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Bovine Liver β-Acetylhexosaminidase Molecular Weight and Subunit Composition

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The molecular weights and subunit compositions of bovine liver β-acetylhexosaminidase A (Hex) and B (Hex B) were studied using enzyme preparations purified by octyl Sepharose chromatography. Analysis by gel filtration showed that Hex A had a molecular weight of *ca.* 310000 (hexamer) at pH 5, 6, and 6.5, and was dissociated into dimers at pH 7 and 8, while the molecular weight of Hex B, *ca.* 310000, remained constant in the pH range of 5—8. The molecular weights of Hex A and B subunits as determined by electrophoresis in sodium dodecyl sulfate were both *ca.* 52000, and this value remained unaltered when the enzyme was treated with a reducing agent or denaturant.

Keywords——β-acetylhexosaminidase; bovine liver; subunit composition; pH-dependent dissociation; gel filtration; polyacrylamide gel electrophoresis

In the previous paper we reported the molecular weights of partially purified bovine liver β-acetylhexosaminidase (hexosaminidase) A and B to be 280000 and 320000, respectively, as determined by gel filtration at pH 5.0.[2] These values seemed rather high as compared with those of the human and some other mammalian enzymes. The present study was carried out to confirm the molecular weights of bovine liver hexosaminidases by gel filtration under more controlled conditions and to investigate their subunit compositions using homogeneous enzyme preparations.

Materials and Methods

Chemicals——Catalase (molecular weight, 240000, from beef liver) and aldolase (molecular weight, 158000, from rabbit muscle) were obtained from Boehringer (Germany), and bovine serum albumin (molecular weight, 67000) from Sigma. Standard markers for molecular weight determination by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis were purchased from BDH Chemicals (England), N-acetylneuraminic acid from Seikagaku Kogyo (Tokyo), and Coomassie brilliant blue R from Sigma. Bovine serum albumin oligomers and lysozyme oligomers were prepared by the method of Payne.[3]

Enzyme——Crude hexosaminidase A and B from bovine liver (*ca.* 2000 units) were separately applied to an octyl Sepharose CL-4B column (1.8×4 cm), and purified as described in the previous paper.[4] The enzymes obtained were further purified by rechromatography on the same column. The specific activities of the hexosaminidase A and B preparations were 239 and 313 units (μmol/min) per mg, respectively, when assayed as described in the previous paper.[2]

Analytical Gel Filtration——The enzyme (150—200 units) and marker proteins were separately applied to a Sephadex G-200 column (1.5×97 cm), and eluted in 2 ml fractions with the equilibration buffer under constant pressure at 4°.

Polyacrylamide Gel Disc Electrophoreses——Intact enzymes (5—15 μg) were applied to gels (0.5×8 cm) and run at 4° with a constant current of 3—4 mA per tube, using Bromphenol blue or Malachite green as a tracking dye. The buffer systems employed were acetate buffer, pH 4.3,[5] and Tris buffer, pH 8.0,[6]

1) Location: *2-2-1, Miyama, Funabashi-shi, 274, Chiba.*
2) M. Tanaka, S. Kyosaka, and S. Murata, *Chem. Pharm. Bull.*, **26**, 1188 (1978).
3) J.W. Payne, *Biochem. J.*, **135**, 867 (1973).
4) M. Tanaka, S. Kyosaka, and S. Murata, *Chem. Pharm. Bull.*, **26**, 3225 (1978).
5) R.A. Reisfeld, U.J. Lewis, and D.E. Williams, *Nature* (London), **195**, 281 (1962).
6) D.E. Williams and R.A. Reisfeld, *Ann. N.Y. Acad. Sci.*, **121**, Art. 2, 373 (1964).

and 9.4.[7] The molecular weight of the intact enzyme was estimated by the method of Hedrick.[8] For the determination of subunit molecular weight, SDS-polyacrylamide gel electrophoresis was performed by the method of Weber *et al.*[9]

Carbohydrate Analysis——Hexosamine was estimated by the method of Rondle and Morgan[10] using glucosamine as a standard after hydrolysis in 2 N hydrochloric acid at 100° for 10 hr. Neutral sugar was estimated by the anthrone method of Horikoshi[11] using glucose as a standard, and sialic acid by the thiobarbituric acid method of Warren.[12]

Results

The purified bovine liver hexosaminidase A and B preparations (Hex A and Hex B) were homogeneous in polyacrylamide gel electrophoresis at pH 9.4, 8.0 and 4.3; both contained carbohydrate, as shown in Table I.

Table I. Carbohydrate Contents of Purified Bovine Liver Hex A and B

	Neutral sugar (%)	Hexosamine (%)	Sialic acid (%)
Hex A	1.9	3.3	0.33
Hex B	5.4	6.2	0.08

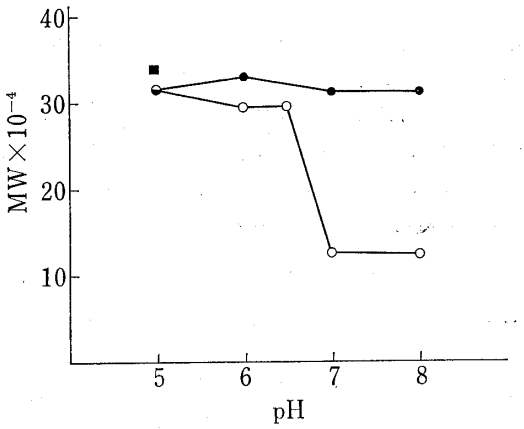

Fig. 1. Effect of pH on the Molecular Weights of Hex A and B as measured by Gel Filtration

Estimated at 4° in 0.05 M buffers: citrate (pH 5), phosphate (pH 6—7) and Tris (pH 8.0). —○—, Hex A; —●—, Hex B. ■: estimated in buffer, pH 5.0, containing 0.15 M NaCl (Hex A and B).

Sephadex G-200 gel filtration was performed under various conditions. Hex B gave a peak of activity corresponding to a molecular weight of *ca.* 310000 at pH 5, 6, 7 and 8. On the other hand, Hex A behaved as a species with a molecular weight of *ca.* 127000 in buffers of pH 7 and 8 while it behaved as one with a molecular weight of *ca.* 310000 in buffers of pH 5 and 6.5 (Fig. 1). The molecular weights were also estimated by polyacrylamide gel disc electrophoresis at various gel concentrations at pH 8.0 according to Hedrick[8] (Fig. 2). The molecular weight obtained for Hex A, *ca.* 100000, was consistent with the values obtained by gel filtration at pH 7.0 and 8.0. Hex B remained at the top of gel at pH 8.0.

Hex A and B were subjected to electrophoresis in 10% polyacrylamide gel containing 0.1% sodium dodecyl sulfate (SDS) after incubating the sample at 100° for 2 min in sodium phosphate buffer, pH 7.0, containing 1% SDS and 1% 2-mercaptoethanol. Both Hex A and B migrated as a single band corresponding to a molecular weight of 55000 (Fig. 3). Similar experiments with 12.5% and 15% gels gave lower values, 53000 and 51400, respectively. With 10% gels, a value of 55000 was again obtained even when a sample dissolved in 0.01 M sodium phosphate buffer, pH 7.0, containing 1% SDS but not 2-mercaptoethanol

7) B.J. Davis, *Ann. N.Y. Acad. Sci.*, **121**, Art. 2, 404 (1964).
8) J.L. Hedrick and A.J. Smith, *Arch. Biochem. Biophys.*, **126**, 155 (1968).
9) K. Weber, J.R. Pringle, and M. Osborn, *Methods in Enzymology*, **26**, 3 (1972).
10) C.J.M. Rondle and W.T.J. Morgan, *Biochem. J.*, **61**, 586 (1954).
11) H. Horikoshi, *Kagaku no Ryoiki*, Zokan, **34**, 36 (1958).
12) L. Warren, *J. Biol. Chem.*, **234**, 1971 (1959).

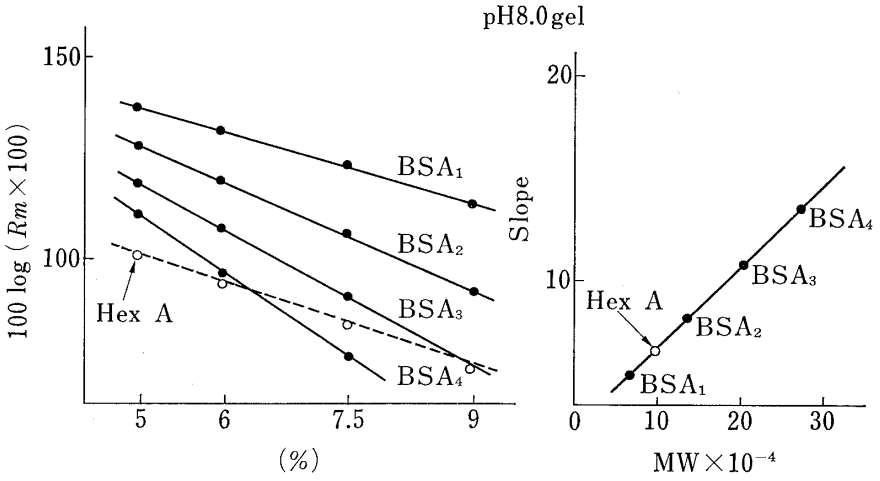

Fig. 2. Estimation of the Molecular Weight of Hex A by Electrophoresis at Various Gel Concentrations

The migration of protein was expressed as a ratio (*Rm*) to that of the tracking dye. The slopes determined on the left are plotted against the molecular weight on the right.

was applied without any preincubation. These values are twice as high as those reported for the human tissue enzymes.[13] To obtain complete dissociation of the enzyme into its subunits, the following procedures were examined: (1) reduction in Tris buffer, pH 8.6, containing 5.4 mM EDTA, 0.17 M 2-mercaptoethanol and 8 M urea for 4 hr under nitrogen at room temperature, followed by S-carboxymethylation with iodoacetate according to the procedure of Crestfield *et al.*,[14] (2) reduction in Tris buffer, pH 8.5, containing 1 mM dithiothreitol and 7 M guanidine hydrochloride for 1 hr at 20°, followed by carboxymethylation and further incubation in phosphate buffer, pH 7.1, containing 1% SDS, 1% 2-mercaptoethanol and 8 M urea at 100° for 5 min according to Marinkovic *et al.*,[15] and (3) reduction with 1 M 2-mercaptoethanol or 30 mM dithiothreitol in 0.01 M phosphate buffer, pH 7.1, containing 1% SDS at 100° for 5 min. Similar molecular weights were obtained by all three procedures: (1), 52100 daltons; (2), 54500; (3), 52400 (2-mercaptoethanol), 51200 (dithiothreitol).

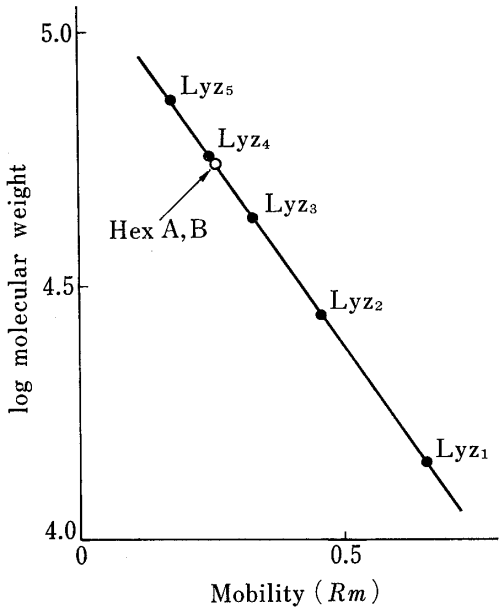

Fig. 3. Molecular Weight Determination of Subunits of Hex A and B by SDS-Polyacrylamide Gel Electrophoresis

The enzymes (5—10 μg) were heated at 100° for 2 min in 0.01 M sodium phosphate buffer, pH 7.0, containing 1% SDS and 1% 2-mercaptoethanol, and then applied to 10% gels, using the method of Weber *et al.*[9] Lyz_n: lysozyme oligomers.

Polyacrylamide gel disc electrophoresis was carried out in gels of pH 9.4 containing 8 M urea (data not shown). Hex A was resolved into three protein bands. The migration of each protein band in gels of various concentrations could be interpreted in terms of the presence of three proteins of different mobility with similar molecular weights of *ca.* 52000, estimating

13) *a*) B. Geiger and R. Arnon, *Biochemistry*, **15**, 3484 (1976); *b*) E. Beutler, A. Yoshida, W. Kuhl, and J.E.S. Lee, *Biochem. J.*, **159**, 541 (1976).
14) A.M. Crestfield, S. Moore, and W.H. Stein, *J. Biol. Chem.*, **238**, 622 (1963).
15) D.V. Marinkovic and J.N. Marinkovic, *Biochem. J.*, **163**, 133 (1977).

the molecular weights according to Hedrick *et al.*[8] On the other hand, Hex B gave a band accompanied by a minor, slowly moving band; the main band showed a mobility similar to that of the most slowly moving band observed in Hex A. This observation suggests that Hex A has a more complex subunit composition than Hex B, as in the case of human placenta hexosaminidase.[13]

Discussion

The molecular weights of Hex A and B, *ca.* 310000, as estimated by gel filtration, are consistent with those reported previously,[2] and are greater than those obtained by the same method for the enzymes from human tissues[16] (*ca.* 100000[13,15]), bovine seminal plasma (*ca.* 200000[17]) and equine kidney (250000[18]). Human placenta hexosaminidases dissociate into subunits of 25000[13] daltons on reductive cleavage of disulfide bonds, while for mammalian hexosaminidase other than the human enzyme, higher subunit molecular weights ranging from 53000[17] to 95000[19] have been reported. The present study suggests that Hex A and B are hexamers composed of subunits with a molecular weight of *ca.* 52000, and that Hex A dissociates into dimers in alkaline media. This conversion seems to be reversible, since overnight dialysis of Hex A against buffer, pH 8.0, did not affect the result of gel filtration at pH 5.0. pH-dependent dissociation-association has previously been reported for glycosidases, such as soybean α-D-galactosidase.[20]

16) In ref. 11, we erroneously described the opposite.
17) A. Khar and S.R. Anand, *Biochem. Biophys. Acta*, **483**, 152 (1977).
18) Y. Seyama and T. Yamakawa, *J. Biochem.* (Tokyo), **75**, 495 (1974).
19) S.J. Wetmore and J.A. Verpoorte, *Can. J. Biochem.*, **50**, 563 (1972).
20) N. Harpaz, H.M. Flowers, and N. Sharon, *Eur. J. Biochem.*, **77**, 419 (1977).