THE SYNTHESIS OF GLUTATHIONE IN *TORULA UTILIS* STUDIED WITH ^{14}C-CARBOXYL LABELED GLYCINE*, **

by

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Torula utilis var. major cells were grown under aeration on a basal medium which contained per liter: glucose, 50 g; $(NH_4)H_2PO_4$, 3 g; $(NH_4)_2SO_4$, 4 g; K_2SO_4, 1.5 g; $MgSO_4 \cdot 7H_2O$, 1 g; and $CaCl_2$, 0.5 g. In each experiment 1.5 ml of packed cells were resuspended in 8 ml of basal medium and 2 ml of this suspension were added to each of three 20 ml beakers which contained 0.2 mg of ^{14}C carboxyl labeled glycine (0.5 mc per mM).

After incubating these beakers in a Dubnoff metabolic shaker at 30° C for 45 minutes, the cells were pooled in a mortar to which 10 ml of 10% trichloroacetic acid containing 40 mg of inert glutathione had been added. Then, the cells were ground with powdered carborundum for one half hour. After centrifuging, the supernatant was transferred to a 50 ml tube and 10 ml of a 10% inert glycine solution was added. From this step on, the procedure for the isolation of glutathione as given by WAELSCH AND RITTENBERG was followed[1]. To insure complete mercaptide formation the cuprous oxide was added in excess, *i.e.*, until the fine white mercaptide precipitate just began to dissolve. The mercaptide was reprecipitated by aerating the solution for 3 to 5 minutes. For the final washings 95% alcohol, rather than absolute alcohol, was used so that any glycine present might be removed. The copper salt of the isolated glutathione was weighed and its radioactivity measured

TABLE I

INFLUENCE OF VARIOUS FACTORS ON THE INCORPORATION OF ^{14}C-GLYCINE INTO GLUTATHIONE BY TORULA UTILIS

Treatment	*Relative radioactivity of mercaptide*
1. No change (basal medium)	100
2. Glucose omitted from basal medium	120
3. $CaCl_2$ omitted from basal medium	100
4. $MgSO_4$ omitted from basal medium	87
5. 0.1 *M* KCN added to basal medium	55
6. 0.016 *M* cysteine and 0.013 *M* glutamic acid added	339
7. O_2 atmosphere	324
8. N_2 atmosphere	0
9. Lyophilized cells	0
10. Cells ground with carborundum:	
a. Cellular debris	21
b. Cellular debris in BLOCH's medium*	39
c. Cellular debris in BLOCH's medium plus 0.001 *M* ATP	70
d. Supernatant	6
11. Cells ground with powdered glass:	
a. Cellular debris	6
b. Supernatant	0

* BLOCH's medium: 1.2 ml of 0.32 *M* glutamate; 0.4 ml of 0.16 *M* cysteine; 1 ml of 0.15 *M* KCl; 2.5 ml of 0.1 *M* phosphate buffer (pH 7.4); and 0.5 ml of 0.15 *M* $MgSO_4$.

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in order to obtain its specific activity. To ascertain that the radioactive glycine was not carried through the extraction, controls were run in which the trichloroacetic acid containing the inert glutathione was added before incubation.

In Table I, the radioactivity of the mercaptide extracted from the cells incubated in the basal medium is taken as 100. The incorporation of ^{14}C carboxyl labeled glycine into the glutathione of yeast is similar to the uptake of labeled glycine into the "protein fraction" of yeast in that both reactions are inhibited by cyanide, anaerobic conditions, lyophilization, and grinding of the cells[2]. (To rule out inhibition by carborundum or its contaminants, cells to which the carborundum was added but which were not ground were also incubated.) Likewise, both reactions are slightly inhibited by the omission of $MgSO_4$, and enhanced by an O_2 atmosphere. The omission of glucose or $CaCl_2$, however, is without effect on the glutathione synthesis by yeast while the omission of either of these two substances causes pronounced inhibition on the incorporation of labeled glycine into the "protein fraction".

BLOCH in a series of eight experiments in which labeled glutathione formation in the presence of O_2 and N_2 was compared, found that the average value for anaerobic synthesis was 17% of that found under aerobic conditions[3]. This investigator also reported that the omission of cysteine or glutamic acid significantly decreased the rate of the reaction. In our experiments on yeast, these amino acids are equally effective.

JOHNSTON AND BLOCH observed that adenosine triphosphate markedly accelerated the aerobic synthesis of glutathione in liver homogenates[4]. By adding adenosine triphosphate to the cellular debris resuspended in BLOCH's medium the synthetic activity may be slightly restored.

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THE INFLUENCE OF ACETATE ON THE FERMENTATION OF BAKERS'. YEAST

by

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In experiments on the stimulation of the anaerobic fermentation of bakers' yeast* by thiamine and ammonium ions it became necessary also to investigate the influence of the pH of the medium.

Living yeast cells suspended in 0.068 *M* sodium acetate solution at pH 5.6 ferment glucose with constant and high velocity during several hours. At pH 3.5 the fermentation rate is equally constant, but very low.

Table I shows that with sodium acetate concentrations between 0.017 and 0.034 *M* at pH 3.5 the fermentation rate is about inversely proportional to the concentration. At pH 5.6 increase of the concentration of sodium acetate causes a weak stimulation instead of an inhibition.

Because at pH 3.5 the undissociated acetic acid molecule is the only component of the solution which is present in a reasonable quantity, whereas at pH 5.6 sodium and acetate ions are the dominating components, it seemed likely that the inhibition of fermentation at pH 3.5 must be attributed to the undissociated acetic acid molecule. That this is indeed true could be demonstrated by replacing the acetate by a succinate solution (Table II).

So far the experiments give no answer to the question whether the observed low rate of fermentation in acetate medium at pH 3.5 is due to a change of internal pH or to a "toxic" influence of acetate ions within the cell.

* "Koningsgist" from the "Nederlandse Gist- en Spiritusfabriek", Delft (Netherlands).