Amino-Terminal Deletions Define a Glutamine Amide Transfer Domain in Glutamine Phosphoribosylpyrophosphate Amidotransferase and Other PurF-Type Amidotransferases†

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Glutamine phosphoribosylpyrophosphate amidotransferase (amidophosphoribosyltransferase) catalyzes the initial reaction in the pathway for de novo purine nucleotide synthesis: 5-phosphoribosylpyrophosphate + glutamine → 5-phosphoribosylamine + glutamate + PPi. Similar to other glutamine amidotransferases, NH₃ can replace glutamine as a substrate in vitro (7) and in vivo (5). The amino acid sequence of amidophosphoribosyltransferase has been derived from the nucleotide sequence of cloned Escherichia coli purF (9, 13) as well as purF cloned from Bacillus subtilis (3) and Saccharomyces cerevisiae ADÉ4 (4). Experiments employing chemical modification (7, 12) and site-directed mutagenesis (5, 6) have identified three amino acid residues, Cys-1, Asp-29, and His-101, that are essential for the glutamine-dependent amidotransferase activity of the E. coli enzyme. Mutant enzymes with replacements of Cys-1, Asp-29, and His-101 retain NH₃-dependent amidotransferase activity in vitro and in vivo (5, 6). These mutations thus serve to identify a NH₃-terminal glutamine amid transfer (GAT) domain in the E. coli amidotransferase. This GAT domain is conserved in a subfamily of amidotransferases that includes glucosamine synthetase (15), asparagine synthetase (1), glutamate synthase (8), and the nodM gene product (11). This subfamily, designated PurF type, is distinct from a subfamily of amidotransferases with a conserved TrpG-type GAT domain (6). An alignment of the first 194 amino acids of the E. coli amidophosphoribosyltransferase sequence with that of glucosamine synthetase (15) indicated 25% identity. This implies that an NH₃-terminal segment of approximately 194 amino acids confers glutamine amid transfer function to PurF-type amidotransferases and that the distal portion of the protein chain constitutes an aminator domain that functions to catalyze the NH₃-dependent reaction by using NH₃ derived from glutamine or from solution. In TrpG-type amidotransferases, distinct GAT and aminator domains are on separate subunits (18) or fused in a single polypeptide chain (16, 17).

We report here a deletion analysis of E. coli purF-encoded amidophosphoribosyltransferase in which truncated enzymes lacking between 135 and 235 NH₃-terminal amino acids have sufficient NH₃-dependent activity to support purine nucleotide biosynthesis and growth. These results support a model for PurF-type amidotransferases in which NH₃-terminal GAT domain of approximately 190 to 200 amino acids is fused to an aminator domain.

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E. coli purF (6, 13) was cloned in pBluescriptIIKS (Stratagene) such that transcription of purF is from the lac promoter. Figure 1 shows a schematic diagram of the translation products. The plasmid encodes a 46-amino-acid lacZ′ fusion peptide and an intact purF-encoded amidophosphoribosyltransferase of 504 amino acids. Unidirectional deletions were constructed by the Henikoff (2) exonuclease III procedure with KpnI and EcoRI restriction sites for generation of 3′ and 5′ protruding ends, respectively. This scheme generates deletions in which the first 19 amino acids of the lacZ′ polycistron product were fused to internal positions in purF. In-frame deletions were screened by restriction mapping and nucleotide sequence analysis. The deletions obtained are listed in Fig. 1. Deletions are designated by the number of the first amidophosphoribosyltransferase amino acid in the fusion protein.

Enzyme function was assessed by functional complementation of a purF mutation in E. coli TX358 (13). This mutant has a lacZ′ Y′ transcriptional fusion (10) to an undefined site in the purF operon that inactivates purF and results in a purine growth requirement. The capacity of plasmids bearing purF deletions to restore growth to strain TX358 reflects the NH₃-dependent activity of mutant enzymes. The obligatory role of Cys-1 in catalysis ensures that glutamine-dependent amidophosphoribosyltransferase activity was abolished in these deletion mutants. Growth rates are given in Fig. 1 for the mutant strain bearing plasmids encoding wild-type enzyme and with purF deletions. The wild-type enzyme conferred a doubling time of 96 min. The addition of adenine increased the growth rate slightly. Plasmids encoding amidophosphoribosyltransferase with deletions up to residue 135 conferred a growth rate that was 50 to 67% of that with the wild-type enzyme. A mutant enzyme with a deletion of amino acids 1 through 237 retained some function, whereas deletions of the amino-terminal 292 and 302 amino acids abolished all enzyme function. In most cases, the addition of adenine restored the wild-type growth rate.
The adenine-supplemented growth rate was marginally slower for strains bearing plasmids that encoded enzymes with deletions of 117 and 238 amino acids. This was not further investigated. Attempts to demonstrate enzyme activity in extracts of strain TX358 bearing purF deletion plasmids were not successful, indicating that the activity of mutant enzymes was probably unstable. Western immunoblot analysis of extracts from strains bearing plasmids with wild-type purF or deletions of 62, 82, 91, 238, and 293 amino acids showed that these strains contained roughly comparable levels of amidotransferase (data not shown). Although this result is qualitative and is influenced by potential differences in immunodetection of enzymes with deletions, it demonstrates that poor growth of strains with deletions of 238 amino acids and lack of growth of those with deletions of 293 amino acids (Fig. 1) is unlikely to be due to rapid proteolysis.

The results in Fig. 1 demonstrate that residues 1 through 237 of E. coli amidophosphoribosyltransferase are not obligatory for NH$_3$-dependent enzyme function and support a structure-function assignment NH$_3$-GAT-aminoribulose-5-P. The apparent lability of deleted enzymes in which the GAT and aminoribulose domains have been dissected suggests that fusion of the NH$_3$-terminal GAT domain is needed not only to provide endogenous NH$_3$ from glutamine but also for interaction with the aminoribulose domain and resultant stabilization. The amidophosphoribosyltransferase GAT and aminoribulose domains are known to interact, since formation of the covalent enzyme-glutamine catalytic intermediate requires initial binding of 5-phosphoribosylpyrophosphate to the aminoribulose domain (7).

This is in contrast to the trpG amidotransferase, anthranilate synthase, in which the trpE-encoded aminoluciferin subunit (anthranilate synthase component I) is stable in the absence of the trpG-encoded GAT subunit (18). Since all PurF-type amidotransferases have a fused NH$_3$-terminal GAT domain, this structural arrangement could be generally important for enzyme stability.

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LITERATURE CITED


catalytic triad is involved in glutamine amide transfer function in purF-type glutamine amidotransferases. J. Biol. Chem. 264:16613–16619.


