Developmental change in activity of N-acetyl-β-glucosaminidase and a comparison of its multiple enzyme activities in the masseter muscle of normal and dystrophic mice

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(Accepted for publication: March 7, 1989)

Key words: N-acetyl-β-glucosaminidase / masseter muscle / muscular dystrophy / mice

Introduction

N-acetyl-β-glucosaminidase (NAG, EC 3.2.1.30) is an enzyme which catalyzes glycoproteins, glycolipids and glycosaminoglycans. This enzyme is widely distributed in a variety of tissues and fluids of mammals. Until now, NAG has been purified from various organs such as human placenta1, bovine spleen2, horse kidney3 and mouse submandibular gland4, and its enzymatic properties have been reported. However, there is little information available on the properties of NAG in skeletal muscle.

Recently, we measured activities of several lysosomal enzymes in the masseter muscle, the major muscle of mastication, of adult normal and muscular dystrophic mice, and found a marked increase of NAG activity in the dystrophic muscle5. In this study, we examined the developmental change of NAG activity in the masseter muscle of normal and dystrophic mice. In addition, we compared the activities of multiple forms of NAG in the muscle of both animals.

Materials and Methods

Male heterozygous normal (C57 BL/6J) and dystrophic mice (C57 BL/6J dy/dy), obtained from Central Institute for Experimental Animals (Kanagawa) were maintained with a solid and powdered chow, respectively, and water ad libitum. At 4, 8, 12 and 16 weeks of age, mice were killed by cervical dislocation and their masseter muscle removed. The muscle from each animal was cut into small pieces and homogenized in 19 volumes of 10 mM phosphate buffer (pH 7.0) using a Polytron P-10 homogenizer. The homogenate thus prepared was taken for enzyme assay.

NAG activity was measured using p-nitrophenyl-N-acetyl-β-glucosaminide as substrate. The assay mixture contained 2.5 μmol substrate, 50 μmol citrate-100 μmol phosphate buffer (pH 4.3), 0.05 ml of homogenate and water to give a total volume of 1 ml. The mixture was incubated at 37°C for 1 to 3 h, and the reaction stopped by the addition of 2 ml of 0.1 M glycine-NaOH buffer (pH 10.5). The p-nitrophenol liberated was measured by the absorbance at 410 nm, and 1 unit of the enzyme was defined as the amount causing the release of 1 μmol p-nitrophenol per min. Protein content in the homogenate was measured by the method of Lowry et al.6.

For the analysis of isoelectric focusing, the masseter muscle removed from 5 animals (8 weeks of age) was combined and homogenized in 9 volumes of 0.15 M KCl. The homogenate was centrifuged at 15,000×g for 30 min,
Table 1  Body weight and protein concentrations in the masseter muscle of normal and dystrophic mice

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Body weight (g)</th>
<th>Protein concentration (µg/mg wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Normal</td>
<td>15.1 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Dystrophy</td>
<td>9.2 ± 0.6*</td>
</tr>
<tr>
<td>8</td>
<td>Normal</td>
<td>24.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Dystrophy</td>
<td>13.1 ± 0.8*</td>
</tr>
<tr>
<td>12</td>
<td>Normal</td>
<td>27.4 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Dystrophy</td>
<td>15.9 ± 1.3*</td>
</tr>
<tr>
<td>16</td>
<td>Normal</td>
<td>31.2 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>Dystrophy</td>
<td>14.7 ± 1.1*</td>
</tr>
</tbody>
</table>

Each value shows the mean ± S.E. of 4 to 5 animals,

* p<0.001,  b p<0.01 and  c p<0.05 as compared with normal values

and the supernatant was used for the sample. Isoelectric focusing was carried out in a column of 50 ml capacity. A sucrose density gradient (0~50%) containing 1% ampholine (pH range, 3.5~10) was prepared, and 1.5 ml of the sample was focused at 700 volts for 24 h. After focusing, 1.2 ml fractions were collected. Prior to the assay of NAG activity, fractions were neutralized with NaOH or HCl.

Results

As shown in Table 1, the body weight of dystrophic mice was apparently lower than that of normal mice at all stages of development. Protein content in the masseter muscle of dystrophic mice was significantly low from 8 weeks until 16 weeks of age, as compared with the normal level. At 4 weeks of age, there was no significant difference in protein content between both groups.

In our previous study, we measured NAG activity in the masseter muscle at only 8 weeks of age. We have now investigated the progressive change of NAG activity in the muscle of normal and dystrophic mice. As shown in Fig. 1, NAG activity of normal mice increased gradually with development. In the dystrophic mice, the activity was found to be markedly higher as early as 4 weeks of age as compared with normal activity, and the increase in activity was retained there after until 16 weeks of age.

By the procedure of isoelectric focusing, NAG in the masseter muscle of mice (8 weeks of age) could be separated into 4 forms of enzymes (designated enzymes I ~ IV) as shown in Fig. 2. The isoelectric points (pIs) of enzymes I, II, III and IV were 4.9, 6.4, 7.2 and about 8.7, respectively, in both normal
and dystrophic mice. In the dystrophic mice, NAG activity of all enzymes was markedly high. Although data are not shown, the pH optima of these normal and dystrophic enzymes were about 4.3 and Km values for the substrate in the range of 0.60 to 0.85 mM.

**Discussion**

Muscular dystrophy is characterized by progressive muscle atrophy, loss of muscle functions and various alterations in muscle enzyme activities. Numerous investigators have suggested that the enhanced catabolism of muscular components is closely involved in the progress of this disease\(^7-13\).

In this study, there was no significant difference in protein content in the masseter muscle at 4 weeks of age between normal and dystrophic mice, suggesting that the degeneration of muscle was not so severe at this stage. On the other hand, NAG activity in the dystrophic muscle increased markedly as early as 4 weeks of age. Therefore, it seems unlikely that the early elevation of NAG activity in the dystrophic muscle may be due to the invasion of phagocytes or other cells.

Since NAG is known to catalyze glycoproteins and other glycoconjugates, the increase of its activity in the masseter muscle from early stage of development may be in part associated with the dysfunction of mastication during muscular dystrophy.

The present study demonstrated the presence of 4 forms of NAGs (enzymes I~IV) in the masseter muscle of mice. The pIs of respective enzymes were the same between normal and dystrophic values. In the dystrophic mice, NAG activity of all enzymes was markedly high. Among the 4 enzymes, the PI of enzyme I was 4.9 and that of enzyme III was 7.2, each value being very similar to that of NAG-A or NAG-B (5.0 or 7.0, respectively), both of which had been reported to be present in the human kidney and urine by Hultberg et al.\(^14\).

It is not known at present as to the localization of NAG and the physiological significance of multiple forms of NAG in the masseter muscle of mice, and thus further study is required to resolve these problems.

**References**


