Vol. 203, No. 1, 1994 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 30, 1994 Pages 640-645

cAMP, ETHANOL, AND CO_2 PRODUCTION WITH THE ADDITION OF D-GLUCOSE ANOMER TO STARVED YEAST CELLS

Kyuboem HAN, Juan HONG*, and Henry C. LIM

Department of Chemical and Biochemical Engineering, University of California, Irvine, CA 92717

Received July 21, 1994

cAMP, ethanol, and CO_2 production in starved yeast cells after the addition of D-glucose anomer was measured and compared over a wide range of anomer concentrations. At 1.0 g/l or higher concentrations, the addition of β-D-glucose resulted in a higher cAMP peak. β-D-glucose was more rapidly metabolized to ethanol and CO_2 than α-D-glucose, although there was no notable difference in the uptake rates of the two anomers. At 0.4 g/l D-glucose anomer, the differences in cAMP and ethanol production rates for the two anomers were not significant. At 0.2 g/l D-glucose anomer or lower concentrations, ethanol production with α-D-glucose was higher than that with β-D-glucose. The uptake rate of α-D-glucose was higher than that of β-D-glucose at this low concentration.

In animal cells, the metabolism of D-glucose anomers has been well studied. The α anomer of glucose is more rapidly metabolized in pancreatic islets (1) and stimulates higher insulin production than the β anomer (2, 3). A higher metabolism rate of α anomer was also observed in rat hepatocytes (4) and rat adipocytes (5). On the contrary, lactate and ^3H_2O are more rapidly produced from β-D-glucose (or β-D-[5-^{3}H]glucose) than from the α anomer in human erythrocytes (6, 7). This may be because the phosphorylation of the β glucose is faster than that of the α anomer by glucokinase in human erythrocytes, resulting in a faster glycolysis (8). It was shown that the anomeric specificity of glucose phosphorylation by glucokinase is responsible for the anomeric preference of glucose utilization and insulin release in pancreatic islets (9).

Few reports have dealt with the anomeric preference in glycolysis in microorganisms. Most studies were concentrated on the uptake of the glucose anomers (10- 16). Only two studies have been reported regarding the anomeric specificity for glycolysis in yeast (17- 18): the CO_2 production rate depends on the anomeric type of glucose and its concentration (17), and β glucose is a more potent activator of cAMP than α glucose (18). Since a high level of cAMP is required for the activation of glycolysis and deactivation of gluconeogenesis (19), it is expected that the β anomer is metabolized faster than the α anomer. However, this is somewhat contradictory to the previous observations that the uptake rate of α glucose is higher than that of β glucose in yeast (10- 14). In this paper, we report the experimental results on glucose uptake rate, CO_2 and ethanol production rates, and cAMP response of starved yeast cells, with respect to anomeric type of glucose and its concentration.

*To whom correspondence should be addressed. FAX : (714) 725-2541.

0006-291X/94 $5.00

MATERIALS AND METHODS

Materials - α-D-glucose (96%), β-D-glucose (97%), and glucose assay kit (510-A) were obtained from Sigma Chemical. Enzyme immunoassay kit (RPN 225) for cAMP measurement was purchased from Amersham (U.K.). Bacto-peptone and Bacto-yeast extract were from Difco Laboratories. Potassium carbonate and perchloric acid were obtained from Aldrich. CO_2 gas (99.998%) was from Liquid Carbonic Specialty Gas Corp. (U.S.A.). All other chemicals were purchased from Fisher Scientific.

Yeast Strain - *Saccharomyces cerevisiae* DC04 (*MATa, leu2-04, ade1, cir0*) was maintained on YEPD (2% Bacto-peptone, 1% Bacto-yeast extract, and 2% dextrose) agar plates.

Incubations of Yeast, Glucose, Ethanol, and cAMP Measurements - One loopful of yeast cells from YEPD agar plates was transferred to 250 ml of YEPD (2% glucose) and cultivated for 24 h at 30°C in the NBS (New Brunswick Scientific Co. Inc.) G24 incubator shaker (250 rpm). Cells were harvested by centrifugation (Beckman GPR centrifuge) at 3,000 rpm for 10 min. Cell pellets were then washed with 250 ml of the medium A (1 g/l Bacto-peptone, 1 g/l Bacto-yeast extract, 0.15 g/l K_2HPO_4, and 0.85 g/l KH_2PO_4), and resuspended in the same volume of medium A (the cell density was adjusted to 6.0 g dry cell weight/l with medium A if necessary). After stirring for 2 h at 25°C, fresh α or β D-glucose solution (which was made 1 min before use) was added to the yeast cell suspension at glucose concentrations ranging from 0.05 to 10 g/l which was then incubated at 25°C. For a typical duration of an experiment (about 5 min), the mutarotation of the anomers was not significant. For cAMP measurement, cell suspension samples (150 μl) were taken and immediately transferred to pre-cooled 1.5 ml tubes in an acetone-dry ice bath. Frozen samples were stored at -80°C for future assays. The frozen samples at -80°C were taken out and 300 μl of 2 M perchloric acid was added before thawing. The mixture was then thawed and frozen five times. The solution was neutralized with 70 μl of 5M K_2CO_3 and then centrifuged. 100 μl of the supernatant was taken for cAMP determination. The acetylation assay protocol for the Amersham cAMP enzyme immunoassay kit was chosen for 1:10 diluted samples. For ethanol and glucose measurements, samples (500 μl) were taken and centrifuged for 20 sec at 10,000 rpm in the IEC Centra-4B centrifuge. The supernatant was heated at 95°C for 5 min to guarantee the equilibrium of mutarotation of glucose anomers. A glucose assay kit (510-A, Sigma Chemicals) was used. A gas chromatograph (Model 3600, Varian) with a FID detector and Poropak N 80/100 column was used for ethanol measurement.

CO_2 Measurement - One loopful of yeast cells from the YEPD agar plate was transferred to 250 ml of YEPD (4% glucose) and cultivated for 24 h at 30°C in the NBS G24 incubator shaker (250 rpm). Cells were harvested by centrifugation at 3,000 rpm for 10 min. Cell pellets were washed with 250 ml YEP medium (2% Bacto-peptone, 1% Bacto-yeast extract, and no dextrose), and resuspended in 100 ml of YEP medium (cell density was adjusted to 20 g dry cell weight/l). The cell suspension was then divided into equal 50 ml portions, stirred, and aerated (1.5 liter/min) at 25°C. After adding α or β glucose powder to the cell suspension at concentrations of 0.4, 1.0, and 10 g/l, the off-gas was continuously monitored by a M200 Quadrupole Gas Analyzer (AMETEK, Thermox Instruments Division, Pittsburgh, PA).

RESULTS

D-Glucose Uptake The specific uptake rate versus glucose concentration is plotted in Fig.1A. The α anomer was consumed more rapidly than the β anomer at low anomer concentrations (less than 0.4 g/l) whereas no significant difference was observed at higher than 0.4 g/l glucose anomer.

Ethanol Production At 0.2 g/l glucose, the ethanol production continued up to 7 min after which the ethanol concentration decreased due to consumption by the starved cells (Fig.2A). The glucose was completely consumed at 7 min of the incubation. The ethanol production was significantly higher with the α anomer. At 0.4 g/l glucose, the time profiles of ethanol concentration were indistinguishable for the α and β anomers (Fig.2B). However, at 1.0 g/l glucose, the ethanol production with the β anomer was greater than that with the α anomer (Fig.2C). A similar result was observed with growing cells (Fig.2D). The growing

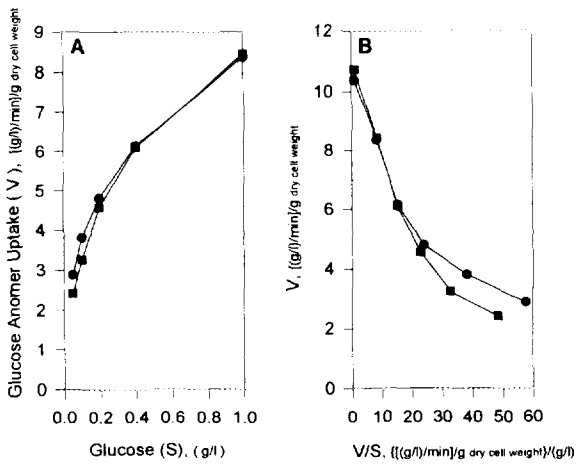

Fig. 1. **Specific uptake rate of D-glucose anomer by starved yeast cells.** - **(A)** Specific uptake rate versus glucose concentration. α-D-glucose (●) is uptaken more rapidly than β-D-glucose (■) at low glucose concentrations (less than 0.4 g/l). **(B)** Edie-Hofstee plot of glucose anomer uptake.

cells were obtained by pre-incubating cells for 1 h in the medium A containing 10 g/l glucose (6.7 g/l of β glucose and 3.3 g/l of α glucose). The cells were centrifuged, resuspended in the glucose free medium A, and glucose was added at 1.0 g/l. The ethanol production with the β anomer was higher than with the α anomer. This was consistent with the result obtained with the starved cells at 1.0 g/l glucose. The ethanol production with the growing cells (Fig.2D) was much higher than that with the starved cells (Fig.2C), indicating that the glycolysis pathway in the starved cells was not fully activated.

CO_2 Production Carbon dioxide production rates were measured at three different glucose concentrations, 0.4, 1.0, and 10 g/l. At 0.4 g/l and 1.0 g/l of glucose, the CO_2 production rates with the α and

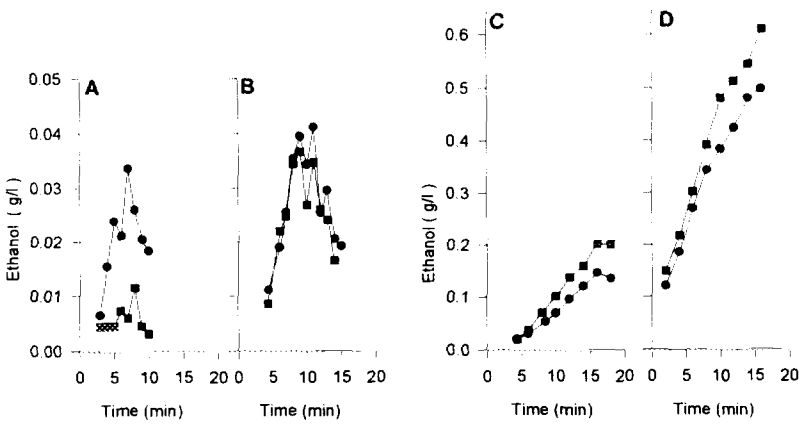

Fig. 2. **Ethanol production with the glucose anomers.** - Ethanol production by starved yeast cells after the addition of 0.2 g/l **(A)**, 0.4 g/l **(B)**, and 1.0 g/l **(C)** D-glucose anomers. As glucose concentration increases, a reversal of α preference (●) to β preference (■) for ethanol production is observed. **(D)** Ethanol production after the addition of 1.0 g/l D-glucose anomers by growing cells pre-incubated in 10 g/l of mutarotated glucose. Ethanol with β-D-glucose is more rapidly produced than that with α-D-glucose. Higher ethanol concentrations than in (C) are due to the pre-activation of glycolysis pathway at 10 g/l of glucose.

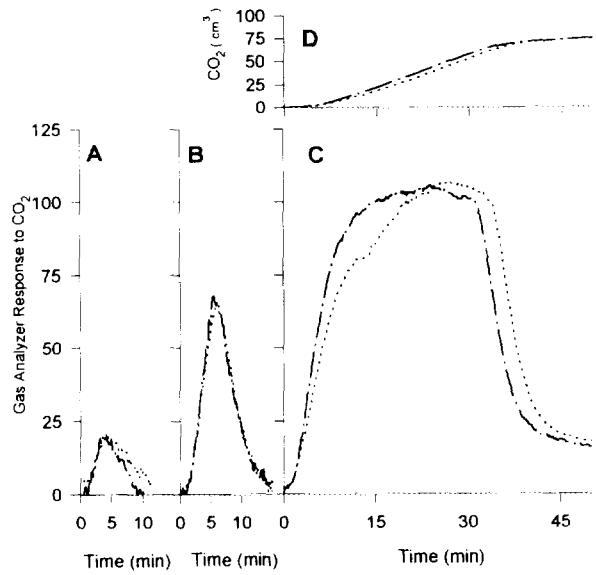

Fig. 3. **CO_2 production with the glucose anomers.** - CO_2 production after the addition of 0.4 g/l **(A)**, 1.0 g/l **(B)**, and 10 g/l **(C)** D-glucose anomers by starved yeast cells. At 10 g/l of glucose, CO_2 production with β-D-glucose (— · —) is faster ,up to 20 min, than that with α-D-glucose (·····). **(D)** The integration of CO_2 responses in (C). It shows that the total amounts of CO_2 generated with both the anomers are same.

β anomers were not significantly different (Figs.3A and B). At 10 g/l of glucose, the CO_2 production rate with the β anomer was significantly greater for up to 20 min after glucose addition than that with the α anomer (Fig.3C). After 20 min of the incubation, the rate with the α anomer was greater. However, the total amounts of CO_2 produced were same (Fig.3D). It should be noted that the gas analyser response (Figs.3A, B, and C) is proportional to CO_2 production rate and the integration of the response with respect to time gives the total amount of CO_2 produced.

cAMP Production The addition of both of the anomers at 10 g/l resulted in a rapid rise of the intracellular cAMP in the starved cells (Fig.4C). The β anomer was more effective in increasing the transient cAMP level than the α anomer. The cAMP peak height obtained with the β anomer was twice that with the α anomer(Fig.4C). At 1.0 g/l glucose anomer, the addition of the β anomer still led to a higher and more rapid increase of cAMP than with the α anomer (Fig.4B). However, at 0.4 g/l, the transient cAMP increases were not significant with either of the anomers (Fig.4A).

DISCUSSION

The experimental results show that, at high glucose concentrations (1.0 g/l or above), the starved yeast cells uptake the α and β anomers almost at the same specific rate and the cells metabolize β-D-glucose more rapidly to ethanol and CO_2 than α-D-glucose. This is rather unexpected since a rapid metabolism of the β anomer to CO_2 and ethanol should be accompanied by a rapid uptake of β-D-glucose. The intracellular cAMP level increase with β-D-glucose is much higher than that with α-D-glucose (Fig.4B and C). It can be speculated that the rapid metabolism of the β anomer to ethanol and CO_2 is the result of the activation of the

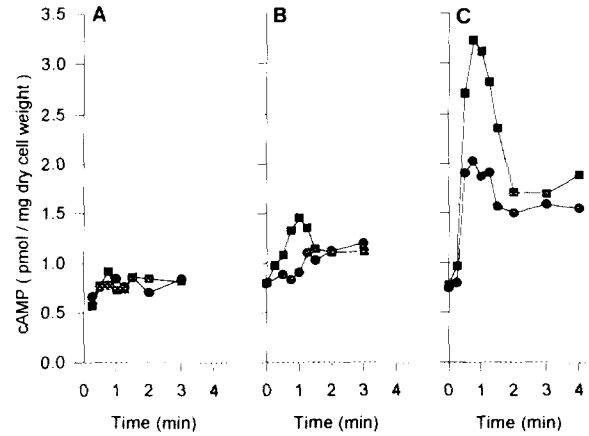

Fig. 4. **Intracellular concentration of cAMP in starved cells.** - cAMP concentration after the addition of 0.4 g/l **(A)**, 1.0 g/l **(B)**, and 10 g/l **(C)** D-glucose anomers. At 1.0 g/l and 10 g/l of glucose, a higher transient peak of cAMP was obtained with β-D-glucose (■) than with α-D-glucose (●). No significant increase of cAMP is observed at 0.4 g/l of glucose.

glycolysis pathway in the starved cells with the increase in the intracellular level of cAMP. The positive effect of cAMP on the activation of glycolysis in yeast is a well known phenomenon (19).

The growing cells pre-incubated at 10 g/l glucose also produce ethanol at a higher rate with the β anomer (Fig.2D). The glycolysis pathway in the growing cells is expected to be fully activated. If so, the cAMP mediated activation of the glycolysis pathway by the β anomer may not be the only factor for the observed higher production rate of ethanol with the β anomer in the growing cells. The anomeric specificity of glucose phosphorylation by glucokinase may be taken into consideration. It has been reported that the most likely rate limiting step in glycolysis is glucose phosphorylation (6, 20-22). Among the three yeast glucose phosphorylating enzymes, hexokinase B is specific to the α and glucokinase for β anomer (23).

At 0.4 g/l glucose, there are no significant differences between the α and β anomers for the glucose uptake rate(Fig.1), the ethanol (Fig.2B), and the cAMP productions (Fig.4A). An anomeric reversal of the ethanol production is observed at 0.2 g/l glucose. At this concentration, the glucose uptake rate and the ethanol production are faster with the α anomer (Figs.1 and 2A), and the cAMP production is negligible with both the α and β anomers. These results suggest that, at 0.2 g/l glucose, the overall rate of glycolysis in yeast is limited by the glucose uptake rate. A similar trend of the anomeric reversal at a critical glucose concentration has been reported for insulin production in rat pancreatic islets (9).

ACKNOWLEDGMENT

The authors gratefully acknowledge support by the National Science Foundation through grant BCS-9101190.

REFERENCES

1. Carruthers, A., and Melchior, D. L. (1985) Biochemistry 24, 4244-4250.
2. Malaisse, W. J., Sener, A., Koser, M., and Herchuelz, A. (1976) J. Biol. Chem. 251, 5936-5943.

3. Niki, A., Niki, H., Miwa, I., and Okuda, J. (1974) Science 186, 150-151.
4. Malaisse, W. J. (1986) IRCS Med. Sci. 14, 609-610.
5. Malaisse-Lagae, F., and Malaisse, W. J. (1986) Eur. J. Biochem. 158, 663-666.
6. Fujii, H., Miwa, I., and Okuda, J. (1986) Biochem. Int. 13, 359-365.
7. Malaisse-Lagae, F., Sterling, I., Sener, A., and Malaisse, W. J. (1988) Clin. Chim. Acta 172, 223-232.
8. Miwa, I., Fukatsu, H., Toyoda, Y., and Okuda, J. (1990) Biochem. Biophys. Res. Commun. 173, 201-207.
9. Miwa, I., Murata, T., and Okuda, J. (1991) Biochem. Biophys. Res. Commun. 180, 709-715.
10. Hopkins, R. H., and Roberts, R. H. (1936) Biochem. J. 30, 76-83.
11. Den Hollander, J. A., Brown, T. R., Ugurbil, K., and Shulman, R. G. (1979) Proc. Natl. Acad. Sci. USA 76, 6096-6100.
12. Ehwald, R., and Mavrina, L. (1981) Folia Microbiol. 26, 95-102.
13. Hong, J., Choi, K. S., and Lim, H. C. (1989) Biotechnol. Lett. 12, 855-860.
14. Benthin, S., Nielsen, J., and Villadsen, J. (1992) Biotechnol. Bioeng. 40, 137-146.
15. Lohmeier-Vogel, E. M., Hahn-Hagerdahl, B., and Vogel, H. J. (1986) Appl. Microbiol. Biotechnol. 25, 43-51.
16. Ugurbil, K., Brown, T. R., Den Hollander, J. A., Glynn, P., and Shulman, R. G. (1978) Proc. Natl. Acad. Sci. USA 75, 3742-3746.
17. Hopkins, R. H., and Roberts, R. H. (1936) Biochem. J. 30, 76-83.
18. Toyoda, Y., Fujii, H., Miwa, I., Okuda, J., and Sy, J. (1987) Biochem. Biophys. Res. Commun. 143, 212-217.
19. Wills, C. (1990) Crit. Rev. Biochem. Mol. Biol. 25(4), 245-280.
20. Bailey, J. E., and Shanks, J. V. (1991) Bioprocess Eng. 6, 273-284.
21. Galazzo, J. L., and Bailey, J. E. (1990) Enzyme and Microb. Technol. 12, 162-172.
22. Reibstein, D., den Hollander, J. A., Pilkis, S. J., and Shulman, R. G. (1986) Biochemistry 25, 219-227.
23. Okuda, J., Miwa, I, and Inagaki, K. (1984) Enzyme 31, 176-180.