and Wang, J.C. (1978) *Proc. Nat. Sci. USA* 75, 2098-2102.
28. Takahashi, M., Blazy, B. and Baudras, A., *Nucl. Acids Res.* (1979) 7, 1699-1712.
29. O'Neill, M.C., Amass, K. and de Combrughe, B. (1981) *Proc. Nat. Sci. USA* 78, 2213-2217.
30. E. Bright, R.H. and Wong, J.R. (1981) *Proc. Nat. Acad. Sci. USA*, 78, 4011-4015.
31. Davidson N. (1972) *J. Mol. Biol.* 66, 307-309.
32. Wang, J.C. (1980) *Trends in Biochem. Sci.* 5, 219-221.
33. Sanzey B. (1979) *J. Bact.*, 138, 40-47.

