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# STIMULATION BY MANGANESE(II) SULPHATE OF A cAMP-DEPENDENT PROTEIN KINASE FROM *ZEA MAYS* SEEDLINGS

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**Key Word Index**—*Zea mays*; Gramineae; maize; cAMP; manganese(II)-sulphate; protein-kinase.

**Abstract**—A cAMP-dependent protein kinase ($M_r$ 36000) was isolated from *Zea mays* seedlings. The enzyme is stimulated six-fold against the control by 0.9 mM $MnSO_4$.

## INTRODUCTION

As described earlier [1], the highest cAMP dependent protein kinase activity of a purified maize coleoptile homogenate by electrophoresis was in the 36000 region. The concentration of $MnSO_4$ in these assay mixtures was less than 0.1 mM; $MgCl_2$ 5 mM and EDTA was 1 mM and the [$\gamma$-$^{32}$P]-ATP activity was 5 $\mu$Ci (185 kBq).

Further investigations surprisingly showed a direct requirement of this cAMP-dependent protein kinase for $Mn^{2+}$. This is the first time that a requirement fo a cAMP dependent protein kinase for $MnSo_4$, as shown in Fig. 1, has been described for a homogenate of a higher plant material.

The conditions for the kinase assays were the same as described in [1] with the following modifications. The buffer used contained 30 mM Tris–HCl, 5 mM $MgCl_2$ and 0.1 mM ascorbic acid(pH 6, 8). The concentration of $MnSO_4$ in the final volume of the reaction mixture of 80 $\mu$l increased from 0 to 2.0 mM.

The dependence of the cAMP stimulation on the $MnSO_4$ concentration showed a rather sharp optimum at *ca* 0.9 mM, with activity falling off rapidly at higher levels of $MnSO_4$. The cAMP dependent protein kinase activity is more than twice of that found earlier [1]. When $MnSO_4$ was replaced by $NiSO_4$, $CoSO_4$ or $FeSO_4$ in the reaction mixture cAMP had only little effect on the protein kinase activity. In the absence of added cAMP, $MnSO_4$ was slightly stimulatory at about 0, 8 mM. Other cyclic nucleotides as cUMP, cCMP, cIMP and cGMP showed no stimuladory effdcts.

In 1969 a very similar requirement for a cAMP dependent protein kinase for $MnCl_2$ was reported in *Escherichia coli* [2]. Whereas the concentrations of cAMP and $Mn^{2+}$ were in the same order of magnitude to stimulate the protein kinase, no $MgCl_2$ was required because in this case $Mn^{2+}$ substituted $Mg^{2+}$. In respect to the discussions about the nature and character of the cAMP dependent protein kinases in higher plants (3), the cAMP-$Mn^{2+}$ dependent protein kinases may be evolutionarily conserved. Another $Mn^{2+}$-stimulated protein kinase has recently been described in *Pisum sativum* [4].

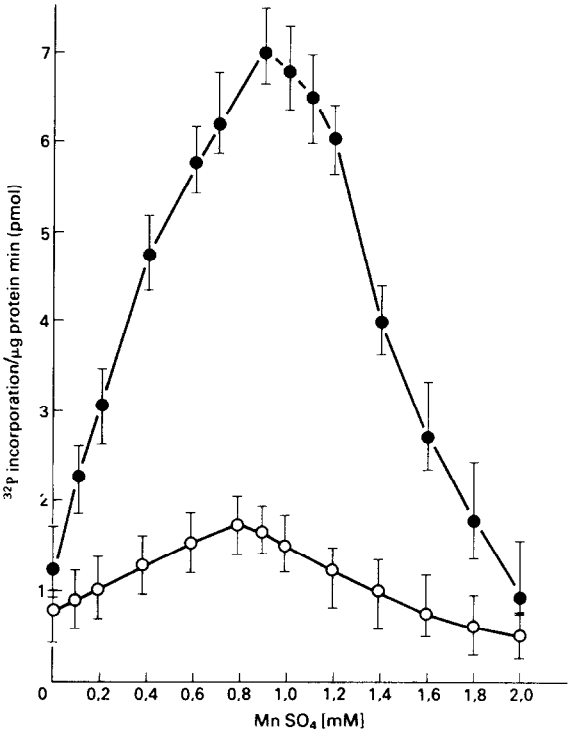


Fig. 1. Effect of $MnSO_4$ on histone II-A phosphorylation catalysed by a isolated protein kinase from maize coleoptile homogenate in the presence (–●–) or absence (–○–) of cAMP($6 \times 10^{-6}$ M). Further information is given in the text. I indicates the SE for three fold measured samples.

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## REFERENCES

1. Janistyn, B. (1986), *Z. Naturforsch.* **41c**, 579.
2. Kuo, J. F. and Greengard, P. (1969), *J. Biol. Chem.* **244**, 3417.
3. Newton, R. P. and Brown, E. G. (1986), in: *Receptors in Plants and Slime Moulds* (Chadwick, C. M. and Garrod, D. R., eds) pp. 115–153. Cambridge University Press, Cambridge.
4. Chiatante, D., Leri, M., Sgorbati, S. and Sparroli, E. (1987) *Plant Sci.* **53**, 271.