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Short Report

Specificity of 4‴-Acetylation by an Aminoglycoside-Modifying Enzyme in Arbekacin-Resistant Strains of Methicillin-Resistant *Staphylococcus aureus*

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FUJIMURA, S., TOKUE, Y., TAKAHASHI, H., NUKIWA, T. and WATANABE, A. *Specificity of 4‴-Acetylation by an Aminoglycoside-Modifying Enzyme in Arbekacin-Resistant Strains of Methicillin-Resistant Staphylococcus aureus.* Tohoku J. Exp. Med., 1998, **186** (1), 67-70 —— Four arbekacin (ABK)-resistant clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) were studied regarding their mechanism of aminoglycoside-resistance. Metabolites of amikacin (AMK) and gentamicin(GM) were obtained by reaction with excess amounts of crude enzyme preparation extracted from the ABK-resistant MRSA strains. Then the metabolites were then isolated and analyzed by means of ^{1}H, ^{13}C-nuclear magnetic resonance. The AMK-modification was 4‴-N-acetylation. However, the site of GM-acetylation may be 6′-N-position by these strains. ——— amikacin; MRSA; NMR; arbekacin-resistance

Arbekacin (ABK) was introduced in Japan in 1990 for the treatment of patients with infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA). Isolation of MRSA strains resistant to this antibiotics was first reported in 1994 (Hashimoto et al. 1994). The mechanism of ABK-resistance results from inactivation principally secondary to phosphorylation of the 2″-OH group and to the acetylation of the 6′-NH_2 group by the bifunctional enzyme (MW = 56 000) (Ubukata et al. 1984; Kondo et al. 1993). Recently we have identified, for the first time, a metabolite of ABK which is mono-acetylated at the 4‴-NH_2 position of the AHB group (Fujimura et al. 1998). In the present study, we examined whether 4‴-N-acetyltransferase (AAC[4‴]) would modify the AHB group of

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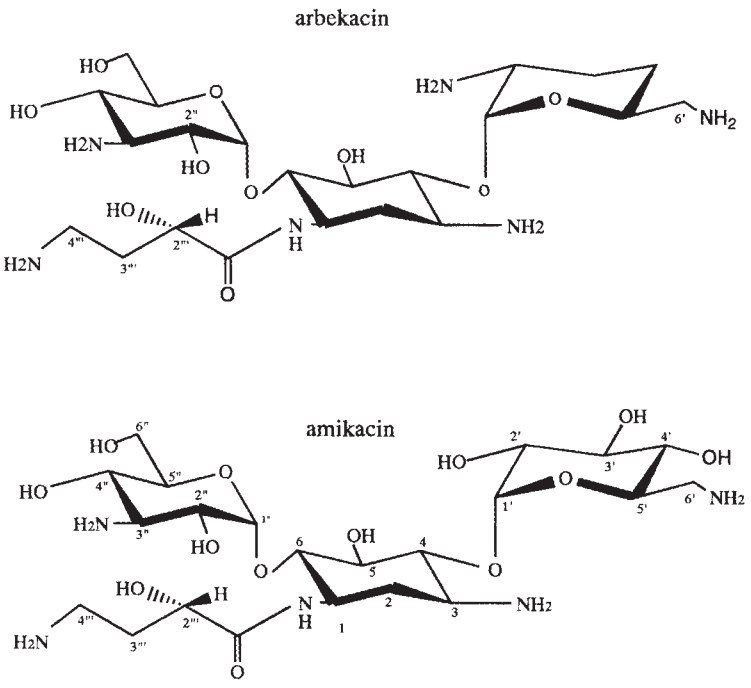

Fig. 1. Structure of arbekacin and amikacin

amikacin (AMK) (Fig. 1) compared to the non-AHB group gentamicin (GM). MRSA strains were used in this experiment and the minimum inhibitory concentrations (MICs) were as follows ABK ($>8\ \mu$g/ml), AMK (32 μg/ml) and GM ($>16\ \mu$g/ml).

We isolated inactivation products of AMK and GM by AAC (4‴) extracts from each strains. First, crude enzyme preparations were obtained by incubating a suspension of each strain in 250 ml of bacto tryptone 1% broth containing 0.5% NaCl and 0.5% yeast extract at 37°C for 8 hours on a rotary shaker. The suspension was centrifuged at 3000 rpm for 20 minutes at 4°C and the supernatant removed. The cell deposit was then washed with 0.05 M Tris-HCl-10 mM $MgCl_2$ buffer (pH 7.8). Lysostaphin, in a concentration of 25 μg/ml, was added to the suspension which was incubated for 30 minutes at 37°C. The cells were disrupted by sonication and the suspension was centrifuged at 30 000 $\times g$ for 30 minutes; the supernatant represented the crude enzyme preparation (Haas and Dowding 1975). AAC (4‴) (MW = 28 000) was purified by fast protein liquid chromatography. Enzyme reactions were carried out in a mixture of AMK (125 μg) or GM (125 μg), AAC (4‴) (42 mg/protein), acetyl CoA (0.2 mmol) and ATP (0.2 mmol) at 37°C for 18 hours (Kondo et al. 1993). The inactivated product in the reaction mixture was purified by Sep-Pak column chromatography (Waters, Milford, MA, USA). Structures of these inactivation products were assigned by nuclear magnetic resonance (NMR). NMR(^{1}H and ^{13}C) spectra were obtained with a JNS-GSX 400 spectrometer (Jeol, Tokyo), with sodium 2, 2-dimethyl-1-2-silapentane-5-sulfonate as an internal standard.

The results of the ^{1}H, ^{13}C-NMR analysis are shown in the Table 1. Proton

TABLE 1. *^{1}H-NMR and ^{13}C-NMR spectra data for AMK and modifying products of AMK*

H-δ ppm (J Hz)			C-δ ppm		
Position	AMK	Modifying products	Position	AMK	Modifying products
C1-H	4.06 m	4.09 m	C1	51.6 d	50.9 d
C2-H (ax)	1.79 m	1.95 m	C2	33.5 t	32.9 t
(eq)	2.17 ddd (12.7, 7.5, 4.0)	2.32 ddd (12.7, 7.0, 4.0)	C3	50.8 d	50.8 d
C3-H	3.43 dd (10.7, 7.5)	3.44 dd (10.9, 7.9)	C4	82.4 d	82.2 d
C4-H	4.01 dd (10.0, 4.0)	4.09 dd (10.3, 4.4)	C5	76.0 d	75.8 d
C5-H	3.88 m	3.91 m	C6	83.0 d	83.0 d
C6-H	3.86 dd (11.3, 8.3)	3.88 dd (11.2, 8.3)	C1′	99.0 d	99.1 d
C1′-H	5.58 d (4.0)	5.58 d (3.9)	C2′	73.6 d	73.5 d
C2′-H	3.68 dd (9.0, 4.0)	3.69 dd (9.0, 4.0)	C3′	74.9 d	75.0 d
C3′-H	3.79 dd (10.0, 9.0)	3.80 dd (10.1, 9.0)	C4′	73.7 d	73.8 d
C4′-H	3.37 m	3.39 m	C5′	71.3 d	71.3 d
C5′-H	4.03 ddd (10.0, 8.6, 3.2)	4.01 m	C6′	43.1 t	43.1 t
C6′-H_2	3.17 dd (13.5, 8.6)	3.17 dd (13.4, 8.6)	C1″	100.7 d	100.2 d
	3.45 dd (13.5, 3.2)	3.46 dd (13.4, 3.5)	C2″	70.9 d	70.8 d
C1″-H	5.17 d (4.0)	5.21 d (4.0)	C3″	57.9 d	57.8 d
C2″-H	3.77 dd (10.0, 4.0)	3.77 dd (10.0, 3.8)	C4″	68.5 d	68.4 d
C3″-H	3.37 dd (10.0, 9.0)	3.37 dd (10.0, 9.0)	C5″	74.7 d	74.7 d
C4″-H	3.67 dd (10.0, 9.0)	3.62 dd (10.0, 8.9)	C6″	62.5 t	62.5 t
C5″-H	4.07 ddd (10.0, 3.2, 3.2)	4.05 m	C1‴	178.1 s	177.2 s
C6″-H_2	3.80 m	3.83 m	C2‴	72.3 d	69.9 d
	3.82 m	3.84 m	C3‴	33.6 t	31.5 t
C2‴-H	4.28 dd (9.0, 4.0)	4.48 dd (9.0, 3.8)	C4‴	39.6 t	37.4 t
C3‴-H_2	1.98 ddd (14.6, 9.0, 7.0)	2.30 m			
	2.18 ddd (14.6, 7.0, 4.0)	2.45 m	$NCOCH_3$	—	177.0 s
C4‴-H_2	3.17 t (7.0)	3.47 t (7.0)	$NCOCH_3$	—	22.6 q
$NCOCH_3$	—	2.20 s			

s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; NMR, nuclear magnetic resonance.

Chemical shift (δ): ppm from sodium 2, 2-dimethyl-1-2-silapentane-5-sulfonate in D_2O at 400 MHz.

signal changes were not identified at position 2″ or 6′, but signals at position 2‴, 3‴ and 4‴ of the AHB group shifted towards the lower magnetic field in AMK. Carbon signals at the same positions of the AHB group were grown shifted towards the higher magnetic field. However, GM which did not have an AHB group in its chemical constitution was shifted towards the lower magnetic field at position 6′ (data not shown). ^{1}H, ^{13}C-NMR demonstrated that a chemical shift of 2.20 ppm (^{1}H) and 177.0, 22.6 ppm (^{13}C) indicated an acetyl group. In the pre-

sent study, aminoglycoside (AGs)-modifying enzyme included in these strains is 6′-acetyltransferase (AAC [6′]), but has specificity at 4‴-NH_2 group of ABK and AMK.

It has not been elucidated whether this AGs-modifying enzyme is a new enzyme or a mutant of AAC (6′) and APH (2″)/AAC (6′) (Le Goffic et al. 1977; Scott et al. 1978; Ubukata et al. 1984). These issues are currently under investigation.

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