Use of glucose analogues to study the mechanism of glucose-mediated cAMP increase in yeast

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The addition of glucose to yeast triggers a transient, several-fold increase in cAMP levels followed by a permanent 2-fold increase. The transient increase can be provoked by a variety of glucose analogues, even those that cannot be phosphorylated, and also by galactose. The extent of the increase appears to be correlated with the affinity of the transport system for the different sugars. The permanent increase in cAMP occurs only with sugars that can be efficiently phosphorylated. The maximal cAMP concentration reached after addition of the sugars determines the extent of inactivation of fructose-1,6-bisphosphatase.

cAMP *Yeast* *Non-metabolizable sugar* *Fructose-1,6-bisphosphatase* *Phosphorylation* *Physiological inactivation*

1. INTRODUCTION

Work from several laboratories has established that the addition of glucose to yeast grown in gluconeogenic conditions causes an increase in cAMP levels [1–4]. The mechanism of the cAMP increase is not known although it has been postulated that it could be mediated by an activation of adenylate cyclase, caused in turn by a depolarization of the plasma membrane [2]. Among other effects the increase in cAMP triggers phosphorylation of fructose 1,6-bisphosphatase which results in partial inactivation of the enzyme [5].

Here, we have used a variety of glucose analogues to determine to what extent a sugar should be metabolized to produce a change in cAMP concentration. In addition, using sugars that induce different changes in cAMP, we have tested the correlation between the increase in cAMP and the degree of inactivation of fructose-1,6-bisphosphatase elicited by the sugars.

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2. MATERIALS AND METHODS

The following strains of *Saccharomyces cerevisiae* were used: X2180 (gal 2), 2611-6D2 (gal 80) and D-308 (hxk 1, hxk 2) obtained from H. Holzer, D.C. Hawthorne and K.D. Entian, respectively.

The yeasts were grown on a complex medium with 1% yeast extract, 2% peptone and 2% glucose. They were collected in the stationary phase of growth, washed with 50 mM Mes (adjusted to pH 6 with 1 M Tris) and resuspended in the same buffer at 20 mg wet wt/ml. They were equilibrated for 15 min at 30°C with shaking and at this time the different sugars were added as indicated in each case.

Sampling of yeast for the determination of metabolites and cAMP measurements were performed as in [6]. ATP was assayed spectrophotometrically with glucose and hexokinase. Cell free extracts were obtained as in [7] with 20 mM imidazole/HCl, pH 7. Assay of fructose-1,6-bisphosphatase was as described [8]. Protein was assayed according to Lowry et al. [9] after precipitation with trichloroacetic acid and us-

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ing bovine serum albumin as standard.

3. RESULTS AND DISCUSSION

When glucose is added to yeast grown on a gluconeogenic carbon source, cAMP concentration changes as follows. First there is a rapid several-fold increase in cAMP levels, afterwards the concentration of cAMP drops and remains at about twice its initial value (fig.1A).

We tested the effect on cAMP levels of 3 types of sugars that share the glucose transport system: metabolizable sugars, sugars that are phosphorylated but not extensively metabolized and sugars that can only be transported into the yeast. Metabolizable sugars elicit both the transient and the permanent increase in cAMP (fig.1B,C). Sugars that can only be phosphorylated show heterogeneous behaviour: glucosamine acts like the metabolizable sugars (fig.1D), 2-deoxyglucose shows the permanent increase in cAMP concentration but not the transient rise (fig.1D) and 3-methylglucose shows only a moderate and somewhat delayed transient increase in cAMP (fig.1E). Sugars that can be transported but not phosphorylated like fructose in a mutant lacking hexokinases, xylose or 6-deoxyglucose trigger only the transient increase in cAMP (fig.1E,F).

The extent of the transient cAMP increase is variable and appears to be correlated with the degree of occupancy of the sugar transport system (fig.2). An exception is 2-deoxyglucose, which has a high affinity for the glucose transport system and does not produce a rapid increase in cAMP. The increase in cAMP triggered by fructose in the hexokinase-less mutant is also lower than expected. Galactose causes a much greater increase in cAMP in a strain with a constitutive specific galactose permease (fig.1B) than in a strain lacking such a permease (fig.1E). Therefore, the effect of galactose is not dependent on an interaction with the glucose transport but can also be mediated by the specific galactose permease.

The permanent increase in cAMP levels occurs only for sugars that can be phosphorylated. Although it has recently been shown that 3-methylglucose can be phosphorylated in vivo by *S. cerevisiae* [15], the rate and extent of phosphorylation should be low as shown by the fact that 3-methylglucose does not deplete intracellular ATP like glucosamine or 2-deoxyglucose (fig.3). This could then explain the fact that 3-methylglucose behaves as the sugars which are not phosphorylated.

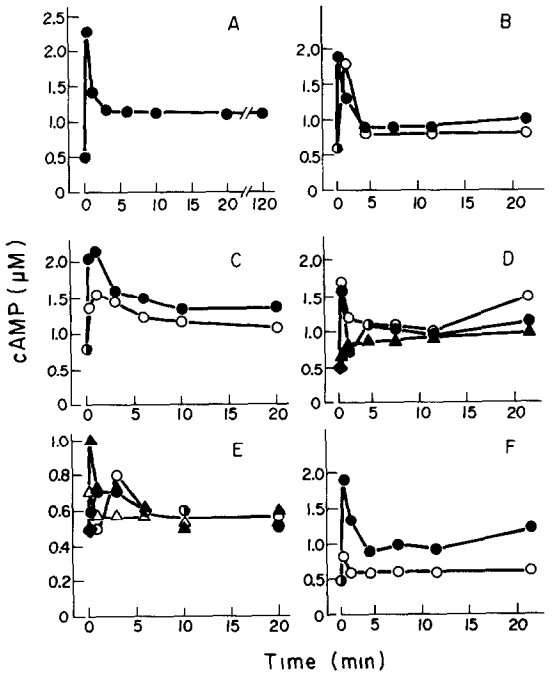

Fig.1. Time course of the effect of the addition of sugars on cAMP concentration in yeast. The yeasts were grown and resuspended in buffer as indicated in section 2. Sugars were added as shown below and samples were taken at different times for the determination of intracellular cAMP as described in the text. (A) *S. cerevisiae* X2180, 2% glucose; (B) *S. cerevisiae* X2180, 2% fructose (●—●), 2% mannose (○—○); (C) *S. cerevisiae* 2611-6D2, 2% glucose (●—●), 2% galactose (○—○); (D) *S. cerevisiae* X-2180, 25 mM glucose (●—●), 25 mM glucosamine (○—○), 25 mM 2-deoxyglucose (▲—▲); (E) *S. cerevisiae* X-2180, 0.1 M 3-methylglucose (●—●), 25 mM 6-deoxyglucose (○—○), 0.1 M xylose (▲—▲), 0.1 M galactose (△—△); (F) *S. cerevisiae* D-308, 2% glucose (●—●), 2% fructose (○—○).

A plausible interpretation for the transient increase in cAMP would be that the glucose permease acts as a sugar receptor and can interact with the adenylate cyclase. The degree of saturation of the receptor would determine the degree of activation of the adenylate cyclase and the subsequent increase in cAMP. Galactose permease would also be able to activate the cyclase. In all

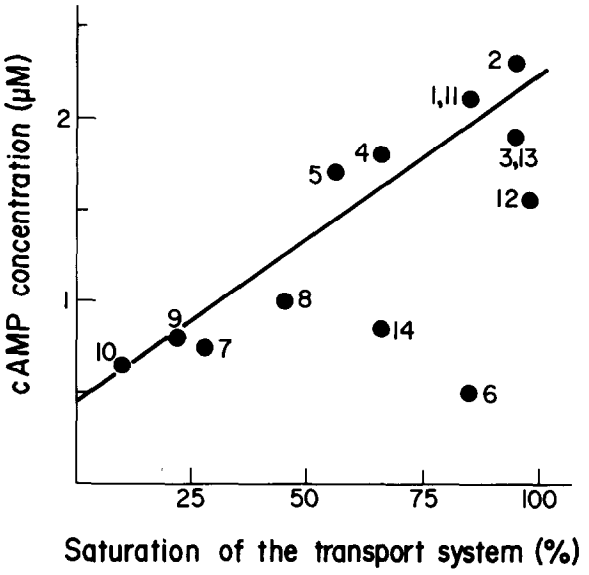

Fig.2. Correlation between concentration of cAMP attained during the transient increase and degree of occupancy of the sugar transport system, after addition of different sugars to yeasts. The yeasts were treated as indicated in fig.1. The % saturation of the transport system was calculated using the affinities given in the literature as follows: glucose, mannose, 2-deoxyglucose, 3-methylglucose [10], galactose (for glucose permease) and xylose [11], fructose [12], galactose (for galactose permease) [13], 6-deoxyglucose [14], glucosamine (20 mM, C. Guijarro, unpublished). Experiments numbered 1–14 as follows: for *S. cerevisiae* X-2180, 25 mM glucose (1), 2% glucose (2), 2% fructose (3), 2% mannose (4), 25 mM glucosamine (5), 25 mM 2-deoxyglucose (6), 0.1 M 3-methylglucose (7), 0.1 M xylose (8), 25 mM 6-deoxyglucose (9), 0.1 M galactose (10); for *S. cerevisiae* 2611-6D2, 2% glucose (11), 2% galactose (12); for *S. cerevisiae* D-308, 2% glucose (13), 2% fructose (14).

cases the activation would be transient. Another possibility is that the sugars alter the activity of adenylate cyclase and/or cAMP phosphodiesterase indirectly, by modifying the membrane potential [2] or the intracellular pH [16,17]. It should be noted, however, that in *S. cerevisiae* glucose is not cotransported with protons and therefore sugars that cannot be phosphorylated or otherwise metabolized are not likely to produce a change in the intracellular pH of the yeast.

The permanent increase in cAMP levels seems to be related with an increase in the level of sugar

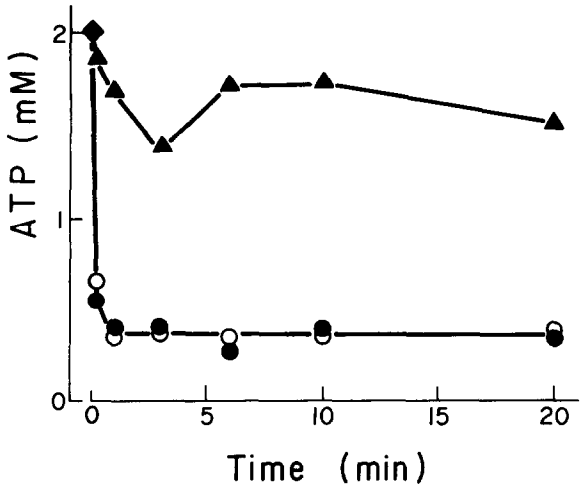

Fig.3. Time course of the effect of addition of non-metabolizable sugars on ATP concentration in yeast. *S. cerevisiae* X-2180 was grown and resuspended in buffer as indicated in section 2. Sugars were added as shown below and samples were taken at different times for the determination of intracellular ATP as described in the text. 25 mM 2-deoxyglucose (●—●), 25 mM glucosamine (○—○), 0.1 M 3-methylglucose (▲—▲).

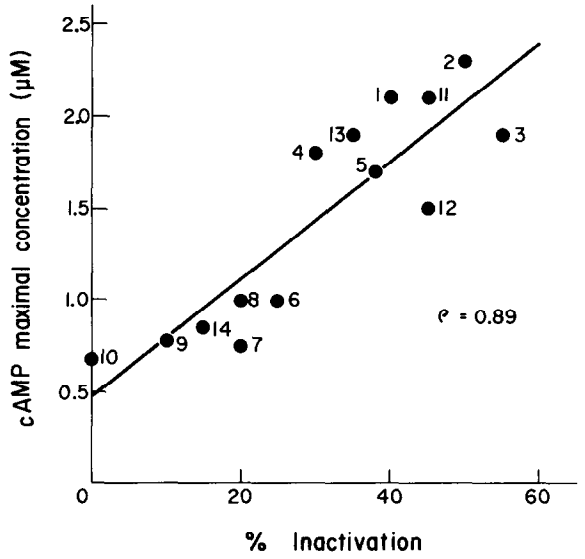

Fig.4. Correlation between maximal concentration of cAMP attained and extent of fructose-1,6-bisphosphatase inactivation, after addition of different sugars to yeasts. The yeasts were treated as indicated in fig.1. Samples were taken in parallel for testing fructose-1,6-bisphosphatase as described in section 2. Maximal concentration of cAMP and % inactivation of fructose-1,6-bisphosphatase after 3–10 min were determined after different additions. Experiments are numbered 1–14 as in fig.2.

phosphates but at present it is not possible to suggest a plausible mechanism.

The addition of the different sugars to yeasts inactivates fructose-1,6-bisphosphatase to a variable extent (fig.4). As can be seen there is a good correlation (p = 0.89) between maximal cAMP reached and % fructose-1,6-bisphosphatase inactivation. Moreover, 2-deoxyglucose, which provokes only a slow increase in cAMP (fig.1D), inactivates the bisphosphatase slower than other sugars. It appears therefore that the extent of fructose-1,6-bisphosphatase inactivation is basically controlled by the increase in cAMP levels.

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REFERENCES

[1] Van der Plaat, J.B. and Van Solingen, P. (1974) Biochem. Biophys. Res. Commun. 56, 580–587.
[2] Mazón, M.J., Gancedo, J.M. and Gancedo, C. (1982) Eur. J. Biochem. 127, 605–608.
[3] Purwin, C., Leidig, F. and Holzer, H. (1982) Biochem. Biophys. Res. Commun. 107, 1482–1489.
[4] Tortora, P., Burlini, N., Hanozet, G.M. and Guerritore, A. (1982) Eur. J. Biochem. 126, 617–622.
[5] Gancedo, J.M., Mazón, M.J. and Gancedo, C. (1983) J. Biol. Chem. 258, 5998–5999.
[6] Eraso, P. and Gancedo, J.M. (1984) Eur. J. Biochem. 141, 195–198.
[7] Funayama, S., Gancedo, J.M. and Gancedo, C. (1980) Eur. J. Biochem. 109, 61–66.
[8] Gancedo, J.M. and Gancedo, C. (1971) Arch. Microbiol. 76, 132–138.
[9] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[10] Cirillo, V.P. (1968) J. Bacteriol. 95, 603–611.
[11] Heredia, C.F., Sols, A. and De la Fuente, G. (1968) Eur. J. Biochem. 5, 321–329.
[12] Bisson, L.F. and Fraenkel, D.G. (1983) Proc. Natl. Acad. Sci. USA 80, 1730–1734.
[13] Cirillo, V.P. (1968) J. Bacteriol. 95, 1727–1731.
[14] Bisson, L.F. and Fraenkel, D.G. (1983) J. Bacteriol. 155, 995–1000.
[15] Gancedo, C. and Gancedo, J.M. (1985) Eur. J. Biochem. 148, 593–597.
[16] Londesborough, J. (1977) Biochem. J. 163, 467–476.
[17] Caspani, G., Tortora, P., Hanozet, M.H. and Guerritore, A. (1985) FEBS Lett. 186, 75–79.