Vol. 143, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 27, 1987 Pages 212-217

Anomeric Specificity of Glucose Effect on cAMP, Fructose 1,6-bisphosphatase, and Trehalase in Yeast

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Received January 13, 1987

The addition of β -D-glucose (final concentration, 50 mM) to a cell suspension of *Saccharomyces cerevisiae* in stationary phase caused a rapid 4-fold increase in the concentration of cAMP, while a 2-fold increase of cAMP was observed by the addition of α -D-glucose. β -D-Glucose was also more effective than α -D-glucose in the inactivation of fructose 1, 6-bisphosphatase and the activation of trehalase. These results, taken together with the previous report that α -D-glucose is transported more rapidly than β -D-glucose in *Saccharomyces cerevisiae,* do not support the view currently proposed by some investigators that cotransport of D-glucose with protons causes the depolarization of the cell membrane, resulting in the activation of adenylate cyclase. The present data, however, provides supporting evidence for the view that cAMP-dependent protein kinase is implicated in the inactivation of fructose 1,6-bisphosphatase and the activation of trehalase.

The addition of D-glucose to stationary phase yeast cells grown on D-glucose is known to cause a rapid increase in the cAMP concentration and also to result in the inactivation or activation of several enzymes [1-11]. The inactivation of fructose1,6-biphosphatase, one of the key enzymes in the gluconeogenic pathway, and the activation of trehalase, the enzyme involved in trehalose hydrolysis, have been studied extensively. The following sequence has been proposed to be involved in these processes [4-8]; D-Glucose transport causes depolarization of the cell membrane because of cotransport with protons; this depolarization activates the membrane-bound adenylate cyclase, resulting in the increase in the cAMP concentration; cAMP-dependent protein kinase is activated and thus phosphorylates fructose 1,6-bisphosphatase and trehalase; phosphorylation induces the inactivation of fructose 1,6-bisphosphatase and the activation of trehalase.

D-Glucose in aqueous solution exists as an equilibrated mixture of its two optical isomers; α -anomer (36 %) and β-anomer (64 %) [12]. There have been numerous

0006-291X/87 $1.50

investigations of the physiological effect, uptake, and metabolism of D-glucose anomers in various cells and organs [13-24]. Here, we have investigated the effects of D-glucose anomers on the intracellular cAMP levels as well as the activities of fructose 1,6-bisphosphatase and trehalase in yeast cells in vivo in order to ascertain the validity of the proposed sequence of glucose-induced events.

MATERIALS AND METHODS

Materials Bakers yeast was obtained from Oriental Yeast Co. Ltd. (Japan). Yeast extract and peptone were from Difco Laboratories (U.S.A). Pure α- and β -D-glucose were prepared as described previously [16]. Glucose-6-phosphate dehydrogenase (yeast) and glucose-6-phosphate isomerase (yeast) were from Boehringer Mannheim (FGR). cAMP radioimmunoassay kit was from Yamasa Shoyu Co. Ltd. (Japan). All other reagents were from Sigma Chemical Co. (U.S.A.).

Incubation of Cells and Preparation of Cell Extracts Yeast cells were grown at 30°C to stationary phase in a medium containing 1% yeast extract, 2% peptone, and 2% D-glucose. The cultures were quickly cooled to 4°C in an acetone-dry ice bath. The cells were harvested by centrifugation, washed twice with 50 mM MES buffer (adjusted to pH 6.0 with KOH), and resuspended in the same buffer to give a concentration of approximately 25 mg wet weight/ml. Following preincubation for 5 min. at 30°C, freshly prepared α- or β-D-glucose solution was added to the suspension of yeast cells (final concentration of D-glucose; 50 mM) and incubated at 30°C. In the control experiment, distilled water was added instead of α- or β-D-glucose. After various time intervals, samples (10 ml) were drawn out, mixed with 30 ml of ice-cold distilled water and centrifuged for 30 sec. The pellets were frozen in liquid N_2 and stored at -20°C until use (no more than 2 days). The pellets were resuspended in 0.5 ml of 50 mM potassium phosphate buffer (pH 7.5) containing 500 mM KCl, 2 mM EDTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride and 20 mM NaF, and were broken with 1 g of glass beads (0.25-0.5 mm diameter) by shaking for five 1-min. periods with 1-min. intervals of cooling in ice. The homogenates were centrifuged at 15,000 x g for 20 min and the resulting supernates were used for enzyme assays.

Enzyme Assays Fructose 1,6-bisphosphatase was assayed as described previously [10]. Trehalase was assayed at 30°C in 70 mM maleate buffer (pH 6.0) containing 50 mM trehalose. The reaction was stopped by heating the reaction mixture for 3 min. in a boiling water bath. Liberated glucose was determined by a glucose oxidase-peroxidase method (25). One unit of each enzyme was defined as the amount of enzyme which catalyzes the conversion of 1 mol of substrate per min. under the assay conditions. Protein was determined by the method of Bradford [26] with bovine plasma immunoglobulin as standard.

cAMP assay After incubation of yeast cells with α- or β-D-glucose for various time periods, cell samples (0.1 ml) were drawn out and mixed with 0.2 ml of 2 M perchloric acid. The mixture was frozen and thawed four times, and then centrifuged at 10,000 x g for 5 min. The supernate was neutralized with $KHCO_3$ and used for the cAMP assay. cAMP was assayed with a radioimmunoassay kit. The concentration of cAMP was calculated by assuming that 1.67 g of wet yeast cells contain 1 ml cell sap [8].

RESULTS

Mutarotation of D-Glucose Anomers The time courses of mutarotation of α- and β-D-glucose in 50 mM MES buffer (pH 6.0) at 30°C are shown in Fig. 1. Each anomer was more than 98% pure prior to incubation. After 5 min. of incubation, the α- and

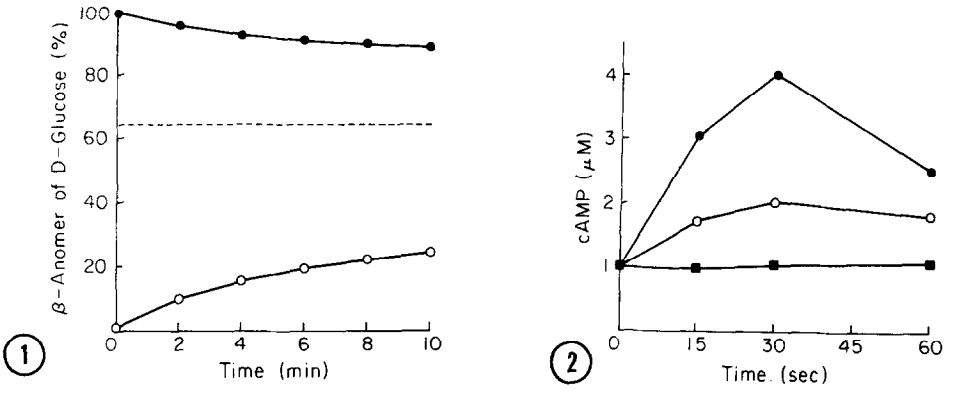


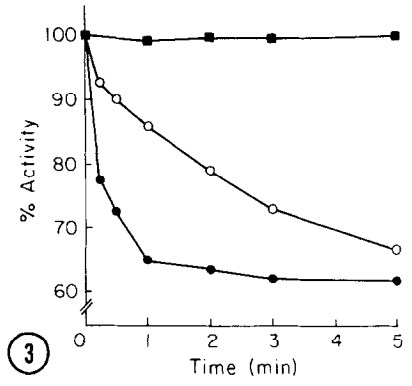
Fig. 1. Mutarotation of α- and β-D-glucose in the incubation medium. The β-D-glucose (●) or α-D-glucose (○) was incubated in MES buffer (pH 6.0) at 30° C. At various times indicated, the percentage of β-anomer of D-glucose was determined by our method using glucose oxidase, mutarotase, and oxygen electrode (27). The dashed line represents the percentage value of the β-anomer of D-glucose at the equilibrium condition.

Fig 2. Effects of D-glucose anomers on the intracellular cAMP levels in *S. cerevisiae*. Yeast cells were treated with α-D-glucose (○), β-D-glucose (●), or distilled water (■). cAMP was extracted and was measured from aliquots taken at the various time indicated as described in Materials and Methods.

β-anomers were mutarotated by only 17 and 9%, respectively. Since the glucose effects in this study are evidenced within 5 min. of glucose addition, the effect of glucose-anomers mutarotation is, therefore, sufficiently slow to be insignificant.

Increase of cAMP The addition of either anomer of D-glucose (50 mM) to yeast cells in stationary phase rapidly increased the cAMP concentration (Fig. 2). The addition of β-D-glucose led to a more rapid transient increase and a higher level of cAMP than the addition of α-D-glucose. Thirty seconds after the addition of α- and β-D-glucose, the intracellular cAMP levels peaked at 2 and 4 μM, respectively. In the control experiment, in which water was added instead of the D-glucose solutions, the cAMP concentration remained at 1 μM.

The Glucose-dependent Inactivation of Fructose 1,6-bisphosphatase. The addition of D-glucose to stationary-phase yeast causes a rapid inactivation of fructose 1,6-bisphosphatase (2). The first phase of this bi-phasic event is a rapid reversible loss of approximately 50% of activity which is due to cAMP-dependent phosphorylation (3, 5). The addition of D-glucose anomers to stationary phase yeast cells caused a rapid inactivation of fructose 1,6-bisphosphatase (Fig. 3). The α- and β-anomers of D-glucose decreased the enzyme activity by 10 and 27%, respectively, at 30 sec. after their addition. Thus, the inactivation of fructose 1,6-bisphosphatase by β-D-glucose was about



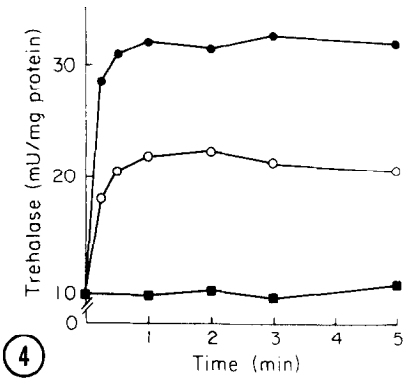


Fig. 3. Effects of D-glucose anomers on the activity of fructose 1,6-bisphosphatase in *S. cerevisiae*. Yeast cells were treated with α -D-glucose (○), β -D-glucose (●), or distilled water (■). Cell extracts were prepared from aliquots taken at the various time indicated and fructose 1,6-bisphosphatase activities were assayed as described in Materials and Methods. Before the addition of D-glucose anomers or distilled water, the specific activity of fructose 1,6-bisphosphatase was 41 mU/mg protein.

Fig. 4. Effects of D-glucose anomers on the activity of trehalase in *S. cerevisiae*. Yeast cells were treated with α -D-glucose (○), β -D-glucose (●), or distilled water (■). Cell extracts were prepared from aliquots taken at the various times indicated and trehalase activities were measured as described in Materials and Methods.

2.7 times greater than that by α -D-glucose at this time of incubation. The inactivation of fructose 1,6-bisphosphatase by β-D-glucose was completed within 1 to 2 min. while that by α -D-glucose gradually progressed throughout the incubation period of 5 min.

The Glucose-dependent Activation of Trehalase The preference for the β -anomer of D-glucose was also observed on the activation of trehalase (Fig. 4). The activation patterns of trehalase by α - and β -D-glucose were similar, showing a plateau in 1-2 min. However, the activation of trehalase by β -D-glucose was about twice stronger than that by α -D-glucose at any time of incubation.

DISCUSSION

There have been many reports concerning the mechanism of D–glucose-induced increase of cAMP in yeast cells. The most probable mechanism at present is believed to be that adenylate cyclase is activated through the depolarization of the cell membrane induced by the cotransport of D-glucose with protons. One of many lines of supporting evidence for this mechanism is that membrane-depolarizing agents such as dinitrophenol and carbonylcyanide m-chlorophenylhydrazone can cause a rapid increase of cAMP [4, 6]. In the present study, we found that β-D-glucose is more effective than α -D-glucose in increasing intracellular cAMP levels. On the other hand, α -D-glucose is known to be

transported more rapidly than β-D-glucose in yeast cells [13]. Therefore, our data do not support the view that transport-dependent membrane depolarization by D-glucose serves as a signal for the activation of adenylate cyclase. Specific glucose receptor with β-anomeric preference which interacts with the adenylate cyclase complex may instead be involved.

We also found that the inactivation of fructose 1,6-bisphosphatase and the activation of trehalase were induced more effectively by β-D-glucose relative to α-D-glucose. This result is the reflection of a greater increase of the intracellular cAMP levels by β-D-glucose as distinct from α-D-glucose, supporting the view that protein phosphorylation catalyzed by cAMP-dependent protein kinase is involved in the inactivation of fructose 1,6-bisphosphatase and the activation of trehalase.

There are numerous studies on the anomeric preference or specificity of physiological effects of D-glucose in higher animals [14-21]. Examples are: The α preference in glucose-stimulated insulin release by pancreatic islets [14] and the β preference in suppression of afferent activity of the hepatic nerve by glucose [19]. With respect to microorganisms, however, there is only one previous study to our knowledge, i.e. the β preference in the germination of dormant spores of a Bacillus [22]. The use of natural glucose anomers may be useful in helping to ellucidate the mechanisms involved in a variety of glucose-induced phenomena such as catabolite inactivation and catabolite repression found in microorganisms.

ACKNOWLEDGEMENT

This work was supported, in part, by a National Science Foundation Research Grant (DMB85-02904).

REFERENCES

1. Van der Plaat, J.B. (1974). Biochem. Biophys. Res. Commun. 56, 580-587.
2. Holzer, H. (1976). Trends Biochem. Sci. 1, 178-181.
3. Lenz, A.-G, and Holzer, H. (1980). FEBS Lett. 109, 271-274.
4. Mazon, M.J., Gancedo, J.M., and Gancedo, C. (1982). Eur. J. Biochem. 127. 605-608.
5. Holzer, H. (1984). Mol. Aspects Cell. Regul. Vol. 3, Cohen P., ed. pp. 143-154. Elsevier Science Publishers, Amsterdam
6. Thevelein, J.M. (1984). J. Bacteriol. 158, 337-339.
7. Thevelein, J.M. (1984). Microbiol. Rev. 48, 42-59.
8. Francosis, J., Van Shaftingen, E., and Hers, H.-C. (1984). Eur. J. Biochem. 145, 187-193.
9. Tortora, P., Burlini, N., Caspani, G., and Guerritore, A. (1984). Eur. J. Biochem. 145, 543-548.
10. Toyoda, Y., and Sy, J. (1984). J. Biol. Chem. 259, 8718-8723.
11. Toyoda, Y., and Sy, J. (1985). Current Microbiol. 12, 241-244.

12. Pigman, W., and Isbell, H.S. (1968). Adv. Carbohyd. Chem. 23, 11-57.
13. Edward, R., Sammler, P., and Goring, H. (1973). Folia Microbil. 18, 102-117.
14. Niki, A., Niki, H., Miwa, I., and Okuda, J. (1974). Science 186, 150-151.
15. Rossini, A.A., Soeldner, J.S., Hiebert, J.M., Weir, G.C. and Gleason, R.E. (1974). Diabetologia 10, 795-799.
16. Miwa, I., Okuda, J., Niki, H., and Niki, A. (1975). J. Biochem. 78, 1109-1111.
17. Okuda, J. (1978). Trends Biochem. Sci. 3, 161-162.
18. Matsuyama, T., Tanaka, R., Shima, K., and Tarui, S. (1979). Horm. Metab. Res. 11, 214-216.
19. Niijima, A., Fukuda, A., Taguchi, T., and Okuda, J. (1983). Am. J. Physiol 244, R611-R614.
20. Sakaguchi, T., Taguchi, T., and Okuda, J. (1983). Biochem. Int. 7, 299-305.
21. Sakaguchi, T., and Shimojo, E. (1984). J. Physiol. 351, 573-581.
22. Miwa, I., Ishihara, H., Fujii, H., Nagano, H., and Okuda, J. (1979). J. Biochem. 85, 315-318.
23. Ishihara, H., Miwa, I., Nagano, H., and Okuda, J. (1979). Experientia 35, 470-471.
24. Okuda, J., and Miwa, I. (1986) J. Clin. Biochem. Nut., in press.
25. Trinder, P. (1969) Ann. Clin. Biochem. 6, 24-27.
26. Bradford, M. (1976). Anal. Biochem. 72, 248-254.
27. Okuda, J., and Miwa, I. (1973) in Methods of Biochemical Analysis Glick, D., ed. Vol. 21, pp. 155-189, John Wiley, New York.