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Polyphosphate glucokinase and ATP glucokinase activities in *Myxococcus coralloides* D

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Abstract. Polyphosphate glucokinase and ATP glucokinase were detected in cell-free extracts of *Myxococcus coralloides* strain D, but pyrophosphate glucokinase was not detected. Both glucokinase activities were separated by chromatography. The approximate molecular weight is 61000 for polyphosphate glucokinase and 47000 for ATP glucokinase. Substrate specificity and pH optimum was studied in the polyphosphate glucokinase. Polyphosphate and ATP glucokinase activities were verified by ^{13}C Nuclear Magnetic Resonance.

Key words: ^{13}C-NMR – Glucokinase – Polyphosphate – Myxococcus

Polyphosphate glucokinase catalyses the specific transfer of the phosphate group from high molecular weight polyphosphate to glucose to form D-glucose-6-phosphate. The discovery of this enzyme in mycobacteria and related microorganisms (Szymona and Ostrowski 1964) has shown that high energy phosphate residues of polyphosphate can be used directly, without the participation of the ADP-ATP system. This enzyme has also been found in other bacteria, such as *Bdellovibrio bacteriovorus* and *Renobacter vacuolatum* (Kulaev and Vagabov 1983) and various species of *Propionibacterium* (Wood and Goss 1985).

Most studies of myxobacterial nutrition have been made with *Myxococcus xanthus*. The inability of *M. xanthus* to metabolize glucose may be explained by its lack of hexokinase (Watson and Dworkin 1968). Hexokinase activity has, however, been found in other myxobacteria such as *Stigmatella brunnea* (McCurdy and Khouw 1969), *Polyangium cellulosum* (Sarao et al. 1985) and *Corallococcus (Myxococcus) coralloides* strain Cc c127 (Irshik and Reichenbach 1985). In these cases, ATP was used as phosphate donor in the hexokinase assays. Although a hexokinase that uses pyrophosphate has been observed in *M. xanthus* (Shimkets 1984), no similar assays with pyrophosphate and polyphosphate as donor have been reported with other myxobacteria.

In this paper we report on glucokinase activity in *Myxococcus coralloides* strain D.

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Materials and methods

Organism and growth conditions

Myxococcus coralloides strain D is a bacterium from our laboratory stock. It was grown at 28°C under shaking (200 rev/min) in a TT liquid medium (0.75% Trypticase peptone-BBL, 0.1% $MgSO_4 \cdot 7H_2O$ in 0.01 M potassium phosphate buffer, pH 6.5).

Preparation of cell-free extracts

The cells from exponentially growing cultures (500 ml) were centrifuged at 12,000 *g* for 20 min at 4°C, then resuspended in 5 ml of 10 mM Tris-HCl buffer pH 7.5 + 10 mM $MgCl_2$ and broken by sonication at 50 W for 10 min in ice water. The cell extract was centrifuged at 40,000 *g* for 20 min at 4°C to remove cell debris.

Gel filtration through Sephacryl-200

Cell-free extracts were chromatographed through a column (2.6 × 35 cm) with Sephacryl-200 (Pharmacia Fine Chemicals). Elution was carried out with 10 mM Tris-HCl, pH 8, containing 10 mM $MgCl_2$, and 4 ml fractions were collected. Absorbance at 280 nm and polyphosphate glucokinase and ATP glucokinase actvities were measured in the fractions.

Molecular weight standards

Molecular weights of the marker protein were: cytochrome *c* (12,400), carbonic anhydrase (29,000) and bovine serum albumin (68,000).