FORMATION OF 2,3-PENTANEDIOL FROM 2,3-PENTANEDIONE AND ACETYLETHYLCARBINOL BY DIACETYL(ACETOIN)REDUCTASE FROM *AEROBACTER AEROGENES*. A POSSIBLE NEW PATHWAY†

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Received 2 February 1973

1. Introduction

Diacetyl(acetoin)reductase catalyzes the reversible reduction of acetoin (acetylmethylcarbinol) to 2,3-butanediol and the irreversible reduction of diacetyl to acetoin [1, 2]. The enzyme has been characterized kinetically and found to follow an ordered bi–bi mechanism [3, 4].

During work with the three enzymes leading from pyruvate to 2,3-butanediol, fig. 1, we have observed that the first enzyme, the pH 6 acetolactate-forming enzyme, in addition to forming acetolactate from pyruvate, is able to form acetohydroxybutyrate from pyruvate and 2-oxobutyrate [5]. This enzyme requires cocarboxylase and Mn^{2+} for its activity [6, 7]. The second enzyme, acetolactate decarboxylase, decarboxylates acetolactate or acetohydroxybutyrate to yield acetoin or acetylethylcarbinol, respectively [8].

In the present report, we have shown that the substrates for diacetyl(acetoin)reductase, diacetyl, acetoin, and 2,3-butanediol, can be replaced by their respective analogues 2,3-pentanedione, acetylethylcarbinol, and 2,3-pentanediol.

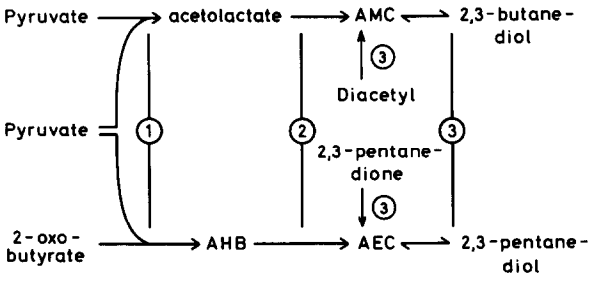

Fig. 1. Formation of 2,3-butanediol and 2,3-pentanediol in *A. aerogenes*. 1 = pH 6 acetolactate-forming enzyme, 2 = acetolactate decarboxylase, and 3= diacetyl(acetoin)reductase. AHB = acetohydroxybutyrate, AMC = acetoin, AEC = acetylethylcarbinol. For simplicity, the coenzymes are omitted in the figure.

2. Materials and methods

1,2-propanediol, 1,3-, 1,4- and 2,3-butanediol, and acetoacetic acid ethylester, were obtained from Fluka, and 2,3-pentanedione and 2,3-pentene from Koch Light. Glycolaldehyde, glyoxylic acid, methyl and ethyl pyruvate, diacetyl, NAD, NADH, were purchased from Sigma Chemical Company, and ethyleneglycol from Merck.

Acetohydroxybutyrate was prepared by T. Stensrud at the University of Oslo [9], and its purity checked as described [8]. Acetylethylcarbinol was

† Code number of enzyme: 2-acetolactate carboxy-lyase (EC 4.1.1.5).
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Table 1
K_m values for the reactions from 2,3-pentanedione to 2,3-pentanediol indicated in fig. 1.

Substrate for K_m determination	Second substrate	K_m (mM)
Acetylethylcarbinol	NADH	1.25
2,3-Pentanediol	NAD	6.3
2,3-Pentanedione	NADH	3.6
NADH	Acetylethylcarbinol	0.0049
NAD	2,3-Pentanediol	0.22
NADH	2,3-Pentanedione	0.012

K_m values were determined by changing the concentration of one substrate while the concentration of the other substrate was held at a constant level. This was repeated at different concentrations of the other substrate. The Lineweaver–Burk plots produced straight lines.

prepared by decarboxylation of acetohydroxybutyrate with sulfuric acid, followed by neutralization with NaOH. 2,3-Pentanediol was prepared from *cis–trans* 2,3-pentene by oxidation with performic acid. Its purity was verified by infrared and nuclear magnetic resonance spectroscopy, and by comparison of its physical properties with those reported in the literature.

The enzyme was purified [1], and assayed as described [3].

Initial reaction rates were measured by observing the rate of change in the optical density at 340 nm. The assay mixture consisted of 1.0 ml 100 μmoles acetate or phosphate, pH 5.8. The amounts of 2,3-pentanedione, acetylethylcarbinol, and NAD, or 2,3-pentanediol and NAD, were appropriate. The reaction was started by adding 0.034 μg enzyme, except in the presence of 2,3-pentanedione where the amount of enzyme was 0.055 μg, which gave a linear change in absorbancy for 2 min.

For spectrophotometric determinations of enzyme activity, the Shimadzu multipurpose recording spectrophotometer Model MPS-50L, with the thermostated cell compartment at 25°, was used.

3. Results and discussion

From Lineweaver–Burk plots for the substrates 2,3-pentanedione, acetylethylcarbinol, 2,3-pentanediol, NAD, and NADH, the K_m values listed in table 1, were found.

K_m for the oxidation of 2,3-pentanediol increases 10-fold at pH 5.8 when the reaction is performed in acetate (not shown in table 1). Similar effects of acetate were observed in the oxidation of 2,3-butanediol [3].

The reduction of acetylethylcarbinol by NADH can only yield one product, namely 2,3-pentanediol (see fig. 1). The oxidation of the latter, however, could theoretically yield two products, acetylethylcarbinol, and propionylmethylcarbinol. Whether both substances are produced is not yet clear. Since acetylethylcarbinol is a substrate for the enzyme, it seems very likely that this substance is produced in the oxidation of 2,3-pentanediol. Propionylmethylcarbinol and acetylethylcarbinol have been detected when yeast is incubated with pyruvate and 2-oxobutyrate [10].

Since diacetyl(acetoin)reductase can catalyze the formation of 2,3-pentanediol, this compound could be a metabolite in *A. aerogenes*. In addition, 2,3-pentanediol can be formed from 2,3-pentanedione, which in turn could possibly be formed from propionyl-CoA (from 2-oxobutyrate) and pyruvate. This would be analogous to the formation of diacetyl from acetyl-CoA and pyruvate in *A. aerogenes* and several other microorganisms and yeast [11, 12]. 2,3-Pentanedione has been detected by gas chromatography in the media during growth of *A. aerogenes* (K. Bryn, personal communication), and it is also produced during growth of yeast [13].

It may be of interest to mention that the following compounds could not be used as substrates in the reaction in a concentration of 1.25 mM in the presence of 0.01 mM NADH: glycolaldehyde, glyoxylic acid, 2,4-pentanedione, methyl and ethylpyruvate, and acetoacetic ethylester.

Similarly, with NAD (0.16 mM) as the second substrate: ethylene-glycol, 1,2-propanediol, 1,3-, and 1,4-butanediol were ineffective as substrates (12.5 mM).

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