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Characterization of Different Plasmid-Borne Dihydropteroate Synthases Mediating Bacterial Resistance to Sulfonamides

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Plasmid-borne resistance to sulfonamides was studied in both newly isolated and earlier characterized R plasmids. Two different classes of drug-resistant dihydropteroate synthases were found to be responsible for most cases of plasmid-mediated sulfonamide resistance. The plasmid-coded enzymes could be completely separated from their chromosomal counterpart and also showed differences in heat stability and molecular size. The resistant and chromosomal enzymes could bind the normal substrate, *p*-aminobenzoic acid, with equal efficiency. In contrast, sulfonamide binding was about 10,000 times lower with the plasmid-coded enzymes than with the chromosomal enzyme. Another substrate analog, *p*-aminosalicylic acid, on the other hand, inhibited chromosomal and plasmid-mediated enzymes to a similar extent. Evidence was also found for the existence of a plasmid-borne resistance mechanism independent of drug-insensitive enzymes.

Plasmid-borne resistance to sulfonamides has been known for a long time. Its biochemical mechanism, however, was not studied until relatively recently. In work by Wise and Abou-Donia (17), it was demonstrated that with clinically isolated R plasmids the sulfonamide resistance could be explained by the formation of a drug-resistant dihydropteroate synthase (EC 2.5.1.15) coded for by the plasmid. Similar results were independently obtained in this laboratory with the earlier well-characterized R-plasmid derivative R1*drd-19* (13).

This bacterial enzyme, which links *p*-aminobenzoic acid (AB) and 2-amino-4-hydroxy-6-pyrophosphorylmethyl-7,8-dihydropteridine (H_2-pteridine-CH_2O-PP) to give the dihydrofolate precursor 7,8-dihydropteroic acid (H_2-pteroate), is the target of sulfonamide drug action.

Chromosomal dihydropteroate synthase was earlier shown to accept sulfonamide as a substrate to form a stable sulfonamide-containing dihydropteroate analog (14). Drug action could thus be explained by both the competitive inhibition of dihydropteroate formation and the drain on the cellular supply of H_2-pteridine-CH_2O-PP.

A direct comparison regarding enzyme saturation was made with the two substrates in spontaneous mutants of *Escherichia coli*, chromosomally resistant to sulfonamides, and in the corresponding parental strain (14). It was observed that in the wild-type strain, the K_m was lower (2.7 μM) for sulfonamide than for the natural substrate AB (5.1 μM); i.e., the enzyme favors the drug over AB. In the mutants, however, the K_m for sulfonamide had increased almost 150-fold and proportionally much more so than that for AB, which increased about 10 times (14). The chromosomal resistance against sulfonamide was thus caused by a mutational change in the dihydropteroate synthase, making the drug less efficient as a substrate and at a cost for the cell of a lower substrate efficiency for the normal metabolite as well.

In the experiments described here, dihydropteroate synthases coded for by plasmids mediating sulfonamide resistance were studied. These enzymes could be completely separated from their chromosomal counterpart by chromatography and showed very high resistance to sulfonamide inhibition without any apparent increase in the saturation concentration for the normal substrate. Furthermore, it was observed that although sulfonamide sensitivity was very different in chromosomal and plasmid enzymes, inhibition by *p*-aminosalicylic acid was quite similar with the two types of enzyme. The plasmid-mediated enzyme thus seems to be very different from the mutationally changed, drug-resistant chromosomal enzyme in the sense that the plasmid enzyme has a much higher substrate specificity.

Evidence was found not only for different types of plasmid-borne dihydropteroate synthases, but also for an R-plasmid-mediated sulfonamide resistance mechanism seemingly independent of drug-resistant, pteroate-forming enzymes.

MATERIALS AND METHODS

Bacterial strains. *E. coli* C strain C-167 *ura str nal* and its thermosensitive derivatives C-167*ts*18 and

C-167*ts*20, used as R-plasmid recipients, were described earlier (13). Strain EC1005 *met nal* is an *E. coli* K-12 derivative (5); it was supplied by K. Nordström, Odense, Denmark. *E. coli* K-12 strain C600 *thr leu thi lac* (16) was also used as a plasmid recipient.

The plasmids investigated are listed in Table 1. Those designated pGS01, pGS02, pGS03, pGS04, and pGS05 were originally isolated as R plasmids transferring sulfonamide resistance from clinical specimens of *E. coli* strains causing urinary tract infections diagnosed at the bacteriology laboratory of a hospital in Stockholm, Sweden. The R1 derivative R1*drd-19* with derepressed transfer functions was described by and obtained from E. Meynell (8). The R plasmid R388 conferring sulfonamide and trimethoprim resistance has been described (1, 15). Plasmid R22259 was also a clinical isolate obtained as described above (15). Plasmids pKT012 and pFC012 are chimeric recombinants containing the sulfonamide resistance gene from plasmid R6-5 borne on a fragment obtained by *Hin*dIII and *Eco*RI restriction endonuclease cleavage, respectively (16).

Chemicals. The radioactively labeled substrate *p*-amino-[carboxyl-^{14}C]benzoic acid (5.64 mCi/mmol) was purchased from New England Nuclear Chemicals GmbH (Dreieichenhain, West Germany). Pteridine-CH_2OH was obtained from Syntestjänst AB (Lund, Sweden); it was pyrophosphorylated by the method of Shiota et al. (12) and purified by a method kindly communicated to us by E. Wise. The charcoal eluate obtained in the procedure by Shiota et al. was evaporated in a flash evaporator at 35°C to 75 ml, and its pH was adjusted to 10.0 with 10 M NH_4OH. It was then introduced into a column of Dowex-AG1-X10 (200 to 400 mesh) and eluted with a linear gradient reaching 0.6 M NH_4HCO_3. The pyrophosphate peak was identified by its absorbance at 350 nm. Pooled fractions were flash-evaporated to 250 ml at 45°C, chilled to 5°C, and added with stirring to 54 g of Dowex-50-H^+. After CO_2 bubbles were stirred away, the suspension was filtered and the filtrate was frozen and finally lyophilized. Reduction of pteridine-CH_2O-PP to its enzymatically active dihydro form was performed before each experiment as described by Shiota et al. (11). Sulfathiazole was from Astra Läkemedel AB (Södertälje, Sweden), and *p*-aminosalicylic acid was from Ferrosan AB (Malmö, Sweden).

TABLE 1. *Characteristics of the plasmids*

Plasmid[a]	Resistance markers[b]	Ts suppression[c]	Reference
R1*drd-19*	Su Ap Km Sm Cm	+	8
pGS01	Su Sm Tc	+	This report
pGS02	Su Ap Sm Cm	+	This report
pGS03	Su Ap Sm	+	This report
pGS04	Su	+	This report
pGS05	Su Sm	+	This report
R388	Su Tp	−	1
R22259	Su Tp Tc Cm	−	15
pKT012	Su	ND	16
pFC012	Su Sm	ND	16

[a] The host for pKT012 and pFC012 was strain C600; all other plasmids were kept in strain C-167.

[b] Resistance markers were determined by replica plating to plates containing the different drugs. Su, Sulfonamides; Ap, ampicillin; Km, kanamycin; Sm, streptomycin; Cm, chloramphenicol; Tc, tetracycline; Tp, trimethoprim.

[c] Ability to support growth of C-167*ts*20 at 42°C in a temperature shift experiment (13) is denoted by +. ND, Not determined.

Transfer of R plasmid. Transfer of plasmids was performed as described earlier (13), and recombinants between sulfathiazole (0.5 mM) resistance and recipient markers were selected for on supplemented minimal agar plates.

Media. The mineral salts medium M9 (2) or Tris medium (3) was used for growth of bacteria and in transfer experiments. Strain C600 was grown in Fraser medium (4).

Preparation of enzyme. Crude bacterial extract was prepared according to Richey and Brown (10) and further purified by either gel chromatography on Sephadex G-100 or ion-exchange separation on DEAE-Sephacel (Pharmacia Fine Chemicals, Uppsala, Sweden). In the latter case, elution was performed with a linear gradient of 0.01 to 0.25 M potassium phosphate buffer, pH 7.0, containing 5 mM $MgCl_2$ and 1 mM dithiothreitol. To obtain reasonable yields of the more labile enzyme activity from plasmids R1, pGS01, and pGS02, another extraction method had to be used. Bacteria were grown in 250 ml of Fraser medium, harvested by centrifugation, and resuspended in 5 ml of 0.1 M Tris-hydrochloride (pH 8.4)–0.05 M KCl–1 mM dithiothreitol. The frozen suspension was treated in a pressure cell (X-press; Biotec, Stockholm, Sweden) to disrupt the cells. After the suspension was thawed, DNase was added to 20 μg/ml, and the crude extract was immediately assayed for activity. For storage, sucrose was added to 20% (wt/vol) before freezing at −80°C. Protein content was determined by the method of Lowry et al. (7), with trypsin as a standard.

Determination of dihydropteroate synthase activity. Enzyme activity was determined by following the reaction between ^{14}C-labeled AB and H_2-pteridine-CH_2O-PP as described earlier (14).

RESULTS

Characterization of plasmids carrying sulfonamide resistance. Earlier studies (13, 17) provided evidence for the existence of sulfonamide-resistant dihydropteroate synthases, coded for by genes on R plasmids. To investigate this resistance mechanism further, several plasmids mediating sulfonamide resistance were isolated from sulfonamide-resistant *E. coli* strains isolated in a hospital laboratory for clinical bacteriology. Of 30 tested isolates, five were able to transfer sulfonamide resistance to *E. coli* C-167 and to its thermosensitive derivative C-167*ts*20. All but one of these five R plasmids also carried resistance to streptomycin (Table 1). In Table 1 are also listed other plasmids carrying sulfonamide resistance and with which the new isolates were compared. Plasmids pKT012 and pFC012 are chimeric recombinants containing drug re-

sistance genes from R plasmid R6-5, which has a sulfonamide resistance determinant apparently identical to that of plasmid R1 (6).

The sulfonamide-resistant mutant C-167*ts*20 has a temperature sensitivity lesion in its chromosomal gene for dihydropteroate synthase (14). When plasmid R1*drd-19* was introduced into this mutant, temperature sensitivity was suppressed (13). All of the newly isolated R plasmids carrying sulfonamide resistance could similarly suppress the temperature sensitivity of C-167*ts*20 (Table 1), which indicated that they all carry genes for dihydropteroate synthase. Similar suppression did not take place with either plasmid R388 or plasmid R22259. This could mean that either the plasmid-borne enzyme in these cases is extremely labile or the resistance mechanism is different. Growth curves with temperature shifts from 30 to 42°C are shown in Fig. 1 for C-167*ts*20 without and with different plasmids mediating sulfonamide resistance. The temperature sensitivity suppression seemed to be very similar in efficiency with pGS01, pGS02, pGS04, and pGS05. With pGS03 the growth was somewhat retarded at the higher temperature, possibly reflecting some degree of heat lability with the enzyme of this plasmid. When similar experiments were carried out with R388 and R22259, no temperature rescue was seen; i.e., the growth curves after temperature shift were congruent with that for C-167*ts*20.

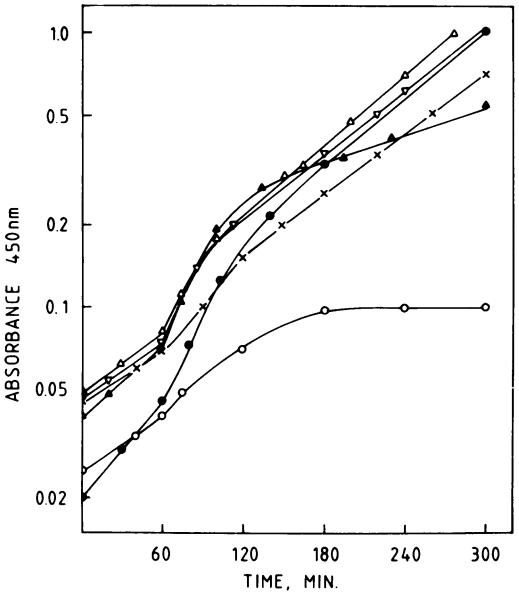

FIG. 1. *Temperature sensitivity suppression of C-167ts20 with plasmids carrying sulfonamide resistance. Cells were grown at 30°C with aeration in M9 minimal medium supplemented with uracil (40 μg/ml). At 60 min the temperature was shifted to 42°C. Cell growth was monitored at 450 nm in a Zeiss PMQ II spectrophotometer. Symbols: ○, C-167ts20; ×, C-167ts20(pGS01); ●, C-167ts20(pGS02); ▲, C-167ts20(pGS03); △, C-167ts20(pGS04); ▽, C-167ts20(pGS05).*

Sulfonamide-resistant dihydropteroate synthase in plasmid-containing bacteria. Dihydropteroate synthase activity was determined in crude extracts from plasmid-containing cells, and resistant enzyme was found in strain C-167 with plasmids pGS03, pGS04, and pGS05 (Table 2). At a concentration of sulfathiazole (100 μM) which inhibited the chromosomal activity of C-167 by 90%, no resistant activity could be seen with plasmids R1*drd-19*, pGS01, and pGS02. This seemed contradictory to the temperature sensitivity suppression data. To investigate this contradiction, two approaches were tried. One was to look for plasmid-borne enzyme in a system where it might be overproduced. In plasmids pKT012 and pFC012, the sulfonamide resistance gene from R6-5 (identical to that from R1) was incorporated into small plasmids with relaxed replication control (16), and its product would then be expected in relatively large quantities by a gene dosage effect. The other approach was to prepare extracts with as mild a procedure as possible. In such extracts of strain C600 containing pKT012, more than 40% dihydropteroate synthase activity remained at a sul-

TABLE 2. *Dihydropteroate synthase activity in plasmid-containing bacteria*

Bacterial strain[a]	Enzyme activity[b]			% Resistance
	−Su	+100 μM Su	+ 250 μM Su	
C-167	4.9	0.51		10.4
C-167(R1*drd-19*)	4.7	0.47		9.9
C-167(pGS01)	8.2	0.76		9.3
C-167(pGS02)	5.4	0.62		11.4
C-167(pGS03)	6.0	3.5		58.3
C-167(pGS04)	6.4	2.4		37.2
C-167(pGS05)	7.4	3.8		50.9
C600	4.5		0.23	5.1
C600(R1*drd-19*)	3.5		1.01	28.7
C600(pGS01)	5.2		0.79	15.3
C600(pGS02)	5.2		1.6	30.6
C600(pKT012)	4.6		1.9	42.3
C600(pFC012)	3.7		1.2	32.8
EC1005(R388)	2.7		0.15	5.8
EC1005(R22259)	3.8		0.18	4.8

[a] C-167 strains were grown in Tris minimal medium, and strains C600 and EC1005 were grown in Fraser medium. Crude extracts were prepared as described in the text, following the procedure for labile enzyme activities.

[b] Enzyme activity was determined as described and is expressed as nanomoles of dihydropteroate formed per hour per milligram of protein. Su, Sulfathiazole.

fathiazole concentration (250 μM) which inhibited chromosomal activity by 95% (Table 2). With plasmids R1*drd-19*, pGS01, and pGS02 resistant activity could be seen under these conditions. With R388 and R22259, on the other hand, no drug-insensitive enzyme was seen, which concurs with the lack of thermosensitivity suppression.

The drug-resistant enzyme activity in crude extracts from cells containing R1*drd-19*, pGS01, and pGS02 was quite labile. The activity was destroyed by freezing and thawing and also by storing on ice for 4 h. When crude extracts from strain C600 without plasmid and with plasmids pKT012 and pGS02 were treated at 50°C (Fig. 2), the extracts from plasmid-containing strains initially showed a high level of dihydropteroate synthase activity in the presence of 250 μM sulfathiazole. This resistant activity rapidly decreased with time at 50°C. In the absence of drug, the chromosomal enzyme activity was stable at 50°C, whereas activities from plasmid-carrying strains gave a biphasic pattern, indicating the presence of two enzymes with different heat stabilities.

Separation of plasmid-borne and chromosomal dihydropteroate synthase activities. Although the drug-resistant enzyme seen with plasmid pKT012 was very labile, a phosphate buffer of pH 8.0 containing 20% (wt/vol) sucrose and 1 mM dithiothreitol stabilized the enzyme enough to permit a separation by ion-exchange chromatography (Fig. 3). Two peaks of enzyme activity were clearly discerned. The first peak contained drug-resistant enzyme, whereas the second peak represented chromosomal activity. The latter peak was larger, which probably reflects the higher lability of the plasmid enzyme.

The dihydropteroate synthase determined by pGS05 seemed to be more stable (see Table 2). This was also reflected when the enzyme was

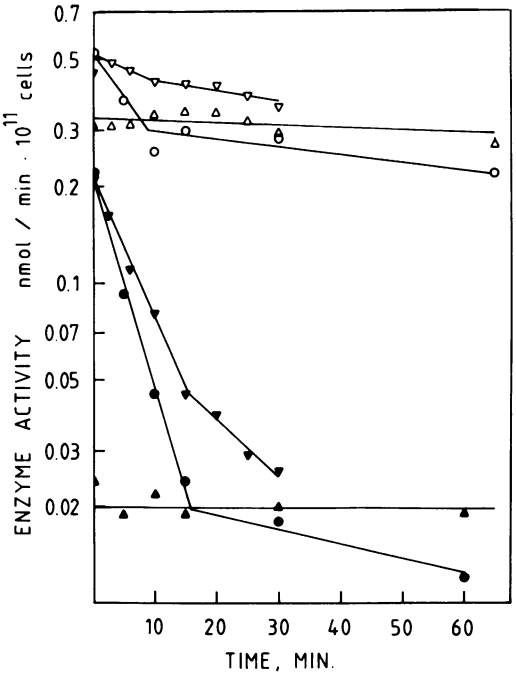

FIG. 2. *Heat stability of dihydropteroate synthase in crude extracts from C600(pKT012) and C600(pGS02). Extracts were prepared from cells grown in Fraser medium. Tubes containing 50-μl portions of crude extract from the different strains were treated at 50°C in the presence of 25 μM H_2-pteridine-CH_2O-PP, 0.1 M Tris-hydrochloride (pH 8.4), 5 mM $MgCl_2$, and 1 mM dithiothreitol. At indicated time points two samples were withdrawn. [^{14}C]AB was added to 50 μM. One of the two samples also received 250 μM sulfathiazole. All samples were then incubated at 30°C for 60 min and assayed for dihydropteroate formation. Enzyme activity is expressed as nanomoles of dihydropteroate formed per minute per 10^{11} cells. Symbols: △, C600; ▲, C600 + 250 μM Su; ▽, C600(pKT012); ▼, C600(pKT012) + Su; ○, C600(pGS02); ●, C600(pGS02) + Su.*

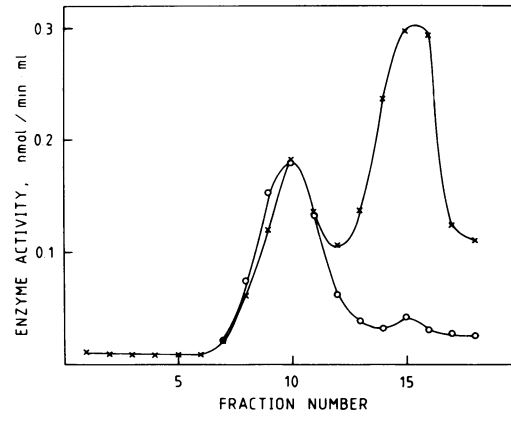

FIG. 3. *Fractionation of extract from C600-(pKT012) on DEAE-Sephacel. Cells of C600(pKT012) were grown in Fraser medium, harvested, and resuspended in 3 ml of 0.01 M phosphate buffer, pH 8.0, containing 1 mM dithiothreitol. After cell disruption, 0.6 g of sucrose was added, and the suspension was thawed, treated with 20 μg of DNase per ml, and centrifuged. Of the supernatant, 2 ml was applied to a column (0.9 by 5 cm) of DEAE-Sephacel equilibrated with 0.01 M phosphate buffer (pH 8.0) containing 1 mM dithiothreitol and 20% (wt/vol) sucrose. The column was developed by a linear gradient, 0.01 to 0.25 M, of phosphate buffer (pH 8.0), 1 mM dithiothreitol, and 20% (wt/vol) sucrose. Fractions of 2 ml were collected at 10 ml/h and assayed for dihydropteroate synthase activity in the absence (×) and in the presence (○) of 250 μM sulfathiazole.*

purified by gel chromatography (Fig. 4), where the peaks for chromosomal and plasmid activities were of about equal size. The drug-resistant enzyme activity was slightly retarded by gel chromatography, indicating an approximately 10% lower molecular weight compared to the chromosomal enzyme, which was judged to have a molecular weight of about 45,000. A complete separation of the two dihydropteroate synthase activities in C-167(pGS05) was achieved by ion-exchange chromatography on DEAE-Sephacel (Fig. 5). The plasmid enzyme was eluted early in the salt gradient. Similar elution profiles were obtained for enzymes determined by plasmids pGS03 and pGS04. In all cases the plasmid-borne enzyme showed a peak equal in size to the chromosomal enzyme peak.

Characteristics of plasmid-borne dihydropteroate synthase. Chromatographically purified chromosomal and plasmid dihydropteroate synthase activities from C-167(pGS05) were compared regarding sensitivity to AB analogs (Fig. 6). An approximately 10,000-fold-higher concentration of the drug was needed to decrease the plasmid activity by 50% compared to that needed for a similar inhibition of the chromosomal activity. Sulfanilamide was a less efficient inhibitor of both chromosomal and plasmid enzymes than was sulfathiazole. The AB analog *p*-aminosalicylic acid had a very similar inhibiting effect on both the chromosomal and the plasmid-coded enzymes. The latter enzyme thus seemed to have an ability to discriminate between the carboxyl groups of AB and *p*-aminosalicylic acid on the one hand and the sulfonyl group of sulfonamides on the other hand. Very similar results were obtained with preparations from C-167(pGS03) and C-167(pGS04).

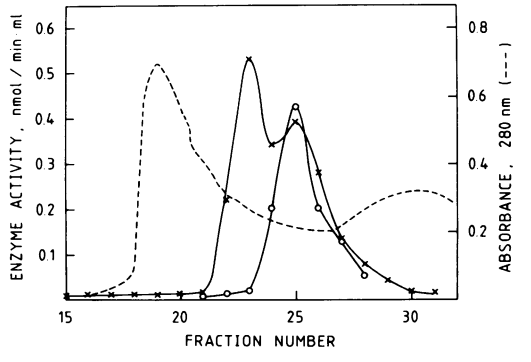

FIG. 4. *Gel chromatography of dihydropteroate synthase from pGS05. Extract from C-167(pGS05) was partially purified. Two milliliters of the 20 to 60% ammonium sulfate fraction containing 12 mg of protein was applied to a column of Sephadex G-100 (1.5 by 80 cm) and eluted with 0.01 M phosphate buffer, pH 7.0. Fractions of 2.8 ml were collected at 8.4 ml/h and assayed for dihydropteroate synthase activity with* (○) *and without* (×) *250 μM sulfathiazole.*

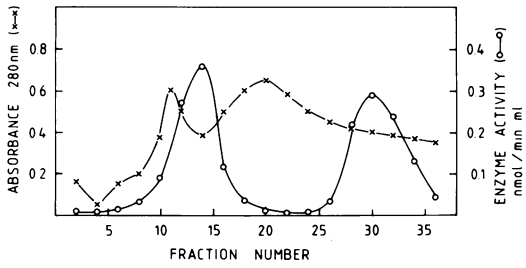

FIG. 5. *Fractionation of dihydropteroate synthases on DEAE-Sephacel. Extract from C-167(pGS05) was partially purified, and 1.5 ml of the 30 to 60% ammonium sulfate fraction containing 60 mg of protein was applied to a column (1.5 by 7.0 cm) of DEAE-Sephacel. Elution took place at 24 ml/h with a linear gradient of potassium phosphate buffer, 0.01 to 0.25 M (pH 7.0), and containing 1 mM dithiothreitol and 5 mM $MgCl_2$. Protein concentration was monitored by absorbance at 280 nm* (×), *and dihydropteroate synthase activity* (○) *was determined in 50 μl from each eluted 4-ml fraction.*

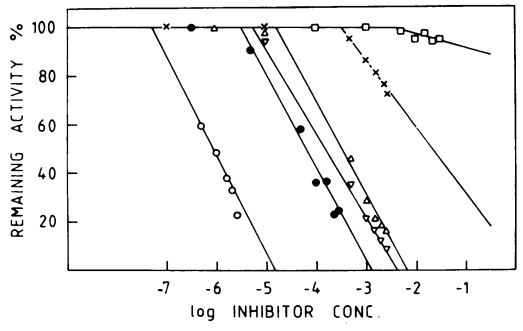

FIG. 6. *Inhibition of dihydropteroate synthases from C-167(pGS05) by AB analogs. Fractions from DEAE-Sephacel chromatography containing the chromosomal and plasmid-coded activity were pooled and concentrated five times by ultrafiltration. Enzyme activities were measured at an AB concentration of 5 μM in the presence of different concentrations of substrate analogs (given as log molarity). Each assay contained 25 μg of partially purified enzyme protein, and 100% activity was 1.04 and 1.26 nmol of dihydropteroate formed per min per mg of protein for the chromosomal and plasmid enzymes, respectively. Chromosomal enzyme: + sulfathiazole* (○); *+ sulfanilamide* (●); *+ p-aminosalicylic acid* (▽). *Plasmid enzyme: + sulfathiazole* (×); *+ sulfanilamide* (□); *+ p-aminosalicylic acid* (△).

The inhibition pattern of the labile dihydropteroate synthase of plasmid pKT012 (purified as described in Fig. 3) was compared with that of the similarly purified pGS05 enzyme. Similar inhibition curves were obtained for the two en-

zymes with both sulfathiazole and *p*-aminosalicylic acid (data not shown).

With all the plasmid-coded sulfonamide-resistant enzymes the K_m for AB was measured and found to be 5 μM, which is the same value found for the chromosomal enzyme (14). Plasmid-coded enzymes did differ, however, when their stability was investigated in a heat inactivation experiment (Fig. 7). At 50°C and in the presence of H_2-pteridine-CH_2O-PP, dithiothreitol, and sucrose, plasmid enzymes from pGS03 and pGS05, respectively, were rapidly inactivated, whereas surprisingly, that from pKT012 was relatively stable. The relative stability of the pKT012 enzyme under these conditions, as compared to those shown in Fig. 2, is explained by the presence of sucrose, which was shown to stabilize the enzyme markedly during purification. The chromosomal enzyme was almost completely stable under these conditions.

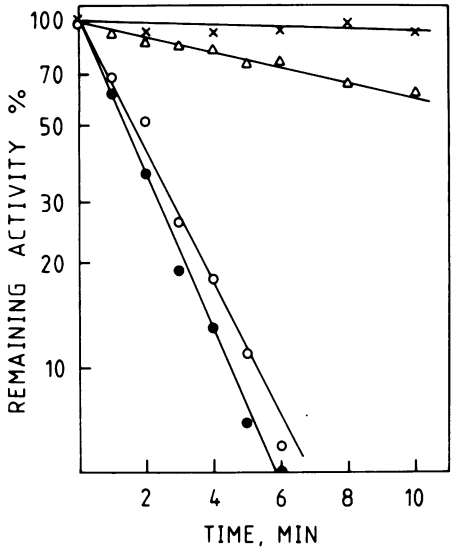

FIG. 7. *Heat stability of chromosomal and plasmid-coded enzymes. Heat treatment was performed at 50°C in the presence of H_2-pteridine-CH_2O-PP as described in the legend to Fig. 2. At the indicated times [^{14}C]AB was added, and the assay tubes were transferred to 30°C, where the enzyme reaction was allowed to take place for 30 min. Each assay contained 25 μg of protein added as chromatographically purified enzyme preparations in 0.1 M phosphate buffer, pH 8.0, containing 1 mM dithiothreitol and 20% (wt/vol) sucrose. C-167 chromosomal enzyme (×), 100%, corresponds to 1.18 nmol of dihydropteroate formed per min per mg of protein; pKT012 plasmid enzyme (△), 100%, corresponds to 0.38 nmol/min per mg; pGS03 plasmid enzyme (○), 100%, corresponds to 0.83 nmol/min per mg; pGS05 plasmid enzyme (●), 100%, corresponds to 1.19 nmol/min per mg.*

DISCUSSION

Plasmid-borne resistance to sulfonamides was due to a sulfonamide-resistant extrachromosomal target enzyme, dihydropteroate synthase (9, 13, 17). It could then be asked if this resistance is represented by one single gene that has spread to give the present situation, where R-plasmid-mediated sulfonamide resistance is very common all over the world. Characterizing the plasmid-mediated enzyme would address the relation of the drug-resistant extrachromosomal activity to the chromosomal dihydropteroate synthase.

In the present study, five transmissible plasmids mediating sulfonamide resistance were isolated from clinical material. In all but one of the plasmids, sulfonamide resistance was linked to streptomycin resistance.

Plasmids were tested for their ability to rescue the growth, at the nonpermissive temperature, of bacterial mutants carrying temperature sensitivity lesions in the dihydropteroate synthase. All but two of the plasmids studied could effect this rescue. The resistance mechanism of these two plasmids could then be of a different type, e.g., an effect on drug permeability. With three of the newly isolated plasmids (pGS03, pGS04, and pGS05), the drug-resistant activity was readily demonstrable in extracts from plasmid-carrying bacteria. With pGS01 and pGS02, as well as with R1*drd-19*, however, the resistant enzyme was quite labile and had to be stabilized by dithiothreitol and sucrose in the extraction buffer. Two different groups of plasmid-coded dihydropteroate synthase activities could thus be discerned. Within the groups no significant differences could be observed between enzymes from different plasmids.

The enzyme from pGS05 was 10,000-fold less sensitive to inhibition by sulfonamide than was the chromosomal enzyme. In spite of this it showed a K_m value for AB of about 5 μM, which is the value found for the normal chromosomal enzyme. This is in contrast to the mutationally sulfonamide-resistant chromosomal enzyme, where the K_m for AB is dramatically increased (14). The pronounced drug resistance of the plasmid-coded enzyme thus seemed to be based on its ability to distinguish very sharply between the carboxyl structure of the normal substrate and the sulfonamido structure of the inhibitor. The same conclusion could be drawn from the inhibition experiments with *p*-aminosalicylic acid, which inhibited chromosomal and plasmid-coded enzymes to a similar extent.

ACKNOWLEDGMENT

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