Changes in Menaquinone Concentration During Growth and Early Sporulation in Bacillus subtilis

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In two strains of Bacillus subtilis, menaquinone-7 has been shown to reach maximal cellular concentrations during early stationary phase. These concentration changes closely parallel the previously reported concentration changes in the cytochromes.

When exponential cultures of Bacillus subtilis, growing on glucose as a carbon source, enter early stationary phase, there appears to be a shift in the pathways of energy metabolism. Fermentative pathways are supplanted by oxidative pathways, the transition being characterized by induction of tricarboxylic acid cycle enzyme activities (10, 12, 18) and increases in the rates of CO₂ evolution and oxygen consumption. The inability of certain tricarboxylic acid cycle mutants to sporulate under normal conditions suggests that this transitional phase is crucial to sporulation (4, 11, 18).

Chaix and Petit (5) observed that changes also take place in the cytochrome concentration of B. subtilis during this period. Concentrations of cytochromes c₁ and o, which are relatively high during exponential growth, decline during late vegetative and early stationary phases, and concentrations of cytochromes a, b, and c increase markedly. These concentration changes occur at approximately the same time as induction of the tricarboxylic acid cycle and the increased rates of CO₂ evolution and oxygen uptake.

We have been interested in the role of menaquinone in growth and sporulation of B. subtilis. This membrane-associated quinone has been implicated in the oxidative metabolism of a number of different bacterial species (1, 2, 13, 20), including B. subtilis (7). Consequently, we investigated the menaquinone-7 (MK-7) content of B. subtilis at various stages of growth and sporulation.

Stains RB 1 (16; trpC2) and SB 202 (trpC2, tyrA, hisB, aroB) were grown in 4-liter volumes of synthetic sporulation medium (SSM) no. 5 (16). Cultures of strain RB 1 were supplemented with tryptophan, whereas those of strain SB 202 were supplemented with either tryptophan, tyrosine, phenylalanine, p-aminobenzoic acid, and histidine, or tryptophan, shikimic acid (a precursor of aromatic amino acids), and histidine. MK-7 concentrations were determined by the method of Salton and Schmitt (17) in membrane preparations from cells harvested at various periods of exponential and postexponential growth. Control experiments showed that 91% of the membrane-associated MK-7 could be extracted by this technique. Figure 1 shows that, for both strains, the quinone content of membranes increased approximately twofold between mid-exponential growth and a time 1 h after the end of exponential growth (T₁). Thereafter the values fell to a constant level by T₄. Since membrane dry weight per optical density unit of culture remained relatively constant when plotted as a function of growth time (Fig. 2), the changes in MK-7 concentration were not merely a reflection of general changes in membrane content of the cells from one phase of growth to another.

Separate experiments, performed in SSM no. 5 supplemented as above indicated that strains RB 1 and SB 202 sporulate at high frequencies in this medium (Table 1).

The rise in quinone concentration to a maximum at T₁ corresponds to increases in cytochromes a, b, and c observed in B. subtilis during late vegetative growth (5, 19) and early sporulation (H. W. Taber, J. R. Coleman, and F. Sherman, unpublished experiments). Similar cytochrome increases during growth and sporulation of B. cereus have been correlated with several electron transport activities (14).

The results obtained when strain SB 202 was grown in the absence of shikimic acid are quite interesting. This strain is an aroB mutant and lacks 5-dehydroquinase synthase, the second enzyme in the common pathway for aromatic amino acid biosynthesis. Menaquinone is synthesized by a series of reactions branching from the common aromatic pathway at the level of chorismic acid (3, 6, 15). Theoretically, strain SB 202 should be unable to synthesize quinone when grown in the absence of the aromatic
precursor. The results suggest that either (i) *B. subtilis* contains an alternate pathway for MK biosynthesis, or (ii) strain SB 202 is bradytrophic at the *aroB* block, allowing sufficient

**Fig. 1.** Changes in cellular menaquinone-7 concentration during growth and sporulation of *B. subtilis*. Growth conditions and MK-7 quantitation were as noted in the text. Symbols: ■, growth (optical density at 540 nm); ○, MK-7 concentration (nanomoles per milligram of membrane).

**Fig. 2.** Dry weight of total membrane recovered per milliliter of culture. Values are expressed as a function of the optical density of the culture at time of harvesting.

**Table 1.** Sporulation characteristics of strains RB 1 and SB 202 in synthetic sporulation medium no. 5

<table>
<thead>
<tr>
<th>Strain</th>
<th>Viable count&lt;sup&gt;+&lt;/sup&gt; (CFU/ml)</th>
<th>Spore count&lt;sup&gt;+&lt;/sup&gt; (heat-resistant CFU/ml)</th>
<th>Sporulation frequency (S/V)&lt;sup&gt;+&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB 1</td>
<td>$1.2 \times 10^4$</td>
<td>$7.0 \times 10^4$</td>
<td>0.58</td>
</tr>
<tr>
<td>SB 202 + <em>Aro</em></td>
<td>$8.5 \times 10^4$</td>
<td>$4.3 \times 10^4$</td>
<td>0.50</td>
</tr>
<tr>
<td>SB 202 + <em>Shk</em></td>
<td>$1.1 \times 10^4$</td>
<td>$5.7 \times 10^4$</td>
<td>0.52</td>
</tr>
</tbody>
</table>

<sup>*</sup> Cultures were grown for 24 h at 37 C with rapid shaking.

<sup>+</sup> Determined by plating on tryptose blood agar base. CFU, Colony-forming units.

<sup>+</sup> Determined by heating a portion of each culture at 80 C for 10 min followed by plating suitably diluted samples on tryptose blood agar base.

* Tryptophan, tyrosine, and phenylalanine were supplied at 50 μg/ml. Para-aminobenzoic acid was supplied at 10 μg/ml. Histidine (50 μg/ml) was also added to each culture of SB 202.

* Shikimic acid was supplied at 50 μg/ml.

* S, heat-resistant CFU per milliliter; V, total viable CFU per milliliter.

substrate through the pathway to allow near normal menaquinone biosynthesis. The isolation of an *aroD* (5-dehydroshikimate reductase) mutant unable to synthesize MK-7 unless grown in the presence of shikimic acid supports the second hypothesis (S. K. Farrand and H. W. Taber, Bacteriol. Proc., p. 68, 1970; reference 8).

By using this menaquinone-deficient *aroD* mutant, we have shown that a decrease by half in the peak MK-7 concentration at T<sub>1</sub> results in a nearly 200-fold decrease in the frequency of sporulation (9). Under such conditions, vegetative growth is unaffected.

Although the reason for the MK-7 requirement is not known, we have determined that a 10-fold higher peak MK-7 concentration is required for maximum sporulation than is necessary to establish normal cellular respiration. This strongly indicates that a lesion in electron transport cannot account for the asporogenic phenotype of this *aroD* mutant. These results
would suggest that the high levels of MK-7 observed at T, are required for some early sporulation event unrelated to the electron transport function of this quinone.

LITERATURE CITED