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Nalidixic Acid-Resistant Auxotrophs of *Escherichia coli*

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purB and *ctr* mutants of *Escherichia coli* are resistant to low levels of nalidixic acid.

Nalidixic acid (Nal; 1-ethyl-7-methyl-1,8-naphythyridin-4-oxo-3-carboxylic acid) has been shown to selectively and reversibly inhibit deoxyribonucleic acid synthesis, with little effect on ribonucleic acid or protein synthesis (2). The molecular basis of inhibition is unknown.

Several prototrophic classes of mutants resistant to Nal (Nal^r) were studied by Hane and Wood (5). We isolated other interesting Nal^r mutants which show new growth factor requirements. In six independent experiments with *Escherichia coli* K-12 and four experiments with *E. coli* B/r, from 2 to 40% of the mutants selected for growth on tryptone-agar containing 10 μg of Nal per ml showed a new requirement for either adenine (Ade^-) or for a mixture of amino acids. The properties of the former class of mutants will be reported here.

The adenine requirement could not be satisfied with inosine, xanthine, hypoxanthine, or guanine, showing that the mutants were probably blocked in the conversion of inosinate (IMP) to adenylate (AMP).

Mating experiments showed that the gene conferring adenine independence was transferred from HfrH to an Ade^- recipient at approximately the same time as a $purB^+$ gene was transferred from the same male into a *purB* recipient (strain 2, Table 1). The Ade^+ recombinants were Nal-sensitive (Nal^s) as were all Ade^+ derivatives obtained by transduction or reversion. Three other Ade^- Nal^r mutations were found to map in this region also. These results show that the mutations are not in the *purA* gene (which maps at 81 min), but are probably in the *purB* gene [at 23 min (7)]. No gene other than *purA* and *purB* has been reported in which mutation leads to a specific adenine requirement.

A bona fide *purB* mutant (strain 2) was resistant to Nal, but derivatives transduced to pur^+ were Nal^s (Fig. 1A). Two Ade^- mutations obtained independently by using the penicillin procedure were mapped in the *purB* region, and the mutants were subsequently found to be Nal^r. Other purine-requiring strains were tested for resistance to Nal, and it was found that all mutants responding to purines other than adenine were sensitive to the drug (Fig. 1). A *purA* mutant, strain 6 (which specifically requires adenine for growth), was shown to be sensitive also (Fig. 1B).

Thus, resistance appears to result from mutation in the *purB* gene and not from mutation in other genes affecting purine synthesis. *purB* is probably a structural gene for adenylosuccinase (4). This enzyme catalyzes two steps in purine synthesis, one step in the main pathway to IMP, the other on the path to AMP (Fig. 2).

In the *purB* mutants, Nal resistance is probably due to accumulation of an adenine precursor prior to the block, or to altered function of adenylosuccinase. Although most Ade^- Nal^r mutants revert, some do not. Presumably the latter contain deletions or other multiple-site mutations, suggesting that resistance stems from complete loss of function and accumulation of an intermediate.

The immediate precursors accumulating in *purB* mutants are 5-aminoimidazol-4-carboxamide ribonucleotide (SAICAR) and adenylosuccinic acid (ASA) [which is formed by conversion from adenine added to the medium (4)]. *purA* mutants are Nal^s (Fig. 1), so resistance cannot result from the accumulation of IMP (*see* Fig. 2). It is unlikely that Nal resistance results from the accumulation of intermediates prior to SAICAR, because no Nal^r auxotrophs with blocks prior to SAICAR formation were obtained (i.e., no resistant mutants that responded to guanine as well as to adenine). We attempted to test the hypothesis that ASA is the compound giving resistance by trying to block ASA formation in a *purB* strain with hadacidin, an inhibitor of adenylosuccinate synthetase (6). Although the *purB* mutant became more sensitive to Nal in the presence of hadacidin, the effect was slight and the hadacidin concentration was large (1 mg/ml); therefore the results were inconclusive. A defini-

TABLE 1. *Escherichia coli strains*[a]

Strain	Other designation	Relevant genotype	Source
1	AB444	*arg aroC purF str thi*	W. Dempsey (1)
2	AB1325	*pro purB his str thi*	A. L. Taylor (7)
3	AB2547A	*ilv argF purF supN ctr*	M. L. Morse (9)
4	B380	*his str*	C. Novotny
5		*his str purB27*	B380, selection with Nal
6	ES4	*purA thi*	E. C. Siegel
7	B96	*purH*	G. R. Greenberg (3)

[a] Symbols as in Taylor (7). All are K-12 strains except B96, which is a derivative of *E. coli* B. Mutations affecting carbohydrate utilization and phage-resistance are not shown.

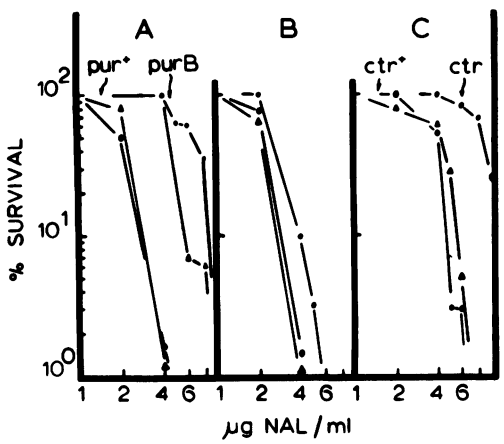

FIG. 1. *Colony-forming ability on tryptone-agar plus nalidixic acid. Single colonies were picked into 1 ml of tryptone broth and grown overnight at 37 C. Appropriate dilutions were spread on the surface of tryptone-agar plates containing Nal at the concentration indicated. In each case, solid symbols refer to pur mutants and open symbols refer to pur+ derivatives obtained by transduction. A, strain 5 and 5 purB+; (●,○); strain 2 and 2 purB+ (▲,△). B, strain 6 and 6 purA+ (●,○); strain 7 (purH) (▲). C, strain 3 (●); 3 ctr+ (▲); 3 ctr+ purF+ (○). Survival of strain 1 (also purF) is similar to that of strain 7, but, for clarity, it is not shown.*

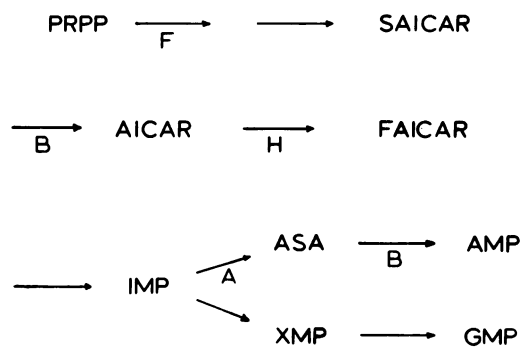

FIG. 2. *Pathway of purine synthesis. Steps between PRPP and SAICAR are omitted; purF mutants are deficient in the first step in this sequence (conversion of PRPP to 5-phosphoribosyl-1-amine). Letters under arrows designate pur genes governing that step. Abbreviations: PRPP, 5-phosphoribosyl-1-pyrophosphate; SAICAR, 5-aminoimidazole-4-N-succinocarboxamide ribonucleotide; AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide; FAICAR, 5-formamidoimidazole-4-carboxamide ribonucleotide; IMP, inosinic acid; ASA, adenylosuccinic acid; AMP, adenylic acid; GMP, guanylic acid.*

tive result could be obtained by constructing a double mutant *purA purB* strain and checking to see whether this is Nal^r or Nal^s.

In the course of these experiments, we identified another class of mutation giving resistance to Nal. A *purF* strain (strain 3) was resistant to the drug (Fig. 1C). This strain fails to grow on most carbohydrates. As a result of a *ctr* mutation, it lacks active transport of many carbohydrates. The loss of active transport appears to be secondary to some more fundamental defect possibly related to catabolite repression (8, 9). Transductants to *ctr*$^+$ simultaneously became Nal^s, and further transduction to *pur*$^+$ did not alter their sensitivity (Fig. 1C). Some spontaneous *ctr*$^+$ revertants (selected for growth on glucose) became Nal^s while others remained Nal^r. This behavior is in accord with the different patterns of utilization of carbohydrates reported among partial revertants of *ctr* (8, 9).

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