AN ALTERED POLYNUCLEOTIDE PHOSPHORYLASE IN E.COLI MUTANT Q13

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Polynucleotide phosphorylase is widely distributed among bacteria. However, its physiological role is still unclear. It has been suggested that the function of this enzyme is the degradation of messenger RNA (Sekiguchi and Cohen, 1963) and, recently a model for this degradation has been postulated (Thang, Guschlbauer, Zachau, and Grunberg-Manago, 1967). A mutant of E.coli Q 13 (Hfr, met$^-$, tyr$^-$, RNase I$^-$, PNPase$_1$) has been isolated by Gilbert* using nitrosoguanidine on E.coli A19, a mutant deficient in RNase I activity (Gesteland, 1966). The Q13 mutant exhibited a very low and unstable polynucleotide phosphorylase activity when assayed with acellular extracts (Gilbert, personal communication). This has been interpreted (Haruna and Spiegelman, 1965; Ben-Hamida and Schlessinger, 1966) as a lack of polynucleotide phosphorylase in addition to RNase I in the mutant. As this seemed very important both for the physiological aspect of the enzyme and for its eventual structural changes resulting from mutation, a study of this mutant has been undertaken. Results on identification and some properties of this enzyme are reported in this communication.

All the activities normally catalyzed by polynucleotide phosphorylase, i.e. polymerization of nucleoside diphosphates, phosphorolysis of polyribonucleotides, and exchange between inorganic phosphate and the terminal phosphate of nucleoside diphosphates, have been demonstrated in the mutant extract (Table I).

* We are grateful to Dr.W.Gilbert for the Q13 strain.

TABLE I

Polynucleotide phosphorylase activities in E.coli mutant Q13 and E.coli B

(mμmoles ADP or $^{32}PO_4$ incorporated/mg protein/15 minutes)

	Mutant Q13	E.coli B
Phosphorolysis poly A	38 - 87	125 - 250
Polymerization (^{32}P) ADP	0.5 - 1	125 - 250
Exchange ADP - $^{32}PO_4$	40	125 - 250

An aluminum-ground cell free extract, dialyzed 3 hours against Tris 0.02 M, pH 8, was used as source of enzyme. 80 μg of mutant proteins, or 40 μg of E.coli B proteins, were added in 0.1 ml mixture. Incubation at 37° C for 15 minutes.

The reaction mixtures (0.1 ml) contained in millimolar concentrations :

- Polymerization : Tris, pH 8,75; $MgCl_2$,2.5; EDTA,0.5; p^{32}pA, 5 (specific activity 2.85 x 10^6 cpm/μmole).
The reaction was stopped by addition of $HClO_4$; the precipitate was then filtered on millipore filter and washed. Radioactivity was determined with a thin window flow-counter.

- Phosphorolysis : Tris, pH 8,75; $MgCl_2$,2.5; EDTA,0.5; poly A, 2.8 (nucleotides); $^{32}PO_4$, 10 (specific activity, 1.37 x 10^6cpm/μmole).
The ^{32}P adenosine diphosphate formed was extracted by the usual procedure, dried on planchets and counted.

- Exchange : Tris, pH 8,100; $MgCl_2$,5; ADP,16; $^{32}PO_4$, 20; (specific activity, 2.64 x 10^6 cpm/μmole).
Same procedure for ^{32}P adenosine diphosphate measurements as for phosphorolysis assay.

NOTE : For the Q13 mutant, the values represent the limits of activity found over 18 extracts. The mean value for phosphorolysis is about 60 mμmoles (^{32}P) ADP phosphorolyzed/mg protein/15 min. while the activity for polymerization is around 1 mμmole ADP incorporated/mg protein/15 minutes. The value for exchange is rather close to that for phosphorolysis. The E.coli B activities in the Table represent values generally obtained in this laboratory over several years.

The validity of these tests to characterize polynucleotide phosphorylase has been discussed elsewhere (Grunberg-Manago, 1963). The product of phosphorolysis, the most specific reaction of the enzyme, has been identified as nucleoside diphosphate. After

dialysis, the polymerization and phosphorolysis activities are unchanged; the exchange activity, however, decreases to one half. The exchange reaction may in part result from other enzymes in addition to polynucleotide phosphorylase.

It should be emphasized that the specific activity of the Q13 enzyme, based on the phosphorolysis assay, is rather high and corresponds to at least one fourth of that of the E.coli B enzyme; in contrast, the polymerization activity is quite low (about one three hundredth that of E.coli B). The assay used for the polymerization, namely measuring the radioactivity of polymers synthesized, is valid; the low activity observed is not due to the presence of RNase II which would degrade the products. In fact, after preincubation at a temperature which inactivates RNase II (Singer and Tolbert, 1965) and is not inhibitory for polynucleotide phosphorylase (55° C) (Lucas and Grunberg-Manago, 1964) the polymerization activity is still very low, even though it becomes more linear as a function of time (fig.1). In the presence of an excess of substrates the rate of phosphorolysis is unchanged. The absence of parallelism for the phosphorolysis and polymerization reactions, with the mutant extract, was the first striking observation, since the E.coli B enzyme always shows parallel activities for both reactions, not only in crude extracts, but also in the various steps of the purification.

The difference, with the mutant, between the rate of phosphorolysis and of polymerization (sometimes up to 100-fold) is probably related to a modification in the enzyme molecule. Indeed, when the extracts of the Q13 mutant enzyme were run on a sucrose gradient by zone density centrifugation (fig.2a), the major activity for phosphorolysis appears in the zone of about 100,000 molecular weight, lighter than the alcohol dehydrogenase taken as a marker. Crude extracts of E.coli B (fig.2b), assayed under identical conditions, show the phosphorolysis activity in a region where polynucleotide phosphorylase from several sources is always sedimented (molecular weight about 200,000). In addition, the light molecules do not exhibit polymerization activity in usual assay; instead this activity is found in the regions where E.coli B polynucleotide phosphorylase sediments. Therefore, a major activity of polynucleotide phosphorylase is associated in the mutant with molecules of only half the molecular weight of normal polynucleotide phosphorylase.

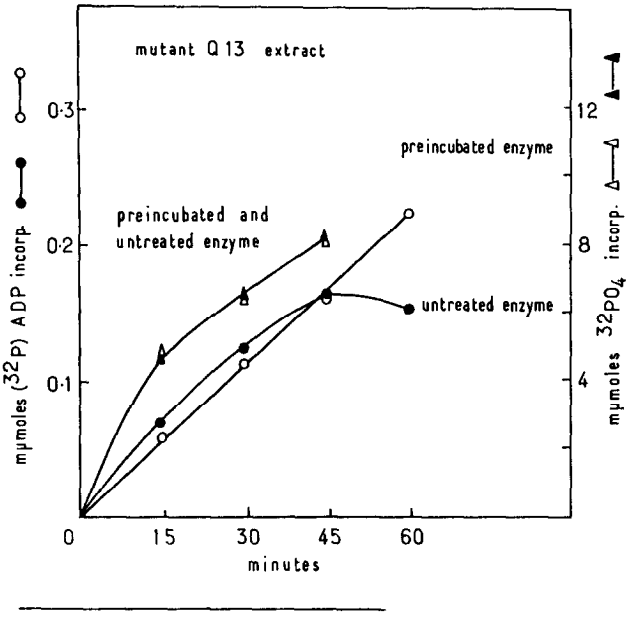


Fig.1

Comparison of polymerization and phosphorolysis activities (with or without preincubation at 55°C)

For preincubation experiment the cell-free extract was pre-incubated at 55°C for 10 minutes in the absence of Mg^{++}, but in the presence of either poly A (for phosphorolysis assay), or ADP (for polymerization assay). Mg^{++} was added after heat treatment.

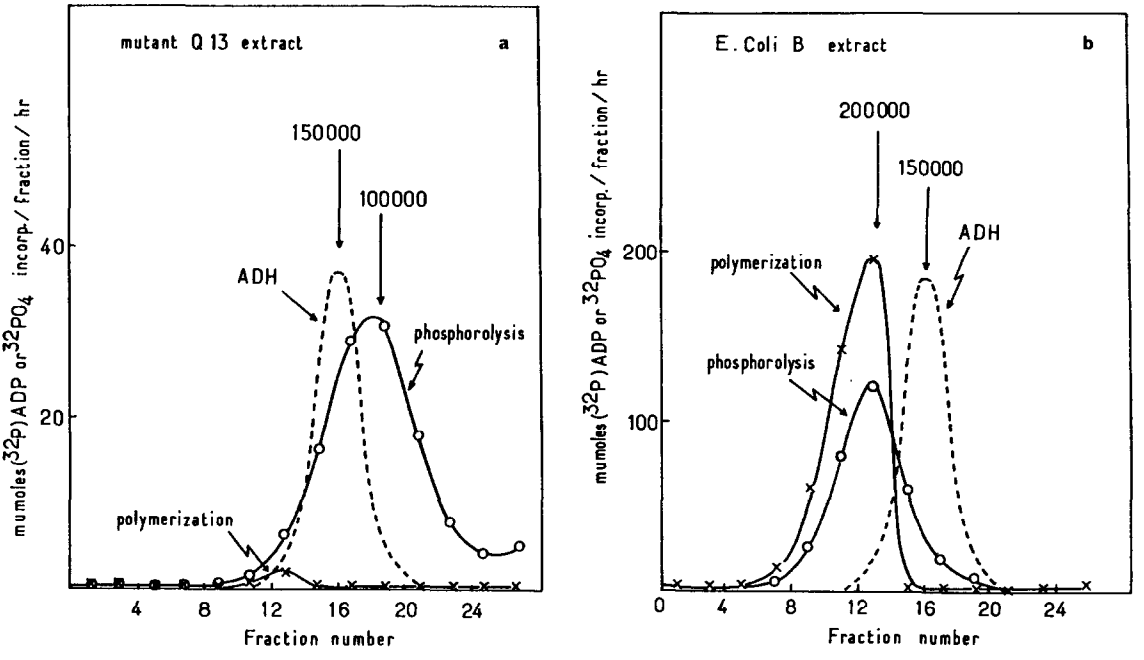
It is, however, necessary to emphasize that in this mutant polynucleotide phosphorylase is not only present in the light form, but also in the "normal" one, i.e. molecules with a molecular weight of about 200,000 and catalyzing both phosphorolysis and polymerization as shown by studies on the purified preparations of the mutant (Thang, Thang and Grunberg-Manago, 1967).

The structural changes undergone by the enzyme present two highly interesting aspects :

1) The lighter form, about one half of the normal size, carries only one of the activities of the conventional polynucleotide phosphorylase (i.e., phosphorolysis). This observation is important for investigating the active sites. Recent studies on E. coli B (see Grunberg-Manago, 1967) indicate that polynucleotide phosphorylase exists in polymeric forms which differ in the ratios of the two activities : polymerization and phosphorolysis. Furthermore, a C. perfringens enzyme (differing from other polynucleotide phosphorylases in its requirement for polylysine in poly A synthesis) could also be separated into two components of different molecular weight. In this case, however, the smaller component (62,000) does not catalyze phosphorolysis but only ADP polymerization (Dietz and Grunberg-Manago, 1967).

2) The alternative conformation of the enzyme is still functional

Fig.2



a b

Activity profiles of polynucleotide phosphorylase from E.coli mutant Q13 and E.coli B on a zone density centrifugation

Mutant Q13 extract (1.8 mg) or E.coli B extract (1.6 mg), mixed with alcohol dehydrogenase as marker, are layered on a 5 to 20% sucrose density gradient made up in Tris buffer 0.1 M, pH 8, and centrifuged for 16 hours at 32,000 rpm in a Spinco SW 39 rotor at +5°C. Fractions are collected by the usual technique. Activities for phosphorolysis and polymerization are assayed as described in Table I. The molecular weights were calculated according to Martin and Ames (1961), using as reference 150,000 for the molecular weight of alcohol dehydrogenase.

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in phosphorolysis of RNA; it could therefore be involved in the degradation of messenger RNA in the mutant.

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