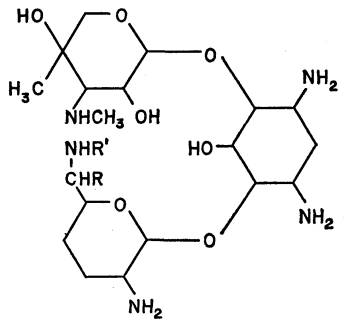
ENZYMATIC INACTIVATION OF GENTAMICIN C COMPONENTS BY CELL-FREE EXTRACT FROM *PSEUDOMONAS AERUGINOSA*

Sir:

Gentamicin C, a broad spectrum antibiotic complex, has been isolated from submerged cultures of *Micromonospora purpurea*[1]. The available form of gentamicin is a mixture of three components called gentamicins C_1, C_2 and C_{1a}[2]. The structures of these components have been determined and were described by COOPER *et al.*[3,4], as shown in Fig. 1.

Fig. 1. Gross structures of the gentamicin C components



gentamicin C_1, R=R'=CH_3
gentamicin C_2, R=CH_3, R'=H
gentamicin C_{1a}, R=R'=H

The lower sugar in the figure is a 2,3,4,6-tetradeoxyamino sugar, a novel compound which has been designated purpurosamine, and contains no hydroxyl groups in the rings. By contrast, several other 2-deoxystreptamine-containing aminoglycosidic antibiotics including kanamycin, neomycin and paromomycin contain a hydroxyl group at position 3 of the amino sugar. This difference is considered to be of biological significance, because several other aminoglycosidic antibiotics were found to be inactivated by the strains of bacteria resistant to the drugs. The inactivating enzymes were known to be capable of specifically phosphorylating[5~10] antibiotics at the 3-hydroxy position of the amino sugar or acetylating kanamycin on the amino group of the amino sugar[5,11]. Similarly, we reported that a kanamycin-phosphorylating enzyme from a resistant strain of *Pseudomonas aeruginosa* inactivated paromomycin, mannosylparomomycin, amikanodeoxykanamycin and neomycin but did not inactivate lividomycin[12] and gentamicin C components, which were devoid of a hydroxy group at position 3 of the amino sugar[13]. TANAKA[14] also reported that gentamicin C components were not inactivated by gentamicin-resistant strains of *P. aeruginosa*; the resistance mechanism being a decrease to the sensitivity of ribosome to the drug.

Recent studies in this laboratory have disclosed that the enzyme from gentamicin-resistant *P. aeruginosa* inactivated the gentamicin C components in the presence of magnesium acetate, adenosinetriphosphate and coenzyme A. Crude cell-free extracts from the organism were prepared by the method described previously[13]. The organism was harvested by centrifugation, washed

Table 1. Inactivation of gentamicin C components by *P. aeruginosa*

Bacterial strain	MIC * (mcg/ml)	Percent inactivation		
		C_1	C_2	C_{1a}
P. aeruginosa 99	>200	99	97	98
P. aeruginosa cape 18	200	70	70	90
P. aeruginosa TK-157	6.25	2	0	0
P. aeruginosa TI-13	3.13	4	0	0

The reaction mixture contained: 0.3 ml of the S-105 fraction (10 mg protein per ml), 0.1 ml of 40 mM disodium adenosinetriphosphate (ATP), 0.1 ml of 2.0 mM coenzyme A(CoA), 0.1 ml of 1.0 mM antibiotic and 0.4 ml of TMK solution. After 3 hours of incubation at 30℃, the reaction was stopped by heating the mixture at 80℃ for 5 minutes. The antibiotic activity in the reaction mixture was determined by the paper-disk method.

* MIC: minimum inhibitory concentration of gentamicin C.

Table 2. Requirement for the inactivation of gentamicin C by the cell-free extract of *P. aeruginosa* 99

Reaction mixture	Percent inactivation		
	C_1	C_2	C_{1a}
Complete system[a]	97.6	97.0	99.0
-(S-105)	0	0	0
-ATP	0	0	0
-(Acetate, CoA)[b]	0	0	0
(S-105), acetyl CoA[c]	97.5	96.2	94.7

The inactivating reaction was carried out at 30℃ for 1 hour and the remaining activity of the antibiotic was assayed.

a: see the footnote of Table 1.

b: Magnesium chloride was used instead of magnesium acetate in TMK solution.

c: ATP, CoA and acetate were replaced by 0.2 ml of 5.0 mM acetyl CoA.

The same buffer as shown in *b* was used.

three times with M/10 TMK solution (M/10 tris buffer containing 0.06 M KCl, 0.01 M magnesium acetate and 0.006 M 2-mercaptoethanol, pH 7.8) and resuspended in the same buffer. The cell suspension was disrupted sonically at 20 Kc for 10 minutes. After treatment with deoxyribonuclease (4 mcg/ml) at 37°C for 15 minutes, the sonicated suspension was centrifuged at $10,000 \times g$ for 20 minutes. The supernatant thus obtained was subjected to further centrifugation at $105,000 \times g$ for 60 minutes and the supernatant was dialyzed for 2 hours against cold TMK solution. The solution was designated the S-105 fraction. As shown in Table 1, gentamicin C components were inactivated by the S-105 fraction prepared from gentamicin-resistant strains but not by fractions from gentamicin-sensitive strains.

Next we examined the requirements for the inactivation of gentamicin C components. As can be seen in Table 2, the inactivating activity was lost without ATP or without both coenzyme A and acetate, but restored completely by addition of the three agents, *i.e.*, ATP, coenzyme A and acetate. But these three agents could be replaced by acetyl coenzyme A. From these results, it is concluded that gentamicin C components are inactivated by acetylation. The inactivation mechanism and the inactivated position of each of the gentamicin C components is still being elucidated and will be described elsewhere.

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