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

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A series of deletions was constructed in cloned *Escherichia coli purF* encoding glutamine phosphoribosylpyrophosphate amidotransferase. These deletions extended into the NH_2 terminus of the protein and removed amino acids that are required for glutamine-dependent enzyme activity. Enzyme function, ascribed to the NH_3-dependent activity, was retained in deletions that removed up to 237 amino acids. This result supports a model in which PurF-type amidotransferases contain an NH_2-terminal glutamine amide transfer domain of approximately 194 to 200 amino acids fused to an aminator domain with NH_3-dependent function.

Glutamine phosphoribosylpyrophosphate amidotransferase (amidophosphoribosyltransferase) catalyzes the initial reaction in the pathway for de novo purine nucleotide synthesis: 5-phosphoribosylpyrophosphate + glutamine → 5-phosphoribosylamine + glutamate + PP_i. Similar to other glutamine amidotransferases, NH_3 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 ADE4* (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 NH_3-dependent amidotransferase activity in vitro and in vivo (5, 6). These mutations thus serve to identify an NH_2-terminal glutamine amide 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 NH_2-terminal segment of approximately 194 amino acids confers glutamine amide transfer function to PurF-type amidotransferases and that the distal portion of the protein chain constitutes an aminator domain that functions to catalyze the NH_3-dependent reaction by using NH_3 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 NH_2-terminal amino acids have sufficient NH_3-dependent activity to support purine nucleotide biosynthesis and growth. These results support a model for PurF-type amidotransferases in which and NH_2-terminal GAT domain of approximately 190 to 200 amino acids is fused to an aminator domain.

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 *Kpn*I and *Eco*RI 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'* polylinker 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 $\lambda lacZ^+Y^+$ 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 NH_3-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.

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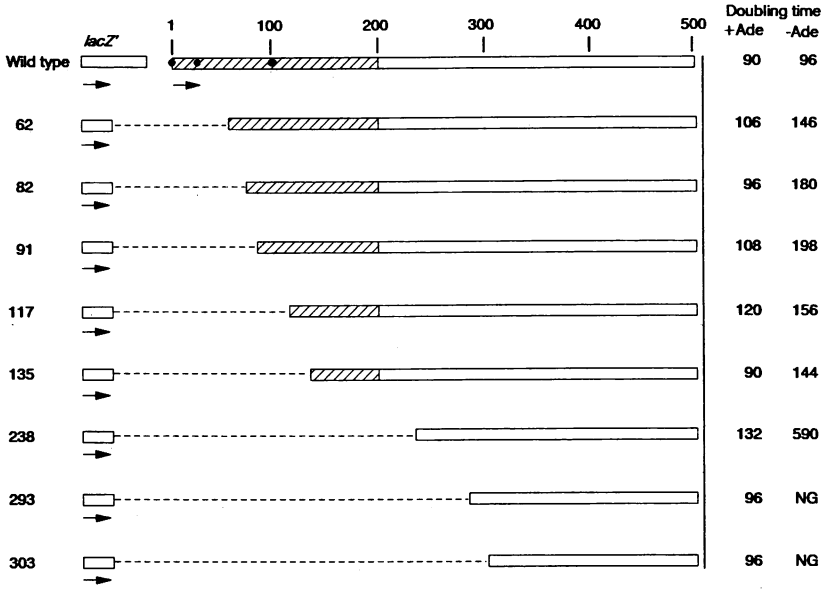

FIG. 1. Schematic diagram showing the capacity for amidophosphoribosyltransferase deletions to support de novo purine nucleotide synthesis and growth of an *E. coli purF* mutant. The wild-type *E. coli purF* gene was cloned into the *Bam*HI polylinker site of plasmid pBluescriptIIKS$^-$. Transcription is from the *lac* promoter. Sites for translation initiation are shown by an arrow. In the wild type, translation yielded a 34-amino-acid *lacZ'* polylinker peptide (not drawn to scale) and an intact amidophosphoribosyltransferase of 504 amino acids. Unidirectional deletions were constructed by the Henikoff exonuclease III procedure (2) by using the *Kpn*I and *Eco*RI polylinker sites to fuse Trp-19 of the *lacZ'* polylinker peptide with various internal positions of amidotransferase. The dashed lines designate the fusions. Deletion plasmids are numbered according to the first amidophosphoribosyltransferase amino acid fused to residue 19 of the *lacZ* polylinker polypeptide. In mutants 91 and 135, Trp-19 is replaced with Cys and Tyr, respectively. Dots represent the positions of Cys-1, Asp-29, and His-101, residues which are essential for GAT function. The hatched region represents the proposed GAT domain of approximately 200 amino acids; the attached open box is the aminator domain. The doubling time, in minutes, of *E. coli* TX358 {*ara* Δ*lac* Φ[*purF200-lac*::λp1(209)] *recA*} (13) with various plasmids was determined in medium containing salts (14), 0.4% glucose, 0.2% acid hydrolyzed casein, 2 μg of thiamine per ml, and 100 μg of ampicillin per ml. Some cultures contained 20 μg of adenine per ml. Isopropyl-β-D-thiogalactopyranoside had no effect on growth rate and was not used. NG, No growth.

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_2-GAT-aminator-CO_2H. The apparent lability of deleted enzymes in which the GAT and aminator domains have been dissected suggests that fusion of the NH_2-terminal GAT domain is needed not only to provide endogenous NH_3 from glutamine but also for interaction with the aminator domain and resultant stabilization. The amidophosphoribosyltransferase GAT and aminator domains are known to interact, since formation of the covalent enzyme-glutamine catalytic intermediate requires initial binding of 5-phosphoribosylpyrophosphate to the aminator domain (7). This is in contrast to the *trpG* amidotransferase, anthranilate synthase, in which the *trpE*-encoded aminator 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_2-terminal GAT domain, this structural arrangement could be generally important for enzyme stability.

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LITERATURE CITED

1. **Andrulis, I. L., M. Shotwell, S. Evans-Blackler, H. Zalkin, L. Siminovitch, and P. N. Ray.** 1989. Fine structure analysis of the Chinese hamster AS gene encoding asparagine synthetase. Gene **80**:75–85.
2. **Henikoff, S.** 1987. Unidirectional digestion with exonuclease III in DNA sequence analysis. Methods Enzymol. **155**:156–165.
3. **Makaroff, C. A., H. Zalkin, R. L. Switzer, and S. J. Vollmer.** 1983. Cloning of the *Bacillus subtilis* glutamine phosphoribosylpyrophosphate amidotransferase gene in *Escherichia coli*. Nucleotide sequence determination and properties of the plasmid-encoded enzyme. J. Biol. Chem. **258**:10586–10593.
4. **Mäntsälä, P., and H. Zalkin.** 1984. Nucleotide sequence of *Saccharomyces cerevisiae* ADE4 encoding glutamine phosphoribosylpyrophosphate amidotransferase. J. Biol Chem. **259**: 8478–8484.
5. **Mäntsälä, P., and H. Zalkin.** 1984. Glutamine amidotransferase function. Replacement of the active-site cysteine in glutamine phosphoribosylpyrophosphate amidotransferase by site-directed mutagenesis. J. Biol. Chem. **259**:14230–14236.
6. **Mei, B., and H. Zalkin.** 1989. A cysteine-histidine-aspartate

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

7. **Messenger, L. J., and H. Zalkin.** 1979. Glutamine phosphoribosylpyrophosphate amidotransferase from *Escherichia coli*: purification and properties. J. Biol. Chem. **254:**3382–3392.
8. **Oliver, G., G. Gosset, R. Sanchez-Pescadore, E. Lozoya, L. M. Ku, N. Flores, B. Becerril, F. Valle, and F. Bolivar.** 1987. Determination of the nucleotide sequence for the glutamate synthase structural genes of *Escherichia coli* K-12. Gene **60:** 1–11.
9. **Sampei, G., and K. Mizobuchi.** 1988. Nucleotide sequence of the *Escherichia coli purF* gene encoding amidophosphoribosyltransferase for *de novo* purine nucleotide synthesis. Nucleic Acids Res. **16:**8717.
10. **Smith, J. M., and J. S. Gots.** 1980. *purF-lac* fusion and direction of *purF* transcription in *Escherichia coli*. J. Bacteriol. **143:** 1156–1164.
11. **Surin, B. P., and J. A. Downie.** 1988. Characterization of the *Rhizobium leguminosarum* genes *nodLMN* involved in efficient host-specific nodulation. Mol. Microbiol. **2:**173–183.
12. **Tso, J. Y., M. A. Hermodson, and H. Zalkin.** 1982. Glutamine phosphoribosylpyrophosphate amidotransferase from cloned *Escherichia coli purF*. NH_2-terminal amino acid sequence, identification of the glutamine site, and trace metal analysis. J. Biol. Chem. **257:**3532–3536.
13. **Tso, J. Y., H. Zalkin, M. vanCleemput, C. Yanofsky, and J. M. Smith.** 1982. Nucleotide sequence of *Escherichia coli purF* and deduced amino acid sequence of glutamine phosphoribosylpyrophosphate amidotransferase. J. Biol. Chem. **257:**3525–3531.
14. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218:**97–106.
15. **Walker, J. E., N. J. Gay, M. Saraste, and A. N. Eberle.** 1984. DNA sequence around the *Escherichia coli unc* operon. Completion of the sequence of a 17 kilobase segment containing *asnA*, *oriC*, *unc*, *glmS*, and *phoS*. Biochem. J. **224:**799–815.
16. **Weng, M., C. A. Makaroff, and H. Zalkin.** 1986. Nucleotide sequence of *Escherichia coli pyrG* encoding CTP synthetase. J. Biol. Chem. **261:**5568–5574.
17. **Zalkin, H., P. Argos, S. V. L. Narayana, A. A. Tiedeman, and J. M. Smith.** 1985. Identification of a *trpG*-related glutamine amide transfer domain in *Escherichia coli* GMP synthetase. J. Biol. Chem. **260:**3350–3354.
18. **Zalkin, H., and D. Kling.** 1968. Anthranilate synthetase. Purification and properties of component I from *Salmonella typhimurium*. Biochemistry **7:**3566–3573.