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

Sir:

Gentamicin C, a broad spectrum antibiotic complex, has been isolated from submerged cultures of *Micromonospora purpurea*. The available form of gentamicin is a mixture of three components called gentamicins C₁, C₂ and C₃. The structures of these components have been determined and were described by Cooper et al., as shown in Fig. 1.

![Gentamicin C Components](image)

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.

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. The organism was harvested by centrifugation, washed and used to inactivate the gentamicin C components. 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°C, the reaction was stopped by heating the mixture at 80°C for 5 minutes. The antibiotic activity in the reaction mixture was determined by the paper-disk method.

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

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>MIC (mcg/ml)</th>
<th>Percent inactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aeruginosa</em> 99</td>
<td>&gt;200</td>
<td>99 97 98</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> cape 18</td>
<td>200</td>
<td>70 70 90</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> TK-157</td>
<td>6.25</td>
<td>2 0 0</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> TI-13</td>
<td>3.13</td>
<td>4 0 0</td>
</tr>
</tbody>
</table>

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°C, the reaction was stopped by heating the mixture at 80°C for 5 minutes. The antibiotic activity in the reaction mixture was determined by the paper-disk method.

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

<table>
<thead>
<tr>
<th>Reaction mixture</th>
<th>Percent inactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete system</td>
<td>97.6 97.0 99.0</td>
</tr>
<tr>
<td>- (S-105)</td>
<td>0 0 0</td>
</tr>
<tr>
<td>- ATP</td>
<td>0 0 0</td>
</tr>
<tr>
<td>- (Acetate, CoA)</td>
<td>0 0 0</td>
</tr>
<tr>
<td>(S-105), acetyl CoA</td>
<td>97.5 96.2 94.7</td>
</tr>
</tbody>
</table>

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

### Notes
- 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 8.0 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 x g for 20 minutes. The supernatant thus obtained was subjected to further centrifugation at 105,000 x 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.

Acknowledgement

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References


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