Tay-Sachs disease, an inborn lysosomal disease featuring a buildup of GM2 in the brain, is caused by a deficiency of β-hexosaminidase A (Hex A) or GM2 activator. Of the two human lysosomal Hex isozymes, only Hex A, not Hex B, cleaves GM2 in the presence of GM2 activator. In contrast, mouse Hex B has been reported to be more active than Hex A in cleaving GM2 (Burg, J., Banerjee, A., Conzelmann, E., and Sandhoff, K. (1988) Hoppe Seyler's Z. Physiol. Chem. 369, 821–829). In two independent studies, mice with the targeted disruption of the Hexa gene did not display the severe buildup of brain GM2 or the concomitant abnormal behavioral manifestations seen in human Tay-Sachs patients. The results of these two studies were suggested to be attributed to the reported GM2 degrading activity of mouse Hex B. To clarify the specificity of mouse Hex A and Hex B and to better understand the observed results of the mouse model of Tay-Sachs disease, we have purified mouse liver Hex A and Hex B and also prepared the recombinant mouse GM2 activator. Contrary to the findings of Burg et al., we found that the specificities of mouse Hex A and Hex B toward the catabolism of GM2 were not different from the corresponding human Hex isozymes. Mouse Hex A, but not Hex B, hydrolyzes GM2 in the presence of GM2 activator, whereas GM2 is refractory to mouse Hex B with or without GM2 activator. Importantly, we found that, in contrast to human GM2 activator, mouse GM2 activator could effectively stimulate the hydrolysis of Gb4 by mouse Hex A and to a much lesser extent also by Hex B. These results provide clear evidence on the existence of an alternative pathway for GM2 catabolism in mice by converting GM2 to GA2 and subsequently to lactosylceramide. They also provide the explanation for the lack of excessive GM2 accumulation in the Hexa gene-disrupted mice.

Human tissues contain two major isoforms of lysosomal β-hexosaminidase (Hex).1 Hex A, a heterodimeric protein composed of α- and β-subunits, and Hex B, a β-subunit homodimer (1, 2). These two isoforms have also been reported to exist in other mammals (3). Human Hex A hydrolyzes the GalNAc from GM2 in the presence of a specific protein cofactor, GM2 activator (4–6). Human Hex B, on the other hand, is not able to hydrolyze GM2 with or without GM2 activator (7–10). A deficiency of Hex A or GM2 activator causes Tay-Sachs disease in humans, a lysosomal storage disease characterized by an excessive buildup of GM2 in the central nervous system (11). Burg et al. (3) reported that, in sharp contrast to human Hex isozymes, the partially purified Hex B prepared from several different mammalian tissues were able to degrade GM2 and that rat Hex B degraded GM2 more effectively than the Hex A. They also reported that the mouse activator preparation made from heat-treated mouse kidney extract was only slightly effective in stimulating the hydrolysis of GM2 by mouse Hex A and inhibited mouse Hex B in the same reaction. Recently, in two independent studies, mice with the targeted disruption of the Hexa gene were found to display neither the severe buildup of brain GM2 nor the concomitant abnormal behavioral manifestations seen in human classical Tay-Sachs patients (12, 13). In both studies, the mild manifestations were attributed to the reported GM2 degrading activity of mouse Hex B (3). Based on the fate of the radioactive Ga4 fed to embryonic fibroblasts derived from Hexa−/− and Hexb−/− mice, Sango et al. (14) proposed the presence of an alternative pathway in mice where siadioside acts upon GM2 to produce GA2 which can be hydrolyzed subsequently by Hex A or Hex B.

To clarify the role of the mouse Hex A and Hex B in the catabolism of GM2 and also to understand better the observed results of the mouse models of classical Tay-Sachs disease (Type B GM2 gangliosidosis), we have purified mouse liver Hex A and Hex B. We have also prepared the recombinant mouse GM2 activator. Using the recombinant human and mouse GM2 activators, we have studied the requirement of these two protein cofactors in the hydrolysis of GM2 and GA2 by mouse Hex A and Hex B. We have also studied the cross-reactivity of human and mouse GM2 activators by studying the stimulation of mouse Hex A by human GM2 activator and of human Hex A by mouse GM2 activator.

**Experimental Procedures**

**Materials**—GM2 was isolated from the brain of a Tay-Sachs patient (15). GA2 was prepared from GM2 by mild acid hydrolysis (16).

**References**

1. The abbreviations used are: Hex, β-N-acetylhexosaminidase; MU, 4-methylumbelliferyl; MUG, 4-methylumbelliferyl-β-GlcNAc; MUGS, 4-methylumbelliferyl-β-GlcNAc-6-SO4; Gm1, Galβ1–3GalNAc–6-sulfate; Gm2, Galβ1–3GalNAc–6-sulfate; Gm3, Galβ1–3GalNAc–6-sulfate; FPLC, fast protein liquid chromatography; SP, sulfopropyl; PAGE, polyacrylamide gel electrophoresis.

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Expression of Murine G{sub}M{sub}2 Activator—A pBluescript vector containing a 1.1-kilobase cDNA encoding the mouse G{sub}M{sub}2 activator (18) was used as a template to generate by polymerase chain reaction a shortened version of the encoding sequence which was homologous to the mature human G{sub}M{sub}2 activator (19). The upstream primer was 5'-ATG-ATG-GAT-CCG-GTG-GCT-TCT-CCT-GGG-ATA-3' and the downstream primer was 5'-CAG-GCA-AGG-CTG-CTG-CCA-GTA-TAT-CTG-3'. This cDNA sequence was cloned into the pT7–7 expression vector at BamHI and HindIII sites, and its sequence was verified to correspond to the 486-base-pair DNA fragment ending at position 232–193 of the mouse G{sub}M{sub}2 activator (18). The recombinant mouse G{sub}M{sub}2 activator was expressed and purified according to the procedures described previously for the human G{sub}M{sub}2 activator (19). The NH2-terminal amino acid sequence of the purified mouse G{sub}M{sub}2 activator was confirmed by a pulse-phase liquid microsequencer equipped with an on-line microbore phenylthiodyantoin-derivative analyzer (Applied BioSystems).

Enzyme Assays—Enzyme activity was determined using fluorescent substrates MUG and MUGS according to Potier et al. (20). The enzyme was incubated with 1.5 mM of substrate in 50 mM sodium citrate buffer, pH 5.0, in a total volume of 50 ml at 37 °C. After a set time, 1.5 ml of 0.2 M sodium borate buffer, pH 9.8, was added to the reaction mixture to stop the reaction. The released MU was determined using a cromaticalfyhydantoin-derivative analyzer (Applied BioSystems).

Molecular Mass Determination—The molecular mass of purified mouse Hex A and Hex B were determined using Superose 6 FPLC gel filtration in 50 mM sodium phosphate buffer, pH 7.0, containing 0.15 M NaCl. The column was first calibrated under the same conditions using ferritin (440,000), catalase (232,000), aldolase (158,000), ovalbumin (49,500), and chymotrypsinogen A (25,000) as molecular weight standards.

Purification of Mouse Liver Hex A and Hex B—All operations were performed at 0–5 °C except the chromatographies on Con A-Sepharose and SP-Fractogel that were carried out at room temperature. Centrifugation was routinely carried out at 10,000 × g for 50 min using a Sorvall RC5C refrigerated centrifuge. Unless otherwise indicated, gel chromatography was carried out with an Amicon stirred cell using a PM-10 membrane. Two hundred frozen mouse livers (391 g) were homogenized using a Polytron (Brinkmann) homogenizer with 5 volumes of cold phosphate-buffered saline (10 mM sodium phosphate, 138 mM NaCl, 2.7 mM KCl, pH 7.4) containing 1 mM EDTA and 1 mM phenylmethylsulfonyl fluoride as protease inhibitors, followed by centrifugation. The supernatant was brought to 30% saturation with solid ammonium sulfate. After 2 h, the precipitate was removed by centrifugation, and the supernatant was further brought to 65% saturation with solid ammonium sulfate. After standing overnight, the precipitate was collected by centrifugation and resuspended in 500 ml of 10 mM sodium phosphate buffer, pH 7.0 (buffer A). The suspension was placed into several dialysis bags and dialyzed against 10 liters of buffer A overnight, changing buffer every 4 h (4 changes). This crude enzyme preparation (780 ml) was centrifuged and applied to a DEAE-Fractogel column (5 × 45 cm) equilibrated with buffer A. The column was washed overnight with buffer A at 2 ml/min, and proteins were eluted with a linear gradient of NaCl from 0 to 0.5 M in the same buffer (total volume 4 liters), and 20-ml fractions were collected. Fractions were assayed for both MUG and MUGS-cleaving activities. Hex B, which cleaves only MUG, was eluted in the nonadsorbed fractions (Fig. 1) and was concentrated by ultrafiltration. As shown in Fig. 1, MUG-cleaving activity eluted with NaCl as a main peak with a leading shoulder. The shoulder contained very low MUGS-cleaving activity, whereas the main peak contained both MUG- and MUGS-cleaving activities. Fractions in the main peak were pooled and concentrated to make a crude mouse Hex A preparation. This preparation was applied to a Sephacryl S-300 column (5 × 90 cm) equilibrated with 50 mM sodium phosphate buffer, pH 7.0, containing 0.15 M NaCl. The column was eluted with the same buffer at 1 ml/min, and 20-ml fractions were collected. MUG- and MUGS-cleaving activities coeluted as a broad peak, and the entire peak was pooled (Fig. 2A) and concentrated to 25 ml by ultrafiltration. The concentrated Hex A was dialyzed thoroughly against buffer A overnight. The crude Hex B preparation (from DEAE-Fractogel column) was diazoyed against buffer A and applied to an SP-Fractogel column (2.5 × 17 cm) equilibrated with buffer A. The column was washed with buffer A at 2 ml/min, followed by buffer A containing 0.5 M NaCl and 17-ml fractions were collected. After the absorption at 280 nm fell to a stable baseline, the column was eluted with buffer A containing 0.5 M NaCl and 17-ml fractions were collected. The fractions eluted by methyl-α-mannoside contained MUG-cleaving activity (elution pattern not shown). This preparation was applied to a Sephacryl S-300 column and eluted under the same conditions as the DEAE-Fractogel purified Hex A (Fig. 2B).

The dialyzed mouse Hex A after gel filtration was applied to a Con A-Sepharose column (2.5 × 33 cm) equilibrated with buffer A. The column was washed with buffer A at 2 ml/min, followed by buffer A containing 0.5 M NaCl and 17-ml fractions were collected. After the absorption at 280 nm fell to a stable baseline, the column was eluted with buffer A containing 0.5 M NaCl and 17-ml fractions were collected. The fractions eluted by methyl-α-mannoside contained MUG-cleaving activity (elution pattern not shown) and were
The two major Hex isozymes were resolved from the elution using buffer A containing 0.5M NaCl and 0.75 M methyl-α-mannoside. Hex B also adsorbed to the column and was specifically eluted using buffer A containing 0.5 M NaCl and 0.75 M methyl-α-mannoside. Hex B after the Con A-Sepharose step was purified about 1500-fold and was used for subsequent studies. Table I summarizes the recovery of Hex A and Hex B from 200 mouse livers according to this purification scheme.

The dialyzed mouse Hex A preparation obtained after the Con A-Sepharose step was centrifuged and applied to an SP-Fractogel column (0.5 x 90 cm). Detailed conditions are described under “Experimental Procedures.” Dotted line, absorbance at 280 nm; filled circles, MUG-cleaving activity; empty circles, MUGS-cleaving activity.

The purified mouse liver Hex A after SP-Fractogel chromatography was used for the subsequent studies. By SDS-PAGE under nonreducing conditions, the purified Hex A showed one broad protein band when stained by Coomassie Brilliant Blue (Fig. 3A, lane 3). Immunostaining with anti-human Hex A revealed two overlapping bands of equal intensity corresponding to molecular sizes of approximately 57 and 59 kDa (Fig. 3A, lane 3). This is in agreement with the postulated makeup of mouse Hex A, which is a heterodimer consisting of an α-subunit and a β-subunit, with molecular sizes before posttranslational processing of 60 and 61 kDa, respectively, as deduced from their cDNA sequences (26–28). In humans, the β-subunit is posttranslationally processed to form two smaller polypeptides, β1 and β2, which are joined by disulfide bonds (29). Fig. 3A, lane 2, shows that similar processing occurs in mouse Hex A, with the appearance under reducing conditions of two overlapping bands of about 27 and 24 kDa, and the concomitant disappearance of the 59-kDa band. Western blot analysis was used to confirm that the protein band visualized by Coomassie Brilliant Blue staining was indeed Hex A. Goat anti-human Hex A recognized both the nonreduced mouse Hex α- and β-subunits and the lower molecular size polypeptide chains after reduction (Fig. 3B). The native molecular sizes of the mouse Hex A and Hex B were determined to be 110 and 120 kDa, respectively, as estimated using Superose 6 FPLC gel filtration. These values suggest that the native structures of mouse Hex A and Hex B consist of dimers as is the case for human enzymes. The isoelectric points of the two isoforms were estimated using Mono P FPLC chromatography to be 5.4–5.8 for the purified mouse Hex A and 6.3–5.8 for the mouse Hex B. When crude mouse liver extract was chromatofocused under the same conditions, MUGS-cleaving activity was detected throughout a broader pH range extending from pH 6.5 to 3.8.

Using MUG or MUGS in 50 mM sodium citrate buffer, both mouse Hex A and Hex B exhibited maximal activity at pH 5.0. This value is slightly higher than the reported human value of 4.4 (8). The $K_m$ value of Hex A toward MUG and MUGS were 0.98 mM and 0.72 mM, respectively. For Hex B, the $K_m$ value toward MUG was 0.90 mM and toward MUGS was 7.8 mM. Similar values were found for the human isozymes (8, 30, 31).

**RESULTS**

**Purification and Characterization of Mouse Liver Hex A and Hex B**—The two major Hex isozymes were resolved from the crude mouse liver extract by DEAE-Fractogel chromatography at pH 7.0 (Fig. 1). The acidic mouse Hex A was purified to near homogeneity using the scheme described under “Experimental Procedures” as summarized in Table I. The Hex A after SP-Fractogel chromatography was used for the subsequent studies. By SDS-PAGE under nonreducing conditions, the purified Hex A showed one broad protein band when stained by Coomassie Brilliant Blue (Fig. 4A, lane 3). Immunostaining with anti-human Hex A revealed two overlapping bands of equal intensity corresponding to molecular sizes of approximately 57 and 59 kDa (Fig. 4B, lane 3). This is in agreement with the postulated makeup of mouse Hex A, which is a heterodimer consisting of an α-subunit and a β-subunit, with molecular sizes before posttranslational processing of 60 and 61 kDa, respectively, as deduced from their cDNA sequences (26–28). In humans, the β-subunit is posttranslationally processed to form two smaller polypeptides, β1 and β2, which are joined by disulfide bonds (29). Fig. 4A, lane 2, shows that similar processing occurs in mouse Hex A, with the appearance under reducing conditions of two overlapping bands of about 27 and 24 kDa, and the concomitant disappearance of the 59-kDa band. Western blot analysis was used to confirm that the protein band visualized by Coomassie Brilliant Blue staining was indeed Hex A. Goat anti-human Hex A recognized both the nonreduced mouse Hex α- and β-subunits and the lower molecular size polypeptide chains after reduction (Fig. 4B). The native molecular sizes of the mouse Hex A and Hex B were determined to be 110 and 120 kDa, respectively, as estimated using Superose 6 FPLC gel filtration. These values suggest that the native structures of mouse Hex A and Hex B consist of dimers as is the case for human enzymes. The isoelectric points of the two isoforms were estimated using Mono P FPLC chromatography to be 5.4–5.8 for the purified mouse Hex A and 6.3–5.8 for the mouse Hex B. When crude mouse liver extract was chromatofocused under the same conditions, MUGS-cleaving activity was detected throughout a broader pH range extending from pH 6.5 to 3.8.

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The enzymatic assays and purification were carried out as described under "Experimental Procedures." Steps 1–5 show the preparation of Hex A and steps 1 and 6–9 describe the preparation of Hex B, starting from 200 frozen mouse livers.

| Step | Purification | Protein | Total activity | Specific activity | Recovery | Purification |
|------|--------------|---------|----------------|------------------|----------|--------------|
| 1    | (NH₄)₂SO₄, 30–65%, Hex A and B | 23010 | 683.2 | 0.03 | 100 | 1 |
| 2    | DEAE-Fractogel (adsorbed), Hex A | 4454 | 677.2 | 0.15 | 99.1 | 5 |
| 3    | Sepharyl S-300-HR, Hex A | 3057 | 841.2 | 0.28 | 123 | 9.3 |
| 4    | Con A-Sepharose, Hex A | 279 | 813.0 | 2.91 | 119 | 97 |
| 5    | SP-Fractogel, Hex A | 2.8 | 121.4 | 43.4 | 17.8 | 1447 |
| 6    | DEAE-Fractogel (non-adsorbed), Hex B | 5135 | 45.8 | 0.014 | 100 | 1 |
| 7    | SP-Fractogel, Hex B | 480 | 32.8 | 0.068 | 71.6 | 4.6 |
| 8    | Sepharyl S-300-HR, Hex B | 151 | 22.6 | 0.150 | 49.3 | 10.7 |
| 9    | Con A-Sepharose, Hex B | 0.94 | 12.9 | 13.7 | 22.2 | 978 |

The molecular mass of the recombinant mouse GM₂ activator determined by SDS-PAGE was 18.5 kDa, which is as expected from the cDNA sequence and is identical to that of the human GM₂ activator. By Western blot analysis, mouse GM₂ activator was recognized by the polyclonal antibodies against human GM₂ activator, indicating similarities in protein structure, although with a weaker interaction than that for the human GM₂ activator.

Hydrolysis of GM₂ by Mouse Hex A and Hex B—The purified mouse Hex A and Hex B were examined for their ability to hydrolyze GM₂. As shown in Fig. 5A, the specificities of the mouse Hex A and Hex B toward GM₂ are the same as their human counterparts. Under the same conditions, mouse Hex A was found to slowly hydrolyze GM₂ in the absence of mouse GM₂ activator,6ho f incubation. B: 1, LacCer; 2, G₄₃ + mouse G₄₃ activator; 3, G₄₃ + mouse Hex A; 4, G₄₃ + mouse Hex A + mouse GM₂ activator; 5, G₄₃ + mouse Hex B; 6, G₄₃ + mouse Hex B + mouse GM₂ activator; 7, G₄₃ + mouse Hex B, 6 h of incubation; 8, G₄₃ + mouse Hex B + mouse GM₂ activator; 6 h of incubation; 8, G₄₃ + mouse Hex B + mouse GM₂ activator, 6 h of incubation.

Hydrolysis of G₄₃ by Mouse Hex A and Hex B—To understand the reported observations on the studies of mouse Hex α-chain disruption (12, 13), we also examined the ability of two mouse Hex isozymes to hydrolyze the GalNAc from G₄₃ (Fig. 5B). Under our assay conditions (30 min of incubation), mouse Hex A was found to slowly hydrolyze G₄₃ in the absence of mouse GM₂ activator (Fig. 5B, lane 3). We found that even though G₄₃ is refractory to human Hex A in the presence of human GM₂ activator (19), mouse Hex A was able to effectively hydrolyze G₄₃ in the presence of mouse GM₂ activator (Fig. 5B, lane 4, 45% hydrolysis). Under the same conditions, mouse Hex B was not able to hydrolyze G₄₃ in the absence of mouse GM₂ activator (Fig. 5B, lane 5), and no detectable hydrolysis was observed in the presence of mouse GM₂ activator (Fig. 5B, lane 6) after 30 min of incubation. However, after extended incubation (6 h of incubation), mouse Hex B was found to be able to slowly hydrolyze G₄₃ in the presence of mouse GM₂ activator (Fig. 5B, lane 8).

It has been shown previously that the ceramide portion of the
Each glycolipid substrate (3 nmol) was incubated with 20 milliunits of Hex at 37 °C for 30 min. The plates were developed with chloroform/methanol/water, 60:35:8 (v/v/v), and stained with diphenylamine reagent. The detailed assay conditions are described under “Experimental Procedures.” A: I, GM2 + human GM2 activator; 2, GM2 + human Hex A; 3, GM2 + human Hex A + human GM2 activator; 4, GM2 + human Hex A + mouse GM2 activator; 5, GM2 + mouse Hex A; 6, GM2 + mouse Hex A; 7, GM2 + mouse Hex A + mouse GM2 activator; 8, GM2 + mouse Hex A + human GM2 activator; 9, GM2: B: I, GM2 + human G M2 activator; 2, G A2 + human Hex A; 3, G A2 + human Hex A + human G M2 activator; 4, G A2 + human Hex A + mouse G M2 activator; 5, G A2 + mouse G M2 activator; 6, G A2 + mouse Hex A; 7, G A2 + mouse Hex A + mouse G M2 activator; 8, G A2 + mouse Hex A + human G M2 activator; 9, LacCer.

G M2 molecule is essential for hydrolysis by human Hex A (10, 19). As is the case for the human enzyme, neither mouse Hex isoforms was able to hydrolyze P-NeuAcGgOseβ, the oligosaccharide derived from G M2, even in the presence of G M2 activator (data not shown).

The mouse Hex A and Hex B are highly homologous to their human counterparts, with the α- and β-chains sharing 86 and 74% identity, respectively, at the amino acid level (28–29). The mouse G M2 activator is also quite similar to the human protein, sharing 75% identity (18). Therefore, we studied the ability of human and mouse activators to cross-stimulate G M2 and G A2 hydrolysis by human and mouse Hex A. Using the same units (20 milliunits) of human and mouse Hex A and the same amount (1 μg) of human and mouse G M2 activator, we found that the mouse activator effectively stimulated the hydrolysis of G M2 by both human Hex A (Fig. 6A, lane 4, 83% hydrolysis) and mouse Hex A (Fig. 6A, lane 7, 72% hydrolysis). It appears that the mouse GM2 activator is more effective in stimulating the hydrolysis of G M2 by human Hex A. Under the same conditions, the human G M2 activator stimulated only 57% hydrolysis of G M2 by human Hex A (Fig. 6A, lane 3). The human GM2 activator was also much less effective in stimulating the hydrolysis of G M2 by mouse Hex A (Fig. 6A, lane 8), which could be seen after extended incubation or in the presence of additional activator protein (data not shown). Of great interest was the observation that the mouse GM2 activator also stimulated the hydrolysis of G M2 by human Hex A (Fig. 6B, lane 4). In contrast, the human activator was not able to promote the hydrolysis of G M2 by human Hex A (Fig. 6B, lane 3) or mouse Hex A (Fig. 6B, lane 8). Extended incubation of the mouse activator with human Hex B resulted in the slow hydrolysis of G A2 (data not shown) as seen for mouse activator with mouse Hex B (Fig. 5B, lane 8).

**DISCUSSION**

To understand the catabolism of G M2 in mouse, we have purified and characterized mouse liver Hex A and Hex B and compared their properties with human Hex A and Hex B. As seen with the recombinantly expressed α- and β-chains (25), the purified mouse liver Hex A was recognized by goat anti-human Hex A. Purified mouse Hex A was determined to be composed of 57- and 59-kDa subunits by SDS-PAGE under nonreducing conditions, and smaller polypeptides were observed in the presence of 2-mercaptoethanol or dithiothreitol (Fig. 4A, lane 2). Therefore, mouse Hex A has a similar subunit composition to human Hex A, with noncovalently linked α- and β-subunits (20). This is also the first direct evidence that one of the subunits is composed of nonidentical cystine-linked polypeptide chains, which, by comparison with the human enzyme, is probably the β-subunit (29).

While the isoelectric points of purified mouse Hex A and Hex B are similar to the isoelectric points of their human counterparts, the presence of mouse Hex A distributed in a wide range of isoelectric points has important consequences for purification. In the past, the separation of the mouse Hex A and Hex B isozymes has been routinely accomplished by passing a preparation over an anion exchange column equilibrated with 10 mM sodium phosphate buffer, pH 6.0–6.5. Hex B is collected in the pass-through fractions, while the retained Hex A is eluted by an NaCl gradient (3, 32). However, these reports followed the method that was originally optimized for the human Hex isozymes (1). We found that in following the previously reported methods (3), the mouse Hex B preparation that was not adsorbed to the DEAE column at pH 6.0–6.5 still contained a small amount of MUGS-cleaving activity. MUGS-cleaving activity has been correlated with the ability to hydrolyze G M2 (33). As reported by Burg et al. (3), we also found that mouse Hex B prepared by this method did contain some G M2-cleaving activity. To ascertain whether this G M2-cleaving activity was inherent in the mouse Hex B or due to contamination by Hex A, we increased the pH of the buffer solution to 7.0 for the separation of the two isozymes by anion exchange chromatography. Interestingly, the amount of MUGS-cleaving activity, as compared with the MUG-cleaving activity, decreased significantly and the resulting Hex B preparation became extremely weak in hydrolyzing G M2 (Fig. 5A), as was observed with human Hex B (11).

From the binding behavior of mouse Hex A to DEAE-Fractogel and also because of its acidic pI, the retention of the enzyme by the SP-Fractogel column at pH 7.0 (Fig. 3) was totally unexpected. This suggests that interactions other than ionic may be involved. This chromatography step was very effective for removing contaminating proteins. Because the Hex B preparation contained other proteins not adsorbed to DEAE-Fractogel at pH 7.0, it is not surprising that the SP-Fractogel chromatography was not as effective for purifying Hex B as for Hex A. Based on the DEAE-Fractogel chromatography, we estimated that approximately 90% of the total MUGS-cleaving activity present in the crude mouse liver extract was Hex A and 7% was Hex B. This is in agreement with previous reports of the level of the two isozymes in mouse liver tissues (32). Because the amount of Hex B in mouse liver is very low compared with Hex A it was not practical to purify Hex B to homogeneity as done for Hex A. However, the final Hex B preparation was free from contaminating glycosidases and proved to be suitable for the studies presented.

The recombinant human and mouse G M2 activators were expressed using the shortened version of cDNAs which encode
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only the mature activator proteins. The cDNA for human GM₂ activator encodes for a protein of 193 amino acids that consists of a signal peptide (23 amino acids), a propeptide (8 amino acids), and a mature protein (162 amino acids). The signal and the propeptides are excised proteolytically to form the mature GM₂ activator protein (5). In the full-length cDNA encoding for the mouse GM₂ activator, the predicted cleavage site is between positions 19 and 20 of the deduced amino acid sequence (34). This site is very close to the cleavage site (positions 23 and 24) of the human sequence (5). Although there is no direct evidence that the first 31 amino acids in the mouse sequence contains a signal peptide and a propeptide, the mouse sequence shows a hydrophobic profile similar to that of the human sequence (18). In addition, the recombinant mouse GM₂ activator and the native human protein were found to have the same specific activity toward the hydrolysis of GM₂, indicating that the mature form of mouse GM₂ activator is very likely to start from amino acid 32 as in the case of humans.

As seen with the human Hex isozymes, mouse Hex A hydrolyzes GM₂, with the requirement of the GM₂ activator, whereas mouse Hex B has only a trace of activity to cleave GM₂ with or without GM₂ activator. To our surprise, in contrast to human Hex A, mouse Hex A was also able to effectively hydrolyze GA₂ in the presence of mouse GM₂ activator (35). Our results complement the recent observation that mouse Hex B (14). The pathogenesis of Tay-Sachs disease, types B, O, and AB of GM₂ gangliosidosis.

Our results provide the explanation for the results generated by targeted disruption of Hex A (36) and Hex B (subunit) (36) genes encoding Hex A (Hexa) and Hex B (Hexb). The model of type O GM₂ gangliosidosis (12, 13) and Hexa/Hexb (12, 13) were also suggested to escape the disease through partial catabolism of GM₂. Mice with defective Hexa-subunits (12, 13). The fact that mouse Hex B cannot hydrolyze GM₂ but can act on GA₂ suggests that in mice GM₂ can be converted to GA₂ that serves as a substrate for human GM₂ activator (35). Our results complement the recent pathological findings of the three mouse models of human Tay-Sachs disease, types B, O, and AB of GM₂ gangliosidosis.

The mouse models of type B (Hexa/–/–) and O (Hexb/–/–) were generated by targeted disruption of Hex A (α-subunit) (12, 13) or Hex B (subunit) (36) genes encoding Hex A (β/α) and Hex B (β/β). The model of type AB GM₂ gangliosidosis (GM₂a/–/–) (GM₂b activator deficiency) was produced by targeted disruption of Gna₂ gene (37). Unlike human type B GM₂ gangliosidosis, the Hexa/–/– mice were asymptomatic (12, 13), while Hexb/–/– mice (36) were severely affected as in the case of human type O GM₂ gangliosidosis. The Hexb/–/– mice accumulated more GM₂ and GA₂ in the brain than the Hexa/–/– mice. The GM₂a/–/– mice (37) showed a phenotype which is intermediate to those of Hexa/–/– (12, 13) and Hexb/–/– (36) with storage of an excess amount of GM₂ and a low amount of GA₂. From these three murine models of Tay-Sachs disease, it has been proposed that Hexa/–/– mice escape the disease through partial catabolism of GM₂ via GA₂ by the combined action of sialidase and Hex B (14). The pathogenesis of GM₂a/–/– mice also suggested a role for the GM₂ activator in GA₂ degradation in mice (37).

Our results provide the explanation for the results generated by the above three mouse models. We have demonstrated the ability of mouse Hex A to participate in the catabolism of GA₂ and a very weak activity of Hex B toward the degradation of GM₂. We have also shown the ability of mouse GM₂ activator to stimulate the hydrolysis of GM₂ by mouse Hex A and to a lesser extent by mouse Hex B. We have also examined the species specificity of the interactions between the mouse and human Hex isoforms and the activators. Previously, crude activator preparations from other mammalian species (3) and purified mullet roe GM₂ activator (38) were found to activate the hydrolysis of GM₂ by human Hex A. We have shown here that purified recombinant mouse GM₂ activator can effectively stimulate the hydrolysis of GM₂ and GA₂ by human Hex A. In reverse, human GM₂ activator was not effective in stimulating the hydrolysis of GM₂ or GA₂ by mouse Hex A.

Although the mouse GM₂ activator is 73.5% identical to the human protein, it also appears that the mouse activator does not share the specificity to the characteristic branched trisaccharide epitope of GM₂ (19) but assists Hex A to hydrolyze GA₂ as well. The observation that mouse GM₂ activator can stimulate the hydrolysis of GM₂ by both human and mouse Hex A, while human GM₂ activator can only stimulate the hydrolysis of GM₂ by human Hex A but not mouse Hex A, provides strong evidence that the GM₂ activator proteins must somehow interact with Hex A. Similarly, the observation that the mouse GM₂ activator can stimulate the hydrolysis of both GM₂ and GA₂ by human Hex A, but the human GM₂ activator can only stimulate the hydrolysis of GM₂ by human Hex A, shows that the GM₂ activators of these two species may have different specificities for the two glycolipids.

Biochemical analysis of enzyme systems is an important complement to molecular and genetic studies in the effort to fully understand the roles of Hex isoforms in mouse. Despite the biochemical similarities between human and mouse Hex isoforms and GM₂ activator proteins, the catabolic pathways for GM₂ in mouse and human are clearly not identical. Therefore, the murine model for Type B Tay-Sachs disease does not truly reflect its counterpart in man.

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