Investigation of 1-Deoxy-D-Xylulose 5-Phosphate Synthase and Transketolase of Bacillus subtilis in Relation to Vitamin B₆ Biosynthesis

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Summary In Escherichia coli, 4-(phosphohydroxy)-L-threonine and 1-deoxy-D-xylulose 5-phosphate are believed to be direct precursors of vitamin B₆ (B₆), and 1-deoxy-D-xylulose 5-phosphate synthase (Dxs) and transketolase could catalyze the formation of each precursor. In this report, the possible involvement of Dxs and transketolase (Tkt) in B₆ biosynthesis in Bacillus subtilis was investigated. The gene disruptant of tkt and conditional mutants of dxs were constructed, and their ability of B₆ biosynthesis was examined. It was found that the tkt disruptants retain the ability to synthesize B₆. The conditional mutant of dxs synthesized the same amount of B₆ per dry cell weight as the wild-type strain. Therefore, it is very likely that neither Dxs nor transketolase is involved in B₆ biosynthesis in B. subtilis.

Key Words vitamin B₆ biosynthesis, transketolase, 1-deoxy-D-xylulose 5-phosphate synthase, Bacillus subtilis

The de novo biosynthesis of vitamin B₆ (B₆) has been mostly studied with a Gram-negative bacterium, Escherichia coli, and strong evidence for the synthetic pathway has been reported. According to enzymatic and isotopic labeling experiments, glycolaldehyde (GA) was identified as a precursor (1-3). 4-(Phosphohydroxy)-L-threonine (4-HTP) and 1-deoxy-D-xylulose 5-phosphate (DXP) were also reported as precursors of B₆ (4) (Fig. 1). 4-HTP is synthesized via an intermediate, D-erythrose 4-phosphate by transketolase (TktA, B), phosphoserine aminotransferase (SerC), and some other enzymes. DXP is synthesized from pyruvate and glyceraldehyde 3-phosphate by 1-deoxy-D-xylulose 5-phosphate synthase (Dxs). However, the possible involvement of 4-(phosphohydroxy)-L-threonine and 1-deoxy-D-xylulose 5-phosphate in B₆ biosynthesis in microorganisms other than E. coli, still remains unknown.

We have reported several lines of evidence regarding the B₆ biosynthetic pathway in Gram-positive bacterium, Bacillus subtilis, yaaD, and yaaE, of which obvious homologues do not exist in E. coli, involved in B₆ biosynthesis (5). GA is probably not a precursor of B₆ in B. subtilis (6). SerC is not directly involved in the biosynthesis in B. subtilis (7). The involvement of the homologues of yaaD was also reported in microorganisms other than B. subtilis (8-10). These reports suggested that there are at least two kinds of B₆ biosynthetic pathway among microorganisms.

We have investigated whether or not the genes involved in B₆ biosynthesis in E. coli are also involved in that in B. subtilis. Here, we report neither Dxs nor Tkt is involved in B₆ biosynthesis in B. subtilis, suggesting that either 4-HTP or DXP is not a precursor of B₆. This also emphasizes our previous suggestion that the B₆ biosynthetic pathway in B. subtilis is different from that in E. coli.

tkt was disrupted by a pMutin 1 (11) in B. subtilis CRK6000 (12) as described previously (7). Primers used for amplification of the internal fragment of tkt were TKT1F (5'-CGCGGATCCAGCAAATTCTGGTCACCGGAC-3') carrying a BamHI restriction site (shown in italic) and TKT1R (5'-GCCGAAGCTTGCTTTCTTTACCAACG-3') carrying a HindIII site (shown in italic). Primers used for confirmation of the integration of a single copy of the plasmids were TKT2F (5'-GCAAGGTACACGTGCTTCTTCC-3') and TKT2R (5'-CGCGAGGTTTCTGGTGAC-3'). It was also confirmed that no transketolase activity was detected in the crude extract of the tkt disruptant.

The wild-type strain and the tkt disruptant of B. subtilis CRK6000 were grown at 37°C in Spizizen minimal medium (0-glucose was replaced with d-sorbitol) supplemented with 0.05 mM adenine sulfate dihydrate, 0.05 mM guanosine, 0.5 mM L-methionine and 0.5 mM L-histidine (SMM). The tkt disruptant did not grow in SMM, but grew in SMM supplemented with 0.5 mM shikimate. The addition of B₆ did not further improve the growth of the tkt disruptant (data not shown).

The amount of B₆ synthesized by the wild-type strain and the tkt disruptant of B. subtilis CRK6000 were grown at 37°C in Spizizen minimal medium (0-glucose was replaced with d-sorbitol) supplemented with 0.05 mM adenine sulfate dihydrate, 0.05 mM guanosine, 0.5 mM L-methionine and 0.5 mM L-histidine (SMM). The tkt disruptant did not grow in SMM, but grew in SMM supplemented with 0.5 mM shikimate. The addition of B₆ did not further improve the growth of the tkt disruptant (data not shown).

The amount of B₆ synthesized by the wild-type strain and the tkt disruptant was quantified in a microbiological assay with Saccharomyces carlsbergensis ATCC 9080 as previously described (7). The wild-type strain and the tkt disruptant were grown in SMM supplemented with 0.5 mM shikimate at 37°C for 20 h. The amounts of B₆ in the culture were determined after 1, 4, 8, and 20 h of....
Fig. 1. Putative biosynthetic pathway of B₆ in E. coli. 4-Phosphohydroxy-L-threonine is formed from D-glyceraldehyde 3-phosphate and D-fructose 3-phosphate by the actions of transketolase, phosphoserine aminotransferase and some other enzymes. 1-Deoxy-D-xylulose 5-phosphate is formed from pyruvate and glyceraldehyde 3-phosphate by 1-deoxy-D-xylulose 5-phosphate synthase. Solid lines stand for single-step reactions; dotted lines stand for multiple-step reactions.

Table 1. Amount of B₆ synthesized.

<table>
<thead>
<tr>
<th>Strain</th>
<th>B₆ (ng/mg dry cell weight)</th>
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<tbody>
<tr>
<td></td>
<td>Cultivation (h)</td>
</tr>
<tr>
<td>Wild-type strain</td>
<td>1.7</td>
</tr>
<tr>
<td>tkt disruptant</td>
<td>1.7</td>
</tr>
</tbody>
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Experiments were repeated three times, and representative data are shown.

cultivation (Table 1). The amount of B₆ synthesized by the wild-type strain and tkt disruptant were almost the same through the 20 h of cultivation, indicating that tkt is not involved in B₆ biosynthesis in B. subtilis.

The possible involvement of dxs in B₆ biosynthesis was also examined. Since dxs is an essential gene in B. subtilis, a conditional mutant of dxs (B. subtilis 168 strain; trpC2) was constructed as previously described (5). Primers used for amplification of the internal fragment of dxs were YQIESDF (5'-GCCGAAGCTTATGGCTGAAAGTGAGTTGATCCG-3') carrying a BamHI restriction site (shown in italic) and YQIESD1R (5'-GGGATCCGATCCCCCTCCGAGCATAGATGAATG-3') carrying a HindIII site (shown in italic). Primers used for confirmation of the integration of a single copy of the plasmids were YQIESD2F (5'-CAGGAGGTATGTTGAAAATGCATACG-3') and YQIE2R (5'-AACATCCGGAGGAGCATG-3'). As a result, the conditional mutant of dxs, of which transcriptions could be induced by isopropyl 1-thio-β-D-galactoside (IPTG), was obtained. The wild-type strain and dxs mutant were grown in Spizizen minimal medium supplemented with 0.5 mM L-tryptophan and 10 mg/L FeSO₄·7H₂O (TMM), and total B₆ amounts were measured at the indicated times. Although the dxs mutant grew poorly in TMM without IPTG, the mutant synthesized almost the same amount of B₆ per dry cell weight (DCW) as the wild-type strain (Fig. 2). The addition of IPTG improved the growth of the dxs mutant, but did not affect B₆ production. The B₆ production per dry cell weight was the same in the dxs mutant and wild-type strain. A higher concentration of IPTG (1.0 mM) did not affect B₆ production of the dxs mutant (data not shown). These results indicate that the inactivation of dxs does not affect B₆ production, and therefore, it was most likely that dxs is not involved in B₆ biosynthesis in B. subtilis.

In the non-mevalonate pathway, DXP is further me-
tabolized to 2-C-methyl-D-erythritol 4-phosphate by 1-deoxy-D-xylulose 5-phosphate reductoisomerase (Dxr) (13). If DXP is a precursor of B₆ in B. subtilis, it could be expected that the inactivation of Dxr would lead to the accumulation of DXP, and DXP flux through B₆ increases. Alternatively, if the expressional level of Dxr is increased, DXP flux through 2-C-methyl-D-erythritol 4-phosphate is increased, and therefore, the amount of B₆ synthesized is decreased. To examine the above speculation, a conditional mutant of dxr was constructed in the same manner as the conditional mutant of dxs, and its characteristics were examined. It was found that the conditional mutant of dxr showed almost the same characteristics as the dxs mutant. The characteristics of the dxr mutant supported the speculation that DXP is not a precursor of B₆ in B. subtilis.

Our results demonstrate that Dxs and Tkt are not involved in B₆ biosynthesis in B. subtilis, and suggest that 4-HTP and DXP are probably not the precursors of B₆ in B. subtilis. Combined with our previous reports, it is most likely that the B₆ biosynthetic pathway in B. subtilis is different from that in E. coli.

REFERENCES