The Neutral and the Acid Proteinase from Calf Bone Marrow

V. Turk, V. Cotič, M. Kopitar, and D. Lebez

Nuclear Institute "Jožef Stefan", Ljubljana, Yugoslavia


A neutral proteinase was found in many organs and cells, among others in thymus\(^1\), mast cells\(^2\), leucocytes\(^3\) and erythrocytes\(^4,5\). Only few data concerning the proteinase of bone marrow are available. Lapersle and Webb\(^6,7\) isolated cathepsin D and E from rabbit bone marrow and spleen and investigated some of their properties. In this laboratory\(^8,9\) investigated cathepsin activity in human and rat bone marrow. They found \(p_H\) optima at 3.5 and 6.0 to 6.5 with hemoglobin as substrate. In our previous experiments the neutral proteinase was not studied in details yet. Some preliminary results of further investigations of neutral proteinase are given.

Bone marrow of one to eight weeks old Jersey strain calves was used in the experiments. Bone marrow was taken away from the medular channel of metatarsal immediately after slaughtering and kept below 0 °C. Purified enzyme was prepared by the modified method of Lapersle and Webb\(^6\) for the preparation of cathepsin D from rabbit spleen. The only modification of this method was, that the precipitation was not carried out with two volume parts of saturated ammonium sulphate solution but with the solid ammonium sulphate until the 70% of saturation was achieved. All further purification on DEAE and CM cellulose was performed according to the method of Lapersle and Webb. Proteolytic activity was measured by the A ns o n’s method\(^10\). The proteolytic activity in dependence of \(p_H\) of the purified enzyme preparation is shown in Fig. 1. Two distinct \(p_H\) optima were found, at \(p_H\) 3.2 and 7.5, respectively. The proteolytic activity of the enzyme was followed at both \(p_H\) optima immediately, four and ten days after preparation. During this period the enzyme was kept at —25 °C. The results are shown in Table I. The measurements have shown that enzyme activity of acid proteinase remains unaltered. This proteinase is cathepsin D, which was isolated from rabbit spleen and bone marrow\(^6,7\), and from bovine spleen\(^11,12\). The neutral proteinase on the contrary shows great instability though it was kept under the same conditions as acid proteinase. A 60% decrease of enzyme activity after 4 days and complete loss of activity after 10 days was found.

On the base of our experiments we cannot draw any conclusions concerning the reasons of instability of neutral proteinase from bone marrow. Unstable neutral proteinase was found in the brain\(^13\) and in peripheral nerves\(^14\).

This work was supported by the Yugoslav Nuclear Energy Commission, Contract No. 03-1063/3a.

2. D. Lagunoff and E. P. Benditt, Amm. N. Y. Acad. Sci. 103, 185 [1963].

Table I. Stability of proteinases from calf bone marrow in different time-intervals after preparation.

<table>
<thead>
<tr>
<th>Proteinase</th>
<th>(E_{150}/0.4) ml of sample ((\gamma \cdot N/ml = 1120))</th>
<th>immediately</th>
<th>4 days</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid</td>
<td>0.335</td>
<td>0.332</td>
<td>0.332</td>
<td></td>
</tr>
<tr>
<td>Neutral</td>
<td>0.582</td>
<td>0.237</td>
<td>0.015</td>
<td></td>
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</tbody>
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Fig. 1. \(p_H\) optima of the purified proteinases from calf bone marrow. Substrate: 2% bovine hemoglobin, prepared in 0.1 M acetate, phosphate and borate buffers.