Purification of Bovine Spleen Collagenolytic Cathepsin (Cathepsin N)

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The lysosomal enzymes cathepsin B and collagenolytic cathepsin are believed to have a physiological role in the turnover and resorption of collagen (Etherington, 1977). Collagenolytic cathepsin has been purified from human placental tissue (Evans & Etherington, 1978), but in previous studies of the bovine enzyme only a partially purified preparation was available. A method has now been developed for the purification of bovine collagenolytic cathepsin of high specific activity and with a good recovery. The enzyme becomes unstable during the later stages of purification, which had frustrated these earlier attempts to obtain pure enzyme. The present method is shown schematically in Fig. 1.

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**Fig. 1. Flow diagram summarizing the purification procedure for bovine spleen collagenolytic cathepsin**

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1978
Table 1. **Purification of bovine spleen collagenolytic cathepsin**

Data obtained for 680g of minced spleen.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Protein (mg)</th>
<th>Collagenolytic activity (units)</th>
<th>$10^{-3} \times$ Specific activity (units/mg of protein)</th>
<th>Purification</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen extract (pH 3.8)</td>
<td>35200</td>
<td>32.7</td>
<td>0.93</td>
<td>(1)</td>
<td>(100)</td>
</tr>
<tr>
<td>NaCl (2.9M)</td>
<td>3150</td>
<td>24.9</td>
<td>7.9</td>
<td>8.5</td>
<td>76.1</td>
</tr>
<tr>
<td>Chromatography on Amberlite CG-50</td>
<td>1450</td>
<td>16.3</td>
<td>11.2</td>
<td>12.1</td>
<td>49.8</td>
</tr>
<tr>
<td>Chromatography on CM-Sephadex C-50</td>
<td>93</td>
<td>14</td>
<td>151</td>
<td>162</td>
<td>42.8</td>
</tr>
<tr>
<td>Affinity chromatography on organomercurial Sepharose</td>
<td>39</td>
<td>14.5</td>
<td>372</td>
<td>400</td>
<td>44.3</td>
</tr>
<tr>
<td>Chromatography on DEAE-Sephadex A-50</td>
<td>12</td>
<td>10.2</td>
<td>850</td>
<td>914</td>
<td>31.2</td>
</tr>
<tr>
<td>Gel filtration on Sephadex G-75</td>
<td>6</td>
<td>7.1</td>
<td>1183</td>
<td>1270</td>
<td>21.7</td>
</tr>
</tbody>
</table>

Spleens were homogenized and the extract acidified to pH3.8 as described previously (Etherington, 1976). The earlier fractionation step with (NH$_4)_2$SO$_4$ was omitted, instead NaCl was used to remove much of the contaminant protein (Ducastaing et al., 1976). The pH of the extract was adjusted to 3.2 with 2M-HCl and solid NaCl added slowly with stirring to a final concentration of 2.9M. After 1 h the precipitated protein was removed by centrifuging at 10000g for 20 min. The supernatant was dialysed and (NH$_4)_2$SO$_4$ added to 90% saturation. The precipitated protein was redissolved and dialysed against 1mM-EDTA and 1mM-2-mercaptoethanol. Collagenolytic cathepsin was separated from cathepsin B by chromatography on Amberlite CG-50 (Etherington, 1976), and then precipitated with (NH$_4)_2$SO$_4$ to 90% saturation.

The concentrated dialysed enzyme was next applied to a column (2.5cm×30cm) of CM-Sephadex C-50, and fractionated by the method of Kirschke et al. (1977a,b) for the separation of cathepsins H and L. Collagenolytic cathepsin was eluted at the position of rat liver cathepsin H and it was confirmed that this protein peak possessed activity against azocasein, which is a substrate for this enzyme.

The peak containing collagenolytic activity was concentrated by affinity chromatography on organomercurial-Sepharose (Barrett, 1973; Etherington, 1976), dialysed against 10mM-sodium phosphate buffer, pH6.5, containing 1mM-EDTA and 0.5mM-dithiothreitol and chromatographed on a column (2.5cm×35cm) of DEAE-Sephadex A-50 as previously described (Etherington, 1976). The enzyme peak was dialysed and concentrated by freeze-drying. A concentrated solution of collagenolytic cathepsin (2ml) was applied finally to a column (1.5cm×94cm) of Sephadex G-75 equilibrated with 0.15M-sodium acetate, pH4.5, containing 0.1M-NaCl, 1mM-EDTA and 0.5 mM-dithiothreitol. For gel filtration the column was calibrated with proteins of known molecular weight in the same buffer (Etherington, 1976). By using this gel-filtration step, collagenolytic cathepsin was separated from a residual trace of activity against Bz-Arg-2-naphthylamide,* which was eluted immediately before the main enzyme.

During purification the fractions were tested for activity against collagen (Etherington, 1972) and Bz-Arg-2-naphthylamide (Cathepsin B) (Barrett, 1972) and for protein with bovine serum albumin as the standard (Lowry et al., 1951). The detailed results for the purification of bovine spleen collagenolytic cathepsin are given in Table 1. The final preparation of enzyme had no detectable activity against Bz-Arg-2-

* Abbreviation: Bz, benzoyl.
naphthylamide, which is a substrate for both cathepsins B and H. Cathepsin B will degrade collagen and part of the measured collagenolytic activity in the spleen extract was due to this enzyme. Therefore, by virtue of this, the recovery value of 21.7% for collagenolytic cathepsin was artificially low.

The molecular weight of collagenolytic cathepsin was calculated from gel filtration on Sephadex G-75 and found to be in the range 18,500–20,000. A single protein band was observed after sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and for which a mol.wt. of 18,000–20,000 was obtained by the method of Weber & Osborn (1970). The pI of the purified enzyme was obtained by isoelectric focusing in an Ampholine gradient (Etherington, 1976) and found to be pH 6.55.

This method for the purification of collagenolytic cathepsin has enabled all final traces of cathepsins B and H to be eliminated. The data for the physicochemical properties of the purified enzyme were almost identical to the previously published values (Etherington, 1976). In order that collagenolytic cathepsin may now be recognized as different from all other known cathepsins, we have renamed the enzyme cathepsin N.


Purification and Compositional Relatedness of Aldehyde Reductase from Several Species

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A low-molecular-weight broadly specific NADPH-dependent aldehyde reductase has been purified and characterized from several mammalian species (Culp & McMahon, 1968; Tabakoff & Erwin, 1970; Bosron & Prairie, 1972; Turner & Tipton, 1972; Ris & von Wartburg, 1973; Kawalek & Gilbertson, 1976; Wermuth et al., 1977). The only systematic study to date on the species and tissue distribution of the enzyme has also been solely concerned with species of the Class Mammalia (Bosron & Prairie, 1973). We have made a wider survey and have found aldehyde reductase present in representative species of several classes (Davidson et al., 1978). In addition, close examination of the reductase from pig kidney (Flynn et al., 1975) and human liver (Wermuth et al., 1977) has revealed a striking similarity between this enzyme and another monomeric oxidoreductase octopine dehydrogenase, which is found in the mollusc Pecten maximus (Thoai et al., 1969). Work in this laboratory has recently been concerned with defining more closely the relationship of aldehyde reductase to octopine dehydrogenase and oligomeric dehydrogenases such as lactate dehydrogenase. We report in the present paper the purification and amino acid compositions of aldehyde reductase from four species and compare these compositions with that of pig kidney aldehyde reductase and octopine dehydrogenase.

The enzyme was purified to homogeneity from rat, chicken, fruit fly and yeast. Methods