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SHORT COMMUNICATION

AMINO ACID PRODUCTION ACCOMPANYING GLUTATHIONE SYNTHESIS BY IMMOBILIZED SACCHAROMYCES CEREVISIAE CELLS

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Abstract

The glutathione synthesis of *Saccharomyces cerevisiae* cells entrapped in polyacrylamide gel was found to be accompanied by amino acid production as a consequence of the immobilization, a partial release of the amino acid pool was also observed.
Key words: immobilized cells, glutathione production, amino acid

Introduction

Immobilized microbial cells containing the intact enzyme system of glycolysis are able to regenerate ATP for the synthesis of glutathione (MURATA et al., 1978). The entrapment of *Saccharomyces cerevisiae* cells in polyacrylamide gel caused a change in the membrane permeability (MURATA et al., 1981). Glutathione and NAD^+ were released from the cells, but ATP was retained. By the addition of NAD^+, the ATP regenerating system was completed and the ATP produced served as energy donor for the continuous production of glutathione. We have detected a significant quantity of different amino acids released together with glutathione owing to *de novo* synthesis, as well as a partial release of the amino acid pool.

Materials and methods

CULTIVATION AND IMMOBILIZATION. *S. cerevisiae* strain IFO 2044 was obtained from the Institute of Fermentation, Osaka, Japan. Cells were cultivated and entrapped in polyacrylamide gel according to MURATA et al. (1981).

ASSAY OF GLUTATHIONE AND AMINO ACID PRODUCTION. 1.2 g of gel particles containing 0.5 g of cells was incubated in 20 ml of the culture medium suggested by MURATA et al. (1981) at 30 °C for 24 hours with continuous shaking. At appropriate times, 0.5 ml of sample was removed and the concentrations of reactants and products were determined.

Glutathione and amino acids were measured by quantitative thin-layer chromatography on Kieselgel 60 F_{254} chromatoplates (Merck AG, Darmstadt, FRG) (ÁBRAHÁM et al., 1983). The spot's were detected in Telechrom OE—974 videodensitometer (Chinoin, Budapest, Hungary). Ethanol was determined by gas chromatography, using a Chrom 4 GC chromatograph (Laboratorni Pristroje Prague, Czechoslovakia) equipped with a flame ionization detector and a Porapak Q (80—100 mesh) column. Nitrogen was used as a carrier gas and methanol as an internal standard.

Results and discussion

A mixture containing the glutathione constituent amino acids (i. e. glycine, glutamic acid and cysteine) in equimolar quantities was incubated with *S. cerevisiae* cells entrapped in polyacrylamide gel. Besides glutathione and the glutathione constituent amino acids, aspartic acid, alanine, glutamine and serine were detected in the reaction mixture (Table 1).

Table 1. Release of metabolites and amino acid pool from *S. cerevisiae* cells immobilized in polyacrylamide gel

Incubation time (hr)	Ethanol (umol)	Glutathione (umol)	Asp (umol)	Ala (umol)	Gln (umol)	Ser (umol)
0	53	0	40	0	0	—
2	174	2	56	56	60	6
4	347	9	34	100	110	12
8	1346	13	34	132	136	40
24	5646	14	34	192	176	60

On this basis it was assumed that the cell membrane became permeable for glutathione and for certain amino acids as a consequence of the immobilization. Aspartic acid was released before the start of fermentation. Its concentration in the effluent remained relatively high and constant during a fairly long period. The concentrations of glutathione, alanine, glutamine and serine increased in parallel with the concentration of ethanol, showing the connection between the synthesis as energy consumer and the glycolysis as energy supplier. The synthesis of alanine, glutamine and serine presumably requires a lower ATP level than does the peptide synthesis. Aspartic acid was released from the amino acid pool of the damaged cells, but its synthesis could not be excluded.

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References

ÁBRAHÁM, M., POLYÁK, B., SZAJÁNI, B. and BOROSS, L. (1983): Analytical Separation of Reduced and Oxidized Forms of Glutathione from Amino Acid Mixtures by Overpressured Thin-Layer Chromatography. — J. Liquid Chrom. *6*, 2635—2645.

MURATA, K., TANI, K., KATO, J. and CHIBATA, I.. (1978): Continuous Production of Glutathione by Immobilized Saccharomyces cerevisiae Cells. — Eur. J. Appl. Microbiol. Biotechnol. *6*, 23—27.

MURATA, K., TANI, K., KATO, J. and CHIBATA, I. (1981): Glutathione Production by Immobilized *Saccharomyces cerevisiae* Cells Containing an ATP Regeneration System. — Eur. J. Appl. Microbiol. Biotechnol. *11*, 72—77.