Glycosidase activities in hog serum, optic nerve, and ocular tissues

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ABSTRACT

Using spectrophotometric assay, we have studied the distribution of α-D-mannosidase, β-D-galactosidase, and N-acetyl-β-D-glucosaminidase in hog serum, the aqueous humor, optic nerve, retina, and uvea (iris, ciliary body, choroid). N-acetyl-β-D-glucosaminidase was the most active glycosidase in all tissues that we studied. The highest activity of α-D-mannosidase was found in the extracts prepared from the iris, while β-D-galactosidase and N-acetyl-β-D-glucosaminidase were the most active in the extracts of the choroid and ciliary body, respectively. The aqueous humor and serum had several times lower specific activity of glycosidases than did the extracts of ocular tissues or optic nerve. There was no significant difference between the activity of these enzymes in the aqueous humor and serum. High activity of glycosidases in the optic nerve suggests their role for intrinsic axonal repair. Retinal extracts had the smallest glycosidase activity of all ocular extracts prepared. The physiological role of these findings is discussed.

Lysosomal enzymes degrade carbohydrates, lipids, nucleic acids, proteins, peptides, and other molecules into smaller fragments. These enzymes digest aged or defective organelles and other constituents — including microorganisms — that are transferred to lysosomes by endocytosis but they may have an extracellular role. Released by exocytosis or lysis of some migratory cells, the enzymes can attack neighboring or distant tissues at acid pH. Under normal conditions, their degrading action is well regulated to hydrolyze regular amount of substrates, which are involved in diverse physiological processes, from fertilization (4) to aging (2). In various pathological conditions, such as tissue damage, inflammation or lysosomal storage disorders, degradation by these enzymes may be excessive or impaired (9, 13, 14).

Glycosidases, a group of lysosomal enzymes, are widely distributed in ocular tissues (6, 7, 10). These enzymes may be involved in various eye diseases, such as storage diseases, retinal degeneration, uveitis, cataract progression, and glaucoma. In aqueous humor (8, 17) and in blood serum (5), glycosidases were separately studied, but we have not found any reference on parallel estimation of these enzymes in two fluids under physiological conditions. In the present study we investigated distribution of three glycosidases, α-D-mannosidase, β-D-galactosidase, and N-acetyl-β-D-glucosaminidase, in hog serum and aqueous humor. We also examined the activities of these enzymes in the optic nerve, retina and uvea (iris, ciliary body, and choroid).

Eyes and blood from freshly slaughtered pigs were obtained from a local slaughterhouse and kept on ice during transfer to the laboratory. The aqueous humor was removed using a needle and syringe through a corneal puncture and centrifuged at 2,000 × g for 5 min and supernatant fluid was used to assay various acid hydrolases. Dissection of the eyeball and all subsequent procedures were performed at 4°C, within 60 min of death. The parts of two
eyes were removed and washed with 0.9% NaCl at 4°C, transferred into a small beakers containing 0.2 M Tris buffer, pH 7.4 and Triton X-100 0.1%, miniced with scissors, and homogenized with a hand-operated glass homogenizer. The detergent Triton X-100 induces complete extraction of the glycosidases and does not interfere with the assay system (11). The homogenate (1 : 50, w/v) was then sonicated for 30 sec. Homogenates were centrifuged at 13,000 × g for 10 min. Samples were assayed within one hour of paracentesis or homogenization.

The substrate solutions used in assaying glycosidase activities were prepared as described by Marinkovic et al. (12): 6 mM p-nitrophenyl α-d-mannoside in citrate/phosphate buffer, pH 5.20; 2 mM p-nitrophenyl β-galactoside in citrate/phosphate buffer, pH 4.00; 2 mM p-nitrophenyl N-acetyl-β-d-glucosaminide in citrate/phosphate buffer, pH 4.50. These substrates were obtained from Sigma (St. Louis, MO). A portion (0.1 ml) of the substrate solution was hydrolyzed with 50 µL of the homogenate, serum (1 : 40 dilution) or aqueous humor. The blank contained the same amount of substrate solution, but water instead of an enzyme source. After incubation at 40°C for 60 min, the reaction was stopped by the addition of 0.1 ml of 2 M NH₄OH and diluted with water to a total volume of 1 ml. The E₅₀₀ of the liberated p-nitrophenol was read in a spectrophotometer. The extinction coefficient for the p-nitrophenol ion at 400 nm was 18.1 × 10⁻³ M and was corrected with the blank. Specific activities were expressed as nmoles/mg protein/h. Protein content was estimated according to the method of Bradford (1). Bovine serum albumin was used as a standard. An unpaired t test was used to compare enzyme activities between plasma and eye tissues. P < 0.05 was considered to be significant.

The results show that the serum, aqueous humor, optic nerve, and ocular tissues of the pig contain α-d-mannosidase, β-d-galactosidase, and N-acetyl-β-d-glucosaminidase (Table 1). The aqueous humor and serum had several times lower specific activity of glycosidases tested than did the extracts of ocular tissues or optic nerve. Although there is no significant difference between specific activities of these enzymes in the serum and aqueous humor, serum has higher total amounts of the enzymes because it has much higher protein content than the aqueous humor, 60 ± 1 mg/ml and 1.2 ± 0.2 mg/ml, respectively. The source of these enzymes in these fluids is not known. Most likely, presence of glycosidases in blood is mainly a reflection of their release from endothelium and circulating phagocytes, while in the aqueous humor they may originate from plasma and ocular tissues surrounding the anterior chamber.

The highest activity of α-d-mannosidase was found in the extracts prepared from the iris, while β-d-galactosidase and N-acetyl-β-d-glucosaminidase were the most active in the extracts of choroid and ciliary body, respectively. The uvea is rich in melanocytes. These cells have high lysosomal enzymatic activity (7) that may play a role in various pathological processes. For example, it was shown that ciliochoroidal detachment is accompanied by accumulation of β-d-galactosidase in the suprachoroidal space (15). However, the exact role of the uveal glycosidases in physiologic conditions remains unclear.

We have found that the hog optic nerve contains high levels of glycosidases, especially β-d-galactosidase and N-acetyl-β-d-glucosaminidase. High activities of glycosidases in the optic nerve of albino rabbits have also been described (18). Although the exact role of lysosomal enzymes in the optic nerve remains unclear, high activities of glycosidases in this tissue suggest that they may participate in the in-

<table>
<thead>
<tr>
<th>Body fluid or tissue extract</th>
<th>N</th>
<th>α-d-mannosidase</th>
<th>β-d-galactosidase</th>
<th>N-acetyl-β-d-glucosaminidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>6</td>
<td>4.6 ± 0.4</td>
<td>14.8 ± 1.3</td>
<td>17.9 ± 1.1</td>
</tr>
<tr>
<td>Aqueous humor</td>
<td>6</td>
<td>3.2 ± 0.6</td>
<td>15.9 ± 2.6</td>
<td>20.9 ± 3.3</td>
</tr>
<tr>
<td>Iris</td>
<td>3</td>
<td>68 ± 5</td>
<td>59 ± 6</td>
<td>305 ± 10</td>
</tr>
<tr>
<td>Ciliary body</td>
<td>3</td>
<td>44 ± 3</td>
<td>55 ± 5</td>
<td>412 ± 13</td>
</tr>
<tr>
<td>Choroid</td>
<td>3</td>
<td>56 ± 4</td>
<td>74 ± 6</td>
<td>402 ± 13</td>
</tr>
<tr>
<td>Retina</td>
<td>3</td>
<td>24 ± 4</td>
<td>52 ± 5</td>
<td>149 ± 10</td>
</tr>
<tr>
<td>Optic nerve</td>
<td>3</td>
<td>62 ± 8</td>
<td>60 ± 6</td>
<td>163 ± 14</td>
</tr>
</tbody>
</table>

All enzyme activities are expressed as nmol/mg protein/h; the values are presented as mean ± SEM. N equals the number of samples prepared.
Lysosomal enzymes in retina is a self-protective mechanism. In physiological conditions, these enzymes are involved in the degradation of photoreceptor outer segments. In pathological conditions, release of these enzymes from the retinal pigmented epithelium (or invaded macrophages) may attack and damage the adjacent tissues, particularly the visual cells (3). In addition, defects of lysosomal degradation may lead to the accumulation of undigested residual material in the retinal pigment epithelium.

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REFERENCES