REACTIVATION OF DENATURED RNA POLYMERASE FROM E. COLI

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SUMMARY

A substantial recovery of enzymatic activity of RNA polymerase which had been dissociated into its inactive subunits by treatment with 6.5 M urea in the presence of dithiothreitol, can be achieved by incubation with DNA in the presence of 0.15 M KCl.

RNA polymerase from E. coli is composed of several different polypeptide chains (1). High concentrations of urea (6 - 8 M) lead to the complete dissociation of the enzyme into its polypeptide components, as can be shown by electrophoresis in polyacrylamide and cellogel (1,2,3). The urea treated enzyme and the isolated subunits lack nucleotide incorporating activity (1,4). In order to study the functions of native and modified subunits in the enzymatic reaction, conditions under which enzymatic activity of dissociated RNA polymerase preparations could be reconstituted, would be highly desirable. Partial recovery of activity of an enzyme preparation which had been treated with low concentrations of urea has been reported (5). At low concentrations of urea, however, the enzyme dissociates into 9 S and 3 S subunits (5) and not into all its polypeptide components. In this communication we wish to describe of conditions leading to a considerable reactivation of RNA polymerase which had been completely dissociated by treatment with 6.5 M urea. The same

procedure can be used to regenerate activity of an enzyme which had been inactivated by repeated freezing and thawing in 1 M LiCl according to the method of Sumper, Riepertinger and Lynen (6).

MATERIALS AND METHODS

All reagents used were of best grade as commercially available. RNA polymerase with and without σ-factor from E. coli strain B or MRE 600 was prepared according to the procedures of Burgess (7) and kept at -20° in the presence of 55 per cent glycerol. For inactivation, 0.28 - 0.34 mg of the enzyme was incubated in presence of 7 mM Tris-Cl pH 7.9, 7 mM $MgCl_2$, 70 mM KCl, 0.07 mM EDTA*, 0.07 mM dithiothreitol, 6.5 M urea and 37 per cent glycerol in a total volume of 0.19 ml for 60 min at 20°. After incubation, an aliquot of the mixture was used to determine the residual enzymatic activity in an assay very similar to that described by Burgess (7). The low concentration of urea (0.26 M) present in the assay did not interfere with the determination of activity. Only background incorporation was found. Total loss of activity was also observed when the inactivation was carried out in the presence of 8 mM dithiothreitol. Electrophoresis was performed according to the general method of Ornstein (8) in gels containing 4.5 per cent acrylamide.

RESULTS AND DISCUSSION

RNA polymerase can be rapidly inactivated by addition of 6.5 M urea. If the inactive enzyme is dialyzed at 4° for 14

*Abbrevation used: EDTA, ethylenediaminetetraacetate

hours against a buffer containing 10 mM Tris-Cl pH 7.9, 10 mM $MgCl_2$, 0.1 mM EDTA, 1 mM dithiothreitol and 5 per cent glycerol to remove the denaturing agent and subsequently incubated at

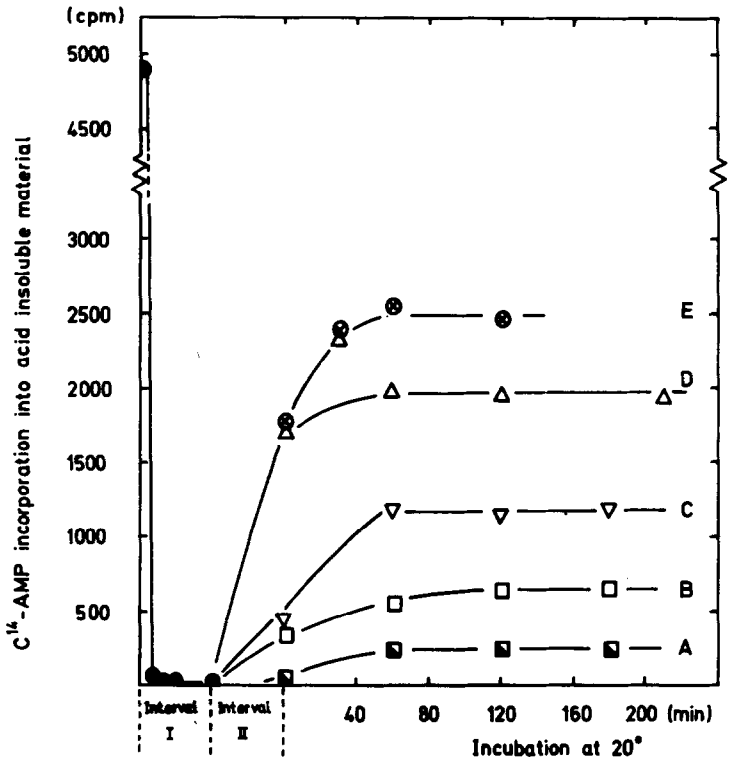

Fig. 1 Restoration of activity of denatured RNA polymerase

Enzyme free of G-factor which had been inactivated for 60 min according to the procedure described under Materials and Methods (indicated by interval I on the abscissa) was dialyzed for 14 hours at 4° (indicated by interval II on the abscissa) against the buffer described in the text. After dialysis 0.13 mg enzyme was incubated at 20° in the same buffer except that the concentration of dithiothreitol was increased to 10 mM (total volume of incubation 0.2 ml). At intervals indicated on the abscissa 25 µl samples were removed for activity measurements in the standard assay (7). The time dependence of incorporation of ^{14}C-AMP in the presence of the four ribonucleoside triphosphates and salmon sperm DNA into acid insoluble material during a 15 min incubation at 37° is indicated on the abscissa. Curve A: only 0.07 mM dithiothreitol present during inactivation of the enzyme; curves B - E: inactivation of enzyme in presence of 8 mM dithiothreitol; curve C: in addition 1.6 mM salmon sperm DNA present during incubation at 20°; curve D: 0.20 M KCl present during dialysis (concentration during incubation at 20° 0.14 M); curve E: conditions as in curve D but 1.6 mM salmon sperm DNA present during incubation at 20°.

20^{o} for two hours, only a very slight recovery of activity as tested by nucleotide incorporation is observed (Fig. 1, curve A). If, however, 8 mM dithiothreitol is present during the treatment of the enzyme with urea, the activity recovered is significantly higher (Fig. 1, curve B). The protecting effect of dithiothreitol is not unexpected since native RNA polymerase contains a large number of HS-groups (1,9) and is rapidly inactivated by mercurials (10) and oxidation (11). It has been observed that DNA stimulates the process of reactivation (5). Indeed, addition of 1.6 mM salmon sperm DNA to the incubation at 20^{o} results in a further increase of activity (Fig. 1, curve C). It is known that RNA polymerase tends to aggregate at low ionic strength (11,12). To prevent formation of enzymatically inactive products 0.14 - 0.20 M KCl was added during dialysis and incubation at 20^{o}. A further increase in activity during incubation is obtained (Fig. 1, curve D). Simultaneous incubation with 1.6 mM salmon sperm DNA results in a 55 per cent restoration of the original nucleotide incorporating activity (Fig. 1, curve E). The presence of 24 per cent glycerol during the incubation at 20^{o} somewhat increases the rate of reactivation but has no effect on the amount of activity recovered. The reconstituted enzymatic activity is stable for at least several hours at all temperatures between 0^{o} and 37^{o}. Similar results have been obtained with enzyme preparations with and without σ-factor.

Native RNA polymerase containing σ-factor migrates in the monomeric (13 S) form as a single band in the polyacrylamide gel electrophoresis (13). After dissociation with urea and removal of the dissociating agent by dialysis several bands appear during electrophoresis. The electrophoretic pattern of

the reconstituted enzyme, however, is the same as that of the native enzyme.

In addition to the normal DNA-directed RNA synthesis RNA polymerase catalyzes the DNA directed synthesis of poly rA (14) and the template-free synthesis of poly rA·poly rU (15). The same reactions are catalyzed by the reconstituted enzyme. The ratio obtained by comparing the different catalytic activities is similar to that observed with the original enzyme. An important property of bacterial RNA polymerase is its strong inhibition by the antibiotic rifampicin (16). The reactivated RNA polymerase shows the same sensitivity towards this inhibitor. A concentration of 5×10^{-8} M of rifampicin is sufficient to obtain 50 per cent inhibition.

A similar degree of reactivation has been observed with RNA polymerase which had been inactivated by repeated freezing and thawing in 1 m LiCl (6) and which was subsequently treated according to the procedure described above.

All these findings are consistent with the notion that after dissociation the different subunits of RNA polymerase can recombine with each other to form active enzyme molecules provided the dissociation occurred in the presence of protecting concentrations of thiols.

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