Chromosomal Location and Nucleotide Sequence of the \textit{Escherichia coli} \textit{dapA} Gene

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In \textit{Escherichia coli}, the first enzyme of the diaminopimelate and lysine pathway is dihydroidipicolinate synthetase, which is feedback-inhibited by lysine and encoded by the \textit{dapA} gene. The location of the \textit{dapA} gene on the bacterial chromosome has been determined accurately with respect to the neighboring \textit{purC} and \textit{dapE} genes. The complete nucleotide sequence and the transcriptional start of the \textit{dapA} gene were determined. The results show that \textit{dapA} consists of a single cistron encoding a 292-amino acid polypeptide of 31,372 daltons.

The \textit{dapA} gene encodes dihydroidipicolinate synthetase (DHDPS) (10), the first enzyme of the diaminopimelate and lysine pathway (23). It has been mapped to 53 min on the \textit{Escherichia coli} chromosome (1). DHDPS activity is sensitive to lysine inhibition (28) as is aspartokinase III (26), the first enzyme of the biosynthetic pathway leading from aspartate to diaminopimelate, lysine, methionine, threonine, and isoleucine. The regulation of expression of several genes of the lysine regulon (19), including \textit{lysC} (26), \textit{asd} (4, 10), \textit{dapB} (13), \textit{dapD} (21), \textit{dapE} (10), and \textit{lysA} (20), has been studied either by directly measuring their gene products or by the use of \textit{lacZ} or \textit{galK} fusions. In contrast to all these genes, whose levels of expression depend on the lysine pool, the synthesis of DHDPS does not appear to be subject to any regulatory control (7, 23). Moreover, it has been shown that DHDPS must catalyze the rate-limiting step in lysine biosynthesis after aspartokinase III (11).

A thorough knowledge of this step is necessary for the understanding of the whole lysine pathway. Consequently, the location of the \textit{dapA} gene on the \textit{E. coli} chromosome has been precisely determined in relation to the neighboring genes. The \textit{dapA} gene has been identified in an \textit{E. coli} \lambda library, purified, and sequenced, and its transcriptional start has been determined.

MATERIALS AND METHODS

Media, strains, and plasmids. Bacterial strains and plasmids are listed in Table 1. Growth conditions were as described previously (21).

DNA manipulations. Plasmid purification; DNA fragment isolation, restriction, and ligation; plasmid transformation; DNA sequencing; and determination of transcriptional start were performed as described previously (21).

Enzyme assays. DHDPS was measured by the O-amino-benzaldehyde assay of Yugari and Gilvarg (28) as modified by Butour et al. (7). Samples of products were taken after 30, 45, and 60 min of incubation at two different protein concentrations. Units are given in increments of optical density (10\(^3\)) at 540 nm per minute per milligram of protein. Protein concentrations were determined by the biuret method. \beta-Lactamase assays were performed by the method of Novick (17).

RESULTS AND DISCUSSION

Isolation and accurate location of the \textit{dapA} gene. Bukhari and Taylor (6) isolated three Dap mutants (AT978, AT984, and AT998) with mutations which were mapped to the \textit{guaA} region (revised map, 33 min [1]) and identified indirectly as \textit{dapE}, \textit{dapA}, and \textit{dapA}, respectively. Recently, Parker (18) located the positions of mutations in strains AT978 and AT984 close to the \textit{purC} gene. We have previously isolated several Dap mutants (22) devoid of DHDPS activity and located close to \textit{purC}.

The \textit{dapA} gene has been isolated on a 2.8-kilobase (kb) \textit{PstI} fragment (22) subcloned from a \lambda bacteriophage (8) into plasmid pBR322 to give pDA1. This plasmid complements all the \textit{dapA} mutants in our collection (22) as well as DO916 (5) and AT998 (6) but fails to complement the two other Dap mutants, AT978 and AT984. The latter two strains contain DHDPS activity at the wild-type level, so they are not affected in the \textit{dapA} gene.

The restriction map of the pLC25-14 plasmid from the Clarke and Carbon library (9) carrying the \textit{purC} gene (16) has been compared with those of the \lambda \textit{dprC} phages studied by

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
Strain or plasmid & Relevant characteristics & Reference or source \\
\hline
RM4102 & \textit{araD139 lacU169 rpsL thia} & 22 \\
RDA1 & \textit{araD139 lacU169 rpsL thia} & 22 \\
8, 14, 25, 26 & \textit{dapA::Mu} & \\
D0916 & \textit{lys+110 lysC1002 dapA1101} & 5 \\
AT978 & \textit{dapA6} & 6 \\
AT978 & \textit{dapE9} & 6 \\
pDA1 & \textit{tc' dapA*} & 22 \\
pBR322 & \textit{Apr} \textit{tc'} & 1 \\
pUC9 & \textit{Apr} \textit{lacZ} & 27 \\
pDA68 & \textit{tc' dapA + Bell deletion of pDA1} & This work \\
pDA2 & \textit{Apr} \textit{dapA + BstNI fragment in pUC9} & This work \\
pDA3 & \textit{Apr} \textit{dapA + BstNI fragment in pUC9} & This work \\
pDA4 & \textit{Apr} \textit{dapA + BstNI fragment in pUC9} & This work \\
pBR322 & \textit{Apr} \textit{dapA + BstNI fragment in pUC9} & This work \\
\hline
\end{tabular}
\caption{Bacterial strains and plasmids}
\end{table}

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TABLE 2. Levels of DHDPS in strain RDA8 harboring various plasmidsa

| Strain | DHDPs sp act Without L-lysine | With 20 mM L-lysine | Plasmid copy no. \\n|--------|-------------------------------|---------------------|-------------------|
| RM4102 | 160                           | 20                  |                   |
| RDA8   | 5                             | NDb                | 120               |
| RDA8(pDA2) | 7,000                     | 700               |                   |
| RDA8(pDA3) | 7,000                      | 725               |                   |
| RDA8(pDA4)c | 3,500                     | 400               |                   |
| RDA8(pDA5)c | 3,500                     | 500               |                   |

a Strains were grown in 63 medium supplemented with 0.4% glucose and thiamine. For RDA8, growth was in the presence of 0.5 mM meso-DL-diaminopimelate. For RDA8 harboring plasmids, growth was in the presence of 25 μg of ampicillin per ml.
b Activities are expressed as increments in optical density (105) at 540 nm per minute per milligram of protein (7).

c ND. Not determined.
d pDA4 and pDA5 are derived from pDA2 and pDA3, respectively (Fig. 2), by cloning the BstNI fragment (with the adjacent HindIII and BamHI sites of the pUC9 linker) into the HindIII and BamHI sites of pBR322.

Nucleotide sequence of the dapA gene. The nucleotide sequence of the 1.2-kb BstNI fragment was determined by the method of Maxam and Gilbert (14). The strategy shown in Fig. 3 allowed complete determination on both strands. The complete sequence is given in Fig. 4. There is a large open reading frame with a potential initiating ATG and a putative ribosome-binding site, GAGG (24). This open reading frame of 292 triplet allows the synthesis of a 31,372-dalton polypeptide, which is in good agreement with the 134,000 molecular weight of the tetrameric form (23) of the native enzyme. The amino acid composition of DHDPS polypeptide deduced from the nucleotide sequencing determination is consistent with the amino acid composition determined by acid hydrolysis (23) except for methionine residues (nine deduced from the nucleotide sequence and none by amino acid analysis; this could be due to destruction of methionine sulfone during acid hydrolysis). Sixteen nucleotides downstream from the translation stop codon, another open reading frame starts, preceded by a strong ribosome-binding site, GGAG, which could be the beginning of another gene cotranscribed with dapA.

Determination of the transcriptional start. The 5' end of the dapA mRNA has been localized by reverse transcription (25). The strategy followed is shown in Fig. 3. The transcriptional start is located at the A 24 nucleotides upstream from the translational ATG (Fig. 5). The same result is obtained by S1 nuclelease protection (2) (data not shown). Upstream of this transcriptional start, the two recognition sequences for E. coli RNA polymerase (12) can be found: TTGCTT 18 base pairs TACCAT (Fig. 4). The strength of this promoter sequence has been determined by the method of Mulligan et al. (15). A score of 46.7% was found, which is relatively low.

Expression of DHDPS. As already reported (22), increases in the dapA gene copy number lead to increases in DHDPS activity. As with the chromosomal gene, expression from multicopy plasmids is not subject to regulation in response to variation of the lysine level in the growth medium (data not shown).

Table 2 gives the value of enzyme-specific activities obtained with strain RDA8 (22) harboring various plasmids carrying the dapA gene. The same 1.2-kb BstNI fragment carrying dapA is present either in pUC9 in both orientations.
FIG. 3. Sequencing strategy for the \( \textit{dapA} \) gene. Only the restriction sites used for the 5' end labeling of plasmid pDA2 are shown. The arrows indicate the direction and extent of sequence analysis. The heavy bar indicates the \( \textit{dapA} \) coding sequence; the dashed bar indicates the position of the \( \textit{BsrNI} \) sites lost from pDA2 during cloning in the \( \textit{HincII} \) site of pUC9. For the determination of the \( \textit{dapA} \) mRNA start, probes used were 5' end labeled at \( \textit{HincII} \). The second restriction cut was with \( \textit{FnuDII} \) for the reverse transcriptase extension method (*RT) and with \( \textit{BsrNI} \) for the S1 mapping method (*S1).

or in pBR322 in both orientations. DHGPS is expressed in pDA2 and pDA3 at the same level, whatever the orientation of the \( \textit{dapA} \) gene with regard to the \( \textit{lac} \) promoter (Table 2). This level is twice that obtained with the pBR322 hybrid plasmid pDA1 (22), pDA4, and pDA5 (Table 2), in agreement with the higher copy number of pUC9 compared with pBR322. The DHGPS activity was inhibited 85 to 90% by lysine in vitro in preparations from strains with either

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FIG. 5. Identification of the \textit{dapA} transcription start. The primer (118 base pairs) used for the reverse transcriptase extension method is shown in Fig. 3 (RT). The extended fragment is 163 nucleotides long; it is shown along with the sequencing reaction products of the \textit{Hinfl}-\textit{BstNI} fragment in Fig. 3.

single-copy chromosomal expression or multicopy plasmids carrying the 1.2-kb insert; this strongly suggests that this 1.2-kb fragment encodes a protein with all the properties (activity and feedback inhibition) expected of DHDPS.

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LITERATURE CITED


