ACTA CHEMICA SCANDINAVICA 20 (1966) 474–478

Metabolism of Acetaldehyde — a Metabolic Branching Point

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Acetaldehyde is formed as an intermediate in alcoholic fermentation. Most of the acetaldehyde formed is reduced to ethanol, but a small fraction is normally oxidized to acetic acid. The metabolism of acetaldehyde thus is an example of a metabolic branching point. The fraction oxidized was found to be a function of the intracellular concentration of acetaldehyde; it decreased as the latter increased. The intracellular concentration of acetaldehyde was affected by the addition of 2,4-dinitrophenol and iodoacetate and by deficiency of niacin and thiamine.

Acetic acid is one of the many by-products formed in alcoholic fermentation. It seems to be formed, at least partly, by oxidation of acetaldehyde since yeast cells contain aldehyde dehydrogenase;[1] an alternative pathway is the hydrolysis of acetyl-SCoA [2] formed from pyruvate by pyruvic oxidase.[3] The relative importance of these two metabolic pathways is not known.

Holzer *et al.*[3–5] have shown that the distribution between oxidation of pyruvate to acetyl-SCoA and decarboxylation to acetaldehyde is a function of the concentration of pyruvate since the Michaelis constants of the two reactions are very different.[3,4] These reactions were used as a model system for a metabolic branching point.[4] The kinetic results, obtained from *in vitro* studies, were then verified in experiments with living cells. The concentration of pyruvate in the cell was increased by 2,4-dinitrophenol and reduced by iodoacetate.

The main reaction of acetaldehyde in alcoholic fermentation is the reduction to ethanol. Thus the metabolism of acetaldehyde in alcoholic fermentation is another example of a metabolic branching point. A study was performed to investigate how the formation of acetate in alcoholic fermentation with *Saccharomyces cerevisiae* was affected by the intracellular concentration of acetaldehyde. The results are reported in this paper.

EXPERIMENTAL

A strain of brewer's top yeast, *Saccharomyces cerevisiae*, was used; it was not deficient in niacin or thiamine, but the fermentation velocity was heavily decreased if the medium contained no thiamine. Niacin deficiency had no significant effect on yeast growth.[6]

Resting-cell fermentations were performed in a medium containing only glucose and citrate (36 mM, pH 5.3); after various times of incubation (continuous stirring) at 20°C the glucose consumption was determined.

The α-keto acid content of the cells was determined as 2,4-dinitrophenyl hydrazones according to Koepsell and Sharpe [7] after preparing the samples according to Holzer *et al.*[5]

In the fermentation experiments a synthetic medium (80 g/l of glucose, ammonium sulphate, salts, trace elements, vitamins, citrate as pH-buffer) was fermented anaerobically under continuous stirring at 20°C as has been described elsewhere.[8,9] After completed fermentation, the media were analyzed titrimetrically for acetic acid [10] and gravimetrically for yeast growth;[9] about 800 mM of ethanol were formed.

RESULTS AND DISCUSSION

Four methods were used to affect the concentration of acetaldehyde in the cell. The reactions involved are shown in the simplified scheme of Fig. 1. The

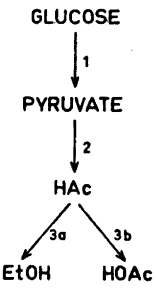

Fig. 1. Simplified scheme for alcoholic fermentation. Reaction 1 is stimulated by 2,4-dinitrophenol and inhibited by iodoacetate, reaction 2 requires thiamine as a cofactor, and reactions 1 and 3 require niacin as a cofactor.

rate of reaction 1 was increased by 2,4-dinitrophenol and decreased by iodoacetate; the former substance is known to stimulate fermentation [11,12] and the latter is a potent inhibitor of the formation of D-1,3-diphosphoglycerate,[13] which is one of the intermediates of reaction 1. Reaction 2 was inhibited by thiamine deficiency or by the addition of the thiamine antimetabolite oxythiamine; thiamine pyrophosphate is a cofactor of pyruvic decarboxylase. Reactions 3a and 3b were affected by niacin deficiency or by the addition of the niacin antimetabolite pyridine-3-sulphonate; niacin adenine dinucleotide is a cofactor of these redox reactions. However, even reaction 1 involves an oxidation. No trial was made to show that the concentration of acetaldehyde was changed, but the concentration of pyruvate in the cell and the velocity of the glucose consumption were used as measures of the effects obtained by the changes of the medium; the former method is relevant only in those cases where a reaction previous to acetaldehyde is involved. The effects obtained agreed with those predicted above from the knowledge of the metabolic effects of the substances studied (Table 1).

The velocity of the reduction of acetaldehyde to ethanol by alcohol dehydrogenase shows a saturation curve when plotted against the aldehyde concentration.[14] Aldehyde dehydrogenase, on the other hand, is inhibited by acetaldehyde above a rather low concentration.[1] Thus, the fraction of acetaldehyde oxidized to acetate will presumably decrease by an increased concentration of acetaldehyde. It can be predicted that an increase in the concen-

Table 1. Effect of some changes in the medium.

Change in the medium	α-Keto acids in the cells (mM) [a,b]	Glucose breakdown (g/g of yeast and day[b])	Formation of [c,f]	
			Yeast (g/l)	Acetate (mM)
None (thiamine.HCl, 0.4 mg/l)	1.57 [e]	—	4.16	3.60
Thiamine deficient	2.20 [e]	—	3.93	7.76
» » , oxythiamine, 0.55 g/l	—	—	1.28	12.69
None (niacin, 0.4 mg/l)	—	—	4.21	4.35
Niacin deficient	—	7.27 [e]	4.10	2.86
» » , pyridine-3-SO_3H, 0.5 g/l	—	7.13 [e]	4.16	1.49
» » » 1 »	—	6.36 [e]	—	—
» » » 2 »	—	5.08 [e]	—	—
None	1.57 [f]	6.3 [f]	4.07	3.29
Iodoacetate, 0.1 mM	0.92 [f]	2.1 [f]	4.10	3.39
» 0.2 »	0.77 [f]	0.5 [f]	[d]	3.80
» 0.3 »	0.58 [f]	0.2 [f]	[d]	4.18
None	—	6.3 [f]	3.71	4.27
2,4-Dinitrophenol, 0.01 mM	—	6.8 [f]	3.45	3.03
» 0.03 »	—	7.3 [f]	2.81	2.69
» 0.05 »	—	7.3 [f]	2.47	2.62

[a]) Samples prepared according to Holzer *et al.*[5]; α-keto acid, *i.e.* essentially pyruvate + α-ketoglutarate was determined.[15]
[b]) Resting-cell fermentations.
[c]) Growing-cell fermentations.
[d]) No reliable results as fermentation was very slow, at 0.3 mM 30 days compared to 4 in control.
[e]) Inoculation yeast cultivated as indicated in the left column; the fermentations reported in the table were performed in the unsupplemented medium.
[f]) Inoculation yeast was cultivated in the unsupplemented medium; the fermentations reported in the table were performed as indicated in the left column.

tration of acetaldehyde in the cell should be obtained by the addition of 2,4-dinitrophenol and at niacin deficiency, and a decrease by the addition of iodoacetate and at thiamine deficiency (*cf.* above and Table 1). The effects obtained in the fermentation experiments agreed quite well with these predictions; the formation of acetate decreased with increasing concentrations of acetaldehyde in the cell as was expected from the kinetic results of Refs. 1 and 14 (*cf.* above).

The addition of pyruvic acid and acetaldehyde to the medium had very little effect on the formation of acetate; these substances were consumed only to about 20 % of the added amounts, indicating an inefficient penetration into the catabolic system.[15]

In the niacin series, the results obtained may also have been affected by the fact that a change in the total amount of NAD + $NADH_2$ may change the velocities of oxidation and reduction of acetaldehyde differently depending upon the respective Michaelis constants.

The increase in acetate formation in the iodoacetate series was lower than could be expected from the effects of this substance on the concentration of

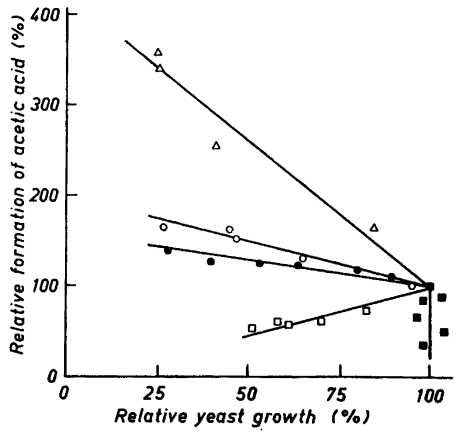

Fig. 2. Correlation between the relative formation of acetic acid and the relative growth; growth was limited by thiamine deficiency and oxythiamine (△), magnesium deficiency (○), potassium dihydrogenphosphate deficiency (●), niacin deficiency and pyridine-3-sulphonate (■),[6] and by the addition of 2,4-dinitrophenol (□).

pyruvate (Table 1); this is not understood, but may be due to the fact that iodoacetate is an inhibitor of many enzymes.[16]

The suitability of the metabolism of acetaldehyde as a model system for quantitative studies of a metabolic branching point is, however, limited as acetate may be formed by the hydrolysis of acetyl-SCoA formed by pyruvic oxidase and by the fact that acetate seems to be used in biosynthetic reactions. If yeast growth is limited, the concentration of acetate after fermentation generally increases (Table I in Ref. 17). In Fig. 2, the relative formation of acetate is plotted against the relative growth. There was a tendency towards an increased formation of acetate when growth was inhibited by deficiency of, respectively, ammonium ion, potassium diphosphate and magnesium.[6] The effects were, however, much more intense in the thiamine, niacin and 2,4-dinitrophenol series, which stresses the view that the formation of acetate is dependent upon the intracellular concentration of acetaldehyde. The iodoacetate series is not included in Fig. 2 since the fermentation time was much prolonged upon the addition of this substance which makes the determination of yeast growth rather inaccurate.[6]

In this connection it may be appropriate to mention that the content of acetate in bottom fermented beer is more than twice that of top fermented beer;[18] the latter is fermented at a considerably higher temperature than the former and hence at a higher rate and, presumably, at a higher intracellular concentration of acetaldehyde.

In summary, the formation of acetate as a by-product of alcoholic fermentation may be used as a model system of a metabolic branching point.

Acknowledgements. Grants from the *Swedish Technical Research Council* are gratefully acknowledged.

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Received November 1, 1965.