SHORT COMMUNICATION

Thymidine Kinase Activity in *Dictyostelium discoideum*

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Thymidine kinase activity is present in soluble and mitochondrial fractions of axenically grown cells of *Dictyostelium discoideum*. The overall activity does not change significantly during development.

INTRODUCTION

The cellular slime mould *Dictyostelium discoideum* has been used for studies of development (Loomis, 1975) and DNA repair (Welker & Deering, 1978). Effective isotopic labelling of DNA requires information on the metabolic processing of exogenous precursors. Thymidine kinase, in the salvage pathway for the utilization of thymidine, is present in some organisms but not in others (Grivell & Jackson, 1968). Here, we demonstrate the existence of thymidine kinase activity in *D. discoideum* and describe some of its properties.

METHODS

Organism, growth and cell preparation. A derivative of *Dictyostelium discoideum* AX3 capable of growing on defined medium was used (Franke & Kessin, 1977). In this medium, 10 ml suspensions in 250 ml flasks incubated at 23 °C on a rotary shaker grew with a doubling time of 14 h. Vegetative amoebae were harvested at 4×10^6 to 8×10^6 ml⁻¹, washed by centrifugation in 0.05 M-Tris/HCl, pH 7, and resuspended at 3×10^8 ml⁻¹ in this buffer for lysis. For development, cells grown as described above were harvested at 1.5×10^7 ml⁻¹, washed, resuspended at 3×10^7 ml⁻¹ in development buffer (20 mM-KCl, 2 mM-MgCl₂, 500 µg streptomycin sulphate ml⁻¹, in 40 mM-KH₂PO₄/Na₂HPO₄ buffer, pH 6.5) and gently aerated for 9 to 11 h at 23 °C. These aggregation-competent cells were again washed, resuspended at 1×10^8 ml⁻¹ in development buffer, and 0.5 ml samples were spread on each of several Millipore filters (MP; AA, 45 mm) supported by MP pads presoaked with 1.2 ml development buffer. These were incubated in Petri dishes at 23 °C. At various times during development the cells were washed from the filters and suspended for lysis as for vegetative cells.

Lysates. The cells were lysed with a Fisher Sonic Dismembrator (Micro Tip) (Fisher Scientific Co., Pittsburgh, Penn., U.S.A.) at a relative output of 0.5 for three 30 s periods in an ice bath. In most cases, lysates were dialysed against 0.05 M-Tris/HCl, pH 7. Lysates were stored at -55 °C.

Subcellular fractionation. Soluble and mitochondrial fractions were prepared by modifications of the procedure of Parish (1975), using two lysis methods. Approximately 0.3 g cells harvested at 5×10^6 to 8×10^6 ml⁻¹ were swirled in 1 ml water for 10 min at room temperature, and then 3 ml 0.5 M-sorbitol, 2.5 % (w/v) Ficoll 400 (Pharmacia) in 0.05 M-Tris/HCl, pH 7.5, was added. For detergent lysis, Triton X-100 was added to give a concentration of 0.05 % (v/v). This suspension was swirled for 2 to 5 min and 3 ml more of the sorbitol/Ficoll solution was added. For mechanical lysis, the cell suspension without added detergent was homogenized in the cold in a Teflon/glass homogenizer for 10 min. Subsequent centrifugation procedures were identical for both lysis methods. The suspensions were centrifuged at 4 °C, 1700 g for 10 min to remove whole cells, nuclei and other large debris. The resulting cell-free homogenate was centrifuged at 4 °C, 3000 g

for 10 min, and the supernatant was centrifuged at 4 °C, 32000 g for 20 min. Fractions were assayed for thymidine kinase activity, succinate dehydrogenase activity and total protein.

Thymidine kinase assay. The procedure of Béndek & Patel (1977) was used. The reaction mixture of 0.1 or 0.2 ml contained 2.5 mM-MgCl₂, 5.0 mM-ATP (Calbiochem), 0.2 mM-[methyl-³H]thymidine [The Radiochemical Centre, Amersham; final sp.act. 50 mCi mmol⁻¹ (1.85 GBq mmol⁻¹)], 50 mM-Tris/HCl, pH 7.8, and 10 to 120 µl cell lysate. Under these conditions at 37 °C, the rate of the reaction was constant up to 20 min and linear with protein concentration up to at least 2 mg per reaction. The phosphorylated product was adsorbed to DEAE-cellulose paper discs (Whatman DE-81) while unphosphorylated substrate was removed by sequential washings in 1 mM-ammonium formate, water and 95 % (v/v) ethanol (Breitman, 1963). The labelled product was eluted from the paper with 1 M-HCl, 0.5 M-NaCl and counted in toluene/Triton X-100 (2:1, v/v) containing 2,5-diphenyloxazole (Fisher, 5.5 g l⁻¹) and 1,4-di-2-(4-methyl-5-phenyloxazolyl)benzene (Fisher, 0.5 g l⁻¹), with a Beckman LS-230 scintillation counter. The product was identified as thymidine monophosphate (dTMP) by chromatography on Eastman cellulose sheets (13254) using *tert*-amyl alcohol/formic acid/water (6:4:1, by vol.) with dTMP as a standard. The *R_F* was 0.45. Non-specific attachment of label to the DEAE-cellulose paper amounted to about 1 % of the total label added. The reaction under our conditions resulted in the conversion of up to 8 % of the initial thymidine to product. One unit of activity was defined as the production of 1 nmol phosphorylated thymidine min⁻¹.

Succinate dehydrogenase assay. The spectrophotometric assay of Bonner (1955) was used.

Protein assay. Protein was determined by the Bio-Rad assay (Bradford, 1976).

RESULTS

Activity in whole cell lysates. The lysate-catalysed phosphorylation product of thymidine was dTMP (see Methods). The specific activity observed in the *D. discoideum* vegetative cell lysates was typically 0.10 to 0.16 units (mg protein)⁻¹, i.e. about 30 % of that of stationary phase *Escherichia coli* B/r assayed under the same conditions (unpublished results). Maximum (control) activity was attained only when Mg²⁺ was included in the reaction mixture; addition of 10 mM-EDTA reduced this activity to 11 %. The activity was reduced to 16 % of the control by the addition of 0.1 mM-deoxythymidine triphosphate (dTTP) and to 6 % by 1 mM-dTTP. No inhibition was observed with 5 mM-NaF. These responses verify that the observed activity is primarily that of a thymidine kinase and not a nucleoside phosphotransferase (Arima *et al.*, 1971).

Thymidine and ATP dependence. Linear Lineweaver-Burk plots were obtained for reaction-rate dependence on thymidine concentration and ATP concentration. Linear regression analysis of these plots yielded *K_m* values of 10.5 µM and 2.1 mM for thymidine and ATP, respectively. *V_{max}* for the reaction was 0.16 nmol dTMP min⁻¹ (mg protein)⁻¹. Of several other triphosphates tested at 5 mM (UTP, GTP, CTP, dATP, dGTP, dCTP), only UTP (39 % of control activity with ATP), GTP (17 %) and dGTP (15 %) showed significant ability to substitute for ATP. Hence, this enzyme is rather specific in its utilization of phosphate donors. As indicated earlier, dTTP was inhibitory in the presence of ATP; dATP, dCTP and dGTP were not inhibitory under these conditions.

pH dependence. The observed activity showed a broad maximum over the pH range from 7 up to at least 10, decreasing at lower pH values to yield 35 % of the maximum activity at pH 5.5.

Temperature dependence. The relative rates of the reaction at 23, 30, 37 and 45 °C were 0.49, 0.54, 1.00 and 0.95, respectively. This optimum was different from the optimum temperature for cell growth, 23 °C.

Stability. Boiling destroyed the activity. When the initial homogenate was stored at 5 °C, the activity decreased to 80 % in 2 d, 40 % in 10 d and 10 % in 35 d. The activity was completely stable in frozen homogenates (−55 °C) for at least 1 month.

Activity in developing cells. The specific activity dropped by 30 to 40 % in stationary phase cells, but then changed by less than 20 % throughout development up to 15 h (preculmination stage). These results indicated that thymidine kinase activity was not under strong developmental control.

Table 1. *Subcellular distribution of thymidine kinase activity*

Fraction	Activity recovered (%)*					
	Detergent lysis		Mechanical lysis		Average	
	Thymidine kinase	Succinate dehydrogenase	Thymidine kinase	Succinate dehydrogenase	Thymidine kinase	Succinate dehydrogenase
Mitochondria-enriched (3000 g pellet)	44	57	23	57	33	57
Other particulate (32000 g pellet)	5	33	9	20	8	27
Soluble (cytoplasmic) (32000 g supernatant)	51	10	68	23	59	17

* Relative to the sum of these three fractions taken as 100%.

Soluble and mitochondrial activities. Table 1 shows the results of several experiments on the localization of thymidine kinase activity in subcellular fractions. Results for the two lysis procedures (detergent or mechanical) were similar. Using succinate dehydrogenase as a mitochondrial marker (Siu *et al.*, 1977), the pellet after the 3000 g centrifugation was identified as the mitochondria-enriched fraction. This is in agreement with the distribution found by Parish (1975). A smaller portion of succinate dehydrogenase activity was also found in the 32000 g pellet. Very little (average 17%) was found in the final supernatant ('cytoplasmic' fraction). On the other hand, more than 50% of the thymidine kinase activity was found in this soluble fraction. Sonication or detergent lysis of the mitochondrial pellet (3000 g) did not change its measured activity. We conclude that *D. discoideum* cells contain both soluble (cytoplasmic) and mitochondrial thymidine kinase activity.

DISCUSSION

In defined axenic medium, exogenous thymidine is not required for the growth of *D. discoideum* (Franke & Kessin, 1977). However, small amounts of exogenous thymidine can be incorporated into both the nuclear and mitochondrial DNAs of this organism (Firtel & Bonner, 1972; C. A. Michrina & R. A. Deering, unpublished results). We have demonstrated here that both mitochondrial and cytoplasmic thymidine kinase activities exist in this organism. No information is available at present as to whether these activities are due to one or more genetically distinct forms of the enzyme (see Littlefield, 1977). These results suggest that attempts to isolate mutants that more effectively incorporate exogenous thymidine into nuclear DNA might be a useful approach to improving the specific labelling of DNA in this organism. We are seeking such mutants.

It is of interest that there is not much decline of thymidine kinase activity during development, a period during which there is little DNA replication or cell division. Thymidine kinase activity in some organisms has been shown to increase markedly during periods of DNA synthesis and decrease thereafter, although the basis for this apparent coupling is unclear (Hildebrandt & Sauer, 1973; Littlefield, 1977).

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