Effect of Carbon Dioxide on the Aspartic Acid Requirement for Proteinase Biosynthesis by Streptococcus faecalis var. liquefaciens

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Non-proliferating cells of Streptococcus faecalis var. liquefaciens required aspartic acid for proteinase biosynthesis in the absence of CO₂ but not in the presence of CO₂. The utilization of CO₂ by various species of streptococci has been reported. Barnes (1) and Wright (9) reported increased dextranucrase synthesis by Streptococcus bovis. Fixation of CO₂ primarily into aspartic acid has been demonstrated in S. anginosus (5) and in S. faecium var. durans (4). Platt and Foster (6) stated that S. faecalis var. liquefaciens incorporated CO₂ into acetate, lactate, and cell material.

Under anaerobic conditions and to a lesser extent under microaerobic conditions, CO₂ stimulates proteinase synthesis by non-proliferating cells of S. faecalis var. liquefaciens in a synthetic medium (8). The present communication describes studies that establish a relationship between CO₂ availability and the aspartic acid requirement for proteinase biosynthesis by non-proliferating cells of S. faecalis var. liquefaciens strain 31 under anaerobic and microaerobic conditions.

Preparation of the cell inoculum has been described (7). Most of the details of incubation have been described (8). The basic medium used to study proteinase biosynthesis was freshly prepared, unsterilized modified optimal adaptation medium [final modified synthetic medium of Hartman et al. (3)], which contained the following components in milligrams per 100 ml: lactose, 200; KH₂PO₄, 1,470; NaCl, 200; MgSO₄·7H₂O, 8; FeSO₄·7H₂O, 0.4; MnSO₄·H₂O, 0.16; CoCl₂·0.12; CaCl₂, 1.5; adenine, 0.5; uracil, 0.5; riboflavin, 0.1; pyridoxal hydrochloride, 0.1; biotin, 0.0001; L-histidine, 6.0; L-lysine·2 HCl, 16.8; L-tryptophan, 3.0; DL-methionine, 13.6; DL-serine, 67.2; DL-threonine, 76.0; L-leucine, 20; DL-isoleucine, 24.8; DL-valine, 74.8; L-glutamic acid, 46.6; L-arginine, 81.6; L-cystine, 0.68; L-tyrosine, 12.0; L-alanine, 10.0; L-proline, 21.2; glycine, 4.0; and L-aspartic acid, 14.2, where noted. The pH of the medium was 6.5.

Table 1 demonstrates the relationship between CO₂ availability and the requirement for aspartic acid. Aspartic acid has no stimulatory effect on proteinase when CO₂ is available to the cells. Aspartic acid is required for a high level of proteinase synthesis when CO₂ is not available to the cells. This relationship between CO₂ availability and the need for aspartic acid exists both in the presence and absence of O₂. Apparently, the cells require CO₂ fixation for the synthesis of aspartic acid, which is needed for protein biosynthesis.

The relationship between the arginine dihydrolase enzyme system and proteinase biosynthesis

<table>
<thead>
<tr>
<th>Medium</th>
<th>Atmosphere and contents of center well</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Na</td>
</tr>
<tr>
<td></td>
<td>No KOH</td>
</tr>
<tr>
<td>17 Amino acids</td>
<td>1,050</td>
</tr>
<tr>
<td>17 Amino acids plus aspartic acid</td>
<td>1,050</td>
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</tbody>
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* Incubation was carried out in Warburg vessels for 5 hr at 37°C. Total volume was 2.5 ml including a 10% (v/v) cell inoculum. Carbon dioxide was absorbed by filter paper impregnated with KOH and inserted in the center well of the vessel.

† Proteinase biosynthesis was measured by the procedure of Rabin and Zimmerman (7). Proteinase synthesis is expressed as micrograms of tyrosine liberated per ml of casein substrate (volume per assay tube) per hr per ml of the supernatant fraction from the cell suspension.
sis was hypothesized by Hartman and Zimmerman (2) to be of a limiting nature, because, under microaerobic conditions, proteinase biosynthesis was found to end when the degradation of arginine was complete. The present work extends the relationship by showing that CO₂, which is a product of the arginine dihydrolase enzyme system, can provide for the synthesis of proteinase. Swiencicki and Hartman (8) have shown that, under conditions similar to those employed in this study, arginine is rapidly degraded during the early phase of the incubation period so that much CO₂ is available to the cells for aspartic acid synthesis prior to the start of proteinase biosynthesis. The arginine dihydrolase enzyme system therefore promotes proteinase biosynthesis by supplying CO₂ and limits proteinase biosynthesis by its rapid, complete degradation of arginine.

LITERATURE CITED