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Search for Inducible Sugar Kinases in *Mycobacterium phlei*

Poszukiwanie indukcyjnych heksokinaz u *Mycobacterium phlei*

Поиски индукционных гексокиназ у *Mycobacterium phlei*

In contrast to yeast which phosphorylates common hexoses by one kinase of broad specificity, mycobacteria seem to possess specific enzymes for the phosphorylation reactions. Separate kinases for gluconate (10), glucose (10, 4) and fructose (6) were found to occur in *Mycobacterium smegmatis* and *Mycobacterium phlei*. Glucose and fructose were attacked by the kinases utilizing inorganic polyphosphates in parallel with those which utilized ATP (4, 6). The results of a previous work (6) suggest the fructokinases of *M. phlei* to be inducible enzymes.

In this paper the phosphorylation of glucose, fructose, mannose and gluconic acid was studied with the use of both ATP and polyphosphate, the attention being paid to the possibility of inducible formation of the corresponding enzymes.

Preliminary results were reported at the Fifth Meeting of the Federation of European Biochemical Societies in Prague, July 1968.

MATERIAL AND METHODS

Reagents. ATP (sodium salt) was obtained from Sigma (St. Louis, U. S. A.). Inorganic polyphosphate (poly-P) was a commercial preparation (Kurrol's salt) which was dissolved in 0.25 M NaCl prior to use. D-glucose was obtained from Toxa (Warszawa, Poland). D-fructose, D-mannose and fructose-6-phosphate were obtained from B. D. H. (Poole, England). D-gluconic acid was furnished by Fluka (Switzerland). It was neutralized with NaOH. Delta-gluconolactone was obtained from Matheson (U. S. A.). NADP was a product of Boehringer (Mannheim, Germany). Sephadex G-100 was purchased from Pharmacia (Uppsala, Sweden). Tris(hydroxymethyl)-aminomethane was from Light (Colnbrook, England). Other chemicals were of highest purity.

Microorganism. The culture of *Mycobacterium phlei* has been kept for a number of years by mass transfers on solid Lowenstein slants.

For experiments *M. phlei* was grown statically at 37° on synthetic media with various sugars (4%) in addition to the basal solution containing glutamate, citrate and mineral salts (2). The media were inoculated with appropriate suspensions of bacilli collected from 7-day old Lowenstein cultures. The organism grew faster with fructose or mannose than with glucose or gluconate. Accordingly, the pellicles were collected after 5 and 7 days of incubation. The cell mass was washed with water by centrifugation and treated with cold acetone as described earlier (4).

Preparation of cell-free extracts. 100 mg of acetone powder were ground for 8 min. in a cold mortar with 100 mg of corundum and 2.5 ml of 0.05 M tris-HCl buffer, pH 7.4. The homogenate was spun down in a refrigerated MSE centrifuge at 10,000 rev./min. for 30 minutes. The separated extract contained about 4 mg protein/ml.

When this extract was deproteinized with 0.1 volume of 60% $HClO_4$ and the supernatant (20 µl), following neutralization with 2 N KOH, chromatographed on Whatman No. 1 filter paper, several reducing spots could be detected. The chromatograms as developed in two different solvents (acetone-pyridine-water, 2:1:1, v/v; and n-butanol-pyridine-water, 3:2:1.5, v/v) revealed among the spots hexose-6-P and glucose, irrespective of the kind of sugar in the culture medium. Contrary to expectations, traces or no amounts of gluconate were found on the chromatograms of the extract obtained from gluconate-grown cells; the concentration of fructose including fructose-6-P, as determined by the resorcinol method (9) in extract from fructose-grown cells was very small and did not exceed 0.3 µmole/ml.

Partial purification. Cell-free extracts contained small but chromatographically detectable amounts of sugars which could interfere in searching for kinases of weak activity. In order to remove endogenous substrates and to concentrate the enzymes studied, the extracts were fractionated with ammonium sulphate (4). The fraction precipitating between 35—75% saturation was collected in minimum water and dialysed against 0.05 M tris-HCl buffer (pH 7.4) for several hours in the cold. The obtained preparation (fraction II-d) was immediately used for assays or kept frozen until needed (a few days). In some cases it was further fractionated on Sephadex G-100 columns (38.5 × 1.1 cm) using 0.05 M tris-HCl buffer (pH 7.4) both to equilibrate the column and elute the enzymes.

Determination of enzyme activities. In most cases the conversion of acid-labile phosphorus of ATP or poly-P into acid-stable ester was the basis of determination. If not stated otherwise, 0.1-ml aliquots of incubation mixtures were transferred into test tubes containing 2.9 ml of water plus 1 ml of 4 N H_2SO_4 and heated for 10 min.

in a boiling water bath. Next the samples were cooled and orthophosphate was measured by the method of Fiske and Subbarow with 1% methol in 3% $NaHSO_3$ as a reducing agent.

ATP glucokinase, ATP mannokinase and ATP gluconokinase were assayed with reaction mixtures which contained in a total volume of 0.25 ml: 2 μmoles of glucose, mannose or gluconate respectively, 1.5 μmole of ATP, 2.5 μmoles of $MgCl_2$, 26 μmoles of NaCl, 20 μmoles of tris-HCl buffer (pH 8.6) and 0.05 ml of extract. At zero time and after 20 min. of incubation 0.1-ml aliquots were taken for analysis. The difference between acid-labile P at zero time and after incubation was a measure of activity.

Polyphosphate glucokinase was assayed with a reaction mixture which contained in a total volume of 0.25 ml: 2 μmoles of glucose, 1.95 μmole of acid-labile P of poly-P, 1 μmole of $MgCl_2$, 25 μmoles of NaCl, 20 μmoles of tris-HCl buffer (pH 8.6) and 0.05 ml of extract. The assay procedure was as above except for the time of boiling which lasted for 15 min.

The above procedure proved satisfactory provided the content of protein in reaction mixtures did not exceed 120 μg per 0.1 ml. If higher amounts of extract were used, the addition of trichloroacetic acid was necessary to remove the protein prior to phosphate determination, in which case inorganic poly-P coprecipitated rendering the results unreliable.

ATP fructokinase was assayed by using a reaction mixture which contained in a total volume of 0.80 ml: 3 μmoles of fructose, 4 μmoles of ATP, 3 μmoles of $MgCl_2$, 100 μmoles of NaCl, 10 μmoles of NaF, 60 μmoles of tris-HCl buffer (pH 8.6) and 0.2 ml of extract. At zero time and after 1 hour of incubation 0.3-ml aliquots were transferred into conical centrifuge tubes which contained 2.0 ml of water and 0.3 ml of 5% $ZnSO_4$. After stirring with a glass rod, 0.25 ml of 0.3 N $Ba(OH)_2$ was added and centrifuged. Next 2 ml of supernatant were taken for the resorcinol reaction.

Polyphosphate fructokinase was assayed using a reaction mixture which contained in a total volume of 0.80 ml: 3 μmoles of fructose, 5 μmoles of acid-labile P of poly-P, 3 μmoles of $MgCl_2$, 10 μmoles of NaF, 60 μmoles of tris-HCl buffer (pH 8.6) and 0.2 ml of extract. The assay procedure was identical as that for ATP fructokinase.

Protein was determined by the method of Lowry et al. (3).

The extinctions were measured in a photoelectric colorimeter.

In some cases the kinase activities were followed by ionophoresis. Whatman filter paper No. 3 (43 × 24 cm) and the buffer consisting of pyridine (15 ml), glac. acetic acid (50 ml) and distilled water (2435 ml) were used for the purpose (1). Ionophoresis was performed at room

temperature for 1 hour at about 600 V and 60 mA. Ionopherograms were dried with hot air and reducing compounds detected with the $AgNO_3$--NaOH reagent as recommended by Trevelyan et al. (7).

RESULTS

Previous observations (5) indicated that the kinases phosphorylating glucose with either ATP or poly-P were constitutive enzymes. Their activities could easily be determined in cell-free extracts from any culture of *M. phlei*. Early attempts to demonstrate phosphorylation of fructose with extract obtained from glucose-grown cells yielded negative or erratic results. However, when fructose-grown cells were taken as a source of extract, fructokinase activity appeared at a level measurable by the resorcinol method. This behaviour suggested induction of the corresponding enzyme.

In the present work five kinase activities were compared in extracts from the cells, which had been grown in four kinds of media i.e., with glucose, fructose, mannose and gluconic acid.

Table 1. Comparison of some kinase activities in extracts from *Mycobacterium phlei* grown on liquid media with various sugars. Figures represent μmoles/hr/mg protein (mean values of three determinations performed with extracts from two or three independent cultures)

Kinase activity with substrates	Cells grown in presense of			
	glucose	fructose	mannose	gluconate
Glucose, ATP	4.1	3.7	4.4	3.2
Glucose, poly-P	11.9	12.3	10.8	10.9
Fructose, ATP	0.3	1.3	1.6	0.3
Fructose, poly-P	0	0.1	0.2	
Gluconate, ATP	11.2	4.0	5.1	10.5

As shown in Table 1, extracts from the glucose- or gluconate-grown cells exhibited only slight activities towards fructose with ATP and no activity with poly-P. However, determination showed a considerable activity increase in extracts from fructose- or mannose-grown cells. Some activity also appeared with poly-P and fructose.

In contrast to the fructokinase activities, the phosphorylation of gluconate with ATP occurred at a high rate comparable to that of glucose. The enzyme was found in all cases studied, its level being markedly higher in extracts from glucose- or gluconate-grown cells.

Attempts to phosphorylate gluconic acid with poly-P failed as judged by the results of the acid-labile P disappearance method, and traces of

activity occasionally found in the extracts could have been due to the presence of endogenous glucose.

The results of experiments with mannose were also unsatisfactory. In fact, the occurrence of a mannokinase in *M. phlei* could not be established with certainty until purified preparations were applied. Figure 1 shows, that fraction II-d did possess mannokinase activity with either ATP or poly-P.

Similarly, fraction II-d from gluconate-grown cells revealed activity toward gluconic acid not only with ATP but also with poly-P (Fig. 2 and 3). However, the poly-P enzyme was found to be absent from glucose-, mannose- or fructose-grown cells.

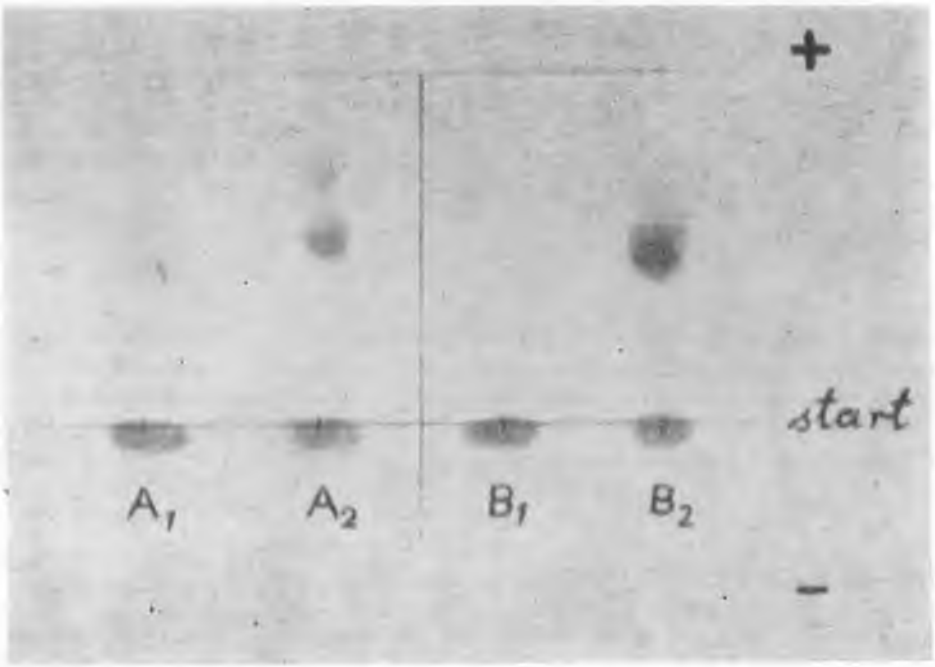

Fig. 1. Ionopherogram showing the phosphorylation of mannose. Reaction mixtures A_1 and A_2 contained: 0.2 ml of enzyme prepared from mannose-grown cells (fraction II-d, 3.2 mg protein), 0.9 μmole of mannose, 1.55 μmole of ATP, 2.4 μmole of $MgCl_2$, 18.4 μmoles of NaCl, 3.1 μmoles of NaF and 30 μmoles of tris-HCl buffer, pH 8.6 in a total volume of 0.4 ml. Reaction mixtures B_1 and B_2 contained: 0.2 ml of enzyme prepared from mannose-grown cells (fraction II-d, 3.2 mg protein), 1.0 μmole of mannose, 2.0 μmoles acid-labile P of poly-P, 0.85 μmole of $MgCl_2$, 40 μmoles of NaCl and 27 μmoles of tris-HCl buffer, pH 8.6 in a total volume of 0.4 ml. Samples A_1 and B_1 were immediately deproteinized with 0.05 ml of 25% trichloroacetic acid and centrifuged. Samples A_2 and B_2 were incubated at 37° for 1 hr and then treated with trichloroacetic acid. Of each supernatant 40 μl were applied to Whatman No. 3 filter paper and ionophoresis carried out as described in Methods

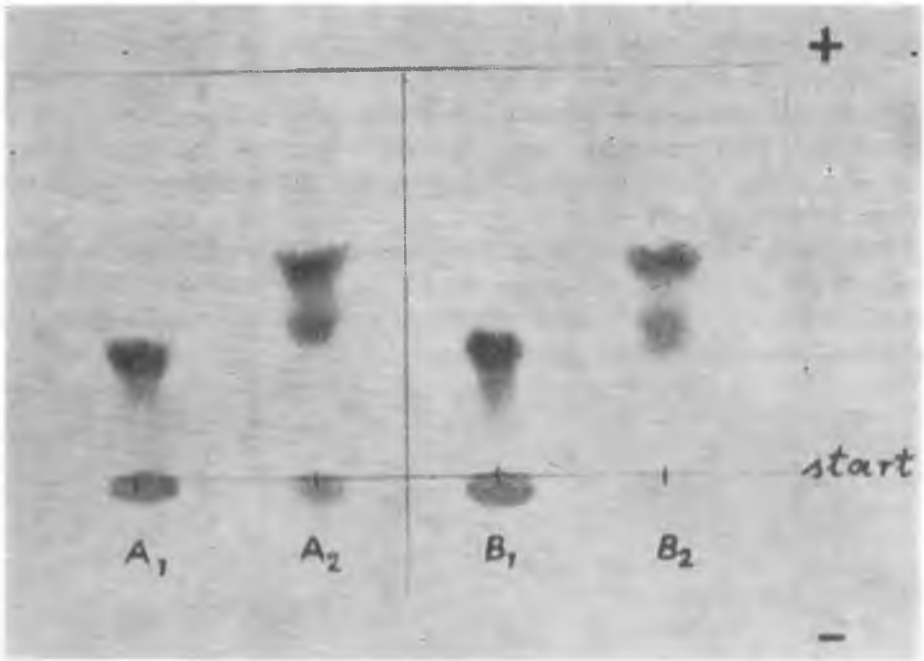

Fig. 2. Ionopherogram showing the phosphorylation of gluconic acid. Reaction mixtures A_1 and A_2 contained in a total volume of 0.4 ml: 0.2 ml of enzyme prepared from gluconate-grown cells (fraction II-d, 3.4 mg protein), 1.0 μmole of sodium gluconate and the other ingredients (ATP, $MgCl_2$, NaCl, buffer) as given in Fig. 1 for A samples. Reaction mixtures B_1 and B_2 contained in a total volume of 0.4 ml: 0.2 ml of enzyme prepared from gluconate-grown cells (fraction II-d, 3.4 mg protein), 1.0 μmole of sodium gluconate and the other ingredients (poly-P, $MgCl_2$, NaCl, buffer) as given in Fig. 1 for B samples. Further treatment of the samples as in Fig. 1

The product of phosphorylation with poly-P was identified in coupled reaction involving NADP and 6-phosphogluconate dehydrogenase. The procedure was as follows. Fraction II-d from gluconate--grown cells (0.4 ml, 4 mg protein) was incubated for 1 hour with 4.5 μmoles of gluconate, 5 μmoles of poly-P, 2.2 μmoles of $MgCl_2$, 100 μmoles of NaCl and 80 μmoles of tris-HCl buffer (final pH 7.8) in a total volume of 0.93 ml. After incubation, 0.05 ml of 60% $HClO_4$ was added to remove the protein and the supernatant neutralized by adding 2 N KOH against a drop of indicator. The obtained solution was tested for the presence of 6-phosphogluconate in a Hilger spectrophotometer at 340 mμ. A control sample was deproteinized at zero time and prepared for spectrophotometric analysis in the identical way.

Figure 4 shows rapid reduction of NADP thus indicating the presence of 6-phosphogluconate formed during preincubation of gluconate with poly-P. Some reduction in the control sample could have been due to small quantity of glucose-6-P possibly present in the extract from glucose-grown cells used as a source of 6-phosphogluconate dehydrogenase.

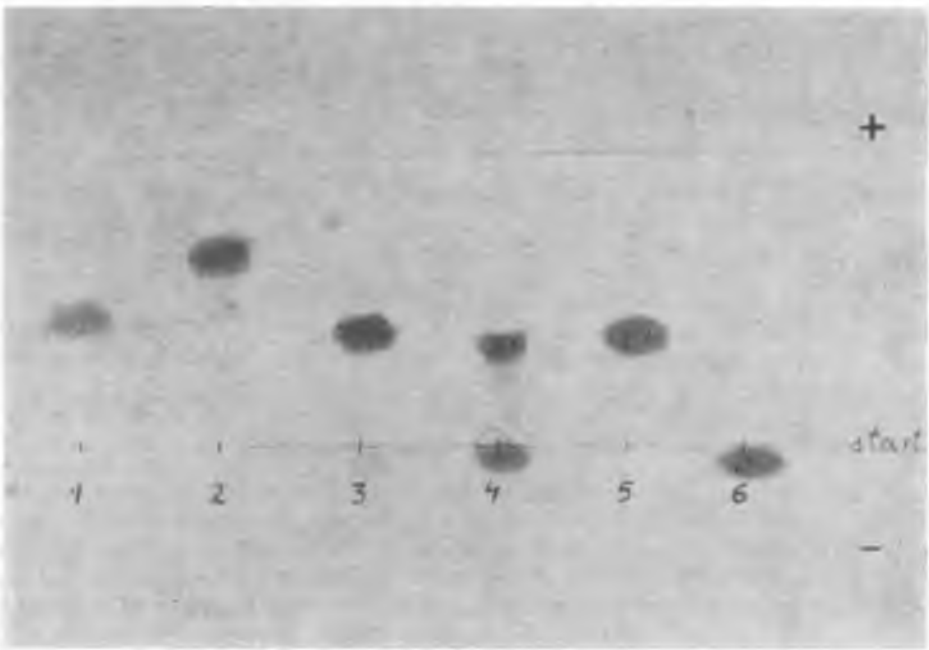

Fig. 3. Reference ionopherogram of: fructose-6-phosphate (1), 6-phosphogluconate (2), potassium gluconate (3), gluconate in solution acidified with trichloroacetic acid (4), delta-gluconolactone in alkaline solution (5), glucose (6)

In further experiments fraction II-d from fructose-grown cells (50 mg) protein/2.5 ml) was chromatographed on a Sephadex G-100 column. The procedure was carried out in a cold room (4°), 2-ml fractions being collected at a speed of about 20 ml per hour. The amount of protein and activities of the five kinases were determined in the eluates. The results are presented in Fig. 5. It is evident that there are four peaks of four distinct enzymes capable of phosphorylating glucose, mannose, fructose and gluconic acid. Fructo- and mannokinase revealed low but measurable activities, and these results were reproduced in three separate fractionations.

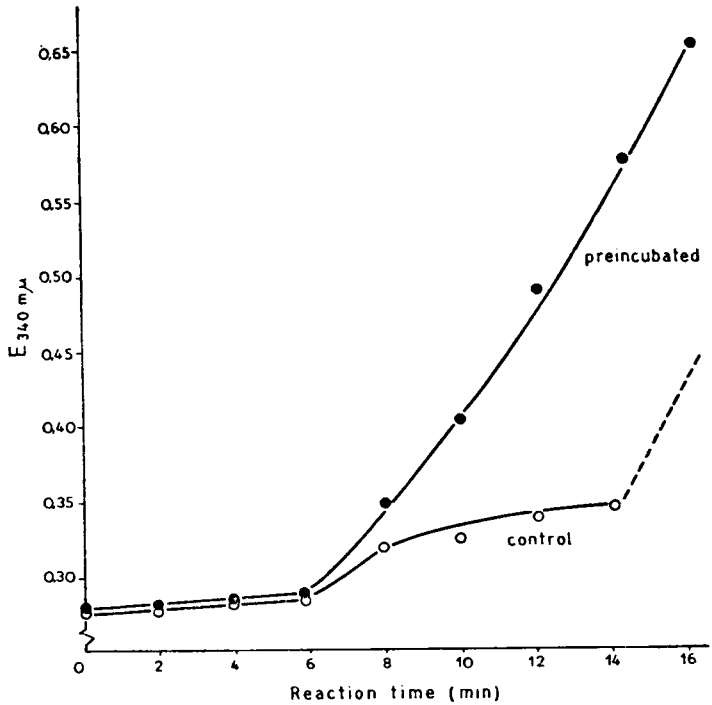

Fig. 4. Reduction of NADP by 6-phosphogluconate which was formed during preincubation of gluconate with poly-P and enzyme from gluconate-grown cells (details in the text). Reaction was followed at 340 mμ in silica cuvettes containing: 2 ml of 0.05 M tris-HCl buffer (pH 7.4), 0.1 ml of 0.1 M $MgCl_2$, 0.5 ml of protein-free test solution, 0.2 ml of cell-free extract from glucose-grown cells (source of 6-phosphogluconate dehydrogenase) and 0.2 ml of 0.01 M NADP. Reaction was started after 6 minutes by the addition of hydrogen acceptor. After 14 minutes authentic 6-phosphogluconate was added to check the system (control sample)

DISCUSSION

The results of the present work demonstrated that *M. phlei* contained separate enzymes for phosphorylation of the sugars examined, their levels, except for those of glucokinases, being dependent on the kind of the substrate in the culture medium.

Thus, fructokinase activity was higher in fructose-grown cells and probably the same was true for mannokinase. The latter enzyme could not be detected by the method used in the cell-free extracts; it was, however, demonstrated in fraction II-d. Similarly, gluconokinase proved to be higher in the glucose- and gluconate-grown cells which indicates that the enzyme was partly induced.

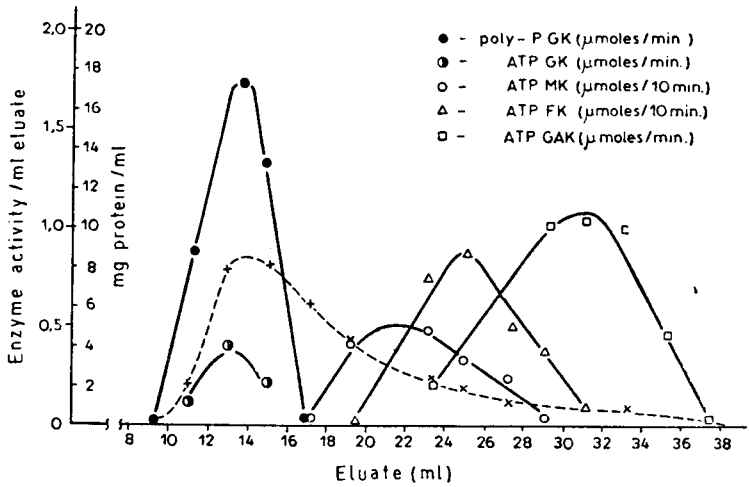

Fig. 5. Filtration diagram of fraction II-d (fructose-grown cells) on Sephadex G-100 column (details in Methods). Dotted line represents protein concentration. Polyphosphate glucokinase (poly-P GK) was assayed with a mixture containing: glucose (6.7 mM), acid-labile P of poly-P (6.7 mM), $MgCl_2$ (3.3 mM), NaCl (133 mM), tris-HCl buffer, pH 8.6 (67 mM) and 0.025 ml of eluate in a total volume of 0.075 ml. Incubation 5 min. ATP glucokinase (ATP GK) was assayed with a mixture containing: glucose (6 mM), ATP (4.5 mM), $MgCl_2$ (10 mM), tris-HCl buffer, pH 8.6 (60 mM), and 0.025 ml of eluate in a total volume of 0.125 ml. Incubation 30 min. ATP mannokinase (ATP MK) and ATP fructokinase (ATP FK) were assayed with mixtures containing: mannose or fructose (7 mM), ATP (2.5 mM), $MgCl_2$ (5 mM), tris-HCl buffer, pH 8.5 (50 mM) and 0.05 ml of eluate in a total volume of 0.10 ml. Incubation 30 min. ATP gluconokinase (ATP GAK) was assayed with a mixture containing: sodium gluconate (4.5 mM), ATP (5.5 mM), $MgCl_2$ (13.3 mM), NaCl (67 mM), tris-HCl buffer, pH 8.6 (80 mM), and 0.05 ml of eluate in a total volume of 0.15 ml. Incubation 5 min. All the above enzymes were determined by the acid-labile P disappearance method using the same test tubes (13×153 mm) in which the reaction mixtures were incubated

It is worthwhile stressing that mannose- or fructose-grown cells, despite of their faster growth on these sugars, revealed higher specific activities towards glucose or gluconate. The content of fructokinase, as increased by induction, though relatively small, was nonetheless sufficient to ensure rapid utilization of fructose. On the other hand, ATP gluconokinase being about three times more active in fructose-grown cells than ATP fructokinase, underwent a further two-fold increase in the presence of gluconate. Thus it appears that the gluconokinase was formed in large excess which was in contrast with the slower growth rate of *M. phlei* on the gluconate medium. In agreement with this assumption are the results of an experiment (unpublished) in which *M. phlei* was starved for 20 h at 37° on a glucose-free medium. The cells

gave extracts with reduced glucokinase activity, yet were able to consume more oxygen upon addition of glucose than unstarved cells.

However, the above comparisons would require support in further studies. First of all, the activities estimated in the cell-free extracts may not fully represent the in vivo content of the enzymes studied, either because of their different lability, inadequate cell disintegration (the enzymes can be partly structure-bound), or of inadequate assay conditions.

It is of interest to note, that regardless of the kind of sugar present in growth medium, there always occurred in the extracts free glucose. This observation suggests that sugars are taken up as phosphate esters being not accumulated in the cell plasm. The presence of glucose could have resulted from a breakdown of endogenous polysaccharides or from phosphatase action toward glucose-6-P which normally occurs in the cell as the key intermediate of carbohydrate metabolism.

Another point to be stressed is the ability of *M. phlei* to utilize inorganic polyphosphates for the phosphorylation of mannose and gluconic acid in addition to that of glucose and fructose reported earlier (4, 6). The phosphorylation of gluconate was established by the use of both ionophoresis and spectrophotometry. The corresponding enzyme was little active and appeared only in response to induction.

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STRESZCZENIE

W bezkomórkowych ekstraktach oraz częściowo oczyszczonych preparatach z *Mycobacterium phlei* oznaczano aktywności kinazowe w stosunku do glukozy, fruktozy, mannozy i kwasu glukonowego.

Otrzymane wyniki wskazują na semikonstytucyjny charakter badanych enzymów z wyjątkiem ATP-owej i polifosforanowej glukokinazy, których poziom był niezależny od rodzaju cukru w podłożu wzrostowym. W przeciwieństwie do wymienionych glukokinaz i glukonokinazy ATP-owej, aktywność glukonokinazowa z polifosforanem występowała tylko w preparatach z hodowli glukonianowej. Produkt fosforylacji — kwas 6-fosfoglukonowy zidentyfikowano na podstawie enzymatycznej redukcji NADP i przy pomocy jonoforezy.

Stosując filtrację na kolumnie sefadeksu G-100 uzyskano rozdział enzymów fosforylujących wszystkie cztery badane substraty.

OBJAŚNIENIA RYCIN

Ryc. 1. Jonoferogram przedstawiający fosforylację mannozy. Mieszaniny reakcyjne A_1 i A_2 zawierały: 0,2 ml enzymu otrzymanego z hodowli mannozowej (frakcja II-d, 3,2 mg białka), 0,9 μmola mannozy, 1,55 μmola ATP, 2,4 μmola $MgCl_2$, 18,4 μmola NaCl, 3,1 μmola NaF i 30 μmoli buforu tris-HCl, pH 8,6 w całkowitej objętości 0,4 ml. Mieszaniny reakcyjne B_1 i B_2 zawierały: 0,2 ml enzymu otrzymanego z hodowli mannozowej (frakcja II-d, 3,2 mg białka), 1,0 μmol mannozy, 2,0 μmole kwasolabilnego P polifosforanu, 0,85 μmola $MgCl_2$, 40 μmoli NaCl i 27 μmoli buforu tris-HCl, pH 8,6, w całkowitej objętości 0,4 ml. Do próbek A_1 i B_1 dodano natychmiast 0,05 ml 25% kwasu trójchlorooctowego i odwirowano. Próbki A_2 i B_2 odbiałczono kwasem trójchlorooctowym po 1-godzinnej inkubacji w 37°. Poszczególne płyny nad osadem naniesiono w ilości 40 μl na bibułę Whatman Nr 3 i wykonano jonoforezę jak w Metodach.

Ryc. 2. Jonoferogram przedstawiający fosforylację kwasu glukonowego. Mieszaniny reakcyjne A_1 i A_2 zawierały w całkowitej objętości 0,4 ml: 0,2 ml enzymu otrzymanego z hodowli glukonianowej (frakcja II-d, 3,4 mg białka), 1,0 μmol glukonianu sodu oraz inne składniki (ATP, $MgCl_2$, NaCl, bufor) jak podano w objaśnieniu ryc. 1 dla próbek A. Mieszaniny reakcyjne B_1 i B_2 zawierały w całkowitej objętości 0,4 ml: 0,2 ml enzymu otrzymanego z hodowli glukonianowej (frakcja II-d, 3,4 mg białka), 1,0 μmol glukonianu sodu oraz inne składniki (polifosforan, $MgCl_2$, NaCl, bufor) jak podano w objaśnieniu ryc. 1 dla próbek B. Dalsze postępowanie z próbkami jak w opisie ryc. 1.

Ryc .3. Jonoferogram wzorcowy: fruktozo-6-fosforan (1), 6-fosfoglukonian (2), glukonian potasu (3), glukonian w obecności kwasu trójchlorooctowego (4), delta-glukonolakton w roztworze alkalicznym (5), glukoza (6).

Ryc. 4. Redukcja NADP przez 6-fosfoglukonian powstały w czasie preinkubacji glukonianu z polifosforanem (szczegóły w tekście). Reakcję śledzono przy 340 mμ w kwarcowych kiuwetach, które zawierały: 2 ml 0,05 M buforu tris-HCl (pH 7,4), 0,1 ml 0,1 M $MgCl_2$, 0,5 ml badanego roztworu, 0,2 ml ekstraktu z komórek wyhodowanych na glukozie (źródło 6-fosfoglukonianowej dehydrogenazy) i 0,1 ml 0,01 M NADP. Akceptor wodoru dodano po 6 minutach obserwacji. Po 14 minutach dodano do próby kontrolnej czystego 6-fosfoglukonianu celem sprawdzenia aktywności enzymu

Ryc. 5. Diagram sączenia frakcji II-d (komórki z hodowli fruktozowej) na kolumnie sefadeksu G-100 (szczegóły w Metodach). Linia przerywana przedstawia stężenie

białka. Glukokinazę polifosforanową (poly-P GK) oznaczano przy użyciu mieszaniny zawierającej w całkowitej objętości 0,075 ml: glukozę (6,7 mM), kwasolabilny P polifosforanu (6,7 mM), $MgCl_2$ (3,3 mM), NaCl (133 mM), bufor tris-HCl, pH 8,6 (67 mM) i 0,025 ml eluatu. Inkubacja 5 min. Glukokinazę ATP-ową (ATP GK) oznaczano przy użyciu mieszaniny zawierającej w końcowej objętości 0,125 ml: glukozę (6 mM), ATP (4,5 mM), $MgCl_2$ (10 mM), tris-HCl, pH 8,6 (60 mM) i 0,025 ml eluatu. Inkubacja 30 min. Mannokinazę ATP-ową (ATP MK) oraz fruktokinazę ATP-ową (ATP FK) oznaczano przy użyciu mieszanin zawierających: mannozę lub fruktozę (7 mM), ATP (2,5 mM), $MgCl_2$ (5 mM), tris-HCl, pH 8,5 (50 mM) i 0,05 ml eluatu w całkowitej objętości 0,10 ml. Inkubacja 30 min. Glukonokinazę ATP-ową (ATP GAK) oznaczano przy użyciu mieszaniny zawierającej w całkowitej objętości 0,15 ml: glukonian sodu (4,5 mM), ATP (5,5 mM), $MgCl_2$ (13,3 mM), NaCl (67 mM), tris-HCl, pH 8,6 (80 mM) i 0,05 ml eluatu. Inkubacja 5 min. Wszystkie w. w. enzymy oznaczano na podstawie ubytku kwasolabilnego fosforu.

РЕЗЮМЕ

В безклеточных экстрактах и в частично очищенных препаратах из *Mycobacterium phlei* определяли киназовые активности по отношению к глюкозе, фруктозе, маннозе и глюконовой кислоте.

Полученные результаты указывают на семиконституционный характер исследованных ферментов за исключением АТФ-ной и полифосфатной глюкокиназы, уровень которых был независим от вида сахара в ростовой среде. Противоположно указанным глюкокиназам и АТФ-ной глюконокиназе, глюконокиназовая активность с полифосфатом проявлялась только в препаратах из глюконовой культуры. Продукт фосфорилирования — 6-фосфоглюконовую кислоту идентифицировали на основании энзиматической редукции НАДФ и при помощи ионофореза.

Применяя фильтрацию на колонке Сефадекса G-100 получили разделение ферментов, фосфорилирующих все четыре исследованных субстрата.

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