lysosomal enzymes. N-acetyl-β-glucosaminidase, β-galactosidase, acid phosphatase, myeloperoxidase, and lysozyme, as well as for cytochrome oxidase, S-ε-nucleotide and neutral α-glucosidase. In addition, the distribution of activity against [3H]leucinein and [3H]acetylated casein and N-benzylxoycarbonyl-β-alanine 2-naphthyl ester, a synthetic substrate used for elastase determinations, was examined. The majority of the activity of each of these enzymes was found within the gradient.

The profiles of the lysosomal enzymes N-acetyl-β-glucosaminidase, β-galactosidase and myeloperoxidase were similar and showed a peak density of 1.21 g/cm³. Interestingly, the lysozyme activity appears to be in a different granule at a modal density of 1.20 g/cm³ and acid phosphatase is in a third granule corresponding to a modal density of 1.17 g/cm³. Although differences were apparent between the distribution profiles of these individual enzymes, they appear distinct from the mitochondria (cytochrome oxidase, modal density 1.16 g/cm³), plasma membrane (S-ε-nucleotide, 1.16 and 1.105 g/cm³) and endoplasmic reticulum (neutral α-glucosidase, 1.145 g/cm³). Myeloperoxidase appears to have a dual localization as a result of a possible association with the endoplasmic reticulum. The modal density of the activity against [3H]acetylated casein, [3H]leucinein and N-benzylxoycarbonyl-β-alanine 2-naphthyl ester was identical with that observed for the lysosomal marker enzymes at density 1.21 g/cm³. Although the acid hydrolase and neutral proteinase activities all appear to be in granules of similar behaviour there are slight differences between all the profiles, which may suggest that these enzymes exist in primary granules rather than in secondary lysosomes. The latency of N-acetyl-β-glucosaminidase activity was approx. 60% in homogenates from human monocytes and a similar value was calculated for neutral proteinase activity against [3H]acetylated casein. These observations indicate that the neutral proteinase activity, as well as the acid hydrolase activity, is normally constrained within the membrane-bound granules termed lysosomes.


The isolation of three thiol proteinases from human kidney that attack glomerular basement membrane

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The lysosomal thiol proteinases are thought to play a major role in the turnover of collagen in mammalian tissues (Burleigh; 1977). It has been reported that highly purified cathepsin B from human liver solubilizes collagen (type IV) from human glomerular basement membrane (Davies et al., 1978). To investigate this finding further, the purification of thiol proteinases from normal human kidney has been undertaken.

Cortex from normal kidneys was homogenized in a solution containing 1% (w/v) NaCl, 2% (v/v) butan-1-ol and 1mM EDTA, adjusted to pH 5.0 with HCl, and centrifuged at 6000 g for 1h. The pellet was extracted with 0.2% Triton X-100 overnight at 4°C and the procedure was repeated until no activity against azocasein at pH 6.0 was detected in the supernatant. The majority of the activity was found in the supernatant. The purified thiol proteinases were characterized by their ability to attack azocasein and low-molecular-weight substrates.

Table 1. Activity of cathepsins B, H and L isolated from human kidney against azocasein and low-molecular-weight substrates

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Conc. (mM)</th>
<th>pH</th>
<th>B</th>
<th>H</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azocasein</td>
<td>1.5</td>
<td>6.0</td>
<td>3.2</td>
<td>0.56</td>
<td>5.6</td>
</tr>
<tr>
<td>Arg-2-NNap</td>
<td>2.5</td>
<td>6.0</td>
<td>0.33</td>
<td>4.34</td>
<td>n.d.</td>
</tr>
<tr>
<td>Leu-2-NNap</td>
<td>2.5</td>
<td>6.0</td>
<td>0.17</td>
<td>1.67</td>
<td>n.d.</td>
</tr>
<tr>
<td>Bz-Arg-2-NNap</td>
<td>2.25</td>
<td>6.0</td>
<td>9.02</td>
<td>3.01</td>
<td>0.05</td>
</tr>
<tr>
<td>Z-Arg-2-NNap</td>
<td>0.0156</td>
<td>6.0</td>
<td>123.5</td>
<td>0.33</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Enzymic activity (units/ml) of cathepsin:

Use of s-ρ techniques in the study of heterogeneity in organelles

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Heterogeneity of rat kidney-cortex lysosomes fractionated by gradient centrifugation in zonal rotors

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Some of the earliest work on the subfractionation of kidney organelles (Straus, 1956, 1964) showed that the kidney lysosomes were heterogeneous in terms of their enzyme content and morphology. Although this heterogeneity has since been noted in fractionation studies on the kidney cortex (Wattiaux-de Guilford, Surrey, GU2 SXH, U.K.) for a gift of Z-Arg-Arg-2-Nap.

Ducastel, A. & Eberthuing, D. J. (1978) Biochem. Soc. Trans. 6, 938–940

The classical reference fractions and the subfractions from the zonal spin were assayed for protein and a range of marker enzymes. Protein, glucose 6-phosphatase, 3'-nucleotidase, succinate dehydrogenase, catalase, acid β-galactosidase, acid ribonuclease, cathepsin D, acid β-galactosidase and acid bis-p-nitrophenolphosphatase were assayed and corrected for sucrose inhibition as previously described (Andersen et al., 1979). N-Acetyl-β-glucosaminidase was assayed as described by Hultberg & Öckerman (1972) and alkaline p-nitrophenolphosphatase by the method of Hübscher & West (1965).

The 'ML' fraction was subjected to rate sedimentation in an HS zonal rotor (MSE Scientific Instruments, Crawley, Sussex, U.K.), which contained a 550 mL exponential sucrose gradient ranging from 0.5M to 1.7M and 150 mL of sucrose as the cushion. After loading the re-suspended 'ML' fraction into the rotor it was spun at 8000 rev./min for 1 h and 20 mL fractions were finally collected for the various assays. The results of the acid hydrolyase distributions indicate that two distinct populations of lysosomes are present in the 'ML' fraction. The faster-sedimenting lysosomal band shows a high purification of acid hydrolyases and an absence of other markers, indicating virtually no contamination with other organelles. The distribution patterns of acid hydrolyases in this region differ only slightly but the range of relative specific activities shows distinct variation, i.e. cathepsin D = 39.9, β-galactosidase = 20.8, N-acetyl-β-glucosaminidase = 14.9. Also, from the total turnover of glomerular basement membrane under normal physiological condition.

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