METAL IONS REQUIREMENT OF POLYNUCLEOTIDE PHOSPHORYLASE

C.Babinet, A.Roller*, J.M.Dubert
Service de Biochimie Cellulaire - Institut Pasteur, Paris

and

M.N.Thang et M.Grunberg-Manago
Service de Biochimie, Institut de Biologie, rue Pierre Curie, Paris

Received February 1, 1965

It is generally believed that Mg^{++} is specifically required for the reaction catalyzed by polynucleotide phosphorylase (cf.Grunberg-Manago, 1963). Manganese has been shown to be inhibitory in the presence of Mg^{++} (Littauer and Kornberg, 1957; Levin et al., 1963). In connection with further studies on the role of Mg^{++} in the mechanism of polymerization (Williams et al., 1964) and phosphorolysis, it became of interest to reinvestigate the action of other divalent metal ions, particularly since highly purified preparations of polynucleotide phosphorylase are now available. (Williams and Grunberg-Manago, 1964; Thang D.C.et al., 1964).

A series of divalent metal compounds : $MnSO_4, 5H_2O$; $CoSO_4, 7H_2O$; $NiCl_2, 6H_2O$; $3CdSO_4, 8H_2O$; $CuSO_4, 5H_2O$; $ZnSO_4, 7H_2O$; and $CaCl_2$, have been assayed with sucrose gradient purified E.coli enzyme (Thang et al., 1964) by both the polymerization and phosphorolysis reactions. Reagent grade $CaCl_2$ was a product of Prolabo (Paris); all other substances used were Matthey "specpure" grade (Johnson, Matthey and Co., London).

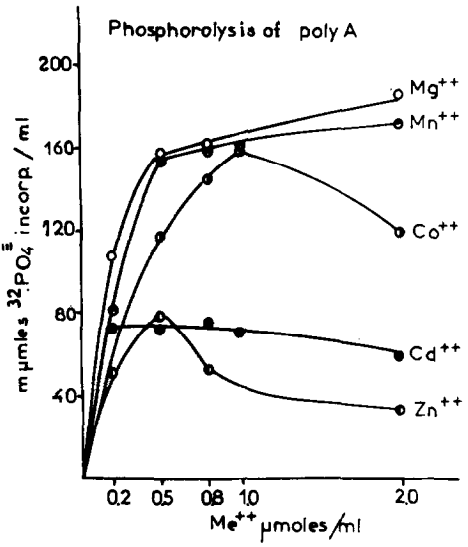
With the exception of the calcium ion, all divalent metals tried are capable of replacing Mg^{++} in the phosphorolysis of poly A (Fig.1 and 3), and four of them could replace Mg^{++} in the polymerization of ADP (Fig.2), although the efficiencies of the various ions are quite different. It appears therefore, that polynucleotide phosphorylase has no absolute requirement for Mg^{++}. Further, in

* Fellow of the Damon Runyon Foundation.

the presence of each of these divalent ions, the behavior of the enzyme differs for polymerization and for phosphorolysis. Mn^{++} is the most suitable metal for replacing Mg^{++}, since at optimal concentration of these two ions, and under conditions of substrate saturation, the amounts of poly A formed (ADP polymerization) or of ADP released (poly A phosphorolysis) are of the same order of magnitude. Co^{++} at concentrations of 1×10^{-3} to 2×10^{-3} M activates the phosphorolysis reaction to the same extent as Mg^{++} and Mn^{++}, but the rate of polymerization of this metal is only 50% of that obtained with Mg^{++}. In the presence of Cd and Zn, the rate of phosphorolysis is 50% of that obtained with Mg^{++}, while the rate of polymerization is greatly decreased. The most striking point is the observation that with Cu^{++} and Ni^{++}, phosphorolysis of poly A occurs readily, while polymerization of ADP is completely inhibited. The poly A used in the experiments has been treated to eliminate Mg^{++} which could be present in the polymer. The method (Massoulié, 1964) consists of dialyzing the poly A against a solution containing 7 M urea and 0.05 M EDTA. The concentration of urea is gradually decreased by adding a solution of EDTA 0.05 M. The final dialysis is against water. The poly A thus treated shows no phosphorolysis in absence of metal ions.

The polymerization of the other nucleoside diphosphates also shows no absolute requirement for Mg^{++}. The action of Mn^{++} has been more carefully studied because of its particular role in RNA-polymerase activity. As shown in Fig.4, the polymerization of all four common nucleoside diphosphates proceeds in the presence of Mn^{++}. The rate of Mn^{++} activated nucleoside diphosphates polymerization under optimal conditions, compared with the Mg^{++} activated reactions, is 70-80% for ADP and 40% for UDP and CDP. In the presence of Mn^{++} a higher amount of (^{14}C)GDP is incorporated; however, the rate of polymerization of GDP is so small that the difference is not very significant. Nevertheless it should be mentioned that at high temperature (60°), the polymerization of GDP in the presence of Mn^{++} yields high molecular weight poly G, a product which is difficult to obtain under normal reaction conditions (Thang et al., 1964; Lucas et al., 1964).

Fig.1
Requirement of divalent ions for the phosphorolysis of poly A



The incubation mixture (0.1 ml) contains in millimolar concentration : Tris, pH 8.1, 100; poly A, 2.5; (^{32}P) orthophosphate, 10; EDTA, 0.5; enzyme, 4 µg/ml. Incubation 10 min. at 37°.

The ability of various divalent metal ions to activate polynucleotide phosphorylase is also true for the enzyme isolated from other species. Assays with the A.vinelandii enzyme show that it is also active in the presence of Co^{++} and Zn^{++} for the polymerization of ADP. M.lysodeikticus and A.vinelandii enzymes can also catalyze the polymerization of ADP in the presence of Mn (Fig.5). However, the optimal ratio of ADP/Mn^{++} for the A.vinelandii enzyme is much lower than for the other two enzymes while the optimal ratio of ADP/Mg^{++} is approximately the same for all three polynucleotide phosphorylase preparations. With the E.coli enzyme, the optimal ratio of ADP/Mn^{++} is about 10 for the polymerization (Fig.6) which indicates that the enzyme has a higher affinity for Mn^{++} than for Mg^{++} for which the nucleotide/metal ratio is 2-3 (Williams et al., 1964). It should be pointed out, however, that at higher concentrations Mn^{++} becomes inhibitory for the polymerization reaction (Table I),

Table I

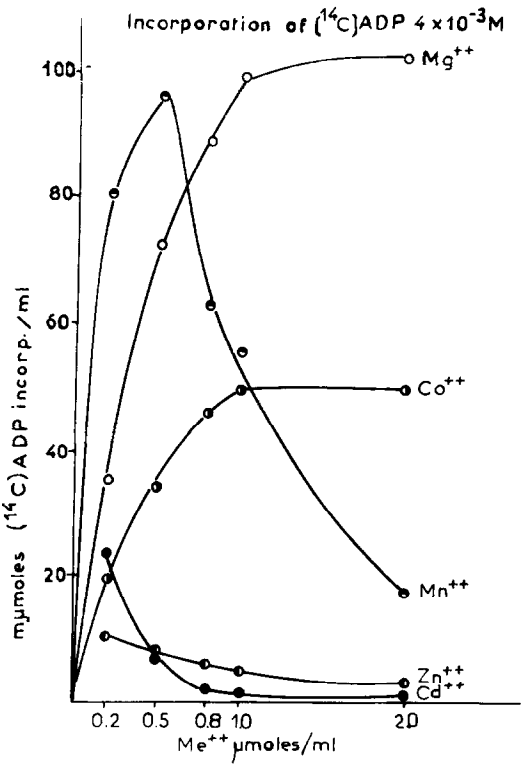
Action of Mg^{++} on the Mn^{++} inhibition of polynucleotide phosphorylase

Mn^{++} µmole/ml	% of inhibition	
	+ Mg^{++} 2 µmoles/ml	- Mg^{++}
0.2	22	10
1	59	39
2	75	82

ADP polymerization reaction under conditions described in Fig. 2. The specific activity in the presence of 2 µm/ml Mg^{++} alone is 220.

Fig. 2

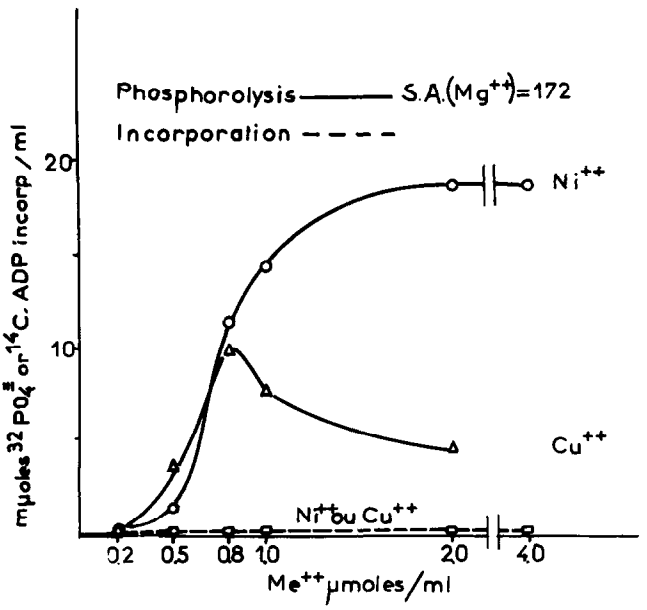
Requirement of divalent ions for the polymerization of ADP



The incubation mixture (0.25 ml) contains in millimolar concentration : Tris, pH 8.1, 100; (^{14}C)ADP; enzyme, 4 µg/ml. Incubation 15 minutes at 37°.

Fig.3

Effect of Ni^{++} and Cu^{++} on the phosphorolysis and polymerization



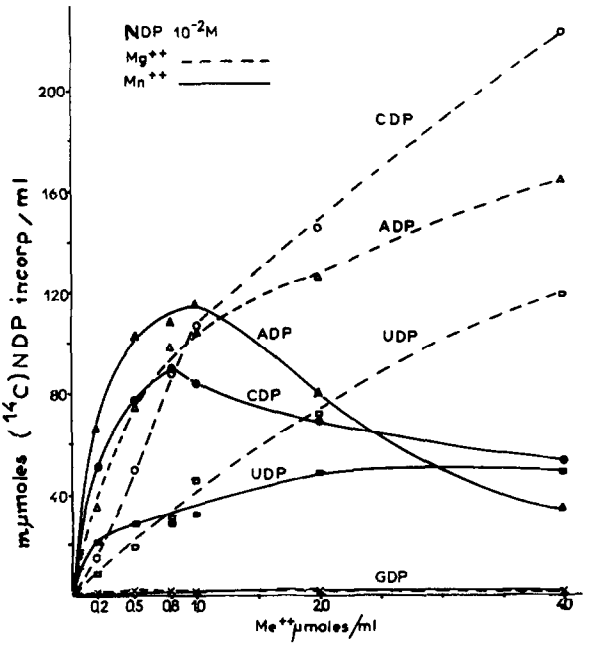
Same conditions as in Fig.1 and 2

even in the presence of optimal concentrations of Mg^{++} (Littauer et al., 1957; Levin et al., 1963). In addition, the presence of these two ions together has a greater inhibitory effect than in the case where each metal is present alone (Table I), suggesting an interaction of two metallic ions in the polymerization reaction.

It has been found that free ADP and free Mg^{++} are the substrate and the activator, respectively, in the polymerization catalyzed by polynucleotide phosphorylase isolated from E.coli and A.vinelandii (Williams et al., 1964); but in the phosphorolysis reaction the action of Mg^{++} is still unknown. Studies of the role of other metallic ions and of their interaction may provide further insight into the mechanism of action of polynucleotide phosphorylase.

Fig.4

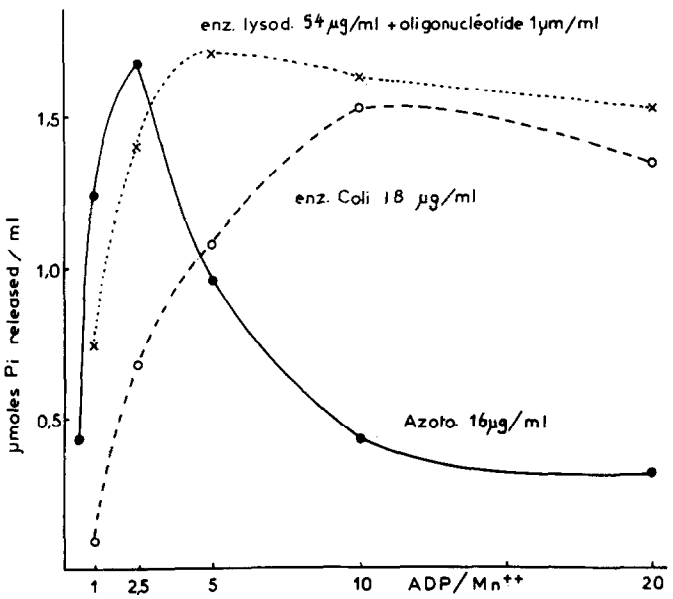
Mn^{++} activated polymerization of all four nucleoside diphosphates



The incubation mixture (0.25 ml) contains in millimolar concentration : Tris, pH 8.1, 100; (^{14}C)NDP, 10; enzyme, 4 μg/ml.

Fig.5

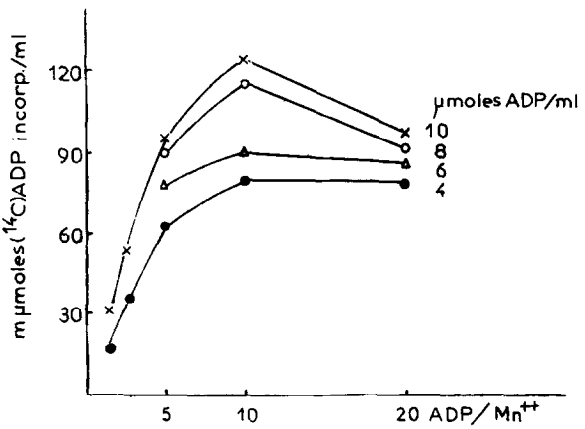
Effect of Mn^{++} on the polymerization of ADP catalyzed by polynucleotide phosphorylase isolated from various sources



The M.lysodeikticus enzyme is isolated according to M.Singer et al.; the A.vinelandii enzyme is purified by a modified procedure set up by D.C.Thang and M.Grunberg-Manago.
Same conditions as in Fig.4

Fig.6

Optimal ratio of ADP/Mn^{++} for E.coli enzyme



Same conditions as for the polymerization of ADP used in other experiments.

Acknowledgements :

This work has been supported by grants from U.S.Public Health Service Research and Délégation Générale à la Recherche Scientifique et Technique (granted to M.Grunberg-Manago and J.Monod) We are grateful to A.Curdel of Biophysics Department (Institut de Biologie) for kindly supplying the divalent metal compounds, and to E.Mery for her preparation of M.lysodeikticus enzyme. We take pleasure in thanking D.C.Thang for her skilled technical assistance.

REFERENCES

M.Grunberg-Manago, Progress in Nucleic Acid Research, 1, 93 (1963)
D.H.Levin, M.N.Thang, M.Grunberg-Manago, Biochim.Biophys. Acta, 76, 558 (1963)
U.Z.Littauer, A.Kornberg, J.Biol.Chem., 226, 1077 (1957)
J.Lucas, M.Grunberg-Manago, Bioch.Bioph.Res.Comm., 17,395(1964)
J.Massoulié (personal communication) (1964)
D.C.Thang, M.N.Thang, M.Grunberg-Manago, unpublished (1964)
M.N.Thang, M.Graffe, M.Grunberg-Manago (in preparation)(1964)
F.R.Williams, M.Grunberg-Manago, Biochim.Biophys.Acta,89,66(1964)
F.R.Williams, T.Godefroy, E.Mery, J.Yon, M.Grunberg-Manago, Biochim.Biophys.Acta, 80, 349 (1964)