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Note

Preparative isolation of the polypeptide chains of human liver β-N-acetylhexosaminidase A by fast protein liquid chromatography–ion-exchange chromatography

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Studies on the fine molecular structure of β-N-acetylhexosaminidase (E.C. 3.2.1.30) from various tissues have revealed that the enzyme molecule exists in two major isoenzyme forms, A and B. β-N-Acetylhexosaminidase A consists of different polypeptide chains, α (M_r 50000) and β (M_r 25000), with composition $\alpha\beta_2$. β-N-Acetylhexosaminidase B is a homopolymer of β-chains with composition $2\beta_2$[1]. From studies on the carbohydrate structures present on β-N-acetylhexosaminidases in tissues it can be concluded that each enzyme molecule contains an average of 1–3 oligosaccharide chains of the N-glycosidic type[2]. Therefore, it is improbable that all polypeptides bear a glycan chain.

In order to investigate the distribution and composition of the oligosaccharides on the individual α and β polypeptide chains, we searched for a method to prepare these chains in high purity and in sufficient quantities. Previous methods include ion-exchange chromatography on DEAE-cellulose[3] or gel filtration[4], both performed under denaturing conditions. These methods yielded insufficient recovery in the separation of the polypeptide chains of β-N-acetylhexosaminidase A and B from human liver. Recently a new anion-exchange resin, Mono Q™, has been introduced for fast protein liquid chromatography (FPLC). In this paper we report our results using FPLC with a prepacked Mono Q column for the isolation of the polypeptides of β-N-acetylhexosaminidase A from human liver.

MATERIALS AND METHODS

Materials

The following materials were purchased as indicated: Mono Q HR 5/5 1-ml prepacked column (Pharmacia Fine Chemicals, Uppsala, Sweden); DL-dithiothreitol and iodoacetamide (Sigma Chemical Company, St. Louis, MO, U.S.A.); bis-Tris (Serva Feinbiochemica, Heidelberg, F.R.G.); Bio-Gel P-2 (Bio-Rad Laboratories, Richmond, CA, U.S.A.). All other chemicals were of the best available grade.

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Preparation of the polypeptide chains

The isoenzyme forms A and B of β-N-acetylhexosaminidase were isolated essentially as described earlier[5]. In a typical experiment, 21 mg of β-N-acetylhexosaminidase A and 19 mg of β-N-acetylhexosaminidase B were obtained from 1010 g of human liver.

Purified β-N-acetylhexosaminidase A was reduced with 10 m*M* dithiothreitol (DTT) and alkylated with 25 m*M* iodoacetamide, both in 5 *M* guanidinium chloride, according to Geiger and Arnon[3]. Prior to the separation of the polypeptides, the mixture was gradually dialyzed against the starting buffer (see below). The complete removal of the guanidine was ascertained by conductivity measurements.

Separation of the polypeptide chains

The starting buffer for the ion-exchange chromatography on Mono Q was 20 m*M* bis-Tris in 8 *M* deionized urea adjusted to pH 6.0 with hydrochloric acid. The limit buffer was the same but with the addition of 1 *M* sodium chloride. All chromatographic runs were performed using an automated FPLC system from Pharmacia, including a peak selective fraction collector. In a typical experiment, 10 mg of the polypeptide mixture were applied in a concentration of 5 mg/ml to a 1-ml Mono Q HR 5/5 prepacked column. The flow-rate was 1.5 ml/min. Unretained material was eluted with starting buffer, and elution was continued with a linear gradient of 0.0–0.3 *M* sodium chloride by gradually mixing with limit buffer. The Mono Q column was regenerated by subsequent washing with limit and starting buffer. The polypeptide containing fractions were placed on a Bio-Gel P-2 column (23 × 0.6 cm) and eluted with distilled water, in order to remove the urea.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

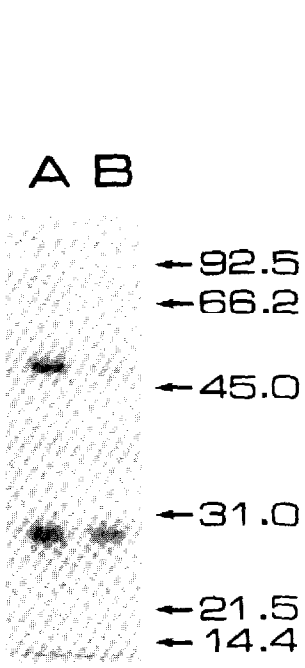
The polypeptides present in the different peak fractions were examined on 13% polyacrylamide slab gels containing SDS according to methods described earlier[5]. Protein bands were visualized with Coomassie brilliant blue.

RESULTS AND DISCUSSION

When examined by SDS-PAGE (Fig. 1), purified β-N-acetylhexosaminidase A migrated as two bands having M_r 29 000 and 50 000, respectively. β-N-Acetylhexosaminidase B yielded one band having M_r 29 000. Some impurities were observed: in β-N-acetylhexosaminidase A at M_r 67 000 and in β-N-acetylhexosaminidase B at M_r 50 000. The impurity in form A disappeared after FPLC of the reduced isoenzyme (see Fig. 3).

After reduction and alkylation of β-N-acetylhexosaminidase A, 10 mg of the polypeptide mixture were applied to a Mono Q anion-exchange column. Five peaks were obtained (Fig. 2). Peaks 1 and 2 were eluted as unretained material, whereas peaks 3, 4 and 5 were eluted at 0.06, 0.08 and 0.10 *M* sodium chloride, respectively. Based on peak areas the total recovery was more than 80%. The whole separation including column regeneration took 16 min.

When examined by SDS-PAGE (Fig. 3), peak 1 (see Fig. 2) did not contain detectable amounts of protein. The polypeptides present in peaks 2 and 3 migrated as one band having M_r 29 000, whereas the apparent molecular weight of peak 4 is



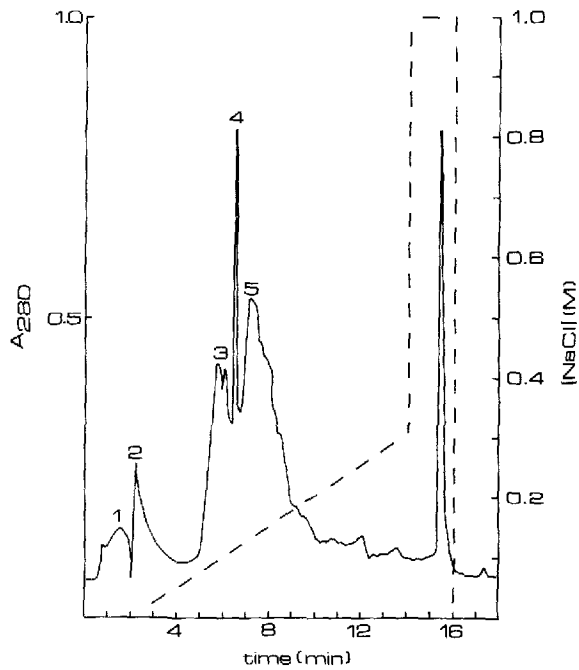


Fig. 1. SDS-PAGE of human liver β-N-acetylhexosaminidase A and B. The electrophoretic and staining procedures were as described earlier[5]. The molecular weight marker proteins are phosphorylase B (92 500), bovine serum albumin (66 200), ovalbumin (45 000), carbonic anhydrase (31 000), soy bean trypsin inhibitor (21 500) and lysozyme (14 400). Their positions are indicated by arrows and their molecular weights given in kD.

Fig. 2. FPLC–ion-exchange chromatography on Mono Q of the polypeptides of human liver β-N-acetylhexosaminidase A. Chromatographic procedure as described in Materials and Methods. Protein was detected by absorbance at 280 nm (A_{280}). There is a time difference between the recording of the salt concentration and that of the UV absorbance. For this reason the actual salt concentrations at which the peaks are eluted are 0.03 *M* lower than indicated. The absorbance peak during the regeneration of the column with 1.0 *M* sodium chloride was mainly due to flow perturbance as a result of the rapid buffer change.

slightly higher. Peak 5 yielded one band having M_r 50 000. No significant impurities could be detected in any of the fractions.

The results indicate that α-chains are eluted at 0.1 *M* sodium chloride, while the peaks eluted at lower salt concentrations concern β-chains. This implies that the β-chains show a heterogeneity with respect to their isoelectric point, in close agreement with the dissimilarity found in the polypeptide chains of the β_2 subunit of β-N-acetylhexosaminidase from normal human placenta[6].

From Figs. 2 and 3 it can be concluded that, for isolation of the polypeptide chains of the isoenzymes of β-N-acetylhexosaminidase, FPLC meets the requirements of high resolution and recovery. Moreover, the procedure is fast, being accomplished within 16 min. Since 10 mg of protein can be applied per chromatographic analysis, the FPLC–ion-exchange method enables us to prepare sufficient of the individual polypeptide chains for carbohydrate structural analyses. To our knowledge, FPLC has not been used for separation of the individual polypeptide chains of β-N-acetylhexosaminidase or those of any other oligomeric protein.

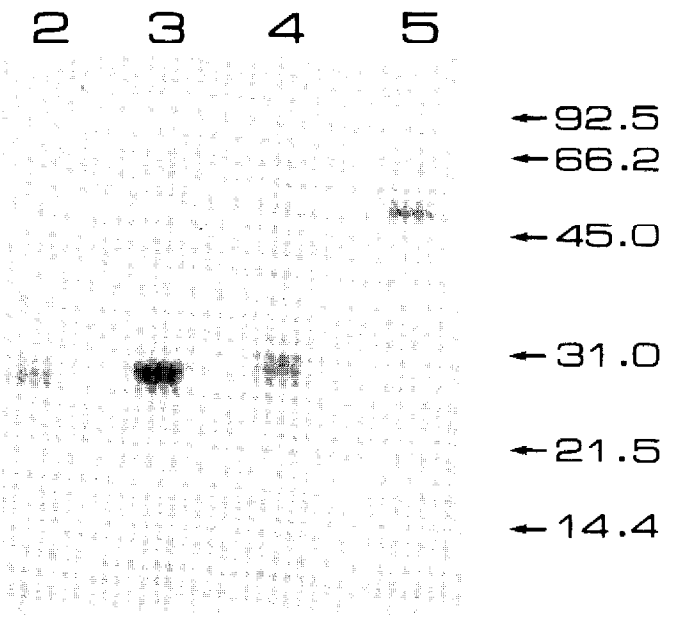


Fig. 3. SDS-PAGE of the polypeptides of human liver β-N-acetylhexosaminidase A. The polypeptide chains were separated by FPLC (see Fig. 2). Lane numbers correspond to the peak numbers of Fig. 2. The electrophoretic and staining procedures were identical to those in Fig. 1. The positions of the molecular weight marker proteins (see Fig. 1) are indicated by arrows and their molecular weights given in kD. Lane 1 (peak 1) is not shown since it did not contain detectable amounts of protein.

It is noteworthy that the initial separation of the A and B forms of β-N-acetylhexosaminidase was performed on DEAE-Sephacel. We have successfully employed FPLC on Mono Q at pH 7.0 and without urea for the separation of the native isoenzymes (data not shown).

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