A SYMPOSIUM ON SOME COMPARATIVE ASPECTS OF INTERMEDIARY METABOLISM IN MICRO ORGANISMS

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Comparative Aspects of Alcohol Formation

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The ability to produce ethanol from glucose is widely distributed in the microbial kingdom, and additionally, higher alcohols are formed by some micro-organisms. The yields vary considerably, from almost 2 moles of ethanol per mole of glucose fermented, characteristic of yeast, to the very much smaller amounts formed by many bacteria. These large variations are attributable to the operation of different metabolic pathways and, at present, four different routes of ethanol formation have been recognized, three of which involve pyruvic acid as an obligatory intermediate.

Pyruvate may be formed from glucose by different metabolic sequences (e.g. Embden–Meyerhof glycolysis or Entner–Doudoroff cleavage) and subsequently may be converted to a C₂ unit by one of two pathways, namely decarboxylation to acetaldehyde or by thioelastic reaction to acetylcoenzyme A. Reduction of either C₂ moiety yields ethanol. The heterolactic fermenters employ an entirely different mechanism: glucose is converted to xylulose-5-phosphate which is split by the enzyme phosphoketolase to acetylphosphate and glyceraldehyde-3-phosphate, the former undergoing reduction to ethanol.

The four different combinations of pathways are:

Type 1. Glycolysis and pyruvate decarboxylase ('carboxylase'; yeast, fungi, protozoa and a few bacteria).

Type 2. Glycolysis and thioelastic reaction (Enterobacteriaceae, Clostridia, Zymosarcina).

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Type 3. Entner–Doudoroff and pyruvate decarboxylase (Zymomonas mobilis, Z. anaerobia).

Type 4. Phosphoketolase (heterolactic bacteria).

To date, the combination of Entner–Doudoroff and thioclastic reactions has not been discovered.

The reductive formation of ethanol, common to all four types, must be coupled with oxidative reactions that occur in the fermentations. Nicotinamide adenine dinucleotides (NAD or NADP) mediate this coupling.

In microbial fermentations the further metabolism of pyruvate can give rise to a variety of products and consequently the occurrence of such reactions (in Types 1, 2 and 3) will divert pyruvate from ethanol formation with corresponding decreases in yield. Controlling factors must be the enzymic constitution and/or the enzymic activity of the organism, regulated by repression, pH or other environmental conditions.

Type 1. Glycolysis may be formulated as:

\[
\text{Glucose} + 2 \text{NAD} + 2 \text{ADP} + 2 \text{Pi} \rightarrow 2 \text{Pyruvate} + 2 \text{NADH} + 2 \text{ATP}
\]

which reveals an effective reducing power equivalent to \(4 \text{H}\), and a net energy yield of \(2 \text{ATP}\). The carboxyl carbons of pyruvate are derived from C-3 and C-4 of glucose. In yeast two enzymes—pyruvate decarboxylase and ethanol dehydrogenase—convert pyruvate to ethanol:

\[
\text{CH}_3\text{C}O\text{OHH} \xrightarrow{\text{Thiamine pyrophosphate}} \text{CH}_3\text{CHO} \xrightarrow{\text{NADH}} \text{CH}_3\text{CH}_2\text{OH} \]

(Carboxylase) (Ethanol dehydrogenase)

Two moles of ethanol can thus be formed per mole of glucose. Ethanol is also formed by these reactions in some fungi (Foster, 1949) and protozoa (Bauchop, 1962), but in very few bacteria, e.g. Zymosarcina ventriculi (Bauchop & Dawes, 1959). The only other examples of ethanol-forming bacteria which possess a carboxylase occur in Type 3.

Type 2. The Enterobacteriaceae and Clostridia cleave pyruvate to acetyl CoA by the following ‘thioclastic’ reactions:

\[
\text{CH}_3\text{C}O\text{OHH} \xrightarrow{\text{Thiamine pyrophosphate}} \text{CH}_3\text{COSCoA} + \text{HCOOH} \]

Enterobacteriaceae

\[
\text{CH}_3\text{COSCoA} + \text{H}_2 + \text{CO}_2 \xrightarrow{\text{Clostridia}} \text{CH}_3\text{CHO} \xrightarrow{\text{NADH}} \text{CH}_3\text{CH}_2\text{OH} \]

The mechanisms are still obscure but the evidence of Mortlock, Valentine & Wolfe (1959) suggests that the \(C_2\) fission product in Clostridium butyricum is at the aldehyde level of oxidation.

Despite earlier suggestions to the contrary (Tikka, 1935), pyruvate is an obligatory intermediate for ethanol formation in Escherichia coli, and acetylcoenzyme A is reduced to acetaldehyde by a coenzyme A-dependent acetaldehyde dehydrogenase, followed by reduction to ethanol by ethanol dehydrogenase (Dawes & Foster, 1956).

\[
\text{CH}_3\text{COSCoA} \xrightarrow{\text{NADH}} \text{CH}_3\text{CHO} \xrightarrow{\text{NADH}} \text{CH}_3\text{CH}_2\text{OH} + \text{CoASH}
\]

As the reduction of 1 mole of pyruvate to ethanol requires \(4 \text{H}\) the maximum possible
ethanol yield by Type 2 reactions would be 1 mole/mole glucose; other reactions which compete for NADH decrease the yield and the highest recorded is 0.8 mole (Stokes, 1949). When glycerol is the substrate, however, the fermentation approximates to

Glycerol → ethanol + formate,

since the conversion of glycerol to pyruvate furnishes the 4H necessary for quantitative ethanol formation. This observation emphasizes a general feature of alcohol production, namely that the yield is higher the more reduced the substrate fermented.

At present, *Zymosarcina ventriculi* is the only organism known to possess both decarboxylase and thioclastic enzymes for pyruvate (Arbuthnott, Bauchop & Dawes, 1960).

Some Clostridia produce butanol when the pH of glucose fermentation has fallen to about 4. The reactions occurring are probably analogous to those in *Escherichia coli*: butyrylcoenzyme A (formed from acetylcoenzyme A via acetoacetylcoenzyme A and reduction) may be reduced to butanol via butyraldehyde (for review, see Barker, 1956). In *Clostridium butylicum* acetone is reduced to isopropanol by the appropriate dehydrogenase.

**CH₃COCH₃ + NADH₂ ⇄ CH₃CHOHCH₃ + NAD.**

**Type 3.** *Zymomonas mobilis* gives a similar fermentation balance to yeast but ethanol derives from C-2, C-3 and C-5, C-6 of glucose, characteristic of the Entner–Doudoroff (1952) pathway (Gibbs & DeMoss, 1954).

\[
\begin{align*}
\text{Glucose} & \longrightarrow \text{glucose-6-phosphate} \xrightarrow{\text{NAD}} \text{gluconate 6-phosphate} \xrightarrow{\text{4H}} \\
2-\text{oxo-3-deoxygluconate-6-phosphate} & \rightarrow \begin{cases} 
\text{pyruvate} \\
\text{glyceraldehyde-3-phosphate} \\
\text{pyruvate}
\end{cases}
\end{align*}
\]

The net reaction is described by the equation

\[
\text{Glucose} + 2\text{NAD} + \text{ADP} + \text{P}_i \rightarrow 2\text{pyruvate} + 2\text{NADH} + \text{ATP},
\]

which reveals that, effectively, it carries out the same overall reaction as glycolysis but with half the energy yield, a conclusion verified experimentally by Bauchop & Elsden (1960). The organism possesses a pyruvate decarboxylase.

*Zymomonas anaerobia* displays a similar fermentation balance (Shimwell, 1950; Millis, 1956), it possesses Entner–Doudoroff and pyruvate decarboxylase enzymes, and has a molar growth yield similar to that of *Z. mobilis* (McGill, Ribbons & Dawes, unpublished).

**Type 4.** The heterolactic organisms, e.g. *Leuconostoc mesenteroides*, ferment glucose as follows:

\[
\text{Glucose} \rightarrow \text{lactate} + \text{ethanol} + \text{CO}_2,
\]

ethanol being derived from C-2 and C-3. The mechanism was illuminated by the discovery of an enzyme phosphoketolase (Heath, Hurwitz & Horecker, 1956) which cleaves xylulose 5-phosphate in accordance with the equation

\[
\text{Xylulose 5-phosphate} + \text{P}_i \rightarrow \text{acetyl phosphate} + \text{glyceraldehyde 3-phosphate}
\]

The triosephosphate is converted to pyruvate, by reactions common to glycolysis, and then reduced to lactate. The 4H made available in the conversion of glucose to
xylulose-5-phosphate are utilized for reduction of acetylphosphate to ethanol, a reaction associated with the loss of biologically useful energy to the organism. A similar loss occurs in the reduction of acetyloenzyme A (Type 2). When pentose is fermented acetylphosphate cannot be reduced to ethanol and an extra mole of ATP is conserved.

Other reactions. Some micro-organisms convert pyruvate to the alcohols acetyl-methylcarbinol (acetoin) and 2,3-butanediol by one of two pathways:

(a) organisms lacking carboxylase, e.g. *Aerobacter aerogenes, Streptococcus faecalis*

\[2 \text{pyruvate} \rightarrow \text{CO}_2 + \alpha\text{-acetolactate} \rightarrow \text{CO}_2 + \text{acetoin};\]

(b) organisms having carboxylase, e.g. yeast

\[\text{Pyruvate} + \text{acetaldehyde} \rightarrow \text{CO}_2 + \text{acetoin}.

The Acetobacter are unique in possessing enzymes for both pathways (De Ley, 1959). Those micro-organisms which possess a 2,3-butanediol dehydrogenase can reduce acetoin to 2,3-butanediol. Clearly these reactions will divert pyruvate from ethanol formation.

The anaerobe *Vibrio cholinicus* (subsequently shown to be indistinguishable from *Desulfovibrio desulfuricans*, Baker, Papiska & Campbell, 1962) ferments choline to trimethylamine, acetate and ethanol:

\[2(\text{CH}_3\text{CH}_{2}\text{OH}) + \text{H}_2\text{O} \rightarrow 2(\text{CH}_3\text{NH} + \text{CH}_3\text{COOH} + \text{CH}_3\text{CH}_2\text{OH}.

Hayward (1960) demonstrated that the C₃ moiety of choline is transformed to acetaldehyde, and that the acetaldehyde then undergoes a dismutation to ethanol and acetyloenzyme A catalysed by NADP-dependent ethanol and acetaldehyde dehydrogenases respectively. Acetyloenzyme A yields acetate and ATP via acetylphosphate. Since the organism grows on choline as the sole carbon and energy source some molar growth yield experiments would be instructive.

Fusel oils. These are higher alcohols produced by yeast from amino acids, in the presence of glucose, and without the release of ammonia. Senth Shanmuganathan & Elsden (1958) showed that tyrosol was produced from tyrosine in a sequence of three reactions: (a) transamination between L-tyrosine and 2-oxoglutarate (formed from glucose) to yield \(p\)-hydroxyphenylpyruvate and L-glutamate; (b) decarboxylation of \(p\)-hydroxyphenylpyruvate to \(p\)-hydroxyphenylacetaldehyde; and (c) its reduction to tyrosol in the presence of alcohol dehydrogenase and NADH₄. n-Propanol is formed from \(\alpha\)-oxobutyrate, an intermediate in isoleucine synthesis, by decarboxylation and subsequent reduction (Guymon, Ingraham & Crowell, 1961).

Regulation. In all reactions leading to alcohol formation the reduction of an aldehyde is the final step, catalysed by an alcohol dehydrogenase. The presence of such an enzyme is therefore essential for alcohol formation and its regulation might be expected under conditions where the alcohol yield is altered. McPhedran, Sommer & Lin (1961) have shown that the ethanol dehydrogenase of *Aerobacter aerogenes* is repressed under anaerobic conditions when a hydrogen acceptor such as fumarate is added to the growth medium; ethanol, acetaldehyde and acetate were without effect. It would be interesting to know if this repression extends to acetaldehyde dehydrogenase.
Comparative aspects of alcohol formation

REFERENCES


