The Effect of DL-β-methylaspartic Acid on the Growth of Escherichia coli

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Aspartic and glutamic acid and their amides have a special position in cellular metabolism. They are not only immediately utilized for protein synthesis but their α-amino group can be transferred by means of a system of transamination reactions to keto acids, giving rise to other amino acids or forming a building block in purine synthesis. Analogues of these amino acids can thus participate very intimately in processes which can be of practical as well as theoretical importance. Various analogues of the two amino acids have been described (Martin, 1951). The DL-β-methylaspartic acid used here was first synthesized by Dakin (1941) and it can represent one of the precursors in the synthesis of glutamic acid in Clostridium tetanomorphum (Barker et al. 1958 a, b). Its effect on the growth of Escherichia coli was examined here on account of its structural similarity to aspartic and glutamic acids.

MATERIALS AND METHODS

β-methyl aspartic acid was prepared by Dakin by condensation of ethylbenzoylaminomalonate and ethyl α-bromopropionate with subsequent hydrolysis of the condensation product. When we were carrying out our experiments, Barker described the preparation of this compound by a similar method, starting from ethylacetaminomalonate. He claims further that for biochemical experiments he used the less soluble three-isomer of DL-β-methylaspartic acid but gives no details, referring only to a paper in the press.

Independently of Barker we succeeded in preparing β-methyl-aspartic acid in the same way. The yield of the synthesis was 25%, m. p. of the non-resolved isomer mixture was 262–4°C. Analysis: C₆H₉O₄N calculated: 40.81% C, 6.16% H, 9.52% N; found: 40.53% C, 6.14% H, 9.55% N. The isomers were then separated by means of paper chromatography on Whatman 1 in phenol-ethylalcohol-water (2:1:1) with the addition of 0.1% 8-hydroxyquinoline, developing three times in succession (Fig. 1). The isomers of the DL-β-methylaspartic acid give a positive reaction with ninhydrin, giving rise to a grey-blue colour similar to that of aspartic acid. The colour is not as intensely blue as with glutamic acid. The isomers were separated in small amounts by using Zerolite E. The column was first washed with sodium acetate and water; the samples were eluted at pH 2.9–2.8 with acetic acid. The first to leave the column was the less soluble isomer. Larger amounts of the individual isomers were prepared by fractional crystallization from water. The products of both the types of separation were chromatographically pure. The melting point of the less soluble isomer was m. p. 261–262°C; that of the more soluble one m. p. 268–271°C.

On the basis of solubility of the two isomers it was concluded that the less soluble isomer prepared here is identical with Barker’s three-isomer, the soluble one with the erythro-isomer.

Culture and Cultivation. A strain of Escherichia coli B from the Carnegie
Institute was used throughout. Bacteria were grown in 500 ml. flasks containing 20 ml. of a synthetic growth medium (Mandelstam, 1958) on a reciprocal shaker (98 osc./min., amplitude 8.9 cm.) at 29—30°C. A test-tube was welded to each cultivation flask so that turbidity could be measured without the flask being opened and samples withdrawn. The density of the culture was determined by absorption at 370 mµ using the Lumetron colorimeter. Concentrations of the analogue shown in tables and graphs are calculated for the L-isomer of β-methyl aspartic acid.

RESULTS

The two isomers of DL-β-methylaspartic acid produced a different effect on the growth of bacterial culture. The threo-form caused a marked inhibition, the erythro-form stimulated growth (Tab. 1).

Table 1. The effect of the threo- and erythro-forms of β-methylaspartic acid on the growth of E. coli

<table>
<thead>
<tr>
<th>Analogue concentration in µmoles/ml.</th>
<th>Growth in % control</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Threo-isomer</td>
<td>Erythro-isomer</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>77</td>
<td>109</td>
</tr>
<tr>
<td>5</td>
<td>67</td>
<td>127</td>
</tr>
</tbody>
</table>

The threo-form, in concentrations between 0.5 and 5.0 µmoles/ml, inhibited growth of Escherichia coli by 25—45%, i.e. without a markedly different effect. The effect of the analogue decreased with the amount of inoculum.

It follows from the growth curve of Escherichia coli in the presence and in the absence of 2 µmoles/ml. of the threo-form of β-methyl-aspartic acid (Fig. 2), that the analogue prolongs the lag phase but is without effect on the rate of multiplication in the logarithmic phase as well as on the final maximal concentration of cells in the medium. The same relationship holds for analogue concentrations of 0.5 and 1.0 µmoles/ml. The general form of the curve suggests that the analogue is detoxicated during the lag phase. This detoxication could take place in basically two ways. Either the β-methyl-aspartic acid is metabolized to glutamic or aspartic acid, or else its effect is reversed by an increased synthesis of the corresponding amino acid. It was, therefore, attempted to determine whether cells of Escherichia coli are capable of bringing about such a degradation or whether they possibly counteract the effect of the analogue by an increased production of glutamic or aspartic acid. As the inhibitory effect of the threo-form of β-methylaspartic acid became manifest in the prolongation of the lag phase it was assumed that the analogue is probably detoxicated by a mechanism of adaptive character which is formed during the lag phase. The stimulating effect of the erythro-isomer appeared immediately, thus suggesting its utilization by an enzyme system present obligatorily in the cell. Therefore, for metabolic studies of the threo-isomer both a normal culture and a culture adapted by growth in a medium containing 1 µmole of the analogue per ml. were used. Washed cells of Escherichia coli grown for 24 hours in a synthetic medium were incubated in an equal volume of