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Nonenzymatic Chloramphenicol Resistance Determinants Specified by Plasmids R26 and R55-1 in *Escherichia coli* K-12 Do Not Confer High-Level Resistance to Fluorinated Analogs

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Plasmids R26 (Inc P) and R55-1 (Inc C) specify inducible nonenzymatic resistance to chloramphenicol. *Escherichia coli* K-12 strains harboring these plasmids encoded low-level resistance to thiamphenicol analogs Sch 25298 and Sch 25393 but failed to specify resistance to the fluorinated chloramphenicol analog Sch 24893. The analogs were efficient inducers of high-level chloramphenicol resistance.

The most frequently encountered mechanism of chloramphenicol resistance (Cm^r) in clinically important bacteria is that mediated by the inactivating enzyme chloramphenicol acetyltransferase (CAT) (3, 6). Chloramphenicol is inactivated by acetylation of the 3'-OH group (13, 15). Fluorinated derivatives of chloramphenicol and thiamphenicol have been designed to evade 3'-*O* acetylation by CAT by the replacement of the 3'-OH group by a fluorine atom (9, 11, 12).

Plasmids specifying Cm^r which does not involve drug inactivation by CAT (hereafter termed nonenzymatic Cm^r) have been reported (5, 7, 8, 10). The resistance mechanism is probably a barrier to drug permeability acting at the level of the cytoplasmic membrane (5). Plasmids R26 and R55-1 do not encode CAT; they specify Cm^r by a mechanism that does not involve detectable drug inactivation or modification (5, 6). Here, we report experiments in which we investigated the possibility that the determinants might confer resistance to the fluorinated analogs.

Cm^r and resistance to the analogs was estimated by two methods: (i) plate minimum inhibitory concentration (MIC) determinations (Table 1) and (ii) growth and challenge tests in broth (Fig. 1). The second technique was used primarily to test the compounds for their ability to induce Cm^r determinants, but it also allowed qualitative confirmation of the MIC results. To estimate induction by growth and challenge tests, a challenging dose was chosen which just prevented growth by the uninduced, challenged culture. The response by the induced, challenged culture to this concentration of drug was then recorded.

It has been reported previously that the nonenzymatic Cm^r determinants are induced by a short exposure to a subinhibitory concentration of chloramphenicol (5, 10). This was confirmed here by the continued exponential growth of induced *Escherichia coli* J5-3(R55-1) cultures in the presence of 35 μg of chloramphenicol per ml (Fig. 1A). The fluorinated analogs also induced the Cm^r determinant. Figure 1A illustrates the induction of Cm^r by 1 μg of Sch 25298 per ml. The cells grew exponentially after challenge with 35 μg of chloramphenicol per ml, but at a lower rate than chloramphenicol-induced cultures. This was due to the inhibitory effect of even 1 μg of the analog per ml. This was demonstrated by the lower growth rate of the analog-induced, unchallenged culture when compared with its chloramphenicol-induced, unchallenged counterpart. The other analogs behaved in a similar fashion, Sch 24893 being the least inhibitory of the three when acting as an inducer (unpublished data).

Plate MIC measurements showed that chloramphenicol-induced cultures of strains J5-3(R26) and J5-3(R55-1) conferred high-level resistance (20- and 40-fold increases in Cm^r, respectively) compared with the plasmid-free strain (Table 1). *E. coli* J5-3 displayed greater intrinsic resistance to Sch 24893 than to chloramphenicol or the other analogs. Plasmids R26 and R55-1 did not confer any additional resistance to this compound, as was shown by the MIC test (Table 1) and by the failure of chloramphenicol-induced cultures to grow any faster than the uninduced culture when challenged with 7.5 μg of Sch 24893 per ml (Fig. 1B).

In contrast, plasmids R26 and R55-1 conferred moderate increases in resistance to Sch 25298 and Sch 25393 (Table 1). Induced strain J5-3(R26) demonstrated 2.5- and 3-fold increases in the MICs of Sch 25393 and Sch 25298, respec-

tively, compared with the plasmid-free host, whereas induced strain J5-3(R55-1) showed a 3.75-fold increase in the MIC of Sch 25393 and a 7.5-fold increase in the MIC of Sch 25298. This was confirmed by the slightly faster rates of growth of chloramphenicol-induced cultures of strain J5-3(R55-1) when challenged with 5 μg of Sch 25298 per ml (Fig. 1C) and 5 μg of Sch 25393 per ml (Fig. 1D) as compared with their uninduced, challenged controls. Strain J5-3(R26) behaved similarly (unpublished data).

The fluorinated derivatives of chloramphenicol and thiamphenicol are not susceptible to inactivation by the commonly encountered CAT mechanism. In principle, the analogs could be used to treat infections caused by Cm^r bacteria. However, it occurred to us that the less frequently encountered inducible, nonenzymatic Cm^r determinants might also confer resistance to the analogs, thus restricting their clinical usefulness. We have shown that these compounds vary in their effectiveness as inhibitors of *E. coli* J5-3 carrying plasmid R55-1 or R26. Neither plasmid conferred resistance to Sch 24893. Low-level (two- to sevenfold) resistance to Sch 25298 and Sch 25393 was displayed. Whether this holds for other species of gram-negative bacteria harboring these or other plasmids carrying nonenzymatic Cm^r determinants awaits further study.

The failure of these plasmids to confer as high a level of resistance to the analogs as to chloramphenicol was not due to their failure to induce the resistance determinant. The MIC data shown here are from experiments in which the cultures were induced by growth in subinhibitory concentrations of chloramphenicol.

In conclusion, it should be noted that a nonen-

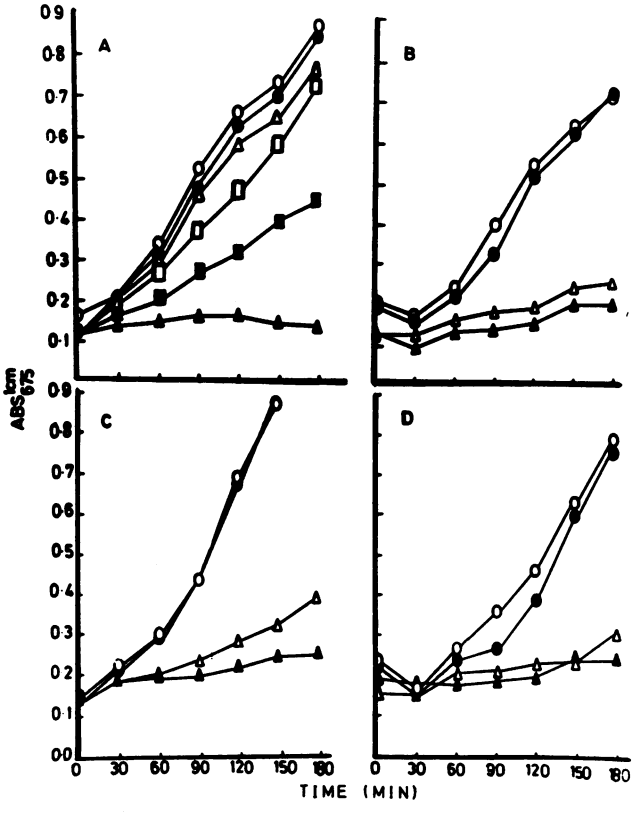

FIG. 1. Growth and challenge tests with strain J5-3(R55-1). Growth and challenge tests with strain J5-3(R55-1) were carried out by growing broth cultures to an optical density of about 0.1 (wavelength, 675 nm; Varian Techtron spectrophotometer) in 250-ml flasks (Gallenkamp orbital incubator, 37°C, 200 rpm). Where appropriate, cultures were induced by adding 1 μg of drug per ml at 60 min before challenge with chloramphenicol (35 μg/ml) (A), Sch 24893 (7.5 μg/ml) (B), Sch 25298 (5 μg/ml) (C), or Sch 25393 (5 μg/ml) (D). Induction in (A) was with chloramphenicol or Sch 25298 (1 μg/ml). In (B), (C), and (D) induction was with chloramphenicol (1 μg/ml). Symbols: ○, uninduced, unchallenged; ●, induced with chloramphenicol, unchallenged; △, induced with chloramphenicol, challenged; ▲, uninduced, challenged; □, induced with Sch 25298, unchallenged; ■, induced with Sch 25298, challenged.

TABLE 1. MIC determinations[a]

Drug[b]	MIC (μg/ml) for:				
	E. coli J5-3[c]	*E. coli* J5-3(R26)[d]		*E. coli* J5-3(R55-1)[e]	
		Uninduced	Induced[f]	Uninduced	Induced[f]
Chloramphenicol	2	20	40	40	75
Sch 24893	5	5	5	5	5
Sch 25298	2	4	6	10	15
Sch 25393	2	4	5	7.5	7.5

[a] MIC determinations were performed by inoculating 10^3 mid-log-phase cells onto Lemco nutrient agar plates (4) containing antibiotics at the following concentrations: 0.5, 1, 2, 3, 4, 5, 7.5, 10, 15, 20, 30, 40, 50, 75, 100, 125, and 150 μg/ml. The lowest concentration which inhibited growth was recorded as the MIC. The numbers are the means of four independent experiments.

[b] Chloramphenicol was purchased from Sigma Chemical Co., St. Louis, Mo.; Sch 24893 (D-*threo*-2-dichloroacetamido-1-*p*-nitrophenyl-3-fluoropropan-1-ol), Sch 25298 (D-*threo*-2-dichloroacetamido-1-*p*-methylsulfonylphenyl-3-fluoropropan-1-ol), and Sch 25393 (D-*threo*-2-difluoroacetamido-1-*p*-methylsulfonylphenyl-3-fluoropropan-1-ol) were gifts from Schering Corp., Bloomfield, N.J.

[c] The host strain for all experiments was a spontaneous nalidixic acid-resistant derivative of *E. coli* K-12 strain J5-3 (*pro met*) (2).

[d] R26 is an Inc P plasmid specifying resistance to chloramphenicol, ampicillin, gentamicin, kanamycin, mercuric ions, streptomycin, spectinomycin, sulfonamide, and tetracycline (14).

[e] R55-1 is an Inc C plasmid which confers resistance to chloramphenicol and sulfonamide (1).

[f] Induction was achieved by adding chloramphenicol (1 μg/ml) to broth cultures and incubating for 1 h before plating. All four drugs proved to be equally effective as inducing agents in plate MIC tests (data not shown).

zymatic Cm^r determinant in *Haemophilus influenzae* does confer resistance to these compounds (16). The relationship (if any) between the resistance in *H. influenzae* and the plasmid-located Cm^r determinants described here is not known.

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LITERATURE CITED

1. **Chabbert, Y. A., M. R. Scavizzi, J. L. Witchitz, G. R. Gerbaud, and D. H. Bouanchaud.** 1972. Incompatibility groups and the classification of *fi*⁻ resistance factors. J. Bacteriol. **112**:666–675.
2. **Clowes, R. C., and W. Hayes.** 1968. Experiments in microbial genetics. Blackwell Scientific Publications, Oxford.
3. **Fitton, J. E., L. C. Packman, S. Harford, Y. Zaidenzaig, and W. V. Shaw.** 1978. Plasmids and the evolution of chloramphenicol resistance, p. 249–252. *In* D. Schlessinger (ed.), Microbiology—1978. American Society for Microbiology, Washington, D.C.
4. **Foster, T. J., and A. Walsh.** 1974. Phenotypic characterization of R-factor tetracycline resistance determinants. Genet. Res. **24**:333–343.
5. **Gaffney, D. F., E. Cundliffe, and T. J. Foster.** 1981. Chloramphenicol resistance that does not involve chloramphenicol acetyltransferase encoded by plasmids from gram-negative bacteria. J. Gen. Microbiol. **125**:113–121.
6. **Gaffney, D. F., T. J. Foster, and W. V. Shaw.** 1978. Chloramphenicol acetyltransferases determined by R plasmids from gram-negative bacteria. J. Gen. Microbiol. **109**:351–358.
7. **Kono, M., and K. O'Hara.** 1976. Mechanism of chloramphenicol-resistance mediated by KR 102 factor in *Pseudomonas aeruginosa*. J. Antibiot. **23**:176–180.
8. **Mitsuhashi, S., H. Kawabe, A. Fuse, and S. Iyobe.** 1975. Biochemical mechanisms of chloramphenicol resistance in *Pseudomonas aeruginosa*, p. 515–523. *In* S. Mitsuhashi and H. Hashimoto (ed.), Microbial drug resistance. University Park Press, Tokyo.
9. **Nagabhushan, T. L., D. Kandasamy, H. Tsai, W. N. Turner, and G. H. Miller.** 1980. Novel class of chloramphenicol analogs with activity against chloramphenicol-resistant and chloramphenicol-susceptible organisms, p. 442–443. *In* J. D. Nelson and C. Grassi (ed.), Current chemotherapy and infectious disease. American Society for Microbiology, Washington, D.C.
10. **Nagai, Y., and S. Mitsuhashi.** 1972. New type of R factors incapable of inactivating chloramphenicol. J. Bacteriol. **109**:1–7.
11. **Neu, H. C., K. P. Fu, and K. Kung.** 1980. Fluorinated chloramphenicol analogs active against bacteria resistant to chloramphenicol, p. 446–447. *In* J. D. Nelson and C. Grassi (ed.), Current chemotherapy and infectious disease. American Society for Microbiology, Washington, D.C.
12. **Schafer, T. W., E. L. Moss, Jr., T. L. Nagabhushan, and G. H. Miller.** 1980. Novel fluorine-containing analogs of chloramphenicol and thiamphenicol: antibacterial and biological properties, p. 444–446. *In* J. D. Nelson and C. Grassi (ed.), Current chemotherapy and infectious disease. American Society for Microbiology, Washington, D.C.
13. **Shaw, W. V.** 1967. The enzymic acetylation of chloramphenicol by extracts of R-factor-resistant *Escherichia coli*. J. Biol. Chem. **242**:687–693.
14. **Stanisich, V. A., P. M. Bennett, and J. M. Ortiz.** 1976. A molecular analysis of transductional marker rescue involving P-group plasmids in *Pseudomonas aeruginosa*. Mol. Gen. Genet. **143**:333–337.
15. **Suzuki, Y., and S. Okamato.** 1967. The enzymic acetylation of chloramphenicol by the multiple drug-resistant *Escherichia coli* carrying R factor. J. Biol. Chem. **242**:4722–4730.
16. **Syriopoulou, V. P., A. L. Harding, D. A. Goldmann, and A. L. Smith.** 1981. In vitro antibacterial activity of fluorinated analogs of chloramphenicol and thiamphenicol. Antimicrob. Agents Chemother. **19**:294–297.