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DISTRIBUTION OF HEXOKINASE ISOENZYMES DEPENDING ON A CARBON SOURCE IN SACCHAROMYCES CEREVISIAE

Haruhiro Muratsubaki and Takurou Katsume

Biochemistry Section, Kyorin College, School of Medical Technology, Shinkawa, Mitaka, Tokyo, Japan

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SUMMARY: DEAE cellulose chromatography and agar gel electrophoresis of glucose-phosphorylating enzymes in Saccharomyces cerevisiae showed the existence of glucokinase and two hexokinase isoenzymes (designated as hexokinase I and II). The distribution of hexokinase isoenzymes was dependent on a carbon source in the medium, while that of glucokinase was not dependent. The cells grown on 3 % ethanol as carbon source showed the isoenzyme pattern with predominant hexokinase I and a little hexokinase II. The isoenzyme pattern of the cells grown on 6 % glucose, which was differnt from that of the cells grown on ethanol, showed that hexokinase I and II were minor and major parts respectively. When the cells grown on 3 % ethanol were incubated on the medium containing 6 % glucose, hexokinase I was repressed and hexokinase II inducted. These facts suggest that two hexokinase isoenzymes, but not glucokinase, are adaptive enzyme.

INTRODUCTION: The existence of two hexokinase isoenzymes (EC 2.7.1.1) (1,2) and glucokinase (EC 2.7.1.2) (3,4) in Saccharomyces cerevisiae was established. Although the kinetic properties (3,5) and the physical states (6) of these enzymes have been reported, the physiological significance is not clear. Glucokinase in mammalian liver is inducible, and the activity changed depending on hormonal and nutritional conditions, while the activity of hexokinase did not change (7,8). In yeasts, glucokinase was constitutive, and the activity did not depend on a carbon source in the medium (9,10).

This paper shows that two hexokinase isoenzymes, but not glucokinase, are adaptive enzyme.

MATERIALS AND METHODS:

Cultivation: The yeast used in this study was a Saccharomyces cerevisiae isolated from commercial baker's yeast (Nisshin Foods CO.,Inc.). The basal medium contained the following components (in grams per liter): KH_2PO_4, 1.0; ammonium sulfate, 1.2; peptone (Difco Laboratories), 10.0; yeast extracts (Difco), 10.0. This medium did not support growth without an additional carbon source. Additions were made, as indicated, to give the following concentration: glucose, 6 % (w/v) and ethanol, 3 %(v/v). The cultures were grown at 28°C with vigorous shaking.

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Assay: The enzyme activity in phosphorylation of glucose or fructose was determined by the spectrophotometric method of Maitra et al. (3). The incubation mixture for glucose-phosphorylating activity contained 40 mM tris-HCl buffer (pH 8.0), 0.2 mM NADP (Sigma), 2 mM ATP (Sigma), 10 mM $MgCl_2$, 30 mM glucose, 1 unit of glucose-6-phosphate dehydrogenase (Sigma) and enzyme in a final volume of 3.0 ml. Fructose phosphorylating enzyme was measured in the same system as that for glucose except that glucose was substituted by 30 mM fructose and 1 unit of phosphoglucose isomerase (Sigma). Incubation was carried out at 30 °C.

Protein was determined by the method of Lowry et al. (11) with bovine serum albumin as a standard.

Preparation of supernatant: The cells were suspended in 2 volumes of 0.25 M sucrose containing 5 mM tris-HCl buffer (pH 7.2) and 1 mM EDTA, disrupted with a French press, and the homogenate was centrifuged at 10.000 xg for 20 min. Then the precipitate was discarded and the supernatant was obtained.

Electrophoresis: Agar gel electrophoresis of glucose-phosphorylating enzymes was performed using gel composed of 1.2 % agar and 10 mM tris-HCl buffer (pH 8.5). Electrophoresis was carried out for 2 hours at 15 mA / 6 cm, and then the gel was stained for glucose -phosphorylating activity with the following solution; 100 ml of 40 mM tris-HCl buffer (pH 8.0) containing 2mM ATP, 0.2mM NADP, 10mM $MgCl_2$, 30 mM glucose, 100 units of glucose-6-phosphate dehydrogenase (Sigma), and 6 mg each of phenazine methosulfate and p-iodonitrotetrazolium violet.

RESULTS: As shown in Table, the difference of glucose-phosphorylating activity between the cells grown on ethanol and those grown on glucose was not observed. The F/G ratio (the ratio of enzymatic phosphorylation of fructose to that of glucose) of the cells grown on ethanol was higher than that of the cells grown on glucose, which shows that the distribution of glucose-phosphorylating enzymes with distinct F/G ratios is dependent on a carbon source in the medium.

To elucidate whether or not there is a difference of isoenzyme pattern between the cells grown on ethanol and those grown on glucose, DEAE cellulose chromatography of glucose-phosphorylating enzyme was performed. The supernatant prepared as described under "Methods" was dialyzed against 200 volumes of 5 mM succinate (pH 6.0) containing 1 mM EDTA, and was applied on a column (2 x 20 cm). The enzymes were eluted by a pH gradient of 5 mM succinate containing 1 mM EDTA between pH 6.0 and 4.2, and then by a linear gradient of NaCl (0 to 0.4 M) in the same buffer of pH 4.2. As shown in Fig.1, the difference of the isoenzyme pattern between the cells grown on glucose and those grown on ethanol was observed. In the case of glucose-grown cells (Fig.1,A), two peaks were eluted by a pH gradient. The F/G ratio of the first peak (designated as hexokinase I, HK I) was 1.9, and that of the secondary peak (hexokinase II, HK II) was 1.2. The ratios of HK I and II to total activity eluted from column were 20 % and 76 %, respectively.

Table. Levels of Glucose-Phosphorylating Activity and F/G ratio in S. Cerevisiae Grown on Different Carbon Source

Carbon source	Activity	F/G ratio
Ethanol	1.02	1.8
Glucose	1.15	1.3

The cells were grown on 3 % ethanol or 6 % glucose as a carbon source for 20 hours. These activities were assayed as described under "Methods". The activity was expressed as μmoles/min/mg protein. The F/G ratio is the ratio of enzymatic phosphorylation of fructose to that of glucose.

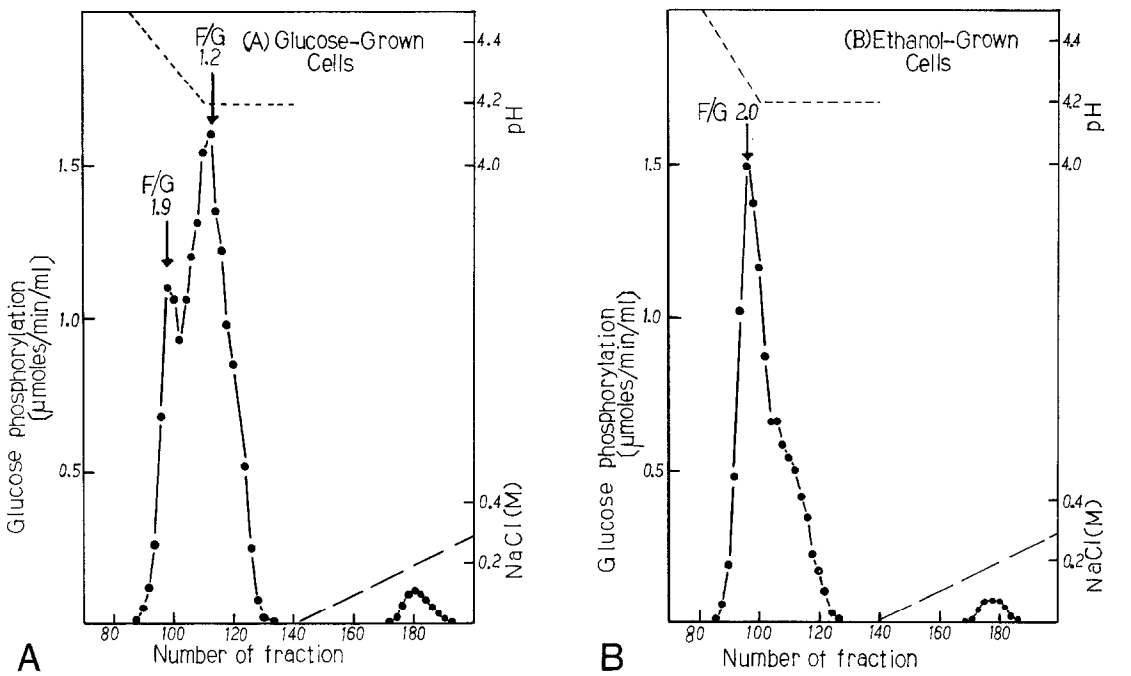

Fig. 1. DEAE Cellulose Chromatography of Glucose-Phosphorylating Enzymes from Cells Cultured on Ethanol or Glucose.

The supernatant was prepared as described under "Methods". The supernatant which had been dialyzed against 200 volumes of 5 mM succinate containing 1 mM EDTA (pH 6.0) was applied on a DEAE cellulose column (2 x 20 cm) equilibrated previously with the same buffer. Amount of the enzymes applied were 230 μmoles/min and 140 μmoles/min, in the case of glucose-grown cells (A) and ethanol-grown cells (B). Fraction of 4.8 ml was collected at a flow rate 50 ml per hour. The activity was determined as described under "Methods" (-●-●-). The pH gradient (- - -)and the NaCl gradient (—— ——) are shown. The F/G ratio for a peak tube is marked.

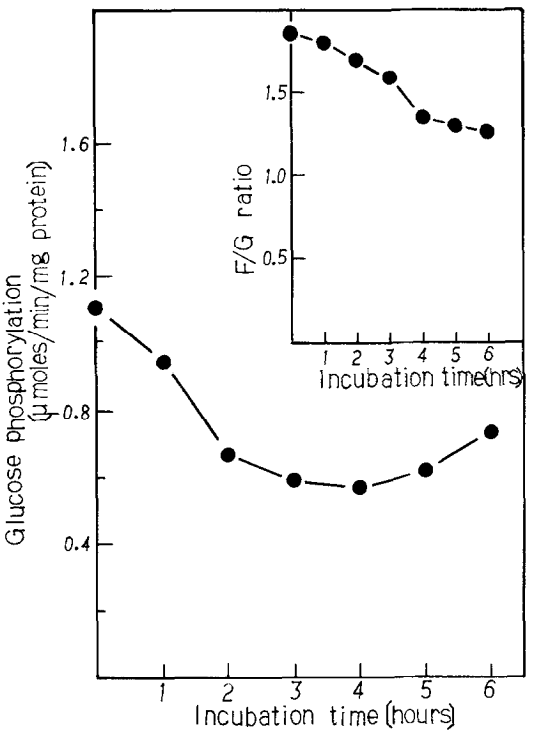

Fig. 2. Time-Course Changes in Glucose-Phosphorylating Activity and F/G ratio of Ethanol-Grown Cells during Incubation on Glucose as a Carbon Source.

Four grams of wet cells grown on 3 % ethanol were suspended in 500 ml of the medium containing 6 % glucose as a carbon source. The suspension was incubated at 28°C under vigorous shaking, and samples were taken at 0, 2, 4 and 6 hours. The activity of glucose phosphorylation and the F/G ratio were determined as described under "Methods".

The isoenzyme pattern of the cells grown on ethanol was apparently different from that of the cells grown on glucose (Fig.1 B). The activity of HK II was small as compared with that of HK I. The ratios of activity of HK I and II to the total activity eluted from column were 60 % and 37 %, respectively. Glucokinase was not eluted by the pH gradient, but was eluted by the NaCl gradient. The ratios of glucokinase to the total activity in case of the cells grown on ethanol and those grown on glucose were only 3 % and 4 % respectively. These facts indicate that the distribution of hexokinase isoenzymes, but not glucokinase, is dependent on a carbon source.

Time-course change in the glucose-phosphorylating activity or the F/G ratio of ethanol-grown cells during incubation on the medium containing glucose was studied. As shown in Fig. 2, the initial activity

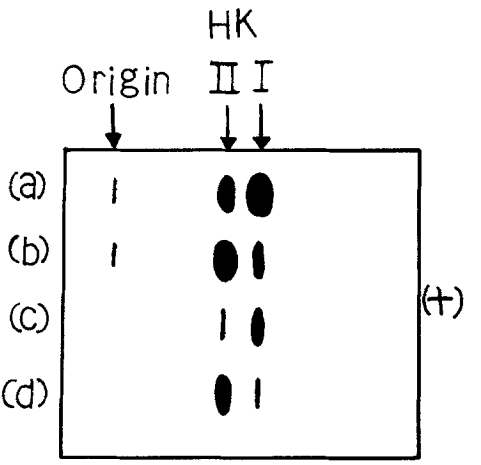

Fig. 3. Agar Gel Electrophoresis of Glucose-Phosphorylating Enzymes from Cells Grown on Ethanol or Glucose as a Carbon Source.
The agar gel electrophoresis of glucose-phosphorylating enzymes was performed as described under "Methods". Five ul of supernatant applied on a gel plate. Electrophoretogram showed the following; (a), ethanol grown cells; (b), ethanol-grown cells incubated on the medium containing glucose during 5 hours; (c), HK I obtained by DEAE cellulose chromatography of ethanol-grown cells; (d), HK II obtained by DEAE cellulose chromatography of glucose-grown cells.

of glucose phosphorylation decreased until 4 hours and thereafter increased gradually. The F/G ratio was 1.8 at initial time, decreased with time, and was 1.3 at 5 hours. The change of the F/G ratio observed during incubation on glucose may be due to the change of distribution of hexokinase isoenzymes with distinct F/G ratios. This possibility was comfirmed by the agar gel electrophoresis of the enzymes. The supernatant from the cells grown on ethanol showed the isoenzyme pattern with HK I of major component and HK II of minor component (Fig. 3). The ethnol-grown cells incubated on a medium containing glucose for 5 hours revealed that HK II was a major component. Glucokinase on agar gel was not detected, because the amount of the enzyme is far smaller as compared with that of hexokinase isoenzymes as shown in Fig. 1. These results show that HK I is repressed and HK II is inducted during incubation on the medium containing glucose as a carbon source.

DISCUSSION: The cells grown on ethanol and on glucose showed two peaks of hexokinase on DEAE cellulose column (Fig. 1). The forms which we have named hexokinase I and II correspond to Colowick's

forms P I and P II (2), and Barnard's A and B (1), respectively. HK I and II were major components in the cells grown on ethanol and on glucose, respectively (Fig. 1). When the ethanol-grown cells were incubated on the medium containing glucose, HK I and II with F/G ratio 1.9 and 1.2 were repressed and induced with time, respectively (Fig.2), which was confirmed by the decreasing of the F/G ratio with time (Fig. 2). These facts suggest that HK I and II play some important role under gluconeogenic and glycolytic conditions respectively.

Glucokinase of liver is a inducible enzyme and the activity changes depending on hormonal and nutritional conditions, while hexokinase is constitutive (7,8). However glucokinase in S. cerevisiae did not depend on a carbon source and was constitutive (Fig. 1). Also it is reported that glucokinase in R. glutinis (10), C. tropicalis (9) and C. lipolytica (9) are constitutive.

When the cells grown on ethanol were incubation on the medium containing glucose; the activity of glucose-phosphorylation decreased during 3 hours and then restored gradually (Fig. 2), which shows perhaps that the decreasing of HK I occurs more rapidly than the increasing of HK II. It is considered that the burst of glucose-phosphorylation is controlled by the rapid decreasing of total activity until a complete induction of HK II.

The distribution of two hexokinase isoenzymes with distinct properties changes depending on a carbon source. This would be important for metabolic control. It is reported that the effects of inhibition by ADP and activation by citrate are much more pronounced with form P II (HK II) than P I (HK I) (12). Thus the induction of HK II with the higher sensitivity for effectors under glycolytic condition is convenient for the control of glucose utilization. Although the regulatory mechanism of sugar metabolism by the enzymes is not clear, this paper showed that two hexokinase isoenzymes, but not glucokinase, are adaptive enzyme. The study will be researched further to elucidate the regulatory mechanism.

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