Chloramphenicol Acetyltransferase May Confer Resistance to Fusidic Acid by Sequestering the Drug

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Enterobacterial chloramphenicol acetyltransferase bound fusidic acid with high affinity, but did not acetylate the drug at an experimentally detectable rate. The enzyme may therefore confer resistance to fusidic acid by sequestering the drug and thereby preventing the drug from binding to translational elongation factor G.

Wild-type gram-negative bacteria are naturally resistant to fusidic acid at therapeutic concentrations; however, Escherichia coli mutant strains have been isolated which are unusually sensitive to the drug, probably due to alterations in the cell envelope (4). In these mutant strains, the enterobacterial R plasmid NR1 (also known as R100 and R222) confers high-level resistance to fusidic acid (4). NR1 and certain related plasmids of the FII incompatibility group also confer resistance to chloramphenicol and several other antibacterial agents (12). The region of NR1 which contains the chloramphenicol resistance gene (cat) and the fusidic acid resistance gene (fus) lies within NR1 EcoRI fragments A and J (9). Neither EcoRI fragment alone confers chloramphenicol or fusidic acid resistance, but both fragments together, in the native orientation, confer both resistances (9). This result shows that cat and fus either are the same gene or are different genes in the same operon. A further observation, consistent with this conclusion, is that deletions in the cat/fus region of NR1 which cause loss of chloramphenicol resistance always cause loss of fusidic acid resistance, and vice versa (12). Similarly, point mutations in the cat gene which result in loss of chloramphenicol resistance also result in loss of fusidic acid resistance (15). This result, in conjunction with DNA sequence information on the wild-type and mutant cat genes (15), shows definitively that the fusidic acid resistance gene is the cat gene itself; i.e., the cat gene product confers resistance to fusidic acid. The cat/fus genes of FII R plasmids and Tn9 code for type I chloramphenicol acetyltransferase (type I CAT) (7); it is not known whether other types of CAT also confer fusidic acid resistance.

The cat gene product confers resistance to chloramphenicol by enzymatic acetylation of the drug (13). However, since chloramphenicol and fusidic acid have no apparent structural similarity, the mechanism by which the cat gene product confers resistance to fusidic acid has remained unclear. It has been suggested that CAT enzymatically inactivates fusidic acid as well as chloramphenicol (15). In this paper, we show that CAT binds fusidic acid with high affinity, but does not acetylate the drug at an experimentally detectable rate. The enzyme may therefore confer resistance to fusidic acid by sequestering the drug and thereby preventing the drug from binding to translational elongation factor G (6).

To determine whether fusidic acid is capable of acting as an inhibitor of the CAT reaction, enzyme kinetic measurements were made. Fusidic acid did, in fact, inhibit the acetylation reaction; inhibition was competitive with respect to chloramphenicol (Fig. 1A) and probably mixed competitive and noncompetitive with respect to acetyl coenzyme A (acetyl CoA) (Fig. 1B). From the variable acetyl CoA concentration data, the dissociation constant (inhibition constant; Kd) for fusidic acid and free enzyme was 3.4 ± 1.9 μM, and that for fusidic acid and acetyl CoA-bound enzyme was 6.2 ± 2.8 μM. The Michaelis constants for chloramphenicol and acetyl CoA were 16 ± 7 and 33 ± 5 μM, respectively. If the Michaelis constants for two-substrate, random-addition reactions, such as the CAT reaction (14), are essentially true dissociation constants for the enzyme-substrate complexes (2), then fusidic acid binds more strongly to the enzyme than does either of the enzyme’s own substrates.

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FIG. 1. Effect of fusidic acid on CAT activity. CAT activity was measured in crude extracts, essentially by the spectrophotometric method of Shaw (13). A 10-ml culture of E. coli K-12 KP245(NR1) (9) was grown overnight to stationary phase in Luria broth (10) plus 20 μg of thymine per ml. The culture was harvested at 4°C, washed with 10 ml of cold 0.1 M Tris-hydrochloride, and suspended in 1.0 ml of cold 0.1 M Tris-hydrochloride. The suspension was sonicated for 30 s, using a Fisher Sonic Dismembrator and microtip at 0.38 maximum power output. Cell debris was removed from the sonicate by centrifugation at 15,600 × g for 15 min. Total protein was measured by the method of Bradford (1). Measurements of CAT activity were made at 37°C and pH 7.8. The lines in the plots of reciprocal of substrate concentration versus reciprocal of specific activity (sp act) were determined by least-squares fittings to the experimental data points, after weighting each data point according to the square of its specific activity (3). Since inhibition was assumed to be linear with inhibitor concentration, each line was determined by using information from all of the data points present in the same set. (A) Effect of fusidic acid (FA) on CAT activity, with variable chloramphenicol (CM) concentration and fixed acetyl CoA (AcCoA) concentration (400 μM). (B) Effect of fusidic acid on CAT activity, with variable acetyl CoA concentration and fixed chloramphenicol concentration (100 μM).

To determine whether fusidic acid can replace chloramphenicol as a substrate for the acetylation reaction, additional measurements were made by using reaction mixtures containing fusidic acid but not chloramphenicol. No acetylation of fusidic acid could be detected, even when fusidic acid was present at 1,400 μM, the highest concentration tested (data not shown). Under similar assay conditions, but with chloramphenicol present and fusidic acid absent, acetylation of chloramphenicol at a concentration of 1 μM would easily have been detected.

The CAT protein binds with high affinity not only to fusidic acid but also to crystal violet (11, 14), even though crystal violet has no apparent structural similarity to either chloramphenicol or fusidic acid. Crystal violet, like fusidic acid, binds to CAT competitively with respect to chloramphenicol. Since bile salts, which are structurally similar to fusidic acid, prevent the binding of crystal violet to CAT in vivo (11), crystal violet, bile salts, and fusidic acid may also bind to CAT competitively with respect to each other. Increased cat gene dosages and increased levels of CAT within enterobacterial cells confer increased levels of resistance to both fusidic acid (8; H. Hashimoto and R. Rownd, unpublished data) and crystal violet (11). Since enterobacterial CAT contains a site capable of binding hydrophobic groups on affinity columns (16), resistance to fusidic acid and crystal violet may occur by the binding of these largely hydrophobic substances to the CAT protein rather than by enzymatic inactivation. The CAT protein does not confer resistance to two derivatives of fusidic acid, helvolinic acid and cephalosporin P1 (15); since these derivatives both contain polar groups on carbons 6 and 7 not present on fusidic acid itself, the additional polar groups may prevent the hydrophobic binding of these derivatives to CAT.

In most cat+ E. coli strains, a large fraction of the total cellular protein, 0.5 to 1.0%, is CAT (13); therefore, in cat+ cells, large amounts of CAT protein are available to bind fusidic acid and prevent its antibacterial activity. Since CAT-mediated fusidic acid resistance seemingly has little selective value to wild-type enterobac-
teria, which are intrinsically resistant to the drug, CAT-mediated fusidic acid resistance may be a fortuitous result of the large quantity of CAT within the cell and the ability of CAT to bind hydrophobic substances.

Four general mechanisms of plasmid-conferred resistance to antibacterial agents have previously been reported (5): chemical modification and concomitant inactivation of antibacterial agents by plasmid-coded enzymes; interference with the net uptake of antibacterial agents into the cell by plasmid-coded proteins; supplementation of host-coded enzymes inhibited by antibacterial agents with plasmid-coded enzymes which are not inhibited; and chemical modification of cellular target sites of antibacterial agents by plasmid-coded enzymes. In this paper, we have presented evidence for a fifth general mechanism of resistance, the sequestering of drugs by plasmid-coded proteins, preventing the drugs from binding to their cellular target sites.

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LITERATURE CITED