Dependence on cyclic AMP of glucose-induced inactivation of yeast gluconeogenetic enzymes

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1. INTRODUCTION

In some yeasts, addition of glucose or metabolically related sugars to cells adapted to a sugar-lacking medium causes a time-dependent disappearance of some enzymes. The proposed physiological role of this 'catabolite inactivation' is the regulation of glucose formation, since nearly all of the involved enzymes take part in metabolic reactions that provide the cells with glucose [1]. In *Saccharomyces cerevisiae*, the gluconeogenetic enzymes fructose-1,6-bisphosphatase [2], cytoplasmic malate dehydrogenase [3,4], and phospho*enol*pyruvate carboxykinase [5] underly catabolite inactivation. Proteolysis has been shown to be involved in the inactivation of all of these enzymes [6–9]; moreover, the native fructose-1,6-bisphosphatase has been reported to be interconverted to a phosphorylated, less active form prior to proteolysis [10–13]. In [14] an abrupt increase in intracellular cAMP within 2 min after glucose addition was seen in *S. cerevisiae*. Such an increase was reduced by precincubation of cells with increasing caffeine concentrations: a strong correlation between cAMP levels and the extent of inactivation could thus be demonstrated for all 3 enzymes, and a possible involvement of the cyclic nucleotide in the degradation process was therefore suggested. Here, catabolite inactivation was tested in the two haploid mutants of *S. cerevisiae*, AM3-4B and AM7-11D [15,16]. The mutant AM3-4B (*α ade6 ade10 amp1 cam1 cam2 cam3*) is adenine auxotrophic (mutations *ade6 ade10*) and able to incorporate 5′-AMP (*amp1*) and cAMP (*cam1 cam2 cam3*) from the medium; the mutant AM7-11D (a *cyr1*) has been isolated from the previous one, and the mutation *cyr1* makes it deficient in adenylate cyclase. In the experiments reported here, the extent of catabolite inactivation of the 3 enzymes and the glucose-induced peak of intracellular cAMP were measured in the mutants AM3-4B and AM7-11D. The absence of the inactivating effect of glucose in the strain defective for cAMP synthesis provides evidence for the involvement of the cyclic nucleotide in the inactivation process.

2. MATERIALS AND METHODS

Yeast growth media were obtained from Merck (Darmstadt). Reagents for enzyme assay were from Boehringer (Mannheim). Blue Sepharose CL-6B was from Pharmacia (Uppsala). Glass beads were purchased from Thomas (Philadelphia, PA). Cellulose nitrate filters were from Sartorius-Membranfilter (Göttingen). Spectrophotometric measurements were carried out in a Gilford 2400 recording spectrophotometer. A PL Prias liquid scintillation counter and Pico Fluor 30 liquid scintillator from Packard (Downers Grove, IL) were employed for radioactivity measurements.

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The haploid mutants of *S. cerevisiae*, AM3-4B and AM7-11D, were kindly supplied by Professor T. Ishikawa, Institute of Applied Microbiology, University of Tokyo. The strain M1 was kindly supplied by Professor H. Holzer, University of Freiburg. Yeasts were grown to the stationary phase for 48 h at 30°C under vigorous shaking. At this time, full derepression of the enzymes under investigation had occurred. The growth was started by inoculating, with 1 ml of a preculture, 100 ml of the growth medium containing 1% yeast extract, 2% universal peptone M66 and 2% glucose; for the mutant AM7-11D, 0.5 mM cAMP was also added. After 48 h growth, cells were collected by centrifugation, extensively washed with distilled water, resuspended to a density of 20 mg/ml in a medium containing 1% yeast extract and 2% universal peptone M66, and incubated for 1 h at 30°C under shaking. Subsequently, cells were again collected, washed and resuspended in 100 mM potassium phosphate buffer (pH 6.0) to a density of 25 mg/ml. After 10 min preincubation, incubation was started by addition of glucose to a 100 mM final concentration, with a decrease in cell density to 20 mg/ml. Preincubation and incubation of cell suspensions were performed at 30°C under shaking. Samples were withdrawn at different times for the required determinations. Zero-time samples were withdrawn immediately before glucose addition. Production of crude extracts, assay of fructose-1,6-bisphosphatase and phospho*enol*pyruvate carboxykinase, as well as cAMP determination were performed as in [14]. Protein was assayed by the Bio-Rad protein assay kit, from Bio-Rad (Richmond, VA) following the standard procedure. Separation of the two isoenzymes of malate dehydrogenase was carried out as follows: suitable amounts of Blue Sepharose CL-6B preswollen in elution buffer (20 mM Tris-HCl, 1 mM EDTA, 1 mM mercaptoethanol, pH 7.0) were placed in Pasteur pipettes to get 0.3-ml bed volumes; aliquots of extracts containing 2.7 mg protein were diluted to 1 ml with elution buffer and applied to the columns. After washing off non-adsorbed material, the cytoplasmic enzyme was eluted with 10 mM NADH in the same buffer, the mitochondrial one with 1 M KCl. Enzyme activity was assayed in 1 ml fractions as reported [4] by measuring the rate of NADH oxidation.

3. RESULTS AND DISCUSSION

The effect of glucose on the intracellular cAMP content in the yeast mutants under investigation was tested (fig.1). Whereas the mutant AM3-4B showed the reported rise in the cyclic nucleotide [14,17], in the adenylate cyclase-defective mutant, AM7-11D, low and constant levels of cAMP were measured throughout the experiment. Such levels are probably due to contaminating extracellular cAMP present in the growth medium. Extensive washing of the cells before glucose incubation, as well as 1 h incubation in medium devoid of cAMP (see section 2), were unsuccessful in removing such contamination. Concurrent with the finding that the initial rise in cAMP was limited to AM3-4B, an extensive inactivation of fructose-1,6-bisphosphatase and of phospho*enol*pyruvate carboxykinase was detected only in this mutant (fig.2). Moreover, fructose-1,6-bisphosphatase lost about 50% of its activity within the first minutes of glucose incubation, but could be fully reactivated by resuspension of the cells in acetate medium with cycloheximide (not shown): this pattern of response corresponds to the well-known reversible interconversion of the enzyme to the less active, phosphorylated form [10–13]. On the contrary, in the mutant AM7-11D, fructose-1,6-bisphosphatase was completely unaffected by incubation with glucose, and phospho-

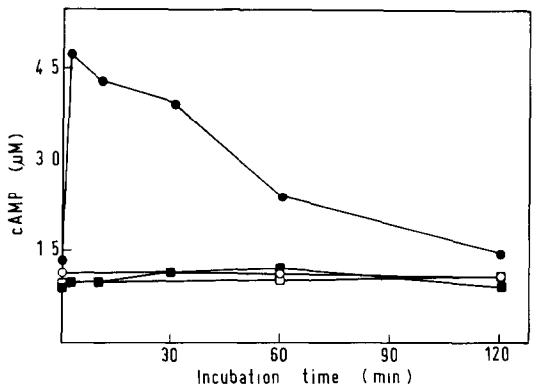

Fig.1. Time course of concentration of cAMP after glucose addition in AM3-4B and in AM7-11D yeast mutants. After addition of 100 mM glucose, samples were withdrawn at the indicated times and treated for intracellular cAMP determination: (●) AM3-4B; (■) AM7-11D. Controls without glucose addition: (○) AM3-4B; (□) AM7-11D.

*enol*pyruvate carboxykinase was only slightly inactivated within 2 h.

For malate dehydrogenase, a rather small decrease in total activity (10–20%) was observed in both mutants by incubation with glucose (not shown). However, two forms of this enzyme exist, the cytoplasmic and the mitochondrial one, and only the former undergoes catabolite inactivation [18,19]. Therefore, in order to separately check the response of each of them to glucose incubation, they were separated by means of Blue Sepharose CL-6B affinity chromatography (fig.3). In the well-characterized yeast strain M1 [20], only the peak eluted with 10 mM NADH had disappeared after 60 min of incubation (fig.3A): this allowed us to clearly identify it as the cytoplasmic enzyme. On the contrary, the non-adsorbed activity, as well as the peak eluted with 1 M KCl, did not decrease in the same time period, and therefore represents mitochondrial enzyme.

It could be thus shown that the cytoplasmic enzyme was extensively inactivated in the mutant AM3-4B, whereas its decrease in activity was small in AM7-11D (fig.3B; C). The reason why no distinct effect of glucose was detectable in AM3-4B when testing the total activity, is the presence of a largely predominant mitochondrial enzyme, as shown in fig.3B. Experiments with the mutant AM3-4B were carried out also with yeast cells grown with 0.5 mM cAMP; however, this did not cause any change in the inactivation pattern.

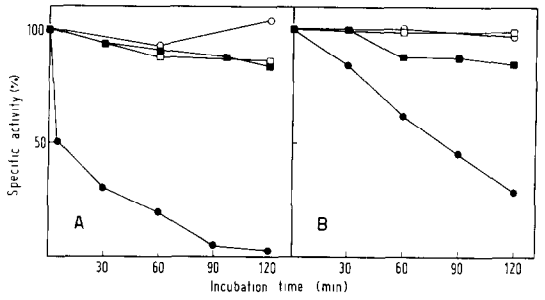

Fig.2. Effect of glucose incubation on fructose-1,6-bisphosphatase (A) and on phospho*enol*pyruvate carboxykinase (B) in AM3-4B and in AM7-11D yeast mutants. After addition of 100 mM glucose, enzyme specific activities were tested at the indicated times and expressed as relative to zero time: (●) AM3-4B; (■) AM7-11D. Controls without glucose addition: (○) AM3-4B; (□) AM7-11D.

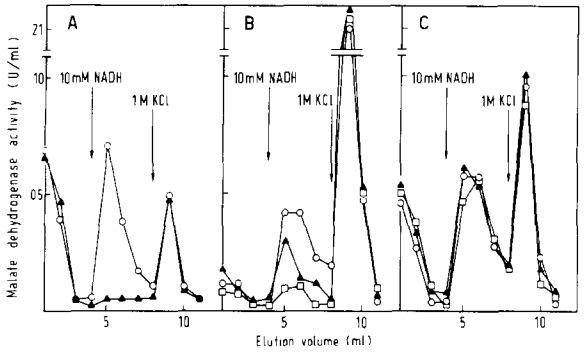

Fig.3. Effect of glucose incubation on cytoplasmic and mitochondrial malate dehydrogenase in the yeast strain M1 (A) and in the mutants AM3-4B (B) and AM7-11D (C). After addition of 100 mM glucose, cell samples were collected at the indicated times and extracts prepared; equal aliquots of extracts (2.7 mg protein) were diluted to 1 ml with elution buffer (20 mM Tris-HCl, 1 mM EDTA, 1 mM mercaptoethanol, pH 7.0) and applied to Blue Sepharose CL-6B columns (0.3 ml bed volume). After washing non-adsorbed material, cytoplasmic and mitochondrial enzymes were eluted with 10 mM NADH and with 1 M KCl, respectively. Incubation times with glucose: (○) 0 min; (▲) 60 min; (□) 120 min.

It may be therefore concluded that the possible mediating effect of cAMP involves all gluconeogenetic enzymes underlying catabolite inactivation. It was recently shown [17] that hexose phosphates exhibit a transient rise preceding the short pulse of cAMP; these catabolites were therefore proposed to be the mediators of the glucose-induced overshoot of cAMP through adenylate cyclase stimulation and/or cAMP phosphodiesterase inhibition. The cyclic nucleotide should in turn trigger the phosphorylation of fructose-1,6-bisphosphatase as shown by in vitro experiments [17,21], via the activation of cAMP-dependent protein kinase. No data are yet available on phosphorylation of cytoplasmic malate dehydrogenase and phospho*enol*pyruvate carboxykinase during glucose incubation. Some preliminary experiments carried out in our laboratory with specific antibodies and (^{32}P)orthophosphate failed however, to detect incorporation of ^{32}P into phospho*enol*pyruvate carboxykinase after glucose addition to yeast cells (unpublished results). Since an involvement of a cAMP-dependent protein kinase is a plausible mechanism of the cyclic nucleotide-triggered inac-

tivation, work is in progress to elucidate a possible role of this kinase for the catabolite inactivation other than phosphorylation of the target enzyme.

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REFERENCES

[1] Holzer, H. (1976) Trends Biochem. Sci. 1, 178–181.
[2] Gancedo, C. (1971) J. Bacteriol. 107, 401–405.
[3] Witt, I., Kronau, R. and Holzer, H. (1966) Biochim. Biophys. Acta 118, 522–537.
[4] Ferguson, J.J., Boll, M. and Holzer, H. (1967) Eur. J. Biochem. 1, 21–25.
[5] Haarasilta, S. and Oura, E. (1975) Eur. J. Biochem. 52, 1–7.
[6] Neeff, J., Hägele, E., Nauhaus, J., Heer, U. and Mecke, D. (1978) Biochem. Biophys. Res. Commun. 80, 276–282.
[7] Neeff, J., Hägele, E., Nauhaus, J., Heer, U. and Mecke, D. (1978) Eur. J. Biochem. 87, 489–495.
[8] Müller, M., Müller, H. and Holzer, H. (1981) J. Biol.Chem. 256, 723–727.
[9] Funayama, S., Gancedo, J.M. and Gancedo, C. (1980) Eur. J. Biochem. 109, 61–66.
[10] Lenz, A.G. and Holzer, H. (1980) FEBS Lett. 109, 271–274.
[11] Tortora, P., Birtel, M., Lenz, A.G. and Holzer, H. (1981) Biochem. Biophys. Res. Commun. 100, 688–695.
[12] Müller, D. and Holzer, H. (1981) Biochem. Biophys. Res. Commun. 103, 926–933.
[13] Mazòn, M.J., Gancedo, J.M. and Gancedo, C. (1982) J. Biol. Chem. 257, 1128–1130.
[14] Tortora, P., Burlini, N., Hanozet, G.M. and Guerritore, A. (1982) Eur. J. Biochem. 126, 617–622.
[15] Matsumoto, K., Uno, I., Toh-e, A., Ishikawa, T. and Oshima, Y. (1982) J. Bacteriol. 150, 277–285.
[16] Matsumoto, K., Uno, I., Oshima, Y. and Ishikawa, T. (1982) Proc. Natl. Acad. Sci. USA 79, 2355–2359.
[17] Purwin, C., Leidig, F. and Holzer, H. (1982) Biochem. Biophys. Res. Commun. 107, 1482–1489.
[18] Witt, I., Kronau, R. and Holzer, H. (1966) Biochim. Biophys. Acta 128, 63–73.
[19] Neeff, J. and Mecke, D. (1977) Arch. Microbiol. 115, 55–60.
[20] Hägele, E., Neeff, J. and Mecke, D. (1978) Eur. J. Biochem. 83, 67–76.
[21] Londesborough, J. (1982) FEBS Lett. 144, 269–272.