Journal of General Microbiology (1984), **130**, 473–482. *Printed in Great Britain*

Resistance to Apramycin in *Escherichia coli* Isolated from Animals: Detection of a Novel Aminoglycoside-modifying Enzyme

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(*Received 19 August 1983; revised 21 October 1983*)

The mechanisms of resistance to apramycin of five isolates of *Escherichia coli* from animals were investigated. Three isolates, which were resistant to all the aminoglycosides tested, did not transfer their resistance and did not produce aminoglycoside-modifying enzymes. The fourth isolate, which was resistant to apramycin, tobramycin, gentamicin, kanamycin and neomycin but not to amikacin, owed its resistance to production of the acetyltransferase AAC(3)IV. The gene specifying this enzyme was carried on a transposon, Tn*800*, on a plasmid designated R1535. The fifth isolate was resistant to apramycin, neomycin and kanamycin but not to gentamicin, tobramycin or amikacin. It produced an acetyltransferase that readily acetylated only apramycin, neomycin and paromomycin, a compound that is closely related to neomycin. Synthesis of this enzyme was specified by a chromosomal gene located near *pyrD* at about 20 min on the map of the *E. coli* K12 chromosome.

INTRODUCTION

The aminoglycoside apramycin is used extensively in veterinary medicine but not in the treatment of humans (Ose *et al.*, 1967; Bowen *et al.*, 1976; Walton, 1978). Resistance to this compound is rare in unselected enterobacteria (Ryden & Moore, 1977) and the frequency of mutations to high-level resistance is extremely low (Bowen *et al.*, 1967; Davies & O'Connor, 1978), but resistant strains are detectable in nature especially after treatment of farm animals with apramycin (Bowen *et al.*, 1976). In some cases conjugative transfer of resistance to apramycin has been demonstrated, implying that this property is plasmid-determined (Bowen *et al.*, 1967; Davies & O'Connor, 1978). In these cases the resistance was mediated by aminoglycoside 3-*N*-acetyltransferase IV [AAC(3)IV], an enzyme with a notably broad range of substrates and the only enzyme reported to modify apramycin (Davies & O'Connor, 1978).

There are two mechanisms of resistance to aminoglycosides that do not involve production of aminoglycoside-modifying enzymes. Firstly, there is ribosomal resistance. Mutations at the *rpsL* locus can confer high levels of resistance to streptomycin but do not affect sensitivity to other aminoglycosides; however, low levels of resistance to these compounds is conferred by mutations at the *nek* and *rplF* loci (Brown & Apirion, 1974; Hull, *et al.*, 1976; Hancock, 1981). These mutations, in which a ribosomal protein component is altered, occur with a very low frequency ($< 10^{-10}$) in *Escherichia coli* K12. Secondly, mutations at a number of loci affecting energy metabolism, including *hemA*, *ubiD*, *uncA,B* and *ecfB*, confer low-level resistance to aminoglycosides by decreasing uptake of the compounds (for review see Hancock, 1981).

As no studies on apramycin resistance have been reported since the work of Davies & O'Connor (1978), we decided to investigate a small collection of apramycin-resistant isolates of

Abbreviation: AAC, aminoglycoside acetyltransferase (position of modification and different enzymes distinguished by suffixes).

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0022-1287/84/0001-1449 $02.00

Table 1. *Derivatives of E. coli K12 used in the study*

Strain no.	Characters	Reference or source
N728	Hfr *nek-728 metB1 purF1 lac*	Brown & Apirion (1974)
HfrH	Hfr prototrophic	J. T. Smith
HfrB9	Hfr *metB1*	Broda (1967)
HfrKL99	Hfr *thi-1 lac*	Low (1973)
HfrKL208	Hfr prototrophic	Low (1973)
HfrKL96	Hfr *thi-1*	Low (1973)
JF568	F⁻ *proC24 aroA357 his-53 purE41 met-65 lacY29 rpsL97*	Foulds & Chai (1978)
JF568-2	Rif^R mutant of JF568	
KL188	F⁻ *pyrD34 trp-45 his-68 thyA25 thi-1 rpsL118*	B. Bachmann
KL188-4	*ompA* mutant of KL188	
AM1248	F⁻ *leu thi his trp pdxA car recB21 recC22 sbcB15 hsdR rpsL*	W. P. M. Hoekstra
X195	F⁻ *pro his trp met purB pyrF tyr lac rpsL*	Pearce & Meynell (1968)
X195-2	Rif^R mutant of X195	
J53	F⁻ *pro met*	Coetzee *et al.* (1972)
J62	F⁻ *pro his trp lac*	Coetzee *et al.* (1972)
J62-1	Nal^R mutant of J62	
J62-2	Rif^R mutant of J62	
W677	F⁻ *thr leu thi lac mal gal xyl ara mtl tonA*	Benveniste & Davies (1971)

E. coli to determine the mechanisms of resistance to this antibiotic in naturally occurring populations of this species.

METHODS

Bacterial strains and plasmids. A collection of five apramycin-resistant isolates of *E. coli* from animals (generously made available by R. Ryden of the Lilly Research Centre, Windlesham, Surrey, UK) was studied. Derivatives of *E. coli* K12 and plasmids used are listed in Tables 1 and 2. Sets of plasmids representative of all incompatibility groups (Hedges & Jacoby, 1980) were also used. *Escherichia coli* TH20914, a clinical isolate from St Thomas' Hospital, was used as a producer of AAC(3)II and *E. coli* TH42662 was chosen as a representative strain that was resistant to gentamicin and other aminoglycosides without producing gentamicin-modifying enzymes. We also used *Providencia stuartii* 133 as a source of AAC(2'): this enzyme has not yet been found to be plasmid-determined.

Genetic studies. Except where otherwise stated, the technique of Datta *et al.* (1971) and Coetzee *et al.* (1972) was used for plasmid transfer by conjugation. In cases where rates of transfer were expected to be low, a few drops of overnight cultures of donor and recipient were poured on a 45 mm diameter filter paper on the surface of a nutrient agar plate. After incubation overnight at 37 °C the filters were washed with saline and the bacterial suspensions plated directly on selective medium. Hfr (or F') crosses with F⁻ recipients were performed as described by Clowes & Hayes (1968). Molecular weights of plasmids were determined by agarose gel electrophoresis as described by Datta *et al.* (1979). The technique described by Coetzee *et al.* (1972) was used for determination of incompatibility groups. Transduction by phage P1 was performed as described by Roth (1970). The technique of Bergmans *et al.* (1981) was used for transformation.

Selection of an ompA mutant. Phage K3hl was used to select an *ompA* mutant of strain KL188 and to distinguish between *ompA* and *ompA*$^+$ recombinants as described by Henning *et al.* (1978).

Susceptibility testing. Minimum inhibitory concentrations (MICs) of aminoglycosides were determined by agar dilution in Oxoid Diagnostic Sensitivity Test Agar (CM 261) with an inoculum of about 10^3 to 10^4 organisms per spot. The MIC was taken as the lowest concentration that completely suppressed growth after incubation at 37 °C overnight. The aminoglycosides tested were apramycin, tobramycin, gentamicin, kanamycin, amikacin and neomycin. Susceptibilities to streptomycin and to antibiotics other than aminoglycosides were assessed by disc diffusion.

Aminoglycoside-modifying enzymes. The cellulose phosphate paper binding method was used (Ozanne *et al.*, 1969). Cell extracts were prepared by suspending the cells from 10 ml nutrient broth cultures in 1 ml of supplemented Tris/maleate buffer, pH 7·8 (Haas & Dowding, 1975) and subjecting them to ultrasonic disintegration. For acetylation the reaction mixture consisted of 10 µl aminoglycoside solution (20 µg ml^{-1}), 10 µl [1-^{14}C]acetyl-coenzyme A [0·2 mM, specific activity 5 Ci mol^{-1} (185 GBq mol^{-1})] and 20 µl cell extract. After 30 min incubation at 37 °C, 35 µl samples were pipetted on to 20 × 20 mm squares of Whatman P81 cellulose phosphate paper. The papers were washed three times in water, dried and the radioactivity measured by liquid scintillation spectrometry as described previously (Shannon *et al.*, 1978). In some experiments the concentration of aminoglycoside was increased to 200 µg ml^{-1} and that of acetyl-coenzyme A to 1 mM. The same procedure was used for

Table 2. *Plasmids used in the study*

Plasmid no.	Characters*	Inc group	Reference or source
F'13	*argF*$^+$ *lac*$^+$ *tsx*^{S} *purE*$^+$	FI	Hirota & Sneath (1961)
RGN238		FI	Matthew & Hedges (1976)
R1411	Ap Sm Cm Su Km	FI	Chau *et al.* (1982)
pGC400	Ap Sm Cm Su Km Tp	FIV	G. Cornelis
JR220	Ap Tc AAC(3)IV production		Davies & O'Connor (1978)
JR221	Ap Tc AAC(3)IV production		Davies & O'Connor (1978)
JR228	AAC(3)IV production		Davies & O'Connor (1978)
R1	Ap Sm Cm Km Su	FII	Jacob *et al.* (1977)
R5	AAC(6') production		Benveniste & Davies (1971)
R27	Tc	H1	Jacob *et al.* (1977)
R726	Sm Tc Cm Su	H1	Jacob *et al.* (1977)
R477-1	Sm Tc Su	H2	Jacob *et al.* (1977)
R478	Tc Cm Km	H2	Jacob *et al.* (1977)
pUZ2	Ap Tc AAC(3) production	M	Gomez-Lus *et al.* (1980)
pTH1	Ap Sm Tc Cm Su Tp AAC(3)I production	M	Datta *et al.* (1979)
RIP135	Sm Tc Su AAC(3)I production	M	Jacob *et al.* (1977)
R446b	Sm Tc	M	Jacob *et al.* (1977)
R64	Sm Tc	I1	Jacob *et al.* (1977)
R483	Sm Tp	I1	Jacob *et al.* (1977)
RA1	Tc Su	C	Jacob *et al.* (1977)
R6K	Ap Sm	X	Jacob *et al.* (1977)
R702	Sm Tc Km Su	P	Jacob *et al.* (1977)
pHH1307	Ap Su Tp AAC(3) production	W	Nugent & Datta (1980)
pCERr35	AAC(3) production		Rubens *et al.* (1981)
R1547	Ap Su Tc Cm Km Su AAC(3)II production		K. E. Price
R1549	Ap AAC(3)III production		K. E. Price

* Abbreviations: Ap, ampicillin resistance; Sm, streptomycin resistance; Tc, tetracycline resistance; Cm, chloramphenicol resistance; Km, kanamycin resistance; Su, sulphonamide resistance; Tp, trimethoprim resistance.

adenylylation, with 200 μg aminoglycoside ml^{-1} and with the acetyl-coenzyme A substituted by [8-^{14}C]adenosine 5'-triphosphate [1 mM, specific activity 5 Ci mol^{-1} (185 GBq mol^{-1})].

The stability of apramycin acetates to alkali was tested by incubation with NaOH (1 M) at 0 °C for 10 min or at 60 °C for 1 h, followed by neutralization with HCl before pipetting on to cellulose phosphate paper: controls were incubated with sodium chloride (0·67 M) at 60 °C for 1 h. Chloramphenicol acetylated by the enzyme from a clinical isolate of Klebsiella was used as a known *O*-acetate: it was separated from unchanged [^{14}C]acetyl-coenzyme A by partition into toluene.

In order to test the ability of modifying enzymes to acetylate various neomycin acetates the following procedure was adopted. The initial reaction mixture consisted of 10 μl neomycin (200 μg ml^{-1}), 10 μl non-radioactive acetyl-coenzyme A (4 mM) and 70 μl AAC(3)IV, AAC(2') or AAC(6'). After 1 h at 37 °C, the mixture was heated to 90 °C to inactivate the enzyme. The product was tested for its ability to serve as substrate for a range of acetyl-transferases by the cellulose phosphate paper binding method, as described above, but with the concentration of [^{14}C]acetyl-coenzyme A increased to 0·5 mM.

RESULTS

Strains that did not modify aminoglycosides

One class of apramycin-resistant isolates, consisting of three isolates represented by P78.351, was resistant to all the aminoglycosides tested, including amikacin (Table 3). In this it differed both from the other apramycin-resistant isolates from animals and from strains of *E. coli* K12 carrying plasmids that specify the various AAC enzymes. In contrast the aminoglycoside resistance pattern of P78.351 resembled that of the *nek* mutant and also that of TH42662, an aminoglycoside-resistant clinical isolate from St Thomas' Hospital that does not acetylate or adenylylate gentamicin.

Attempts to transfer the aminoglycoside resistance of P78.351 to *E. coli* K12 failed. No acetylation or adenylylation of apramycin or gentamicin by extracts of P78.351 was detected.

Table 3. *Susceptibility to aminoglycosides of apramycin-resistant organisms and organisms with known mechanisms of aminoglycoside resistance*

Organism*		MIC values (μg ml^{-1})					
		Apramycin	Tobramycin	Gentamicin	Amikacin	Kanamycin	Neomycin
E. coli J53		2	0·5	0·25	1	2	1
E. coli J62		4	1	0·5	2	4	4
E. coli P78.351		128	32	16	128	128	32
E. coli P377		>1024	256	128	4	>1024	1024
E. coli J53(R1535)		>1024	256	128	2	>1024	1024
E. coli AP92:B15		128	1	2	4	>1024	1024
E. coli AM1284 transformed to apramycin resistance		128	1	1	2	8	64
E. coli J53(RIP 135)	[AAC(3)I]	4	2	64	4	4	4
E. coli TH20914	[AAC(3)II]	4	32	>128	4	16	4
E. coli J53(R1549)	[AAC(3)III]	4	>512	256	2	>1024	64
E. coli J62(JR228)	[AAC(3)IV]	>1024	32	256	1	8	8
P. stuartii 133	[AAC(2')]	16	512	512	8	8	128
E. coli W677(R5)	[AAC(6')]	16	64	4	128	>1024	16
E. coli N728	[*nek* mutant]	64	4	8	32	32	16
E. coli TH42662		1024	128	256	512	512	256

* Mechanisms of resistance of standard strains are shown in square brackets.

P377, a strain that produced AAC(3)IV

The second class of apramycin-resistant isolates, consisting of a single isolate P377, was resistant to all the aminoglycosides tested except amikacin (Table 3). The aminoglycoside resistance of this strain, together with the resistance it possesses to chloramphenicol, sulphonamide and trimethoprim, was transferable to *E. coli* K12 and was carried on a plasmid, designated R1535, of about 70 MDal. The plasmid was incompatible with R64 and hence assignable to incompatibility group I1. The pili determined by R1535 were characteristic of group I1 (D. E. Bradley, personal communication).

Although R1535 was efficiently eliminated by entry of R64, one transconjugant was noted, among 40 tested, that had lost resistance to chloramphenicol and trimethoprim and suffered a decrease in its level of resistance to kanamycin (MIC decreased from > 1024 to 32 μg ml^{-1}), but retained resistance to sulphonamide and the aminoglycosides gentamicin, tobramycin and apramycin. Plasmid R64 was freely transferred from this strain with an efficiency of about 10^{-3} per donor h^{-1} and with no alteration in molecular weight or resistance characteristics. The resistances derived from R1535 were transferred at about 10^{-9} per donor h^{-1}. Transconjugants that received the aminoglycoside resistance derived from R1535 also received sulphonamide resistance (10 out of 10 tested), and both resistances subsequently cotransferred with the resistances specified by R64 as part of a single I1 group plasmid (subject to elimination by R483) that was slightly larger than R64.

Apramycin resistance, together with resistance to gentamicin and tobramycin, was transduced by phage P1kc from this derivative of R64 into the plasmid-free *E. coli* strain X195-2. By conjugation of the transductant with HfrH the genetic determinant of apramycin resistance was found to have inserted into the bacterial chromosome near the *trp* operon, since over 70% of *trp*$^+$ recombinants were susceptible to apramycin.

P377 did not adenylylate gentamicin. However, it produced an enzyme that acetylated all the compounds tested except amikacin and so was indistinguishable from AAC(3)IV (Table 4).

AP92:B15, a strain that produced a new acetyltransferase

The single isolate, AP92:B15, in the third class of apramycin resistance was resistant only to apramycin, neomycin, kanamycin and streptomycin of the aminoglycosides tested (Table 3) and also to sulphonamides. However, only the resistance to neomycin and kanamycin was readily

transferable to *E. coli* K12. This occurred with an efficiency of about 10^{-3} per donor h^{-1} on a plasmid of group I1 designated R1539. Resistance to sulphonamide and streptomycin was frequently transferred along with R1539 and was presumed to be determined by a non-self-transmissible plasmid mobilized by R1539. Although it was not tested, the resistance to neomycin and kanamycin determined by R1539 is assumed to be due to aminoglycoside 3′-*O* phosphotransferase.

Apramycin resistance was transferred at very low frequency to *E. coli* J62-1: after overnight conjugation on filter paper an apparent rate of transfer of 10^{-9} to 10^{-10} was observed. Almost all the apramycin-resistant transconjugants were also resistant to kanamycin, that is possessed plasmid R1539. The two, of more than 30 tested, that were kanamycin-susceptible carried cryptic plasmids that seemed to be derivatives of R1539. The determinant of resistance to apramycin appeared to be more closely associated with the *trp* locus than with any other of the markers tested, since in three separate experiments a proportion of the recombinants (3 of 8, 5 of 10 and 6 of 15) was trp^+ whereas all had the other nutritional markers (*pro, his, lac*) of the J62-1 parent.

None of the transconjugants contained any DNA supercoil other than R1539 (approximately 60 MDal) or the slightly smaller cryptic derivative. Elimination of R1539 by entry of R64 did not lead to loss of apramycin resistance and transfer of R1539 did not involve transfer of apramycin resistance. One recombinant resistant to both apramycin and kanamycin and having all the nutritional markers of J62-1 was designated J62-1 Apr^R(R1539). Conjugal transfer of apramycin resistance from J62-1 Apr^R(R1539) to other strains of *E. coli* K12 occurred with a frequency of 10^{-9} per donor h^{-1} or less and was always accompanied by transfer of R1539. Since other plasmids of incompatibility group I1 have been reported to mediate transfer of chromosomal genes (Datta & Barth, 1976) it was concluded that apramycin resistance was determined by a chromosomal gene mobilized by R1539.

In attempts to map the site of the apramycin resistance determinant in J62-1 Apr^R(R1539) it was found that Hfrs H, B9, KL99, KL208 and K196 transferred nutritional markers into J62-1 Apr^R(R1539) with kinetics similar to those when J62-1 was the recipient. Thus the segment derived from AP92:B15 did not greatly influence the recombination distribution. The determinant was located at about 20 min on the *E. coli* K12 map (Bachmann & Low, 1980) by selecting for nutritional markers and scoring for loss of apramycin resistance.

The presence of R1539 prevented growth of phage P1 and J62-1 Apr^R(R1539) so transduction of apramycin resistance was impossible. However, transfer of the apramycin resistance determinant into a plasmid-free strain was accomplished by introduction of F′13 into J62-1 Apr^R(R1539) and conjugation of this strain with JF568-2. Recombinants resistant to both rifampicin and apramycin were selected and from amongst these kanamycin-sensitive, lactose-non-fermenting clones were found. One of these, designated JF568-2 Apr^R, had all the auxotrophic markers of JF568-2 but was resistant only to apramycin and neomycin and contained no plasmid DNA.

Phage P1 was grown on JF568-2 Apr^R and the lysate used to map the resistance determinant by transduction to KL188 and KL188-4. Selection for apramycin resistance gave 65% cotransduction of $pyrD^+$ whereas selection for $pyrD^+$ gave 36% apramycin-resistant progeny. With the *ompA* recipient K1188-4, it was found that the $ompA^+$ gene was cotransduced to 80% of apramycin-resistant transductants. Three-point mapping showed that the *ompA* gene lay between *pyrD* and the determinant of apramycin resistance.

There was no evidence for transposition since attempts to mobilize apramycin resistance by a range of plasmids including R1, R64, R483, R446b, RA1, R6K and R702 produced either no recombinants or exclusively chromosomal recombinants. Chromosomal transformation allowed the transfer of the apramycin resistance of AP92:B15 into AM1284 with an efficiency of about 10^{-9} per recipient cell. This was significantly above the rate of spontaneous mutation to apramycin resistance, about 10^{-10}, and, whereas all five mutants tested were resistant to a wide range of aminoglycosides, the transformants were resistant to apramycin and neomycin alone. One transformant was allowed to conjugate with various Hfrs and its resistance determinant was found to map at about 20 min.

Table 4. *Substrate profiles of aminoglycoside acetyltransferases*

Acetyltransferase activity was measured by the cellulose phosphate paper binding assay described in Methods. NT, Not tested.

Strain no. or enzyme	Substrate concn (μg ml $^{-1}$)	Radioactivity bound (c.p.m.)								
		No antibiotic	Apramycin	Tobramycin	Gentamicin	Gentamicin C_1	Amikacin	Kanamycin	Neomycin	Paromomycin
P377	5	52	2641	2355	2100	2233	66	136	866	1002
	50	250	32278	34757	28955	NT	276	2655	27317	NT
AP92:B15	5	88	2815	77	129	84	95	123	3038	2061
	50	342	25139	530	1022	NT	409	648	46904	NT
AAC(3)I	5	102	78	121	2473	2883	87	100	100	NT
AAC(3)II	5	46	46	1624	2683	3037	67	141	56	NT
AAC(3)III	5	68	94	2327	2164	2398	63	2648	945	NT
AAC(3)IV	5	61	2507	2166	1966	2425	76	239	803	1301
AAC(2')	5	27	45	3246	2701	3386	35	42	1535	2204
AAC(6')	5	64	68	3460	1742	114	2470	3402	1795	79

Table 5. *Ability of acetyltransferases to acetylate neomycin acetates*

Substrate	Radioactivity bound (c.p.m.) by:			
	AAC(AP92:B15)*	AAC(3)IV	AAC(2')	AAC(6')
No antibiotic	215	220	125	211
Neomycin	2076	1356	1123	1379
Neomycin 3-*N*-acetate	247	201	278	189
Neomycin 2'-*N*-acetate	2041	208	126	1551
Neomycin 6'-*N*-acetate	1175	1050	1223	250

* AAC(AP92:B15) is the acetyltransferase from strain AP92:B15.

The extract from AP92:B15 did not adenylylate gentamicin but an enzyme was detected that acetylated neomycin, paromomycin and apramycin, but not other aminoglycosides, at the low substrate concentration usually used (Table 4). However, some other aminoglycosides were acetylated, albeit poorly, at a 10-fold higher substrate concentration. Strain AM1248 transformed to apramycin resistance produced an acetyltransferase that was indistinguishable from that of AP92:B15.

The ability of the enzyme from AP92:B15 and of standard aminoglycoside-modifying enzymes to acetylate various neomycin acetates, prepared by incubation of neomycin with known acetyltransferases as described in Methods, is shown in Table 5. The enzyme from AP92:B15 was able to acetylate neomycin 2′- and 6′-*N*-acetates but not neomycin 3-*N*-acetate. Other enzymes differed in their ability to acetylate neomycin acetates (Table 5).

The apramycin acetates produced by the enzymes from AP92:B15 and P377 were stable to incubation at 60 °C in the presence of NaOH, whereas chloramphenicol acetate was almost completely destroyed in 10 min, even at 0 °C.

Incompatibility testing of previously described apramycin resistance plasmids

Incompatibility groups were determined for the plasmids conferring resistance to apramycin described by Davies & O'Connor (1978). JR228, which confers resistance only to aminoglycosides, was incompatible with plasmids R27 and R726 (of group H1) and R477-1 and R478 (of group H2). JR220 and JR221, which confer resistance to ampicillin and tetracycline in addition to aminoglycosides, were incompatible with RGN238 and R1411 (of group FI) and pGC400 (of group FIV).

Selection of apramycin-resistant derivatives of strains that produce AAC(3) enzymes

We had no success in attempts to obtain apramycin-resistant derivatives, able to grow in the presence of 25 μg ml^{-1}, from large populations ($> 10^{11}$ cells) of strains of J53 carrying plasmids pUZ2, pTH1, pHH1307, RIP135, R1547 and R1549, all of which specify aminoglycoside acetyltransferases.

DISCUSSION

The acetyltransferase produced by strain P377 closely resembles that described by Davies & O'Connor (1978) and designated AAC(3)IV. The plasmid specifying the enzyme in P377 belongs to incompatibility group I1, whereas the plasmids reported by Davies & O'Connor (1978) belong to the F and H complexes. That plasmid JR220 was incompatible with reference plasmids of both groups FI and FIV is not too surprising since the compatibility relationships between plasmids assigned to group FI are complex and correlated with the numerous replication origin specificities demonstrated in the group (Bergquist *et al.*, 1982): it may be that group FIV should be regarded merely as one section of an FI complex.

The lack of correlation of AAC(3)IV production with a particular plasmid group suggests transposability (Hedges & Jacob, 1974; Hedges *et al.*, 1974). We have shown this for the aminoglycoside and sulphonamide resistance from P377, which can enter the *E. coli* chromosome and also insert into plasmid R64 to produce the slightly larger derivative that has acquired the genes for aminoglycoside and sulphonamide resistance. We designate this transposon Tn*800*.

It has previously been shown that determinants for AAC(3) enzymes are transposable (Nugent & Datta, 1980; Rubens *et al.*, 1981) and there are indications that the genes for AAC(3)I and AAC(3)III are related (Rubens *et al.*, 1981). Thus it is interesting to ask whether AAC(3)III and AAC(3)IV are related. A simple explanation would be an alteration in the gene for AAC(3)III that reduces the specificity of the enzyme so that apramycin can be acetylated. However, we have failed to detect mutation to apramycin resistance by this mechanism in strains carrying genes for AAC(3)I, AAC(3)II or AAC(3)III.

The acetyltransferase from AP92:B15 is less easy to identify than that from P377. The acetylation of paromomycin, which differs from neomycin in having a hydroxy rather than an amino group attached to the carbon atom at the 6′ position, indicates that it is not an AAC(6′).

There is nothing in its substrate profile to distinguish clearly between AAC(3) and AAC(2'). The ability of the enzyme to acetylate neomycin 2'- and 6'-*N*-acetates suggests that it is not an AAC(2') or an AAC(6'). However, the inability to acetylate neomycin 3-*N*-acetate does not indicate that it is an AAC(3). The stability of the product of its acetylation of apramycin to sodium hydroxide suggests that the enzyme may be an *N*-acetyltransferase, not an *O*-acetyltransferase (Dowding, 1979): however, in the absence of known aminoglycoside *O*-acetates for comparison, the evidence is not conclusive. Thus while we have been unable to locate the site of acetylation of aminoglycosides by this enzyme, it is clearly distinct from aminoglycoside acetyltransferases previously described. Identification of its site of action will require determination of the structures of its products which, unfortunately, is beyond our capabilities. This enzyme could be more common than we think since apramycin susceptibility is seldom tested and neomycin resistance is likely to be ascribed to production of aminoglycoside 3'-*O*-phosphotransferase – a common enzyme.

The genetic determinant of this enzyme is interesting in that it has a unique chromosomal location, although it is hard to understand how such a gene could evolve *in situ*. It is more plausible for the determinant to have been introduced as a transposon but subsequently to have lost transposability. Such defective transposons have been reported (Cornelis *et al.*, 1981; Fayet *et al.*, 1982; Link & Reiner, 1982). Loss of transposability may be an advantage to a replicon since the selectively advantageous character cannot be acquired by a competitor. The asymmetrical transduction results also suggest that the apramycin resistance determinant might be carried on a DNA sequence for which no homologue exists in the *E. coli* K12 chromosome.

In principle, chromosomal transformation provides a most valuable technique for the analysis of genetic variation between strains of *E. coli*. The ability to transfer the whole range of enterobacterial plasmids into a common genetic (chromosomal) background has been most valuable in our understanding of plasmid organization and diversity (for example, Datta, 1974), but at present no similar technique is available to handle chromosomal diversity. The main problem with transformation is the very low efficiency of the process. This means that gene transfer may be undetected against the mutational background. The apramycin resistance of AP92:B15 provides an excellent test case since apramycin-resistant mutants are very rare and phenotypically distinguishable from transformants. Our evidence suggests that the transformed resistance determinant has integrated at a specific chromosomal site and behaves as a regular, mappable genetic marker. Thus, the transformation technique has proved usable and, if the efficiency of transformation could be improved, may be of value in surveying the range of genetic characters in *E. coli*.

We have not established the mechanism of resistance in strain P78.351. It is possible that it is a *nek* mutant since such mutants show similar broad-spectrum aminoglycoside resistance. On the other hand, it is at least equally likely that the strain owes its resistance to defective uptake of aminoglycosides, since this has been shown to be the most common mechanism of 'non-enzymatic' aminoglycoside resistance in clinical isolates of enterobacteria (Price *et al.*, 1981).

Ose *et al.* (1976) found less than 1% of isolates of *E. coli* and salmonella, mostly from pigs and cattle, to be apramycin-resistant. However, the faeces of 8 out of 10 pigs contained small numbers (< 350 organisms g^{-1}) of apramycin-resistant *E. coli* after, but not before, treatment with apramycin for 5 d (Bowen *et al.*, 1976). The question remains as to whether apramycin resistance is rare in isolates from humans and, if so, why? Since production of AAC(3)IV confers resistance to gentamicin and tobramycin it is somewhat surprising that it has not been reported as a cause of resistance to these drugs in hospital isolates, although it could be that this is merely a failure to distinguish AAC3(IV) from AAC(3)III through omission of apramycin from the range of compounds tested. Since the gene specifying AAC(3)IV occurs on transmissible plasmids of incompatibility groups well represented in hospital isolates and is, at least in one case, transposable, it is unreasonable to suppose that it could not spread under hospital conditions. The only apparent reason for the failure to observe AAC(3)IV in hospital isolates is ecological separation of the bacteria of farm animals from the hospital flora.

We are grateful to Mr R. Ryden, Lilly Research Centre Limited, Windlesham, Surrey, for providing the

apramycin-resistant isolates of *E. coli*. Drs A. E. Jacob and W. P. M. Hoekstra provided help and advice on transformation procedures. Mrs K. Ellis (Hammersmith Hospital) helped with the determination of plasmid molecular weights. Drs W. P. M. Hoekstra, J. E. Davies (Biogen S. A., Geneva), B. Bachmann (Yale University), C. Rubens (Washington University) and D. G. Bobey (Bristol Laboratories, Syracuse, New York) provided bacterial strains and helpful advice. Aminoglycosides were provided by the following companies: apramycin and tobramycin by Lilly Research Laboratories, gentamicin by Nicholas Laboratories, amikacin and kanamycin by Bristol Laboratories, neomycin by Upjohn and paromomycin by Parke Davis.

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