THE ROLE OF CYCLIC AMP IN THE THERMOSENSITIVE LESION OF THE FORMATION OF CLOSED COVALENT CIRCULAR Rts 1 DNA[+]

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Received November 18,1974

Summary: The formation of closed covalent circular DNA of R factor, Rts 1, does not take place at non-permissive temperature, 42°C, in E. coli 20SO. However, when Rts 1 was placed in mutants having a low level of cyclic AMP or lacking cyclic AMP receptor protein, the thermosensitive lesion is overcome. Addition of cyclic AMP caused inhibition of the formation of ccc DNA in mutants with low cyclic AMP level, but not in mutants lacking cyclic AMP receptor protein.

The thermosensitive drug resistance factor, Rts 1, confers to its host kanamycin resistance and causes the thermosensitive growth pattern of the host organism (1,2). In addition to the effect on the host, it has been shown that the formation of closed covalent circular (ccc) form of Rts 1 DNA was thermosensitive (3). During a search for a host mutant which overcomes the temperature dependent effect of Rts 1, it was found that mutants which have lower cellular cyclic AMP levels were rather insensitive to the temperature dependent effect of Rts 1. These results suggest strongly direct involvement of cyclic AMP in the thermosensitive block of the circularization of Rts 1 DNA.

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[+]Supported by U.S.P.H.S. Grant GM-12,053

MATERIALS AND METHODS. The properties of the R factors and the conditions for culture used in this paper have been described previously (2). The original strain harboring Rts 1 was E. coli W3630/Rts 1 (1). For preparation of E. coli AB1157/Rts 1 (used as a donor of the Rts 1 factor), overnight trypticase soy broth cultures (grown at 30°C, 0.1 ml) of E. coli W3630/Rts 1 and E. coli AB1157 (proC, thr, leu, his, argF, thi, str, lac, gal, mtl, xyl, ara, F^- (4))were mixed in 0.8 ml of trypticase soy broth. After 1 hour of incubation of 30°C, the mating mixture was diluted and plated on MacConkey agar plates containing 1000 μg/ml streptomycin and 50 μg/ml of kanamycin. For preparation of E. coli Hfr H harboring Rts 1, E. coli AB1157/Rts 1 and E. coli Hfr H (thi, Hfr (5)) were mixed as above. The mating mixture was diluted and plated on glucose M9 medium (6) containing 1 μg/ml thiamine and 50 μg/ml kanamycin. The conjugant isolated represented E. coli Hfr H/Rts 1. The strain E. coli GP1/Rts 1 was prepared in a similar fashion by mating E. coli GP1 (cya, met E, ilv, thi, Hfr (7)) and the donor cell (E. coli AB1157/Rts 1) and selecting conjugants on the M9 glucose medium containing 1 μg/ml thiamine, 50 μg/ml each of isoleucine, valine and methionine, and 50 μg/ml kanamycin. E. coli CA7902/Rts 1 was similarly prepared by the use of M9 glucose medium containing 1 μg/ml thiamine and 50 μg/ml kanamycin from the mating mixture of E. coli CA7902 (cya, thi, F^- (8)) and the donor cell for Rts1. The strain E. coli PP47/ Rts1 was selected on the M9 glucose plate containing 1μg/ml thiamine and 50 μg/ml kanamycin from the mating mixture between E. coli PP47 (crp, thi (9)) and the Rts 1 donor cell in a similar fashion. E. coli AB1157/R100 (used as a donor of R100 plasmid for the preparation of various strains harboring R100) was prepared

by conjugation between E. coli JE948 (10)(containing R100) and E. coli AB1157, and conjugants were selected on MacConkey containing 25μg/ml chloramphenical and 1000 μg/ml streptomycin. Cells harboring R100 such as E. coli Hfr H/R100 and E. coli GPI/R100 and E. coli CA7902/R100 were similarly prepared using the nutritional and the chloramphenical resistance marker as described above.

Preparation of lysates in the analysis for closed covalent circular DNA was performed as described previously (3).

RESULTS. The effect of cyclic AMP on the emergence of R^- segregants at 42°C. It has been shown that bacterial strains containing Rts 1, when grown from small inoculum size, give emergence of R^- segregants at non-permissive temperature (11,3). This was interpreted to indicate that Rts 1 may be eliminated at high temperature (11,12) but the possibility was raised that the emergence of R^-cells may be a result of overgrowth of R^- cells at the non-permissive temperature (3). In the experiment indicated in Table 1, T-1 this emergence of R^- strain at high temperature was studied with various E. coli strains harboring Rts 1 or R100. It is clear from this table that in confirmation of the previous results (11,3), E. coli Hfr H/Rts 1 gave a population of cells which had lost Rts 1 at 42°C. The identical strain with R100 (Hfr H/R100) did not lose R100 plasmid at the non-permissive temperature. On the other hand, when Rts 1 was placed in strains whose intracellular cyclic AMP level was lower (E. coli GP1 and E. coli CA7902), no appreciable loss of Rts 1 was observed indicating that replication of ccc Rts 1 was not hindered in these mutant strains. However, the addition of cyclic AMP to these strains resulted in the loss of Rts 1 at the non-permissive temperature supporting the concept that cyclic AMP hinders the formation of plasmid Rts 1 at non-permissive temperature. Of particular

TABLE 1. EFFECT OF CYCLIC AMP ON EMERGENCE OF R^- SEGREGANTS FROM STRAIN HARBORING Rts 1

Strain	1 mM cyclic AMP	R^+ frequency after grown at 27°C	42°C
Hfr H/Rts1	-	1.00	<0.01
Hfr H/R100	-	1.00	1.00
GP1/Rts 1	-	1.00	0.94
GP1/Rts 1	+	1.00	<0.01
GP1/R100	-	1.00	1.00
GP1/R100	+	1.00	1.00
CA7902/Rts 1	-	1.00	0.97
CA7902/Rts 1	+	1.00	<0.01
CA7902/R100	-	1.00	1.00
CA7902/R100	+	1.00	1.00
PP47/Rts 1	-	1.00	0.95
PP47/Rts 1	+	1.00	0.96

Various E. coli strains were grown overnight at 27°C in trypticase soy broth and diluted to the final concentration of 10^3 cells/ml of trypticase soy broth. The emergence of R^- strains after growth at 42 or 27°C for 24 hours was examined as described previously (3).

interest in this table is the observation that strain PP47/Rts 1 which lacks the cyclic AMP receptor protein did not lose this plasmid at 42°C even in the presence of cyclic AMP. This observation establishes the fact that complex of cyclic AMP and cyclic AMP receptor protein is involved for the loss of this plasmid at the high temperature.

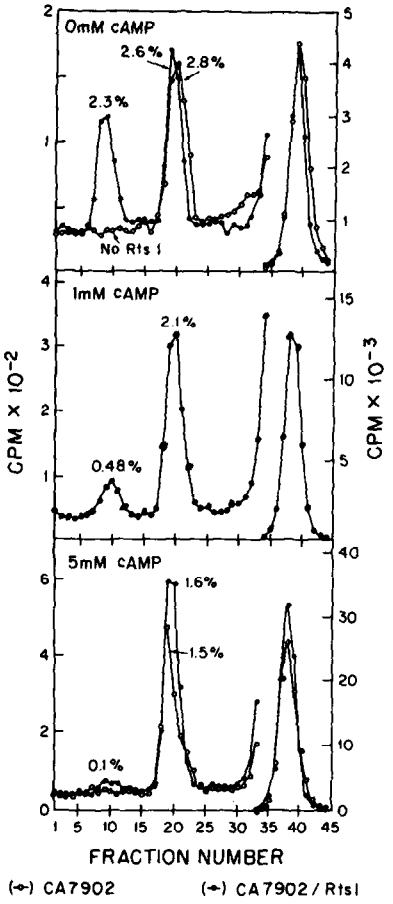

Figure 1. Alkaline sucrose density gradient analysis of DNA from E. coli CA7902/Rts 1 labeled at 42°C, -Effect of cyclic AMP. (a) Exponential phase cultures (grown at 27°C) were inoculated into glucose minimal medium at 2 to 4 x 10^7 cells/ml. The culture was incubated at 42°C for 1 hour. At this point, ^{3}H-thymidine (27.8 mCi/mg, New England Nuclear) at 25 µCi/ml and 2'-deoxyadenosine at 250 µg/ml were added. The cells were labeled for 30 min. Lysates were prepared and subjected to alkaline sucrose gradient centrifugation at 40,000 rpm for 20 min as described in Methods. The sedimentation is from right to left. The fast sedimenting peak on the left represents Rts 1 and a peak in the middle is an unknown plasmid. Note change in scale for the host DNA peak. (b)(c) Experimental conditions were identical to (a) except that cells were grown overnight in the presence of 1 mM cyclic AMP, and diluted into a medium containing 1 mM (b) or 5 mM (c) cyclic AMP at 42°C. •——•, E. coli CA7902/Rts 1 and o——o, E. coli CA 7902.

Loss of thermosensitivity of formation of ccc Rts 1 DNA in E. coli CA7902 and reversal of this effect by the addition of cyclic AMP.

The data shown in Table 1 suggested that the formation of ccc Rts 1 DNA

was not influenced even at non-permissive temperature if the plasmid was placed in E. coli CA7902. This was indeed shown in the experiment illustrated in Figure 1. In this experiment, the cells were grown at 27°C and the temperature was raised to 42°C, and the labeled thymidine was given. The formation of ccc Rts 1 was determined by the use of alkaline sucrose density gradient centrifugation as described previously (3). It is clear from this figure that, in the absence of cyclic AMP, the ccc Rts 1 DNA was formed at 42°C (Figure 1a), and the ratio of this plasmid to the host DNA was not appreciably different from the control value where the Rts 1 DNA was formed at the permissive temperature (data not shown). In the presence of 5 mM cyclic AMP, however, complete loss of labeled Rts 1 DNA was observed (Figure 1c). It is noted in this figure that these strains harbor an unknown plasmid which sedimented slower than Rts 1 DNA. The addition of cyclic AMP did not appreciably change the synthesis of this unknown plasmid at 42°C indicating the specific nature of the inhibitory effect of cyclic AMP on the formation of ccc Rts 1 DNA. Similar results were observed with strain GP1 and PP47 except that the addition of cyclic AMP did not influence the formation of ccc Rts 1 DNA in PP47. In the control experiments, the addition of cyclic AMP had no effect on formation of ccc Rts 1 DNA at low temperature in these strains.

DISCUSSION. Possible roles of cyclic AMP in the circular DNA synthesis has been briefly suggested by the observation that it has stimulatory effec on replication of colicinogenic factor (13, 14) but the exact mechanisms through which the multi-potential compound exhibits its effect on the formation of ccc DNA is not known. The thermosensitive lesion of Rts 1 which is perhaps at the circularization of the precursor molecule into ccc DNA was overcome by a lower intracellular concentration of cyclic AMP. This observation

strongly suggest some kind of involvement of this compound at this step of ccc DNA formation. It should be pointed out that the participation of cyclic AMP in this process must go through the complex of cyclic AMP and cyclic AMP binding protein. The exact biochemical mechanism of the participation of cyclic AMP in the thermosensitivity of Rts 1 is currently under study and will be reported in detail elsewhere.

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