Lytic Effect of Mycobacillin and Its Derivatives on Erythrocytes

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Mycobacillin as well as its O-acetyl (tyrosyl and seryl) and ester derivatives is known to have antifungal activity. The antibiotic also possesses hemolytic activity. This latter property is not appreciably altered by partial or complete esterification of its free carboxyl groups. On the other hand, acetylation of the molecule at the two tyrosyl hydroxyl groups nullifies selectively the hemolytic activity.

Mycobacillin, an antifungal antibiotic (5), is inactivated to more than 90% in the presence of serum (1). A few ester and two acetyl derivatives of this cyclic peptide were prepared to overcome this serum inactivation, and their antifungal activity was studied (2, 3). It has recently been observed that mycobacillin causes hemolysis. This communication describes the effect of mycobacillin and a number of its derivatives on erythrocytes.

Mycobacillin was purified from the culture filtrate of the producer organism, Bacillus subtilis B₃ (6). Its ester derivatives were prepared with acidic alcohols as esterifying agents, and their purity was checked by thin-layer chromatography and paper electrophoresis (3). Diacet- ylation at two tyrosine hydroxyl groups of mycobacillin was carried out with acetic anhydride at a constant pH of 7.4 and at 1 to 2°C. The triacetyl derivative was prepared by acetylating all the hydroxy groups (two from tyrosine and one from serine) of mycobacillin with acetic anhydride in the presence of pyridine at 100°C. The acetyl derivatives were homogeneous by paper chromatography (2). Antifungal activity of the compounds was tested against Aspergillus niger spores in liquid media as described earlier (2). Erythrocytes were collected from albino rats, and diluted suspensions of the cells in physiological saline were prepared as described by Dimick (4) for measuring hemolytic activity. Stock solutions (10 mg/ml) of the test compounds were prepared in 95% ethanol, and the solutions were diluted with the same, as required. A 0.5-ml portion of a diluted solution of the compounds was added to 10 ml of erythrocyte suspension, and hemolysis was determined with a Klett-Summerson colorimeter at 660 nm by the method of Dimick (4).

Table 1 shows the minimum concentrations required for complete hemolysis by mycobacillin and its derivatives. Complete hemolysis is defined as a fall of 80 Klett units within 3 min in an erythrocyte suspension having an initial optical density of about 85 Klett units at 660 nm. Hemolysis occurred very quickly and was almost complete within 2 min. It can be seen in Table 1 that the esters were fairly hemolytic. All the esters retained 79 to 90% of the parental hemolytic activity, except the methyl ester. The methyl ester was the only fully esterified product of mycobacillin, and it retained 63% of the latter's hemolytic activity. It was reported earlier (3) that all the ester derivatives have antifungal activity. The methyl ester and mycobacillin had minimum inhibitory concentrations of 24 to 28 mg/ml and 20 mg/ml, respectively, as compared with A. niger, i.e., the derivative was approximately 70% as active as mycobacillin. Thus, the properties of this ester derivative strongly suggest that the seven carboxyl groups of mycobacillin may not be required for its antifungal and hemolytic actions.

The effect on erythrocytes of two acetyl derivatives and mycobacillin at different concentrations is shown in Fig. 1. Mycobacillin caused complete hemolysis at a concentration of 25 μg/ml, but the acetyl derivatives showed only slight activity at 60 μg/ml, remaining almost constant at higher concentrations (Fig. 1). It is also evident that the change in hemolytic activity of mycobacillin was mainly due to blocking of the tyrosyl hydroxyl groups in the diacetyl derivative; additional blocking of the serine hydroxyl group in the triacetyl derivative had an insignificant effect on activity. Therefore, it may be assumed that the tyrosyl hydroxyl groups play a key role in the expression of the hemolytic activity of mycobacillin.

Previously it was reported that the minimum
Table 1. Minimum concentration of mycobacillin and its derivatives required for complete hemolysis

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mol of ester or acetyl groups/mol of derivative</th>
<th>Concentration (μg/ml)</th>
<th>Concentration (nmol/ml)</th>
<th>Hemolytic activity retained (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycobacillin</td>
<td></td>
<td>25.0</td>
<td>16.3</td>
<td>100</td>
</tr>
<tr>
<td>Methyl esterc</td>
<td>6.88</td>
<td>42.5</td>
<td>26.1</td>
<td>63</td>
</tr>
<tr>
<td>Ethyl esterc</td>
<td>3.05</td>
<td>30.0</td>
<td>18.6</td>
<td>88</td>
</tr>
<tr>
<td>Ethyl esterd</td>
<td>5.22</td>
<td>32.5</td>
<td>19.5</td>
<td>84</td>
</tr>
<tr>
<td>n-Propyl esterc</td>
<td>3.00</td>
<td>30.0</td>
<td>18.1</td>
<td>90</td>
</tr>
<tr>
<td>n-Propyl esterd</td>
<td>5.10</td>
<td>32.5</td>
<td>18.7</td>
<td>87</td>
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<tr>
<td>n-Butyl esterc</td>
<td>2.86</td>
<td>32.5</td>
<td>19.2</td>
<td>85</td>
</tr>
<tr>
<td>n-Butyl esterd</td>
<td>5.30</td>
<td>37.5</td>
<td>20.7</td>
<td>79</td>
</tr>
<tr>
<td>iso-Amyl esterc</td>
<td>2.85</td>
<td>35.0</td>
<td>20.1</td>
<td>81</td>
</tr>
<tr>
<td>iso-Amyl esterd</td>
<td>5.10</td>
<td>37.5</td>
<td>20.0</td>
<td>81</td>
</tr>
<tr>
<td>Diacetyl derivatice</td>
<td>1.92</td>
<td>170</td>
<td>124.0</td>
<td>13</td>
</tr>
<tr>
<td>Triacetyl derivatice</td>
<td>3.06</td>
<td>180</td>
<td>120.8</td>
<td>13</td>
</tr>
</tbody>
</table>

* a A fall of 80 Klett units in 3 min from an initial optical density of 85 Klett units was taken for complete hemolysis.
* b Calculated from the concentrations in nanomoles per milliliter.
* c Soluble in water.
* d Insoluble in water.
* e Complete hemolysis by the acetyl derivatives was not obtained at concentrations of up to 200 μg/ml.

It is now found that these acetyl derivatives are not hemolytic. Thus, the two acetyl derivatives of mycobacillin, primarily the diacetyl one, demonstrate a complete segregation of antifungal and hemolytic activities. This segregation of properties suggests a difference in the mode of action of the antibiotic towards erythrocytes and fungi.

LITERATURE CITED