PROPERTIES OF POLYNUCLEOTIDE PHOSPHORYLASE FROM *E. COLI* POLYNUCLEOTIDE PHOSPHORYLASE-DEFICIENT AND THERMO-SENSITIVE MUTANTS

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1. Introduction

E. coli mutants, deficient in polynucleotide phosphorylase or thermosensitive for that enzyme, have been isolated by Reiner [1,2] and by Aprion's group [3]. The latter's group proposed a function for polynucleotide phosphorylase in vivo: this enzyme would degrade the stable RNAs and would be required to induce certain enzymes. Polynucleotide phosphorylase would thus be essential for *E. coli* growth.

In time, these mutants appeared to be not completely deficient in polynucleotide phosphorylase [3]. It was therefore important to have precise data on the amount and properties of these polynucleotide phosphorylases. It is already known that the activity of the enzyme depends on the presence of metal ions [4]. Moreover, the relationship between phosphorolysis and polymerization activities could also yield some data on the role of this enzyme in vivo. Furthermore, comparing phosphorolysis of polymers and of oligonucleotides, one might confirm or infirm the hypothesis of the dual sites (catalysis and fixation) of polynucleotide phosphorylase. Finally, the study of these modified polynucleotide phosphorylases is important with respect to the relationship between structure and function.

We therefore made a study with three mutants isolated by Reiner: PR 7, PR 13, and PR 27 (the latter being thermosensitive) [1,2], and with a PNPase like mutant isolated recently by Apirion [3].

2. Materials and methods

2.1. *Strains and conditions of growth*

Polynucleotide phosphorylase mutations were isolated from strongly mutagenized strains and transducted by phage P1 in isogenic RNase$^-$ strains.

Growth was performed in an AM_3-Difco medium and stopped when the optical density at 650 nm reached 1. This was followed by centrifugation at 7000 *g* for 10 min; the pellet was suspended in 20 mM Tris buffer (pH 8.3) and centrifuged 10 min at 37 000 *g*. The bacteria from the different mutants were then sonicated twice for 3 min in an MSE sonicator, 0.35 g at a time, in the presence of 1 ml of the following buffer: 20 mM Tris, 0.1 mM EDTA, and 100 mM β-mercaptoethanol. The extracts were then centrifuged 20 min at 37 000 *g* to eliminate the membrane debris. All the experiments were then performed with the corresponding supernatant.

2.2. *Chemicals*

Poly A and poly U were prepared in our laboratory as well as the oligonucleotide $(pU)_7$. ADP and UDP were from Sigma, and the labelled nucleoside diphosphates from Amersham.

The products for electrophoresis were from Canalco; the solvants for the chromatography were from Merck (*n*-propanol) and from Prolabo (ammonia).

3. Results and discussion

3.1. *Phosphorolysis*

All the mutants showed a measurable activity, albeit weak for poly A phosphorolysis, the weakest being that of mutant PR 7 (4% of that of a wild-type crude extract which is 0.5 U/mg protein), and the strongest that of PR 27 which is comparable to that of the wild-type (0.45 U/mg). With respect to poly U all four mutants have a comparable activity (less than 0.1 U/mg under standard conditions) much weaker than that of the wild-type.

The affinity of the mutant enzymes for poly A and poly U is much weaker than that of the E. coli wild type polynucleotide phosphorylase (1000-fold less for PR 7).

In contrast, phosphorolysis of an oligonucleotide (i.e. $(pU)_7$) occurs almost normally: 0.20 to 0.60 U/mg as compared to 0.80 U/mg for the wild type enzyme. We checked with mutant PR 7 that the phosphorolysis product of $(pU)_7$ was indeed UDP which confirms that all the residual activities found do correspond to a polynucleotide phosphorylase activity, as had been shown by Reiner [2] with another mutant PR 13.

The difference observed in the behavior of the mutant enzymes for polymer phosphorolysis and oligonucleotide phosphorolysis confirms the theory of two sites on the enzyme, one for catalysis and one for attachment [6]. The weak affinity of the enzymes for the polymers suggests that the attachment sites for the latter are not intact on the mutant enzymes, and that these have either been degraded or are in an inadequate conformation. In contrast, the almost normal phosphorolysis of an oligonucleotide suggests that the catalytic site is only slightly affected.

3.2. *Polymerization*

Polymerization of ADP or UDP (table 2) by the mutant enzymes (except, again, for PR 27) is low or very low (3 to 300 times lower than with the wild-type enzyme). However, there does not appear to be any clear correlation between the phosphorylysis and the polymerization activities of the three mutants, other than PR 27.

In the presence of Mn^{2+} (table 3) the four mutant enzymes behaved quite differently from the wild-type polynucleotide phosphorylase. Whereas the wild-type enzyme is much more active in the presence of Mg^{2+} than of Mn^{2+}, the contrary occurs with the mutant enzymes. They showed an equivalent or stronger activity (up to 17-fold) in the presence of Mn^{2+}. This should be compared to the behavior found in this laboratory of the Q 13 mutant (from which originates PR 13) [4].

Table 1
Phosphorolysis reaction by mutant and wild-type polynucleotide phosphorylases

Source of enzyme	Poly A (mU/mg)		Poly U (mU/mg)		K_M approx. poly A (10^{-5} M)	poly U	Oligo U (mU/mg)
PR 7	21	(4)*	63	(13)	140	160	250
PR 13	65	(13)	90	(18)			270
PR 27	450	(90)	78	(15)	35	7.5	620
N 1112	58	(12)	46	(9)		300	200
Wild-type	500	(100)	500 abt	(100)	0.15	0.15	800

* Percentage as compared to the activity of the wild-type enzyme.
Phosphorolysis was performed as follows: the incubation medium (final volume 50 μl) contained Tris–HCl (pH 8.3) 100 mM; $MgCl_2$ 2 mM; poly A, poly U or oligo U $(pU)_7$ 1 mM; 10 mM labelled KH_2PO_4 (spec. act. 10 μCi/μmol); and about 100 μl enzyme. The reaction was stopped by addition of 550 μl of 2.5% $HClO_4$ and the radioactivity of the ADP (or UDP) formed was measured in a Tracerlab counter (the mineral phosphate which had not reacted was first eliminated, as usual [5] by using a phospho-molybdate complex). The wild-type enzyme was from an *E. coli* ML304G strain 1 mU corresponds to the formation of 1 nmol of nucleotide in 1 hr)

Table 2
Polymerization of ADP and UDP by mutant and wild-type polynucleotide phosphorylases

Source of enzyme	UDP (mU/mg)		ADP (mU/mg)		Activity (UDP) / Activity (ADP)
PR 7	19	(0.65)*	59	(4.5)	0.3
PR 13	16	(0.55)	29	(2.2)	0.55
PR 27	1800	(62)	2200	(170)	0.8
N 1112	200	(7)	130	(10)	1.5
Wild-type	2900	(100)	> 1300	(100)	2.2

* Figures between parentheses correspond to the percentage of activity as compared to that of the wild-type enzyme. The polymerization incubation medium contained (final volume 50 μl): Tris (pH 8.3) 100 mM; $MgCl_2$ 4 mM; labelled nucleoside diphosphate (spec. act. about 0.5 mCi/mmol) 14 mM for ADP or 12.5 mM for UDP; and about 100 μg of enzyme. The reaction was stopped by addition of 2 ml perchloric acid 7% and one drop of 0.5% albumin. The test-tube was then left for 10 min at 0°C prior to filtering the precipitate on Millipore filters with 8 times 2 ml perchloric acid 1% and twice 2 ml 0.01% perchloric acid. 1 mU corresponds to the incorporation in 1 hr of 1 nmol of nucleoside diphosphate.

3.3. *Electrophoresis profiles*

Migration of proteins is revealed by formation in situ of poly A during ADP polymerization [7]. Electrophoresis of PR 7, PR 13 and PR 27 (not shown) revealed the existence of a major species, corresponding to *E. coli* polynucleotide phosphorylase and minor species which represent either an associated enzyme, or an enzyme containing a larger proportion of the β subunit [8] which has been shown to have no catalytic activity. With the N 1112 mutant, the electrophoresis profile showed that the major species is most probably proteolyzed which implies, in this mutant, the existence of a non-negligible proteolytic activity for polynucleotide phosphorylase.

3.4. *Thermosensitivity*

Polynucleotide phosphorylase from PR 27 was known to be thermolabile, but we also found that PR 7 whose phosphorolysis activity for poly A is the weakest was also more thermosensitive than the wild type enzyme; moreover, both mutant enzymes are protected against thermal inactivation by the presence of polymers.

This study shows, once more, that no mutants are truly PNPase⁻. Nevertheless, our work shows that the PNPase⁻ mutants we studied have only a very weak polynucleotide phosphorylase activity as compared to that of the wild-type enzyme. Moreover, we have shown that the polynucleotide phosphorylases from the four mutants we investigated exhibit properties differing from those of the wild-type enzyme. In particular, polymerization, albeit weak, is less so in the presence of Mn^{2+} than of Mg^{2+}, in contrast to what occurs with wild-type polynucleotide phosphorylase; secondly, while N 1112 has a slightly proteolyzed enzyme, the three others contain, in addition to the normal enzyme, a heavier component (as shown by electrophoresis profiles). Furthermore, we found that not only was the enzyme of PR 27 thermosensitive (which could be expected since this mutant is thermo-

Table 3
Polymerization of ADP by mutant and wild type polynucleotide phosphorylases in the presence of either Mg^{2+} or Mn^{2+}

Source of enzyme	+ Mg^{2+}		+ Mn^{2+}		Activity + Mn^{2+} / Activity + Mg^{2+}
	Amount (mM)	Activity (mU/mg)	Amount (mM)	Activity (mU/mg)	
PR 7	6	6	8	13	2.2
PR 13	10	36	15	650	17.5
PR 27	4	2200	0.8	1750	0.84
N 1112	3–4	130	10	470	3.7
Wild-type	4–6	> 1300	1	130	0.1

Polymerisation as in table 2, except for the amount of cation which was optimum and was as indicated, and for ADP which was 14 mM in the presence of Mg^{2+}, and 8 mM in the presence of Mn^{2+}.

sensitive), but that PR 7 also had a thermosensitive polynucleotide phosphorylase as compared to the wild-type enzyme. Finally, phosphorolysis experiments have shown that the affinity of the mutant enzymes for polymers is lower than that of the wild-type, but that the reaction was almost normal for an oligonucleotide. This last evidence confirms the theory of two sites of action for polynucleotide phosphorylase.

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