L-IDURONIDASE IN CULTURED HUMAN FIBROBLASTS AND LIVER*

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SUMMARY

Extracts of normal human livers and skin fibroblasts cultured from normal individuals and patients with the Hurler syndrome released L-iduronic acid when incubated with desulfated dermatan sulfate. Enzyme extracts of normal fibroblasts liberated larger amounts of L-iduronic acid, as judged by paper chromatography, than did homogenates from Hurler fibroblasts. Preliminary studies with desulfated heparan sulfate using the same enzyme systems have also shown material with the R_f of iduronolactone on paper chromatography.

L-Iduronic acid is a constituent of the acid mucopolysaccharides, dermatan sulfate, heparan sulfate and heparin (1, 2). The degradative pathway of these polysaccharides in tissues are not known, but partially degraded dermatan sulfate and heparan sulfate have been isolated from urines and tissues of patients with the mucopolysaccharidoses, Hurler's, Hunter's and Sanfilippo's syndromes (3, 4). Degraded products of heparin have been observed in urine of patients following injection of heparin (5). In vitro studies demonstrated the breakdown of heparan sulfate by rat liver lysosomes (6), and depolymerization of dermatan sulfate by high concentrations of testicular hyaluronidase (7). In none of these investigations has the release

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of free L-iduronic acid been observed. This communication reports the demonstration of L-iduronidase activity in homogenates of normal human liver and of fibroblasts cultured from normal humans and patients with the Hurler syndrome. The L-iduronidase activity of normal fibroblasts appeared to be higher than that of Hurler fibroblasts.

MATERIALS AND METHODS

Fibroblast cultures from biopsies of skin of normal individuals and from patients with the Hurler syndrome were established and maintained as previously described (8). Enzyme extracts were prepared from 2-3 week old confluent cultures containing 1.4 - 1.6 x 10^7 cells per 100 mm Falcon tissue culture dish. Cells from 10 plates were removed by a rubber policeman, suspended in 5 ml of a 0.05 N acetate buffer, pH 4.5, containing 0.15 N NaCl, and disrupted with a Dounce glass homogenizer. Normal human livers were obtained at autopsy 4-8 h after death and kept frozen until used. Extracts were prepared by homogenization of 2 gm of liver in 5 ml of acetate - NaCl buffer following which the homogenates were centrifuged at 600 x g for 10 minutes. The supernatant fractions were used as enzyme preparations. All procedures for the enzyme extraction were carried out at 4°.

For enzymic study, extracts were adjusted to 10 mg of protein per ml for the fibroblasts and 15 mg of protein per ml for the liver. The substrates for measurement of iduronidase activity were desulfated dermatan sulfate and desulfated heparan sulfate prepared by the method of Kantor and Schubert (9). The desulfated dermatan sulfate contained less than 2% sulfate and desulfated heparan sulfate less than 4% sulfate. Each incubation mixture contained 10 mg of substrate, 5 ml of enzyme extract, 1 mg of testicular hyaluronidase (Sigma type VI, 3500 NF units/mg) and 10,000 Fishman units of β-glucuronidase (Sigma type B-3). Controls were prepared by boiling enzyme extracts for 2 min

at pH 6.5, and readjusting the pH to 4.5 before addition of substrate, hyaluronidase and β-glucuronidase. After 36 hours of incubation at 37°, the mixtures were centrifuged at 10,000 x g for 10 min. The supernatant fluid was concentrated to 1.5 ml and chromatographed on Sephadex G-25 columns (superfine, 100 x 0.6 cm) using H_2O as eluent. The effluent fractions obtained between 17 and 25 ml were pooled, passed over Dowex 50, H^+, and then adjusted to pH 2.0 with Dowex 3, $CO_3^=$. The solution was further adjusted to pH 6.8 with sodium carbonate and applied to a Dowex 1, acetate column. The uronic acid fraction was eluted with 3.0 M acetic acid, and the eluate concentrated and chromatographed on Whatman No. 1 paper, using two solvent systems; A) tertiary amyl alcohol-isopropyl alcohol-water (4:1:1.5), and B) ethyl acetate-acetic acid-water (3:1:3). Spots were visualized by a silver reagent. Uronic acid was estimated by the carbazole and orcinol methods and protein was determined as described previously (8). Uronic acid was also determined by gas liquid chromatography (10). Standard L-iduronolactone was prepared by acid hydrolysis of dermatan sulfate and purification by paper chromatography using solvent A.

RESULTS AND DISCUSSION

When incubated with desulfated dermatan sulfate, extracts from normal liver liberated a substance which on paper chromatography showed a mobility identical with authentic iduronolactone in both solvent systems (Fig. 1). No such product was present in boiled controls. A similar spot was observed on incubation of fibroblast extracts with desulfated heparan sulfate. So far insufficient amounts of the product from desulfated heparan sulfate have been isolated for identification. The material obtained from incubations with desulfated dermatan sulfate was isolated on a preparative scale by paper chromatography with solvent system A and was found to have a

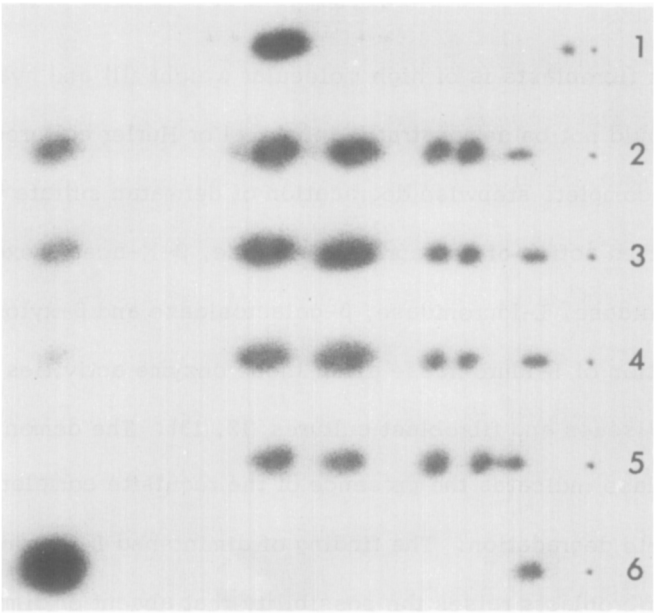

Figure 1. Chromatogram illustrating the production of iduronic acid from desulfated dermatan sulfate by enzyme extracts from liver and fibroblasts. (1) glucuronolactone standard, (2) normal liver extracts (3) normal fibroblast extracts (4) Hurler fibroblast extracts (5) boiled normal fibroblast extracts, (6) iduronolactone standard. The fast moving spot is iduronolactone.

carbazole:orcinol ratio of 0.24, in agreement with the previously reported ratio for iduronic acid (2). Gas liquid chromatography of this fraction showed 2 peaks with retention times similar to authentic iduronolactone (10). Preparations from normal and Hurler fibroblasts also showed iduronidase activity although the activity of Hurler extracts as judged by the intensity of the iduronolactone spots after paper chromatography appeared to be much lower than that found in extracts from normal fibroblasts (Fig. 1).

The most prominent feature of the mucopolysaccharidoses is the accumulation of the L-iduronic acid-containing polysaccharides, dermatan sulfate and heparan sulfate. It is known that dermatan sulfate is partially degraded by hyaluronidase (7), which may account for the small molecular size of mucopolysaccharides deposited in tissues and excreted in urine in the mucopolysaccharidoses. However, the dermatan sulfate accumulated

in Hurler's fibroblasts is of high molecular weight (11) and hyaluronidase activity could not be demonstrated in normal or Hurler cultured fibroblasts (12). The complete stepwise degradation of dermatan sulfate would require the concerted action of appropriate sulfatase, β-N-acetylhexosaminidase, β-glucuronidase, L-iduronidase, β-galactosidase and β-xylosidase. With the exception of L-iduronidase all of these enzyme activities have been shown in tissues and fibroblast cultures (12, 13). The demonstration of L-iduronidase indicates the presence of the requisite complement of enzymes for complete degradation. The finding of diminished L-iduronidase activity in Hurler fibroblasts raises the possibility that absent or diminished L-iduronidase activity plays a role in the pathogenesis of mucopolysaccharidoses. This question is being examined further.

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