ON THE POSSIBLE HYDROLYSIS OF THE α-L-IDURONIDE LINKAGE BY β-D-GLUCURONIDASE (EC 3.2.1.31)

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1. Introduction

The Hurler, Scheie and Hurler-Scheie syndromes are all characterized as the deficiency diseases of α-L-iduronidase (EC 3.2.1.76) [1,2]. For understanding their different clinical manifestations [1–3], however, more detailed characterization of this enzyme will be required. During the course of purification of α-L-iduronidase from the commercial bovine liver β-glucuronidase preparation, we found a hitherto undescribed enzyme species active toward 4-methylumbelliferyl α-L-iduronide.

This paper describes the presence of two distinct species of α-L-iduronidase in the bovine liver preparation and raises the possibility that β-glucuronidase may play a role for the catabolism of α-L-iduronic acid-containing glycosaminoglycans.

2. Materials and methods

2.1. *Chemicals*

4-Methylumbelliferyl α-L-iduronide was prepared as in [4]. Phenyl α-L-iduronide was purchased from Zeria Shinyaku Kogyo (Tokyo). An authentic specimen of 4,6-*O*-isopropylidene-β-L-idofuranurono-6,3-lactone was commercially obtained from Nakarai Chem. (Kyoto). 4-Methylumbelliferyl β-D-glucuronide was from Koch-Light Labs. D-Saccharic acid-1,4-lactone (saccharolactone) was from Sigma Chem. Bovine liver β-glucuronidase was a preparation of Worthington Biochem. (GL 35A991).

2.2. *Enzyme assay*

4-Methylumbelliferyl α-L-iduronidase activity was measured in 0.2 ml total vol. of 0.1 M sodium acetate buffer (pH 4.0) containing 0.9% NaCl, 50 nmol substrate and 50 μl enzyme solution. After incubation at 37°C for 30 min, the reaction was stopped by adding 3 ml 0.2 M sodium carbonate buffer (pH 10) and fluorescence was measured as in [4]. Phenyl α-L-iduronidase activity was determined according to the manufacturer's instruction using 125 μmol substrate and 0.2 ml solution to be assayed (see also [5]). β-Glucuronidase activity was assayed as in [4] using 4-methylumbelliferyl β-D-glucuronide except that incubations were at 37°C.

2.3. *Fractionation of the bovine liver β-glucuronidase preparation*

The commercial preparation with specific glucuronidase activity of 1.25 μmol $.h^{-1}.mg\ protein^{-1}$ (102 mg) was dissolved in 5 ml 0.1 M sodium acetate buffer, (pH 5.0) and fractionated by gel-filtration through an Ultrogel AcA22 column (2.5 × 138 cm) equilibrated with the same buffer. The fraction containing β-glucuronidase activity was further purified by a DEAE-cellulose column (1.5 × 9 cm) in 0.02 M Tris–HCl buffer (pH 7.3). With a linear gradient elution from 0–0.3 M NaCl (200 ml each) a single peak fraction containing β-glucuronidase activity was eluted at 0.12 M NaCl. This fraction was gel-filtered through an Ultrogel AcA22 column (1.4 × 46 cm) and the active fraction to the β-glucuronide was concentrated with a Diafilter and dialyzed against 0.1 M sodium acetate buffer (pH 4.5). The protein was determined

as in [4]. Apparent molecular weights were determined by Sephadex G-150 column as in [6], using *Escherichia coli* β-galactosidase, bovine serum albumin, ovalbumin and horse cytochrome *c* as standards.

3. Results and discussion

Preliminary experiments showed that the commercial β-glucuronidase preparation contained several glycosidases including β-galactosidase, β-glucosidase and *N*-acetyl-β-glucosaminidase. Also fluorescence was observed when 4-methylumbelliferyl iduronide was incubated with this preparation. A chromatographic pattern of this preparation through Ultrogel is seen in fig.1. Two peak fractions (fractions I, II) were active toward 4-methylumbelliferyl iduronide but only fraction II had phenyl iduronidase activity. Fraction I was further purified as mentioned above and a final preparation with specific β-glucuronidase activity of 2.8 μmol $.h^{-1}$ .mg $protein^{-1}$ was designated as fraction PI. During these chromatographic separations 4-methylumbelliferyl iduronidase activity was always co-chromatographed with β-glucuronidase activity.

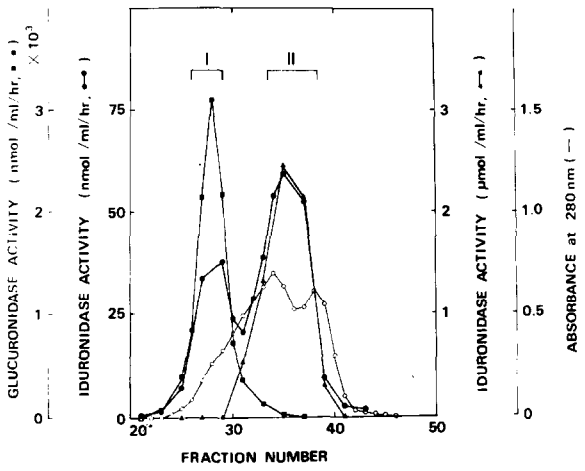

Fig.1. Gel-filtration of the commercial β-glucuronidase preparation through Ultrogel AcA 22. The column (2.5 × 138 cm) was equilibrated with 0.02 M Tris–HCl buffer (pH 7.3) and elution was performed with the same buffer. Each fraction contained 11 ml and was monitored by A_{280} (○). Enzymatic activities for 4-methylumbelliferyl β-D-glucuronide (■), 4-methylumbelliferyl α-L-iduronide (●) and phenyl α-L-iduronide (▲) were determined utilizing 50 μl of each fraction.

Fraction PI contained 4-methylumbelliferyl iduronidase activity corresponding to 1.8% of β-glucuronidase activity but was devoid of phenyl α-L-iduronidase activity.

Although the purity of 4-methylumbelliferyl α-L-iduronide was not rigorously established [4], several lines of evidence supported the idea that fraction PI did hydrolyze this substrate. We showed [4] by gas-chromatographic examination that a maximum contamination of the β-glucuronide was 0.1%, if at all. The present gas-chromatographic study of commercially obtained 4,6-*O*-isopropylidene-iduronolactone confirmed the assignments of peaks to those due to iduronic acid [4]. A molar extinction coefficient of the cyclohexylammonium salt of the same substrate prepared by a different method [7] was reported to be 14 900 which agreed with our value of 14 400 [4].

More convincing evidence came from the identification of the product as seen in fig.2. Fraction PI (110 μg protein) and 4-methylumbelliferyl iduronide (1420 nmol) were incubated at 25°C for 24 h. Under this condition, 4.2% of the substrate was hydrolyzed. Control incubation (without enzyme solution) gave a value corresponding to 0.4% of hydrolysis. The reaction mixture was separated by DEAE-cellulose chromatography as in [4]. The reducing compound eluted first was identified to be iduronic acid (fig.2). Paper chromatography [8] of this product after lactonization confirmed this finding (data not shown). The unhydrolyzed substrate was recovered in another fraction. The hydrolyses of the original substrate and the recovered one by fraction PI with total β-glucuronidase activity of 46.5 nmol $.h^{-1}$ were both linear up to at least 3 h at 37°C and the initial rates were comparable (0.84 and 0.87 nmol/h, respectively).

The pH-dependent activity curves for combinations of enzymes with substrates are shown in fig.3. α-L-Iduronidase of fraction PI was different from that of fraction II in that the former showed no activity at pH < 3.0 (fig.3). The hydrolysis of 4-methylumbelliferyl iduronide by fraction PI was inhibited by saccharolactone which is an inhibitor for β-glucuronidase [9]. Apparent inhibition was 88% and 94% in the presence of 10 μM and 50 μM inhibitor, respectively. In contrast, no appreciable inhibition by saccharolactone was observed in the system of fraction II and 4-methylumbelliferyl α-L-iduronide under similar conditions.

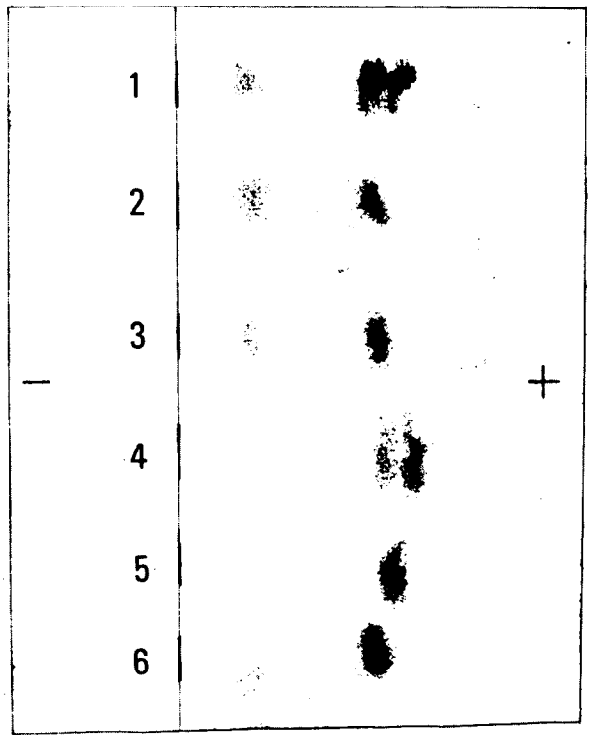

Fig.2. Paper electrophoretic examination of the reaction product obtained after incubation of 4-methylumbelliferyl α-L-iduronide with fraction PI, followed by separation with DEAE-cellulose [4]. Electrophoresis was performed with pyridine–acetic acid–water–formic acid (pH 3.0) [16] for 2 h at 20 V/cm and spots were visualized with an alkaline silver nitrate reagent [4]. (1) A mixture of glucuronic acid and iduronic acid; (2, 6) iduronic acid; (3) the reaction product; (4) the reaction product plus glucuronic acid; (5) glucuronic acid.

Apparent molecular weights of enzyme (s) of fraction PI and fraction II were estimated to be 290 000 and 78 000, respectively. Those for bovine liver β-glucuronidase [10], human urine and kidney α-L-iduronidases [6,11] have been reported to be 290 000, 87 000 and 60 000 ± 6500, respectively.

These data suggest that at least two distinct enzyme species hydrolyzing 4-methylumbelliferyl iduronide are present in the bovine liver preparation. The possibility that fraction PI contained an aggregated form of fraction II cannot be excluded but seems less likely in view of their different behavior toward pH and the inhibitor.

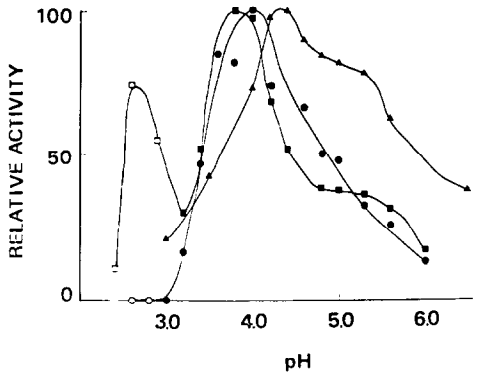

Fig.3. Enzymatic activity curves depending on pH for combinations of fractions with the substrates. Activities were determined in 0.1 M sodium formate buffer (open symbols) and in 0.1 M sodium acetate buffer (closed symbols). (▲) Fraction PI versus 4-methylumbelliferyl β-D-glucuronide. (○, ●) Fraction PI versus 4-methylumbelliferyl α-L-iduronide. (□, ■) Fraction II versus 4-methylumbelliferyl α-L-iduronide.

These observations led the authors to the suggestion that β-glucuronidase may share α-L-iduronidase as an intrinsic property. If so, the residual activity in the Hurler and Scheie syndromes [4,12,13] as detected with this substrate might be in part due to β-glucuronidase activity.

Since phenyl α-L-iduronide was not a substrate for the β-glucuronidase fraction, the conformation of the iduronic acid residue may differ from that in the 4-methylumbelliferyl glycoside. Although the relationship among steric structures of this residue in these substrates and native glycosaminoglycans is not clear at present, this study raises the possibility that β-glucuronidase may play a role in the degradation of certain iduronic acid-containing mucopolysaccharides. This suggestion could provide an answer to the unsettled question as to why β-glucuronidase deficient patients accumulate dermatan sulfate and heparan sulfate [1,14,15].

Acknowledgements

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