Vol. 136, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 14, 1986 Pages 404-410

BINDING OF recA PROTEIN FROM E. Coli TO DOUBLE-STRANDED DNA : INFLUENCE OF THE DEGREE OF SUPERHELICITY

Marie CHABBERT*, Christian CAZENAVE, Tula SAISON-BEHMOARAS and Claude HELENE

Laboratoire de Biophysique, INSERM U.201 & CNRS UA.481, Muséum National d'Histoire Naturelle, 61, Rue Buffon, 75005 PARIS, France

Received February 25, 1986

The binding of the recA protein from E. coli to supercoiled double-stranded DNA is strongly dependent upon the superhelical density of the DNA molecule. A threshold of superhelical density is required for strong binding in the presence of ATP. This finding is consistent with a model in which recA protein first binds to unpaired regions and then polymerises on the contiguous double-stranded lattice.

The recA gene product of E. coli is involved in two important events : general genetic recombination and induction of the SOS response (for reviews see references 1 and 2). The different activities of the recA protein require its binding to single-stranded and double-stranded DNA. However these two effectors (or substrates) are not equivalent. Under physiological conditions recA protein binds more strongly to single-stranded DNA than to double-stranded DNA (3) although the effect can be reversed at low salt (4). In the presence of ATP, binding to single-stranded DNA stimulates further binding to homologous double-stranded DNA (5). Binding of recA protein to a duplex DNA is very dependent upon the topology of the DNA molecule. At low Mg^{2+} concentration binding to supercoiled DNA molecules in the presence of ATP and subsequent unwinding do not require homologous single-stranded DNA (6). In this case, the ATPase activity of recA protein is two-third that induced by single-stranded DNA of identical length whereas it is reduced ten-fold when it is induced by

* Present address : Faculté de Pharmacie, Université Louis Pasteur STRASBOURG , France

0006-291X/86 $1.50

a linear duplex DNA (7). Formation of D-loops is also greatly dependent on the topology of the double-stranded molecule (8). Anomalous kinetics are observed when the double-stranded molecule is negatively supercoiled implying that specific interactions occur between recA protein and the double-stranded lattice starting from D-loop (9).

A better knowledge of the mechanism of recA protein binding to DNA and especially of the role of DNA superhelicity is required in order to understand the ubiquitous properties of this protein. For this purpose we examined the binding of recA protein to negatively supercoiled DNA molecules (form I DNA) as a function of superhelical density. We found that binding is strongly dependent upon superhelical density of the DNA molecule and that a minimum degree of unwinding is obviously required for strong binding.

MATERIALS AND METHODS

Chemicals : recA protein was purified from strain KM1842 as already described (10). The pAO_3 plasmid was purified from strain **E. coli** C 600 (a generous gift from Dr. Oka) by cesium chloride centrifugation. Creatine kinase, creatine phosphate, adenosine triphosphate (ATP) and adenosine-5'O-γ thiotriphosphate (ATPγS) were purchased from Boehringer Mannheim. The concentration of the plasmid was expressed as moles of phosphate residues per liter and determined from its absorbance at 260 nm using $\varepsilon = 6500\ M^{-1}\ cm^{-1}$

Binding assay : Reactions were carried out in 20 mM Tris-HCl (pH 7.5), 4 mM $MgCl_2$, 0.1 mM EDTA and 1 mM 2-mercaptoethanol (18 µl final volume). Unless stated otherwise, an ATP-regenerating system consisting in a mixture of creatine phosphate and creatine kinase was also present in the reaction mixture. When present, the final concentrations of pAO_3, recA, ATP, creatine kinase and creatine phosphate were 130 µM, 25 µM, 2.4 mM, 100 U/ml and 4.1 mM respectively. The reaction was initiated by addition of recA protein to the mixture of all other components. Samples were subsequently incubated at 37°C for 30 minutes (first incubation) then, when stated, ATPγS was added to a final concentration of 230 µM and the reaction allowed to proceed for 5 minutes (second incubation). At the end of the second incubation, samples were chilled in an ice-water bath and 8 µl of loading buffer (20 mM Tris-HCl pH 7.5, 20 % (v/v) glycerol, 4 mg/ml bromophenol blue) were added. Then samples were loaded on a composite acrylamide-agarose gel.

Composite acrylamide-agarose gel electrophoresis : Samples were run on a 2 % acrylamide, 0.5 % agarose gel in a buffer containing 36 mM Tris-HCl (pH 7.7), 30 mM NaH_2PO_4, 1 mM EDTA at 5 V/cm for 12 hours at room temperature according to Germond **et al.** (11). At the end of the electrophoresis, the gel was stained with 2.5 µg/ml ethidium bromide for 30 minutes at room temperature, extensively rinsed and photographed under UV light through a red filter. The negative of the gel photograph was analyzed with a Helena densitometer.

RESULTS

Native pAO_3 DNA was resolved into topoisomers by gel electrophoresis (figure 1, lane 8). The linking number difference of the fastest topoisomer (at the bottom of the lane) was nine as compared to relaxed DNA (12). Six different topoisomers ranging in linking number differences from 9 to 4 were clearly separated on the gel. Topoisomers with smaller linking numbers were not present in appreciable amount in the natural population of plasmids extracted from **E. coli** C600. The major band at the top of the lane corresponds to the open circular molecule (form II DNA).

In control experiments where pAO_3 plasmid was incubated at 37°C for 30 minutes with all components except recA protein (figure 1, lane 1)

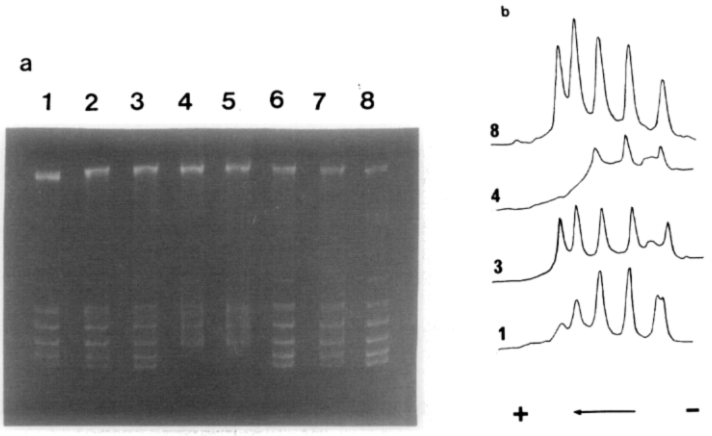

Figure 1 : a) Electrophoresis in 2 % acrylamide - 0.5 % agarose gel of pAO_3 DNA incubated with recA protein, ATP, ATPγS as described in the following table whose columns correspond to the different lanes of the gel. When present, ATPγS was added just before the second incubation. Concentrations and experimental conditions are described in Materials and Methods.

	1	2	3	4	5	6	7	8
pAO_3	+	+	+	+	+	+	+	+
recA	-	+	+	+	+	+	+	-
ATP	+	-	+	+	+	-	-	-
ATP regenerating system	+	-	+	+	-	-	+	-
1st incubation (30 minutes)	+	+	+	+	+	-	-	-
ATPγS	+	-	-	+	+	-	+	-
2nd incubation (5 minutes)	+	+	-	+	+	+	+	-

b) Densitometer tracings of lanes 1, 3, 4 and 8 of the gel.

the intensity of the two fastest-moving topoisomers decreased. This effect was particularly striking for topoisomer 9. A new extra band appeared just below the band which corresponds to the topoisomer having five supercoils. This observation is consistent with the existence of a transition to a cruciform structure occurring in the largest palindrom of pAO_3 DNA which is 31 bp long (13, 14). Cruciform formation was demonstrated by 2D-gel electrophoresis (results not shown). During incubation some cleavage occurred converting supercoiled molecules to form II DNA. This phenomenon might be due to a nuclease contamination.

When recA protein was added in the absence of ATP or ATPγS (Figure 1, lane 2) a very faint band appeared between bands 4 and 5. The relative decrease of the intensity of the fastest-moving band was less important than in the absence of recA protein. However the cruciform structure appeared very clearly. When pAO_3 plasmid was incubated with recA protein in the presence of ATP, the relative intensities of the different bands did not change as compared with pAO_3 alone except for the faint band that appeared between topoisomers 4 and 5 (Figure 1, lane 3). The most supercoiled topoisomer was not converted into the cruciform structure. The most likely explanation is that, under these conditions, recA protein interacts with this topoisomer preventing it to undergo the transition. However such complexes between recA protein and pAO_3 plasmid might be instable and dissociate during loading (3). When ATPγS, a non-hydrolysable ATP analog was used instead of ATP, the intensities of all the bands of form I DNA decreased dramatically (figure 1, lane 7). Increase in the amount of form II DNA was not sufficient to account for such a decrease (compare lanes 6 and 7 of figure 1). A diffuse fluorescence of ethidium bromide was seen on the gel. When the incubation time increased, all the discrete bands vanished and a continuous diffuse zone situated above the band corresponding to topoisomer 8 increased in intensity (data not shown). Therefore the complexes recA protein- pAO_3 DNA formed in the presence of ATPγS are stable enough to alter the migration

of the plasmid. Nevertheless no striking selective effects linked to superhelical density were noticed under these conditions.

As shown on lanes 4 and 5 of figure 1, a striking selective effect of linking number was observed when recA protein was first incubated with pAO_3 plasmid in the presence of ATP and then for a short time in the presence of ATPγS. In this case, the two fastest-moving bands of supercoiled DNA disappeared selectively while a diffuse region appeared above the position of the band corresponding to topoisomer 8. The faint band between topoisomers 4 and 5 was still observed. A similar procedure for stabilizing recA protein-DNA interactions was previously used by Soltis and Lehman (15). It allowed the visualization of the complexes preformed during the first incubation. When the ATP regenerating system was omitted (compare lanes 4 and 5 of figure 1), the band corresponding to cruciform DNA was observed. Such an effect was probably due to ADP resulting from DNA-dependent hydrolysis of ATP catalyzed by recA protein which inhibited further binding of recA protein to DNA.

DISCUSSION

This study clearly demonstrates that a threshold of superhelical density is required for strong binding of recA protein to double-stranded DNA in the presence of ATP. This threshold corresponds to the density of the topoisomer having eight negative supercoils i.e. - 0.049. The torsional stress associated with negative supercoiling facilitates separation of the two DNA strands. Theoretical and experimental studies (16-18) have shown that the number of unpaired base pairs increases markedly when superhelical density increases over a threshold comprised between - 0.03 and - 0.08 depending on the plasmid and on the experimental conditions. The presence of a protein might shift the threshold value. A plausible explanation for our findings is that recA protein first binds to unpaired regions and then invades the contiguous double-stranded lattice via a nucleation-polymerisation process. The polymeric structure of recA protein bound to a supercoiled plasmid has been previously re-

vealed by electron microscopy (19, 20). The threshold of superhelicity required for binding is expected to depend upon ionic conditions since lowering the ionic strength destabilizes the double helix. As a matter of fact, binding of recA protein to a supercoiled DNA is easier at low rather than high Mg^{2+} concentration (6). A similar binding mechanism with a nucleation step on a single-stranded region, followed by polymerization on the adjacent lattice, might take place when recA protein binds to gapped DNA (21) or on DNA bearing D-loops (9). However, studies with monoclonal antibodies clearly demonstrate differences in the structure of recA protein bound to double-stranded and single-stranded lattices (22). Single-stranded regions act as "activator" allowing cooperative binding on the double-helix. The recA-DNA complex is then able to promote recombinational events (23).

In vivo, single-stranded regions could result from the action of the recBC enzyme (24) or from the replication of damaged DNA which leaves gaps opposite lesions (25). The requirement of a single-stranded region to induce recA protein binding may greatly increase the number of recombination events when DNA has been damaged and thus allow repair by recombinational mechanisms (26).

ACKNOWLEDGMENTS : We wish to thank Dr. Oka for a gift of the **E. coli** C 600 strain containing the pAO3 plasmid used in the above studies and Dr. K. McEntee for a gift of the **E. coli** KM 1842 strain overproducing the recA protein. We acknowledge the skillful assistance of L. Perrouault in the preparation of plasmids and gels.

REFERENCES

1. Little, J.W., & Mount, D.W. (1982) Cell, **29**, 11-22.
2. Dressler, D., & Potter, H. (1982) Ann. Rev. Biochem., **51**, 727-761.
3. McEntee, K., Weinstock, G.M., & Lehman, I.R. (1981) J. Biol. Chem., **256**, 8835-8844.
4. Cazenave, C., Chabbert, M., Toulmé, J.J., & Hélène, C. (1984) Biochim. Biophys. Acta, **781**, 7-13.
5. Ohtani, T., Shibata, T., Iwabuchi, M., Watabe, H., Iino, T., & Ando, T. (1982) Nature, **299**, 86-89.
6. Iwabuchi, M., Shibata, T., Ohtani, T., Natori, M., & Ando, T., (1983) J. Biol. Chem., **258**, 12394-12404.
7. Shibata, T., Das Gupta, C., Cunningham, R.P., & Radding, C.M. (1979) Proc. natl. Acad. Sci. USA, **76**, 1638-1642.
8. Shibata, T., Das Gupta, C., Cunningham, R.P., Williams, J.G.K., Osber, L., & Radding, C.M. (1981) J. Biol. Chem., **256**, 7565-7572.

9. Shibata, T., Ohtani, T., Chang, P.K., & Ando, T. (1982) J. Biol. Chem., 257, 370-376.
10. Cazenave, C., Toulmé, J.J., & Hélène, C. (1983) EMBO J., 2, 2247-2251.
11. Germond, G.E., Hirt, B., Oudet, D., Grossbellard, H., & Chambon, P. (1975) Proc. Natl. Acad. Sci. USA, 72, 1843-1847.
12. Panyutin, I.G., Lyamichev, V.I., & Lyubchenko, Y.L. (1982) FEBS Lett., 148, 297-301.
13. Lyamichev, V.I., Panyutin, I.G., & Frank-Kamenetskii, M.D., (1983) FEBS Lett., 153, 298-302.
14. Panyutin, I., Klishko, V., & Lyamichev, V. (1984) J. Biomol. Struct. Dyn., 1, 1311-1324.
15. Soltis, D.A., & Lehman, I.R. (1983) J. Biol. Chem., 258, 6073-6077.
16. Benham, C.J. (1979) Proc. Natl. Acad. Sci. USA, 76, 3870-3874.
17. Vologodskii, A.V., Lukashin, A.V., Anshelevich, V.V., & Frank-Kamenetskii, M.D. (1979) Nuc. Ac. Res., 6, 967-982.
18. Vologodskii, A.V., & Frank-Kamenetskii, M.D. (1981) FEBS Lett., 131, 178-180.
19. Staziak, A., & Di Capua, E. (1982) Nature, 299, 185-186.
20. Dunn, K., Chrysogelos, S., & Griffith, J. (1982) Cell, 28, 757-765.
21. West, S.C., Cassuto, E., Mursalim, J., & Howard-Flanders, P. (1980) Proc. Natl. Acad. Sci. USA, 77, 2569-2573.
22. Shibata, T., Makino, O., Ikawa, S., Ohtani, T., Iwabuchi, M., Shibata, Y., Maeda, H., & Ando, T. (1984) Cold Spring Harbor Symp. Quant. Biol., 49, 541-551.
23. West, S.C., & Howard-Flanders, P. (1984) Cell, 37, 683-691.
24. Taylor, A., & Smith, G.R. (1980) Cell, 22, 447-457.
25. West, S.C., Cassuto, E., & Howard-Flanders, P. (1981) Nature, 294, 659-662.
26. Walker, G.C. (1985) Ann. Rev. Biochem., 54, 425-457.