Original Article

Cytosolic cAMP-dependent protein kinase of *Polysphondylium violaceum*: developmental regulation and properties

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Summary

The cellular slime mould *Polysphondylium violaceum* contains a cAMP-dependent protein kinase resembling the mammalian type I enzyme. The appearance of this enzyme is developmentally regulated. The level of kinase activity is very low in vegetative cell and increases more than tenfold during differentiation.

The catalytic subunit of this cAMP-dependent protein kinase has a native molecular weight of 60–80 kDa, an isoelectric point of 5.7 and an apparent $K_m$ for ATP and Kemptide of 50 and 13.4 $\mu$M respectively. It is characterised by its sensitivity to a synthetic inhibitor specific for cAMP-dependent protein kinase. The regulatory subunit has a molecular weight of 50 kDa.

Abbreviations: HEPES – N-2-Hydroxyethylpiperazine-N'-2-ethane sulphonic acid, EDTA – ethylenediamine tetraacetic acid, EGTA – ethyleneglycol-bis-(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid, SDS – sodium dodecyl sulphate

Introduction

The control of various metabolic pathways by reversible phosphorylation of key enzymes is well documented [1, 2]. The involvement of phosphorylation in the control of gene expression and differentiation is less well understood. However, phosphorylation of chromatin histone and nonhistone proteins has been shown to change during the cell cycle in yeast [3] and *Physarum* [4] and the activity and subcellular distribution of cAMP-dependent protein kinase also changes during chemically induced differentiation of tissue culture cells [5–7].

The mechanism of differentiation in higher eukaryotes is difficult to determine due to their complexity and simpler organisms such as cellular slime moulds are often used as model systems. *Dictyostelium discoideum* is the most commonly used cellular slime mould and a developmentally regulated cAMP-dependent protein kinase [8–11], as well as cAMP-independent protein kinases [12, 13] have been characterised. cAMP is also used as a chemoattractant (acrasin) in this organism and these effects are mediated by a cell surface receptor. It is thus difficult to distinguish the effects of cAMP which are associated with its role as achemotactrant (acrasin) from those which are associated with any intracellular role. However, other species of slime moulds do not use cAMP as their acrasin and hence the situation is simpler to investigate. We have selected *Polysphondylium violaceum*, in which the acrasin is a modified dipeptide [14], and report here the presence of a developmentally regulated cAMP-dependent protein kinase in this organism.
**Experimental procedures**

**Materials**

Dephosphorylated casein and histones were purchased from Sigma; DE-52 and P81 paper from Whatman; Matrex-gel Blue A from Amicon; cAMP-Sepharose (Sepharose 4B-NH-CH₂-CH₂-HN₆-cAMP), phenyl-Sepharose, Sephacryl S-200 and the chromatofocussing kit from Pharmacia; [γ-³²P]-ATP was prepared by the method of Johnson and Walseth [16]. [³H]-cAMP was obtained from Amersham. All other reagents were of the highest purity available.

The synthetic substrate peptides and inhibitor used were a generous gift of Dr. Bruce Kemp. Their sequences are shown in Table 1. The catalytic subunit of beef heart cAMP-dependent protein kinase was purified as described [15].

**Cell culture**

Polysphondylium violaceum (Strain Tokyo, kindly provided by Prof. K. Williams) was grown at 22°C with Enterobacter aerogenes on SM agar [17] in the dark. To determine the developmental regulation of protein kinase activity we developed the following method for synchronous development of *P. violaceum*. 39 hours after inoculation the spores had germinated and they were all vegetative. The amoebae were then washed off the surface of the agar with distilled water, freed of bacteria by 2 – 3 low speed centrifugations (200 × g for 10 minutes) and spread on SM agar at a density of 0.5 – 1.0 × 10⁸ cells/cm². They were then incubated at 22°C with fluorescent lighting. Under these conditions differentiation was > 80% synchronous, reaching late culmination stage after 15 hours. When they reached the appropriate stage of differentiation they were harvested by drawing a glass slide across the agar and used immediately.

**Buffers**

Buffer A: 10 mM HEPES, 1 mM EGTA, 2 mM EDTA, 5 mM β-mercapto-ethanol (pH 7.5). Buffer B: Buffer A containing 1M ammonium sulphate. Buffer C: Buffer A containing 50% propylene glycol. Buffer D: 20 mM HEPES, 2 mM EGTA, 4 mM EDTA, 5 mM β-mercapto-ethanol, 20% propylene glycol.

**Partial purification of protein kinase subunits**

Cells differentiated for 15 hours as described above were used. The moulds (approximately 15 grams) were suspended in 4 volumes of buffer A containing 10 mM benzamidine. 100 μg antipain/ml and 0.1% Triton X-100 and homogenised by sonication (2 × 30 seconds). The homogenate was spun at

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Table 1. Sequences of synthetic peptides used

<table>
<thead>
<tr>
<th>Peptide*</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>1. Kemptide</td>
<td>Leu-Arg-Arg-Ala-Ser-Leu-Gly</td>
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<tr>
<td>2. PDH</td>
<td>Tyr-His-Gly-His-Ser-Met-Ser-Asn-Pro-Gly-Val-Ser-Tyr-Arg</td>
</tr>
<tr>
<td>3. GS-3</td>
<td>Arg-Tyr-Pro-Arg-Pro-Ala-Ser-Val-Pro-Pro-Ser-Pro-Leu-Ser</td>
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<tr>
<td>5. Histone H4 (43-49)</td>
<td>Val-Lys-Arg-Ile-Ser-Gly-Leu</td>
</tr>
<tr>
<td>6. CS MLC2 (1-17)</td>
<td>Pro-Lys-Lys-Lys-Arg-Arg-Ala-Ala-Glu-Ser-Ser-Ser-Val-Phe-Ser</td>
</tr>
<tr>
<td>7. CG MLC2 (6-23)</td>
<td>Lys-Ala-Lys-Thr-Phe-Lys-Lys-Arg-Pro-Gln-Ala-Thr-Ser-Asn-Val-Phe-Ser</td>
</tr>
<tr>
<td>8. MBP (148-157)</td>
<td>Gly-Thr-Leu-Ser-Lys-Ile-Phe-Lys-Leu-Gly</td>
</tr>
<tr>
<td>9. PKI (5-24)</td>
<td>Thr-Thr-Tyr-Ala-Asp-Phe-Ile-Ala-Ser-Gly-Arg-Thr-Gly-Arg-Asn-Ala-Ile-His-Asp</td>
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