Myokinase activity in microorganisms

Myokinase (adenylate kinase, adenosine diphosphate phosphomutase) which catalyses the reaction

2 adenosine diphosphate (ADP) $\rightleftharpoons$ adenosine triphosphate (ATP) + adenylic acid (AMP) (1)

was first discovered in muscle by COLOWICK AND KALCKAR[1] and has since been reported in other mammalian tissues and in insect muscle (reviewed by KALCKAR AND KLENOW[2]). Myokinase activity has also been found in preparations from yeast (KOTEL'NIKOVA[3]; KORNBERG AND PRICER[4]) and the experiments of KNIVETT[5] indicate that the enzyme is present in *Streptococcus faecalis* but, as far as we are aware, these are the only two microorganisms in which the enzyme has been demonstrated, to date.

Recently, one of us has described a rapid and convenient method for the assay of myokinase (OLIVER[6], *cf.* KORNBERG AND PRICER[4]) in which reaction 1 is coupled with reactions 2 and 3 by the addition of a preparation from yeast containing hexokinase and "zwischenferment". The rate of reduction of triphosphopyridine nucleotide (TPN) is followed spectrophotometrically by

$$\text{ATP} + \text{glucose} \longrightarrow \text{ADP} + \text{glucose-6-phosphate} \quad (2)$$

$$\text{Glucose-6-phosphate} + \text{TPN}_{ox} \longrightarrow \text{6-phosphogluconate} + \text{TPN}_{red} \quad (3)$$

measuring the increase in absorption at 340 mμ and, under suitable conditions, is proportional to the myokinase activity. This assay technique has been used to demonstrate myokinase activity in cell-free extracts of several microorganisms.

Cultures of the organisms were harvested in the late logarithmic phase of growth and washed in distilled water. With four exceptions, extracts were then prepared by crushing 1–3 g wet wt of the cell paste (or mycelial mat in the case of *Rhizopus nigricans*) in a bacterial press (HUGHES[7]). The crushed material was suspended in 1.5–5 ml 0.01 *M* $KH_2PO_4 - K_2HPO_4$ pH 6.8/g wet wt original cell paste, and centrifuged for 20 min at 9000 *g*. The supernatant was used for assays. In the case of *Thiobacillus denitrificans* the extract was kindly provided by Dr. P. A. TRUDINGER and was prepared in a similar fashion except that the cell paste was mixed with an equal volume of powdered glass before crushing and the phosphate buffer was replaced by 1.5 ml 1% KCl/g wet wt cell paste. The extracts of *Clostridium kluyveri*, *Escherichia coli* and the rumen organism LCl were obtained by extracting vacuum-dried cells for 1 h with 10 ml 0.01 *M* phosphate buffer/g dried cells. Before use, 2–3 ml samples of all extracts were dialysed with agitation against 1 l 0.001 *M* $KH_2PO_4 - Na_2HPO_4$ pH 7.4 for 2–3 h at 0°.

Fig. 1 shows a typical spectrophotometric reaction curve obtained with an extract of *Mycobacterium phlei*. Initially a slow increase in optical density due to a slight reduction of TPN by the hexokinase-zwischenferment preparation was observed. Upon addition of the bacterial extract, a rapid reduction of TPN began which proceeded at a linear rate (Curve B, Fig. 1). The reduction ceased abruptly when excess AMP was added, a further indication that the TPN reduction was indeed due to myokinase activity. A control experiment showed that there was no reduction of TPN by the bacterial extract in the absence of ADP (Curve A, Fig. 1).

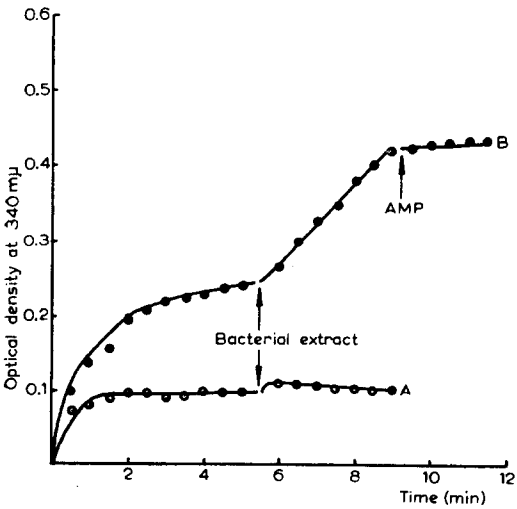

Fig. 1. Spectrophotometric demonstration of myokinase activity in extract of *Mycobacterium phlei*. Cuvettes initially contained in a total volume of 3.4 ml: 0.05 *M* barbitone-HCl buffer pH 8.6, 0.01 *M* $MgCl_2$, 0.1 *M* KCl, 0.05 *M* glucose and 10^{-4} *M* TPN. In addition, 0.001 *M* ADP was present in expt. B. In both experiments, 0.2 ml hexokinase-zwischenferment preparation (containing 8 mg dry matter) was added at time 0 and 0.02 ml of extract of *M. phlei* (containing 0.12 mg dry matter, representing the material extracted from 1.4 mg dry weight of whole cells) was added at 5.5 min; in expt. B only, 0.1 ml of 0.2 *M* AMP was added at 9.5 min. Optical density was measured with the Unicam SP 500 spectrophotometer, the blank cell containing barbitone buffer, $MgCl_2$ and KCl in the above concentrations. Temperature 30°.

Myokinase activity in *M. phlei* was confirmed by using the paper chromatographic method of KREBS AND HEMS[8] to follow changes in the adenosine phosphates (Fig. 2). Incubation of the bacterial extract with ADP or with ATP plus AMP resulted in the formation of a mixture of AMP, ADP and ATP. From the combined evidence there can be no doubt of the occurrence of myokinase activity in the organism.

Fig. 2. Chromatographic demonstration of myokinase activity in extract of *Mycobacterium phlei*. Initially, replicate tubes each contained in a total volume of 0.5 ml: 0.05 *M* barbitone-HCl buffer pH 8.6, 0.01 *M* $MgCl_2$, 0.1 *M* KCl and either 0.01 *M* ADP (expt. A) or 0.008 *M* ATP plus 0.008 *M* AMP (expt. B). After 5 min preincubation, 0.02 ml extract of *M. phlei* (details as in Fig. 1) was added at time 0. At times 0 and 60 min, in both experiments, the reaction was stopped in one tube by the addition of 0.06 ml of 30% trichloracetic acid and the tube cooled in ice. After centrifuging, approx. 0.03 ml samples of the supernatants were used for chromatographic examination of the adenosine phosphates as described by KREBS AND HEMS[8], except that the spots applied to the paper were dried in a stream of cold air. The photograph of the U.V. absorbing spots was printed from a negative obtained as described by MARKHAM AND SMITH[10].

The myokinase contents of all the microorganisms so far tested are listed in Table I. These are expressed (a) relative to the dry weight content of the extract and (b) relative to the dry weight of cells subjected to extraction. In the latter case, myokinase content was calculated as

$$\frac{\text{Enzyme units/ml extract} \times \text{Combined vol. cells plus buffer during extraction (in ml)}}{\text{Dry weight of cells extracted (in g)}}$$

TABLE I

MYOKINASE ACTIVITY OF SOME MICROORGANISMS

Organism	*Myokinase content*	
	(a) Units/mg dry matter in extract	*(b) Units/mg dry wt cells extracted*
Clostridium kluyveri	9.8	2.1
Rumen organism LC1*	9.1	3.4
Escherichia coli	1.5	0.33
Thiobacillus denitrificans	35	8.2
Streptococcus faecalis	6.7	0.72
Saccharomyces cerevisiae	5.8	2.4
Clostridium butyricum	6.2	1.0
Corynebacterium hofmanii	6.4	0.58
Proteus vulgaris	4.1	0.65
Rhodospirillum rubrum	22	2.0
Pseudomonas strain CO1**	15	1.9
Pseudomonas strain CO2**	5.6	1.1
Bacillus sphaericus strain CO8**	7.3	2.7
Mycobacterium phlei	12	1.0
Rhizopus nigricans	12	2.7

The unit of enzyme activity is that required to produce 1 μmole ATP from ADP in 1 h.

* Described by ELSDEN, GILCHRIST, LEWIS AND VOLCANI[9].

** These organisms were isolated from soil by one of us (J. L. P.). The two *Pseudomonas* species have been tentatively identified as strains of *Pseudomonas fluorescens*.

where the unit of myokinase activity is defined as that required to bring about the formation of 1 μmole ATP/h under the conditions of the spectrophotometric assay. So far, myokinase activity has been found in all the organisms tested. These comprise one yeast, one mould and several bacteria of diverse metabolic and morphological types, including both flagellated and non-flagellated species. All the extracts, with the exception of those obtained from *Clostridium butyricum*, *Corynebacterium hofmanii*, *Proteus vulgaris* and *Rhodospirillum rubrum*, were also tested qualitatively by the chromatographic technique and in each case myokinase activity confirmed.

For an accurate comparison of the activities of different organisms it would be necessary to establish that a quantitative extraction had been achieved. This has not been done in the present case, but bearing in mind that myokinase is a relatively stable enzyme, at least in animal tissues, certain general conclusions seem justified. The main conclusion to be drawn from these experiments is that myokinase activity is widely distributed in microorganisms as well as in animal tissues. Furthermore, the myokinase contents of the whole organisms are of the same order of magnitude as those observed in animal tissues other than muscle (*cf.* OLIVER[6]). In addition, the relatively high activity found in *T. denitrificans* is considered to be significant and may possibly be connected with the fact that this organism is a strict chemoautotroph, in contrast to the other species examined.

Biochemistry Department, Sheffield University (England) I. T. OLIVER

Agricultural Research Council Unit for Microbiology, Sheffield University (England) J. L. PEEL

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The absolute value for the electrophoretic mobility of *Aerobacter aerogenes*

The calibration of a modified micro-electrophoresis apparatus (to be described elsewhere) with human erythrocytes in *M*/15 mixed phosphate buffer solution has resulted in a revised value for the absolute electrophoretic mobility of *Aerobacter aerogenes*. The absolute value, determined using both the original Abramson type and the modified apparatus, in *M*/150 mixed phosphate buffer solution (pH = 7.00, ionic strength = 0.013) is now found to be $2.37 \pm 0.04 \cdot 10^{-4}$ cm/sec/volt/cm, corresponding to a ζ-potential of -0.029 volts and a charge density of 3060 e.s.u., (compare BARRY AND JAMES[1]).

The error in the previous value was found to be due to a faulty component in the electrical circuit used. In this, the part of the circuit in parallel with the electrophoresis cell, concerned with the measurement of the applied voltage, (although not used for the accurate determination of field strength) was of much lower resistance than previously believed.

The graph showing the variation of the ζ-potential with salt concentration[1], whilst being of the correct shape, will in consequence be displaced along the concentration axis. The general qualitative conclusions regarding the effect of proflavine (JAMES AND BARRY[2]) and crystal violet (LOWICK AND JAMES[3]) on the electrophoretic mobility of this organism are in no way invalidated, since all the observations were made in buffer solutions of constant ionic strength and hence under conditions of identical field strength. The only effect will be the displacement of the histograms toward higher mobility values, the distributions remaining otherwise unchanged.

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Department of Chemistry, Chelsea Polytechnic, London (England) J. H. B. LOWICK
D. E. LOVEDAY
A. M. JAMES

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