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Substrate Specificities and Structure–Activity Relationships for Acylation of Antibiotics Catalyzed by Kanamycin Acetyltransferase†

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ABSTRACT: Antibiotic resistance caused by the presence of the plasmid pMH67 is mediated by the aminoglycoside acetyltransferase AAC(6′)-4, also known as kanamycin acetyltransferase. Bacteria harboring the plasmid are resistant to the kanomycins plus a broad range of other deoxystreptamine-containing aminoglycosides but not to the gentamicins XK62-2 and C_1 which are substituted at the 6′-position. Substrate specificity studies on the purified enzyme, however, now show that the enzyme acetylates an even broader range of aminoglycosides, including the gentamicins XK62-2 and C_1. The enzyme also accepts several acyl-CoA esters, which differ in nucleotide as well as in acyl chain length. Application of the method of analysis of structure–activity data developed earlier for gentamicin acetyltransferase [Williams, J. W., & Northrop, D. B. (1978) *J. Biol. Chem. 253*, 5908-5914] to the kinetic data obtained for AAC(6′)-4 shows that the turnover of the acylation reaction is limited by catalysis and not by the rate of release of either the acetylated antibiotic or CoA. Most structural changes in aminoglycosides cause changes in rates of release, and only drastic changes, near the 6′-amino group, affect catalysis. The structural requirements on aminoglycosides for enzymatic activity run parallel to the structural requirements for antibacterial activity.

Kanamycin acetyltransferase (EC 2.3.1.55) catalyzes the $N^{6'}$-acetylation of several deoxystreptamine-containing aminoglycosides (see Figure 1). It was first identified as the biochemical basis of R-factor mediated resistance to kanamycin by Okamoto & Suzuki (1965), who demonstrated a requirement for acetyl-CoA in kanamycin interaction by cell-free of *Escherichia coli* K12 containing plasmid R5. This report was the first ever of an aminoglycoside-modifying enzyme in bacteria and second only to that of penicillinases as an instance of bacterial resistance being caused by enzymatic modification of an antibiotic. Kanamycin acetyltransferase is now designated AAC(6′)-4 by the Plasmid Group nomenclature (Mitsuhashi, 1971), because unlike other 6′-N aminoglycoside acetyltransferases, it acetylates the clinically important amikacin.

The catalytic features of the modifying enzyme must be the determining characteristics of the bacterial resistance mediated

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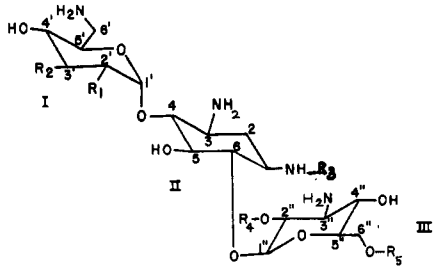


COMPOUND	R_1	R_2	R_3	R_4	R_5
KANAMYCIN A	OH	OH	H	H	H
AMIKACIN	OH	OH	L-AHBA	H	H
KANAMYCIN B	NH_2	OH	H	H	H
TOBRAMYCIN	NH_2	H	H	H	H
2"-O-ADENYLYL TOBRAMYCIN	NH_2	H	H	ADENYLYL	H
NEBRAMYCIN 4	NH_2	H	H	H	$CONH_2$

FIGURE 1: Structures of aminoglycosides related to kanamycin, analogues, and derivatives. AHBA is 4-amino-1-hydroxybutyryl.

by it. Despite the 34 years since microbial resistance to kanamycin was noted in Japan and the 19 years since the biochemical mechanism of such a resistance was identified, no study that correlates the actual kinetic characteristics of AAC(6′)-4 to the structures of its substrates has been reported. The relative "efficiency" of different aminoglycosides to accept the [^{14}C]acetyl group from acetyl-CoA had been reported by measuring the amount of radioactivity transferred to the antibiotics in a fixed time in the presence of partially purified AAC(6′)-4 (Benveniste & Davies, 1971). Differences observed, therefore, could be due to either differences in maximal velocities (V) or Michaelis–Menten constants (K). Lately, this assay method itself has been reported to be unreliable even for screening bacteria for the presence of aminoglycoside-acetylating enzymes (Kagan & Davies, 1980).

AAC(6′)-4 has been purified only recently to a state equivalent to homogeneity (Radika & Northrop, 1981, 1984a). The enzyme has been tested with a variety of antibiotics and coenzymes, leading to the discovery of a new kinetic diagnostic of enzymatic mechanisms (Radika & Northrop, 1984b), the establishment of a random kinetic mechanism for the enzyme (Radika & Northrop, 1984c), and a correlation between V/K values and expression of antibiotic resistance (Radika & Northrop, 1984d). The present report examines substrate kinetic characteristics and correlates these to the structural variations in the substrates.

Materials and Methods

Materials. Lithium salts of acetyl-CoA, *n*-propionyl-CoA, *n*-butyryl-CoA, isovaleryl-CoA, *n*-valeryl-CoA, *n*-hexyl-CoA and 1,N^6-ethenoacetyl-CoA, 2-(*N*-morpholino)ethanesulfonic acid (MES), and 3-[[tris(hydroxymethyl)methyl]amino]-propanesulfonic acid (TAPS) were purchased from Sigma Chemical Co. The chromogenic 4,4′-dithiodipyridine (Aldrithiol-4) was purchased from Aldrich. Sisomicin, *N*-ethylsisomicin, and gentamicins C_{1a}, B, and B_1 were gifts from A. Waitz of Schering Corp. Tobramycin was a gift from M. Gorman of Eli Lilly Co. Neomycins B and C were gifts from J. E. Grady of Upjohn Chemical Co. F. Leitner of Bristol Laboratories donated nebramycin factor 4. Paromomycin I and kanamycin C were gifts from D. Perlman and H. Umezawa, respectively. *E. coli* W6777 containing plasmid pMH 67 [which is a chimeric plasmid constructed from plasmid R5 (Haas & Davies, 1980)] was provided by M. J. Haas of J. Davies' laboratory. Kanamycin acetyltransferase (6′)-4 was purified 112-fold from *E. coli* W677/pMH67 as described elsewhere (Radika & Northrop, 1981, 1984a), by a modification of a previously described method (Scarbrough et al., 1979). The enzyme was the more abundant and the more active of the two forms isolated and had been purified through chromatography on DEAE-agarose, and had a specific activity of 1.9 international units/mg at pH 7.8, corrected for substrate inhibition.

Enzyme Assays. The coenzyme A released from acyl-CoA upon acylation of the antibiotic was detected by its reaction with the sulfhydryl reagent Aldrithiol-4, as described previously (Williams & Northrop, 1978a; Scarbrough et al., 1979). The formation of the chromophoric pyridine-4-thiol (λ_{max} 234 nm, ϵ_M = 19 800) was monitored continuously on a Gilford Model 240 spectrophotometer, equipped with a Leeds & Northrup Speedomax recorder. Cuvette chambers were maintained at 25 °C by Haake circulating water baths. All assays at pH 7.8 were conducted in 1-cm path-length, self-masking quartz cuvettes, employing a slit width of 0.35 mm and a full-scale value of 0.04–0.2 absorbance unit. All assays at pH 6.0 were conducted in 5-cm path-length optical glass cuvettes, employing a slit width of 0.9 mm and full-scale values of 0.02–0.1 absorbance unit.[1] Assay mixtures at pH 7.8 contained 0.05 M potassium phosphate, 0.1 M potassium chloride, 0.1 mM ethylenediaminetetraacetic acid (EDTA) and 0.12 mM Aldrithiol-4 plus the substrate and the cofactor in a total volume of 0.99 mL. Assay mixtures at pH 6.0 contained 0.1 M MES, 0.1 M potassium chloride, 0.1 mM EDTA, and 0.12 mM Aldrithiol-4 plus the substrate and the cofactor in 1.49 mL. Reactions were initiated by the addition of 0.01 mL of enzyme, which had been stored at −20 °C and diluted into a pH 8.5 buffer of 0.025 M TAPS.

Aminoglycosides, coenzymes, and Aldrithiol-4 were prepared in filtered, deionized and distilled water just prior to the assays. Concentrations of stock solutions of aminoglycosides and coenzymes were determined by enzymatic assays with AAC(6′)-4 using an excess of enzyme (at least 0.03 IU).

Data Analysis. Substrate kinetic data were fitted to eq 1 and 2 by using Fortran programs SUBIN and HYPER (Cleland, 1978), representing the presence and absence of substrate inhibition, respectively:

$$v = VA/[K + A + (A^2/K_I)] \quad (1)$$

$$v = VA/(K + A) \quad (2)$$

where v represents measured reaction velocity, V is the maximal velocity, A is the concentration of antibiotic or coenzyme, K is the Michaelis–Menten constant for antibiotic or coenzyme, and K_I is the substrate inhibition constant.)

Results

Apparent kinetic constants for acetyl-CoA and various aminoglycosides, obtained at pH 7.8, are presented in Table I. All the aminoglycosides studied showed substrate inhibition[2] as illustrated in Figure 2A for netilmicin and by the finite K_I terms in Table I. However, no substrate inhibition was

[1] The latter cuvettes were necessary for the determination of very low Michaelis–Menten constants and required a specially constructed cuvette chamber equipped with adjustable pinholes to prevent light from falling on the sides of the long, narrow cuvettes. Adherence to Beer's and Lambert's laws was demonstrated prior to kinetic studies.

[2] Amikacin exhibited two enzymatic activities, one at low substrate concentrations and subject to substrate inhibition and a second at very high substrate concentrations.

Table I: Apparent Kinetic Constants at pH 7.8[a]

substrate	K (μM)	V (IU/mg)	V/K	K_i (μM)
acetyl-CoA	0.76 ± 0.08	0.33 ± 0.01	0.43 ± 0.04	
kanamycin A	0.13 ± 0.03	0.81 ± 0.004	6.23 ± 1.25	24.73 ± 5.66
kanamycin B	0.18 ± 0.05	0.78 ± 0.08	4.33 ± 0.70	2.73 ± 0.62
amikacin[b]	0.16 ± 0.06	(0.46 ± 0.04)	2.88 ± 0.92	11.43 ± 4.32
amikacin[c]	115.24 ± 23.7	0.78 ± 0.07	0.01 ± 0.001	
tobramycin	0.32 ± 0.07	0.93 ± 0.05	2.91 ± 0.51	24.67 ± 3.38
nebramycin 4	0.11 ± 0.03	0.65 ± 0.05	6.03 ± 1.43	8.39 ± 1.78
gentamicin B	0.50 ± 0.13	1.35 ± 0.08	2.70 ± 0.54	65.49 ± 15.23
gentamicin B_1	0.22 ± 0.04	0.25 ± 0.01	1.14 ± 0.16	46.06 ± 6.50
gentamycin C_{1a}	0.51 ± 0.06	1.35 ± 0.06	2.65 ± 0.22	20.55 ± 2.56
sisomicin	5.56 ± 0.86	3.33 ± 0.25	0.60 ± 0.05	81.07 ± 13.66
N-ethylsisomicin	0.29 ± 0.06	1.90 ± 0.14	6.55 ± 0.60	32.47 ± 5.96
neomycin B	0.06 ± 0.03	0.53 ± 0.05	8.33 ± 3.57	3.72 ± 0.90
neomycin C	0.01 ± 0.02	0.36 ± 0.03	36.00 ± 86.34	3.71 ± 0.94

[a] Acetyl-CoA kinetic constants were determined with 50 μM amikacin. Aminoglycoside kinetic constants were determined with 60 μM acetyl-CoA. [b] Kinetic constants were calculated from data obtained between 0.21 and 10.66 μM amikacin; estimate of V value is not reliable because the second K value is present. [c] Substrate constants were calculated from data obtained between 53.32 and 213.26 μM amikacin.

Table II: Aminoglycoside Kinetic Constants at pH 6.0

aminoglycoside	K (μM)	V (IU/mg)	V/K
kanamycin A	0.65 ± 0.09	0.35 ± 0.01	0.54 ± 0.06
kanamycin B	−0.34 ± 0.06	0.40 ± 0.02	−1.20 ± 0.24
tobramycin	0.70 ± 0.07	0.70 ± 0.13	1.00 ± 0.08
amikacin	1.73 ± 0.24	0.59 ± 0.02	0.35 ± 0.04
gentamicin C_{1a}	0.46 ± 0.04	0.64 ± 0.01	1.39 ± 0.12
sisomicin	2.62 ± 0.63	0.59 ± 0.04	0.23 ± 0.04
neomycin B	0.05 ± 0.03	0.22 ± 0.01	4.35 ± 2.52
neomycin C	0.10 ± 0.04	0.33 ± 0.01	3.35 ± 1.41

Table III: Nucleotide Kinetic Constants at pH 6.0[a]

nucleotide	K (μM)	V (IU/mg)	V/K
acetyl-CoA	4.98 ± 0.36	0.55 ± 0.01	0.112 ± 0.006
n-propionyl-CoA	6.25 ± 0.59	0.28 ± 0.01	0.034 ± 0.002
n-butyryl-CoA	14.76 ± 1.48	0.04 ± 0.001	0.0030 ± 0.0003
1,N^6-ethenoacetyl-CoA	27.56 ± 4.56	0.74 ± 0.06	0.031 ± 0.003

[a] Acetyl-CoA constants were measured by using 40.6 μM and all others, by using 255.5 μM amikacin.

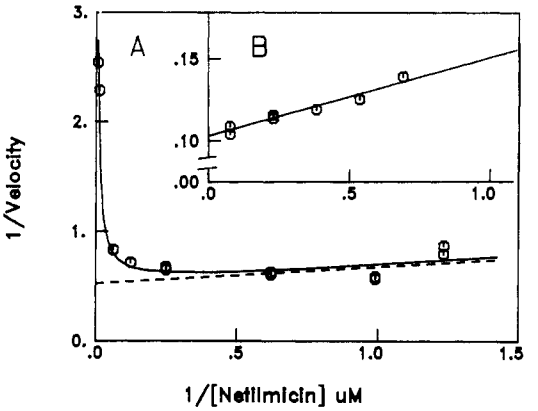


FIGURE 2: Double-reciprocal plots of initial velocity vs. netilmicin. The data in (A) were obtained at pH 7.8 and fitted to eq 2; the straight line was generated by computer using only the kinetic constants V and V/K, and not all data points are shown in order to illustrate substrate inhibition. The data in (B) were obtained at pH 6.0 and fitted to eq 1.

found at pH 6, while at pH 7 and 9, some antibiotics gave inhibition and some did not. Apparent kinetic constants for aminoglycosides, obtained at pH 6, are presented in Table II. The absence of substrate inhibition is graphically presented in Figure 2B, again with netilmicin as an example. Apparent kinetic constants for nucleotides, also obtained at pH 6 and free of substrate inhibition, are presented in Table III.

Not listed in the tables are gentamicins XK62-2 and C_1 which were found to be substrates with relative activities of 3% and 1%, respectively, compared to V of amikacin. The former carries an *N*-methyl group on the 6′-carbon, and the latter carries both an *N*-methyl group and a methyl group on the 6′-carbon. These and other aminoglycosides carrying similar modifications at the 6′-carbon had been reported not to be substrates for this enzyme (Price et al., 1974; Cox et al., 1977). No detectable acetylation of either kanamycin C or paraomomycin I (both lacking a 6′-amino group) was observed, indicating the absence of other aminoglycoside acetyltransferase activities contaminating the preparation of AAC(6′).

Discussion

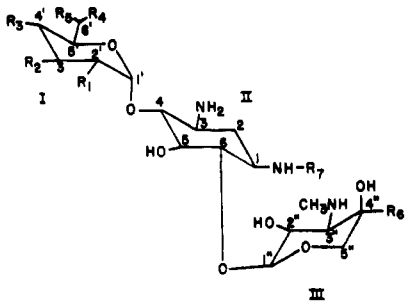
Structure–activity relationships of substrates for enzymes have been interpreted, traditionally, by evaluating changes in K and V values. This approach is dependent upon the implied assumptions that K is a measure of the binding of the substrate to the enzyme and V is a measure of the rate of chemical catalysis. Such assumptions, however, are valid only under rapid equilibrium conditions. Alternatively, substrate specificity can be evaluated in terms of V/K, the apparent first-order rate constant for the combination of the substrate with the enzyme during catalytic turnover, as has been done for gentamicin acetyltransferase, AAC(3)-1 (Williams & Northrop, 1978b), the only other aminoglycoside-modifying enzyme for which such a detailed study has been reported so far. Williams and Northrop argued that structural changes in substrates which alter catalysis will affect V and V/K similarly, but not necessarily by the same amount, while changes affecting binding will affect V and V/K differently. The latter interpretation stems from the following assumptions: bimolecular rate constants are diffusion controlled, and therefore, binding varies primarily as a function of rates of release; the chemical groups that govern the rate of release of a product and contribute to V also govern the rate of release of the parent substrate and contribute to V/K. When this approach was used, changes in V and V/K for each of the structural modifications on the substrates of AAC(6′)-4 were calculated from the data shown in Table I. The results are presented in Table IV. The structures of the antibiotic substrates of AAC(6′)-4 are given Figures 1, 3, and 4.

Case A in Table IV is most striking: an ethyl substituent on the 1-amino group has produced more than a 10-fold increase in V/K, while V decreased less than 2-fold. A decreased rate of substrate release increases V/K, while a decreased rate of product release decreases V, and hence, binding has been reduced. Moreover, because V/K changed more than V, the

Table IV: Kinetic Effects of Structural Differences between Aminoglycosides[a]

case	antibiotic	position	group	ΔV (x-fold)	$\Delta V/K$ (x-fold)
A	sisomicin	1-N	H	1.8	1
	N-ethylsisomicin		CH_2CH_3	1	10.9
B	kanamycin A	1-N	H	1.8	2.2
	amikacin		L-AHBA[b]	1	1
C	tobramycin	6″-O	H	1.4	1
	nebramycin-4		$C(O)NH_2$	1	2.1
D	tobramycin	3′-C	H	2.5	1
	kanamycin B		OH	1	1.5
E	sisomicin	4′–5′	C=C	2.5	1
	gentamicin C_{1a}		C–C	1	4.4
F	gentamicin B	6′-C	H	5.4	2.4
	gentamicin B_1		CH_3	1	1
G	kanamycin A	2′-C	OH	1	1.4
	kanamycin B		NH_2	1.0	1

[a] Data derived from Table I. [b] L-AHBA = 4-amino-1-hydroxybutyryl.



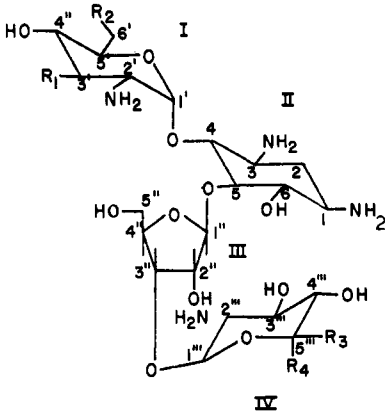
COMPOUND	R_1	R_2	R_3	R_4	R_5	R_6	R_7
GENTAMICIN B	OH	OH	OH	H	NH_2	CH_3	H
GENTAMICIN B_1	OH	OH	OH	CH_3	NH_2	CH_3	H
GENTAMICIN C_{1a}	NH_2	H	H	H	NH_2	CH_3	H
SISOMICIN	NH_2	H	$\Delta_{4'}$	H	NH_2	CH_3	H
N-ETHYL SISOMICIN	NH_2	H	$\Delta_{4'}$	H	NH_2	CH_3	CH_2CH_3

FIGURE 3: Structures of aminoglycosides related to gentamicin and sisomicins.

rate of turnover of sisomicin is more dependent on the rate of catalysis than the rate of product release. An explanation for tighter binding lies in the basicity of the 1-amino group. Due to an inductive effect, the ethyl substituent on the 1-amino group makes the secondary amine more basic than the primary one, resulting in a more positive charge at pH 7.8, to give tighter binding.

The basicity of the 1-amino group is changed in case B also, but in an opposite manner. Due to a resonance delocalization effect, a carbonyl substituent (of the 4-amino-1-hydroxybutyryl group) makes the amide less basic than the primary amine. The lowered basicity reduces the charge at pH 7.8 and gives weaker binding expressed as a small decrease in V/K. A decrease in V appears to be inconsistent reasoning, but this change is suspect because of unusual kinetics of high concentrations of amikacin.[2] (At pH 6.0, where more reliable data were obtained at high concentrations of amikacin, there is a positive change in V and negative change in V/K between kanamycin A and amikacin.)

In cases C and D, both esterification of 6″-OH and hydroxylation of the 3′-carbon facilitate binding slightly because the effects on V are negative and those on V/K, positive. Similarly, case E shows a decrease in V and increase in V/K, but unlike case A, the two changes are of comparable magnitude. This could arise if the rate of turnover of sisomicin were more dependent on the rate of catalysis than on the rate



COMPOUND	R_1	R_2	R_3	R_4
NEOMYCIN B	OH	NH_2	H	CH_2NH_2
NEOMYCIN C	OH	NH_2	CH_2NH_2	H

FIGURE 4: Structures of aminoglycosides related to neomycin.

of product release, which would contradict case A. Alternatively, a combination of effects on catalysis and binding are being expressed in case E; the double bond of sisomicin is close to the site of enzymatic reaction and is likely to affect both catalysis and binding. Thus, the single bond in gentamicin C_{1a} is more favorable for substrate binding but less favorable for catalysis than the double bond.

Case F is the only instance where both V and V/K are definitely decreased by a single structural modification. As both V and V/K change in parallel fashion, there must be definitely a reduction in catalysis. That the rate of catalysis would be affected considerably is not surprising when it is the 6′-carbon, very close to the position of enzymatic modification, that has been modified. Consistent with this interpretation are the very low activities of gentamicins XK62-2 and C_1.

Case G shows a decrease in V/K accompanied by no change in V, which suggests slightly poorer binding but little change in catalysis. The replacement of the hydroxyl group by a charged amino group at the 2′-position is deleterious to V/K and substrate binding but not to V because product release is not rate limiting with kanamycin. Despite being a modification on ring I, this modification does not affect catalysis, perhaps because it is far from the position of enzymatic modification.

The enzyme also accepts a variety of cofactors, as shown in Table III. When acetyl-CoA is compared to propionyl- and butyryl-CoA, both V and V/K decrease as the transferred acyl group itself is changed, indicating a change in catalysis, but the changes in V/K are greater than that on V, and more so with butyryl than propionyl, indicating some effect on binding as well as catalysis. When acetyl-CoA is compared to ethenoacetyl-CoA, V and V/K change differently, suggesting that it is binding and not catalysis which is affected by the addition of the five-membered ring to the adenylyl moiety. Moreover, the change in V/K is 3 times that on V, meaning that the change in the rate of substrate release is more fully expressed than the change in the rate of product release. Hence, the overall rate of the reaction is limited by catalysis and not by CoA product release.

In summary, the above analysis reveals that the turnover rate of this reaction is limited primarily by catalysis and not by the rate of release of either the acetylated aminoglycoside or CoA. Most structural differences in aminoglycosides cause

changes in rates of release. Only drastic changes in ring I, close to the reaction center, affect catalysis. Catalysis is also affected by changes in the transferrable acyl group of cofactors.

Because kanamycin acetyltransferase has the capacity to modify aminoglycosides of considerable structural variation but does not accept all aminoglycoside antibiotics, it is important to consider what structural features are required for activity. The enzyme does not seem to have an absolute requirement for hydroxyl groups at any particular position of its substrates, though all of the compounds listed do have some hydroxyl groups. Among the amino groups, the two at positions 3 and 6′ are common to all the substrates listed in Table I. It was found earlier that 3-acetylgentamicin was not a substrate for AAC(6′)-4 (Scarbrough et al., 1979) and, as noted under Results, modifications at the 6′-carbon reduce but do not abolish substrate activity.

One current approach to obtain new antibiotics for clinical use in the face of bacterial resistance involves chemical modification of existing drugs (Nara et al., 1975; Umezawa, 1982). The underlying presumption in this approach is that structural modifications can make the antibiotics invulnerable to enzymatic inactivation by resistant bacteria while allowing them to retain their antibiotic properties. Unfortunately, most of the structure–activity relationships of this enzyme parallel the antibacterial activity. For example, it has been found that 1,2-substituted deoxystreptamines (such as neomycins) are better antibiotics than 1,3-substituted deoxystreptomycins (such as kanamycins) (Benveniste & Davies, 1973). AAC(6′)-4 modifies both classes of antibiotics but has a higher V/K for the former than the latter, and thus greater resistance. Similarly the presence or absence of hydroxyl groups on ring I does not affect antibiotic activity (Benveniste & Davies, 1973) or enzymatic activity. The amino groups, on the other hand, play a predominant role in the antibiotic activity, which decreases, depending on the number and positions of amino groups, as for example (Benveniste & Davies, 1973, Cox et al., 1977)

2′,6′-diamino > 6′-amino > 2′-amino > no amino

All the good substrates of AAC(6′)-4 also have two amino groups, but at the 3- and 6′-position. Among the poorer are those which are methylated at the 6′-amino position or are methylated both at the 6′-carbon and amino positions (gentamicins XK62-2 and C_1). These are also the only modifications at the 6′-position that do not abolish antibacterial activity. Acetylation of the 3-amino group abolishes both enzymatic and antibiotic activity.

From a few differences between enzymatic and antibacterial activity, it had been suggested that alkylation at the 6′-position and hydroxylation at the 4′-position would be the most promising modifications to lower V/K. Methylation at the 6′-carbon does again lower V/K (case F, Table IV), but 4′-hydroxylation has little effect on AAC(6′)-4 (cf. Figure 1 and Table I).

The earlier study of gentamicin acetyltransferase also indicated various similarities between enzymatic and antibacterial structure–activity relationships (Williams & Northrop, 1978b). The present study strengthens the conclusion that, in the search for a means to overcome resistance, chemical modifications of existing aminoglycoside antibiotics should not be based on a reduction of enzymatic activity measured in the presence of excess antibiotic. Because the kinetic property of the enzyme that correlates with microbial resistance is V/K (Radika & Northrop, 1984d), structural modifications of aminoglycosides that reduce V/K are more promising.

Registry No. EC 2.3.1.55, 50864-41-0; acetyl-CoA, 72-89-9; kanamycin A, 59-01-8; kanamycin B, 4696-76-8; amikacin, 37517-28-5; tobramycin, 32986-56-4; nebramycin 4, 51736-76-6; gentamicin B, 36889-15-3; gentamicin B_1, 36889-16-4; gentamicin C_{1a}, 26098-04-4; sisomicin, 32385-11-8; *N*-ethylsisomycin, 56391-56-1; neomycin B, 119-04-0; neomycin C, 66-86-4; *n*-propionyl-CoA, 317-66-8; *n*-butyryl-CoA, 2140-48-9; 1,N^6-ethenoacetyl-CoA, 64755-21-1; netilmicin, 56391-56-1.

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