REGULATION OF GLUCONEOGENESIS IN THE YEAST *SACCHAROMYCES CEREVISIAE*

Evidence for conversion of enolase isoenzymes

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1. Introduction

Regulation of gluconeogenesis is quite complex in *Saccharomyces cerevisiae*. Two mechanisms have been described for controlling the level of gluconeogenic enzymes: carbon catabolite repression, another term is glucose repression, which prevents synthesis of certain enzymes [1]; and carbon catabolite inactivation (reviewed in [2]), which leads to destruction of gluconeogenic enzymes within 1 h after addition of glucose. Investigations with antibodies have suggested a proteolytic process for inactivation [3–5]. In [6], inactivation of fructose-1,6-bisphosphatase remained reversible for ⩾3 min after glucose addition. Hence, the authors suggested that an interconversion of fructose-1,6-bisphosphatase preceeds irreversible proteolysis. During this investigation another phenomenon which is probably involved in the regulation of gluconeogenesis was observed for enolase isoenzymes. Contradictory results on the number of yeast enolase isoenzymes have been reported [7–10]. Whereas multiple forms of enolase were interpreted as the result from in vitro modification of the enzyme [7,10], 3 isoenzymes were demonstrated in [8]. Two of them were serologically identical, but without cross reaction with antibodies against the third enzyme. In our studies 3 isoenzymes, enolase I, enolase II and enolase III, were detected in cells growing with ethanol as carbon source, whereas only 2 isoenzymes were observed with glucose as carbon source. Addition of glucose to ethanol growing cells led to a rapid decrease in enolase I activity, whereas enolase II activity increased to the same extent. This indicated that enolase I was converted to enolase II under conditions of glucose repression.

2. Materials and methods

Haploid strain cat1.S3-14A carried the genes *a his4 MAL3 SUC3 MAL2-8^c* (for gene symbols see [12]). Cells were grown on media containing 1% yeast extract, 2% bacto peptone and 4% D-glucose, 3% ethanol, respectively as carbon source. Logarithmically growing cells were harvested at a titer of 5×10^7–1×10^8 cells/ml. Stationary phase cells were harvested 4 h after exhaustion of glucose. Cells were washed twice with KPO_4 buffer (pH 6.5) and frozen until use. Crude extracts were prepared with glass beads [13]. Cell debris was centrifuged at $5000 \times g$ for 15 min at 4°C. The supernatant fluid was used as crude extract. Protein was determined as in [14] at 290 nm. Enolase was assayed in 48 mM triethanolamine buffer (pH 7.6) with 1 mM 2-phosphoglycerate, 7.4 mM $MgCl_2$, 0.73 mM fructose-1,6-bisphosphate (as an activator of pyruvate kinase), 1 mM ADP and 0.85 mM NADP. Pyruvate kinase (2 U/ml), hexokinase (1.4 U/ml) and glucose-6-phosphate dehydrogenase (0.85 U/ml) were used as indicator enzymes.

Discontinuous gel electrophoresis was done in the Ultra Phor apparatus (Colora, Lorch) as in [15,16]. Separator gel had 10% acrylamide and collecting gel 3.75%. Gels were overlayed with 1 mg protein/cm gel breadth. After electrophoresis, the gels were cut to slices of ~1 mm. Each slice was incubated with 0.5 ml enzyme assay mixture using incubation plates (Costar, Greiner, Nürtingen) and incubated for 15 min. Afterwards 0.5 ml formazane mixture (100 mg MTT 3(4,5-dimethyl-thiazoyl-2)-2,5-diphenyl tetrazoliumbromide and 17.5 mg phenazine methosulfate, both Serva (Heidelberg) per 100 ml KPO_4 buffer (pH 6.5) were added. The formazane mixture was as in [17]. The

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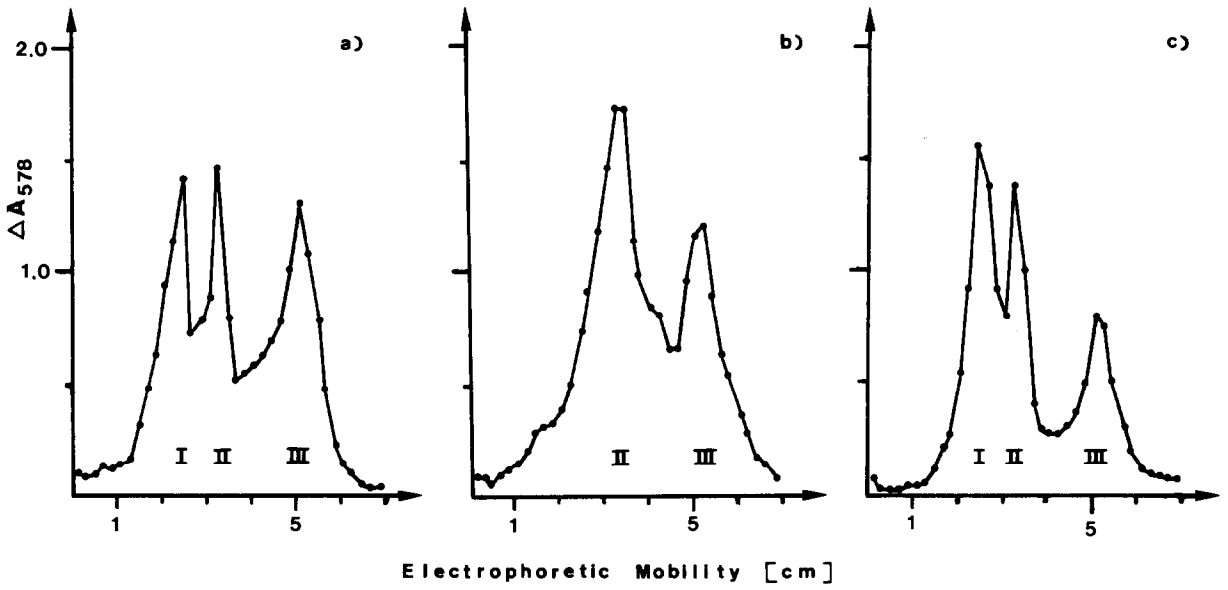

Fig.1. Detection of enolase activity in polyacrylamide gels after electrophoresis (16 h at 130 V): (a) cells grown with ethanol as carbon source; (b) cells grown with glucose as carbon source; (c) stationary phase cells.

solution was immediately transferred to another vessel. Enzymatic reactions stopped immediately, because the enzyme remained in the gel. The dye reaction needed ~30 s and was stable for ⩾10 min. Extinction was measured at 578 nm.

3. Results and discussion

Enolase activities were determined in crude extracts after polyacrylamide gel electrophoresis. Three enzymes enolase I, enolase II and enolase III, were detected in cells growing with ethanol as carbon source (fig.1a). Cells growing logarithmically with glucose as carbon source had enolase II and enolase III (fig.1b) only. Enolase II activity was markedly higher in the D-glucose grown cells. Four hours after the glucose was exhausted, the stationary phase cells had again 3 enzymes (fig.1c). Clearly, regulation of the activities of the enolase isoenzymes depended on the availability of glucose. When glucose was added to cells growing with ethanol as carbon source, enolase I activity decreased after 15 min; simultaneously, enolase II activity increased (fig.2). Assuming symmetrical peaks, as found for single enzymes with the electrophoretic method used, band height can be used as a measure of enzyme activity. Additionally, overlapping of bands must be considered and this plotting method illustrated the observed effects (fig.3). No changes in activity

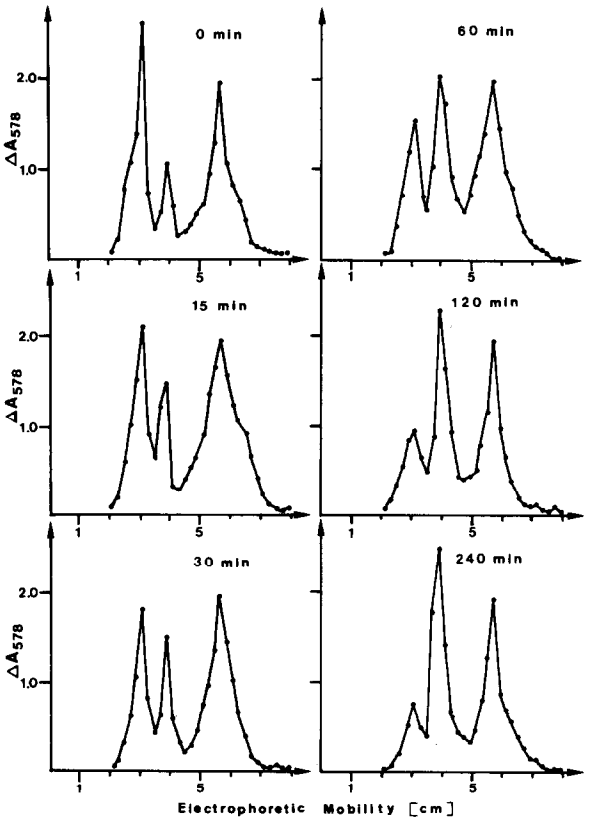

Fig.2. Detection of enolase activity in polyacrylamide gels after electrophoresis (20 h at 130 V). Samples were investigated different times after adding glucose to cells growing with ethanol as carbon source.

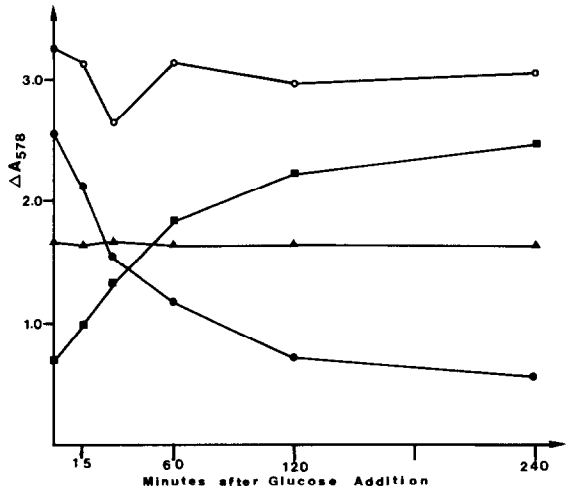

Fig.3. Bed height diagram of enolase activity after adding solid glucose (final conc. 2%) to cells growing with ethanol as carbon source: (●) enolase I; (■) enolase II; (▲) enolase III; (○) sum of enolase I and enolase II bed height.

were observed for enolase III. The sum of enolase I and enolase II activity remained constant after adding glucose. No further changes in overall specific activity (2.5 U/mg protein) were observed. Enolase I seemed to be converted into enolase II without loss of catalytic activity. Hence, enolase I was interpreted as a gluconeogenic enzyme. Conversion of this enzyme would give the cell another regulatory mechanism for preventing gluconeogenesis if glucose is present in the cell. That enolase is the first enzyme during gluconeogenesis which is also necessary for glycolysis is consistent with this hypothesis whose physiological importance is now under further investigation.

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