# SOME PROPERTIES OF THE POLYNUCLEOTIDE PHOSPHORYLASE AND RIBONUCLEASE II OF ESCHERICHIA COLI 1113B

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A series of mutants derived from Escherichia coli, strain AB 301, and selected for abnormalities in enzymes that degrade RNA, have been isolated in the laboratory of W. Gilbert. Mutants D-10 and A-19 have low RNase I activity (Gesteland, 1966). Mutant Q-13, a derivative of A-19, has a modified polynucleotide phosphorylase (PNPase) (Thang and Grunberg-Manago, 1967; Hsieh and Buchanan, 1967). Mutant 1113B*, a derivative of Q-13, is the subject of this report. As expected, it appears to have the abnormalities characteristic of the strains from which it is derived. Furthermore, as indicated by Gilbert*, the potassium-activated phosphodiesterase (RNase II) of this organism is more sensitive to heat than is the RNase II of its parent, strain Q-13. The stability of the RNase II of Q-13 is the same as that of the unrelated strain, E. coli MRE-600, which lacks RNase I (Cammack and Wade, 1965; Singer and Tolbert, 1965).

Preparation of Extracts. All cell strains were grown, harvested, and stored as described previously (Singer and Tolbert, 1965) except that strain 1113B was grown below 35°. On agar plates containing casamino acids-glycerol medium (Gesteland and Boedtker, 1964) strain 1113B grew at 29° and 37° but not at 41°. Crude extracts were prepared either by grinding cells with alumina or

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* E. coli 1113B was isolated in the laboratory of W. Gilbert, Harvard University. We are grateful to Dr. Gilbert for providing us both unpublished information indicating the presence of a temperature sensitive RNase II in this strain, and the strain itself.

passing cell suspensions through a French press and centrifuging at 20,000 x g (Singer and Tolbert, 1965). The suspending medium contained 0.01 M Tris, pH 7.8, 5 mM $MgCl_2$, and 1 mM β-mercaptoethanol (standard buffer).

RNase I. RNase I activity was not detectable in crude extracts of strain 1113B. The assay procedure of Neu and Heppel (1964) was used.

RNase II. RNase II was assayed at 30° by the method of Singer and Tolbert (1965) using poly A as substrate. The specific activity (μmoles soluble nucleotide formed per hr per mg protein) of crude extracts of strains MRE-600, Q-13, and 1113B were 11.8 ± 1.5, 10.1 ± 1.0, and 3.9 ± 0.5, respectively. The activity of the crude extracts is stable for 2 weeks at 4°. After 3 weeks extracts of 1113B lose about 1/3 of their original activity. The following observations indicate that the activity measured in 1113B is indeed RNase II. 1) More than 90% of the activity is lost if $K^+$ is omitted from the assay mixture. 2) The product of the reaction with poly A is 5'-AMP. 3) If the poly A is present in the triple helix composed of 2 moles of poly U and 1 mole of poly A, it is not degraded. 4) RNase I is missing. 5) Degradation cannot reflect PNPase activity since $P_i$ was not present and furthermore the phosphorolysis activity of the PNPase of 1113B is almost undetectable (see below).

Fig. 1 shows that the RNase II activity of 1113B is more sensitive to heat inactivation than the RNase II from Q-13 or MRE-600, when crude extracts are compared. There is a 6 to 6.5° difference between the temperatures for 50% inactivation. The data in Fig. 1 correspond to values obtained after 5 min of heating. However, curves generated by data obtained at either 10, 15, or 30 min of heating at various temperatures are similar to Fig. 1 both in shape and in the temperature difference for points of 50% inactivation. When RNase II activity is measured as a function of time at any given temperature, a biphasic curve is obtained. Control experiments showed that neither inactivation nor reactivation of RNase II of 1113B or MRE-600 occurs at 30°, the temperature of the assay. Mixing experiments demonstrated that crude extracts of 1113B

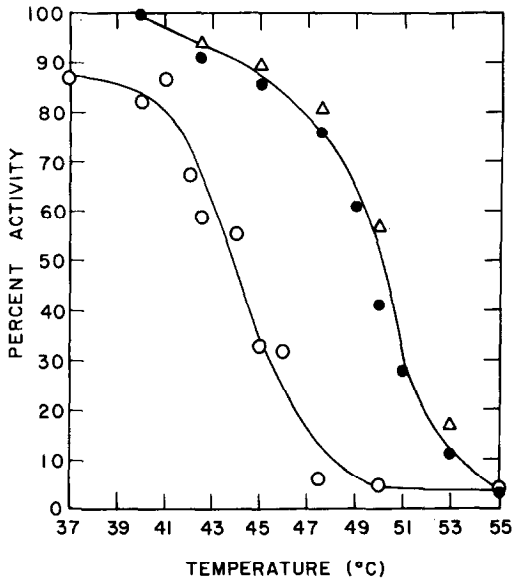


FIG. 1. Heat inactivation of RNase II. Crude extracts of E. coli MRE-600 (•), Q-13 (▲), or 1113B (o) were diluted to 0.8 mg of protein per ml with standard buffer and heated for 5 min at the indicated temperatures. RNase II activity was determined with $^{3}$H-poly A as the substrate by the method of Singer and Tolbert (1965) except for an incubation temperature of 30°; the results are the average of assays at four protein concentrations. The data are expressed as percent of the activity of the untreated sample.

do not increase the heat sensitivity of RNase II of MRE-600 and conversely, extracts of MRE-600 do not contain a protective agent.

PNPase. It was of some interest to demonstrate the identity of the PNPase of 1113B with the PNPase of Q-13, its parent. Because of the difficulties in estimating polymerization of nucleoside diphosphates in crude extracts, disc gel electrophoresis was used to obtain a qualitative characterization of polymerization. Fig. 2 shows photographs of gels obtained with crude extracts of MRE-600, Q-13, and 1113B. The bands correspond to polymerization activity (Thang, Thang and Leautey, 1967). The activity of MRE-600 is somewhat higher with $Mg^{++}$ than with $Mn^{++}$ as expected for normal PNPase (Babinet et al., 1965). Q-13 and 1113B differ markedly from MRE-600. Activity with $Mn^{++}$ is much higher than activity with $Mg^{++}$ (the latter is only barely detectable on the

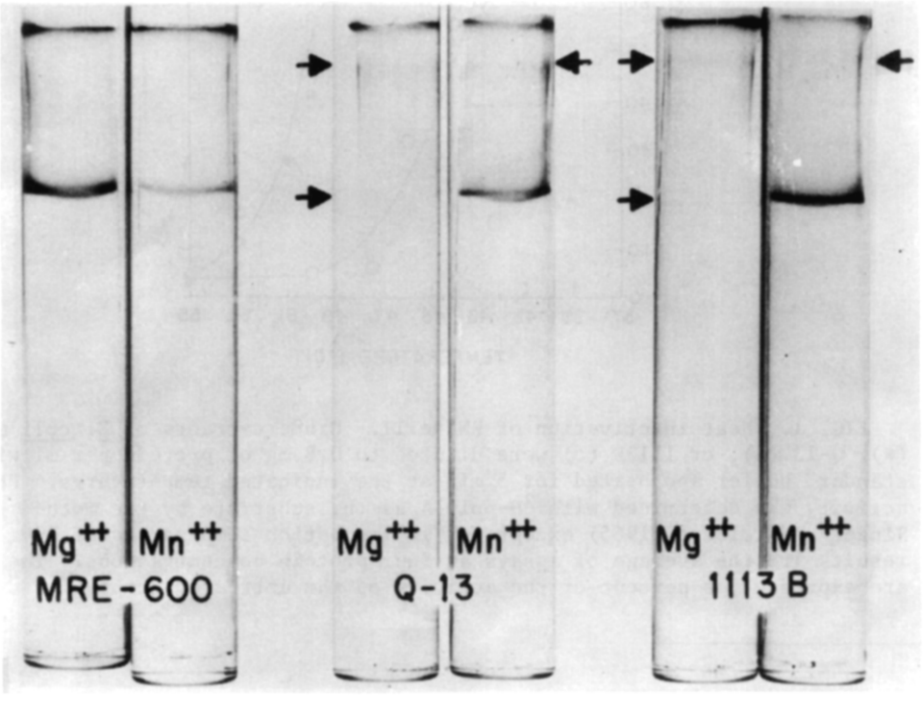


FIG. 2. Polymerization activity on gel electrophoresis. 100 μg of crude extract from the indicated organism was subjected to polyacrylamide gel electrophoresis as described by Klee (1967). After electrophoresis the gels were split longitudinally and incubated for 1 hr at 37° in 0.1 M Tris HCl, pH 9.0, containing 5 mM ADP. Either 2 mM $MgCl_2$, or 10 mM $MnCl_2$, was included, as indicated. Polyribonucleotide formation was located by staining with acridine orange (Thang, Thang and Leautey, 1967). The arrow indicates light bands which are not very apparent on the reproduction.

photographs). Hsieh and Buchanan (1967) reported that the PNPase of Q-13 requires $Mn^{++}$ as the divalent cation. It should be noted (Fig. 2) that with all three strains the mobility of the activity measured in the presence of $Mn^{++}$ is the same as the mobility of the activity measured in the presence of $Mg^{++}$.

In crude extracts, phosphorolysis of poly A usually provides a reliable assay for PNPase over a wide range of protein concentrations, and this is the case for MRE-600. Using the method of Singer and Guss (1962) the activity in MRE-600 extracts was found to be 33 mμmoles of ADP produced per 15 min per mg of protein. However, assays performed with extracts of strain 1113B or Q-13 were neither linear, nor reproducible when carried out in the presence of either 5 mM $Mg^{++}$ or 10 mM $Mn^{++}$. The values obtained were, in any case, far below that found with MRE-600 extracts. A mixing experiment between crude

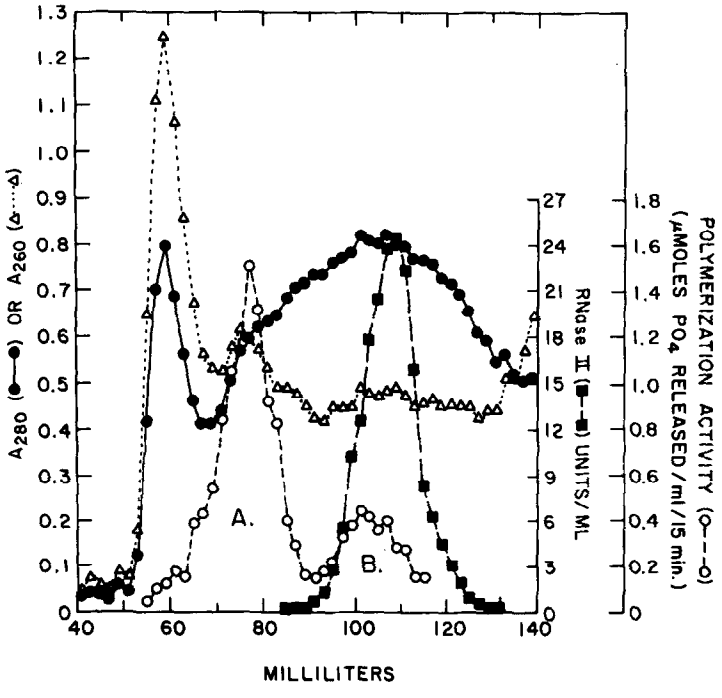


FIG. 3. *Separation of various enzymes on Sephadex G-200.* 6 g of E. coli 1113B were dispersed in 30 ml of standard buffer and disrupted in a French pressure cell. After centrifugation at 20,000 x g for 1 hr, the supernatant fluid was made 0.5% in streptomycin sulfate and centrifuged again. The pellet was discarded. Ribosomes were removed by centrifugation of the streptomycin supernatant fluid at 105,000 x g for 2 hr. The fraction of the supernatant fluid insoluble between 30% and 60% $(NH_4)_2SO_4$ was collected and dissolved in 0.01 M Tris, pH 7.8, containing 0.1 M KCl, 1 mM $MgCl_2$, and 1 mM β-mercaptoethanol. 2 ml of this solution, containing 66 mg of protein, were passed through a Sephadex G-200 column (2.5 x 35 cm) equilibrated with the same buffer. 1-ml fractions were collected at a flow rate of 6 ml per hr. The absorbance at 280 and 260 mμ was determined for every other fraction. Aliquots (0.02 ml) were assayed for polymerization activity and RNase II activity at 30°. Polymerization was measured by the procedure of Singer and Guss (1962) using 10 mM $MnCl_2$ rather than $MgCl_2$. RNase II activity was determined according to Singer and Tolbert (1965).

extracts of MRE-600 and 1113B did not suggest the presence of an inhibitor in 1113B or an activator in MRE-600.

Separation of Various Activities on Sephadex G-200. Crude extract of strain 1113B was partly purified and passed through a column of Sephadex G-200 (details are given in the legend to Fig. 3). The fractions were assayed for RNase II, and for PNPase (Fig. 3). Two discrete peaks of polymerization activity were detected when assays were carried out in the presence of $Mn^{++}$. The activity of peak A is decreased by more than 90% when the assay is carried out in $Mg^{++}$ (Table I). The activity of peak B is the same, regardless of which cation is used. Sucrose density gradient centrifugation indicated that the sedimentation rate of peak A is twice that of peak B. This is consistent with the relative mobilities of the two peaks on Sephadex G-200. The presence of $(Ap)_4A$ in the assay does not stimulate the activity of either peak. Both peaks appear to catalyze the phosphorolysis of poly A (Table I) but the activity

TABLE I. Polynucleotide phosphorylase activity in peak fractions of Sephadex G-200 column

| Fraction | Divalent cation in assay | Phosphorolysis | Polymerization |
|---|---|---|---|
| | | μmoles $^{32}P_i$ incorporated into ADP/ml enzyme/15 min | μmoles $P_i$ Released/ml/enzyme/15 min |
| 77 | $MnCl_2$ - 10 mM | .01 | 1.5 |
| | $MgCl_2$ - 5 mM | .01 | 0.1 |
| 101 | $MnCl_2$ - 10 mM | .01 | 0.4 |
| | $MgCl_2$ - 5 mM | .01 | 0.4 |

Assays were performed as described in the legend to Fig. 3, with the divalent cations as indicated. In the phosphorolysis assay, the usual charcoal procedure (Singer and Guss, 1962) was not used. Instead, $^{32}P$-labeled ADP and $^{32}P_i$ were separated by the method of Sugino and Miyoshi (1964). Radioactive ADP was determined in a liquid scintillation spectrometer. The values for phosphorolysis represent the average of several determinations made by carrying out the reaction for different time periods. Because the phosphorolysis values were all low and not strictly linear, they should be considered approximations.

is very low and the determinations were variable. Two peaks of PNPase activity differing in molecular size were previously noted with preparations from strain Q-13 (Thang et al., 1967), E. coli B (Kimhi and Littauer, 1968) and Clostridium perfringens (Dietz and Grunberg-Manago, 1967).

RNase II activity appears as a single peak slightly behind and overlapping with peak B.

Comments. Thus far it appears that the potassium-activated phosphodiesterase (RNase II) of E. coli strain 1113B has all of the properties of the normal RNase II (Spahr, 1964; Singer and Tolbert, 1965) except for its increased sensitivity to heat. Additional purification is necessary in order to characterize the mutant enzyme further. The temperature sensitivity may be useful in elucidating the physiological role of the enzyme. Strain 1113B should also be of value as a source of nuclease-free components for studies on in vitro protein synthesis (see, for example, Hershey and Thach, 1967). Although strain 1113B fails to grow at 41°, the cause of the temperature sensitivity is unknown, and there is no evidence available relating this growth characteristic and the RNase II instability.

The low phosphorolysis activity in 1113B is consistent with earlier reports of low (Thang et al., 1967) or undetectable (Hsieh and Buchanan, 1967) phosphorolysis in extracts of strain Q-13, the parent of 1113B. The other properties of the PNPase of 1113B described in this report also suggest that it is identical to the enzyme in Q-13. Peak A is similar to the Q-13 enzyme described by Hsieh and Buchanan (1967). Peak B, on the other hand, may correspond to the low molecular weight Q-13 PNPase reported by Thang et al. (1967). Indeed, the experiments reported here may explain the apparent disagreement between the work described in these two reports.

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