Communications to the Editor

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FLUOROMETRIC DETERMINATION OF UNSATURATED DISACCHARIDES PRODUCED ENZYMATICALLY FROM GLYCOSAMINOGLYCANS AND SEPARATED BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Unsaturated disaccharides produced enzymatically from glycosaminoglycans were converted to fluorescent derivatives by treatment with dansylhydrazine. They were separated by high performance liquid chromatography with a TSK-gel NH_2-60 column (Toyo Soda Co. Ltd.) using acetonitrile-0.1 M acetate buffer (pH 5.6)(90/20: v/v) as the mobile phase. The detection limit was a few pmol.

KEYWORDS ——— unsaturated disaccharide; glycosaminoglycan; high performance liquid chromatography; dansylhydrazine

Although paper chromatography has been used for many years to analyze unsaturated disaccharides produced from glycosaminoglycans (GAGs) by digestion with enzymes such as chondroitinase AC and ABC, high performance liquid chromatography (HPLC) has been shown to be a faster and more sensitive method.[1] Recently, Kodama et al.[2] developed a sensitive determination method using HPLC with a pre-column derivatization process. In their method, unsaturated disaccharides were converted to the corresponding pyridylamino derivatives and submitted to HPLC equipped with a fluorometric detector. However, this method was very complicated and the derivatizing was time-consuming.

In this communication, we describe the chromatographic separation of unsaturated disaccharides using dansylhydrazine (N-dimethylaminonaphthalene-5-sulphonic acid hydrazide) as a prelabeling reagent. This process was applied to the determination of reducing sugars[3,4,5] and uronic acids.[6]

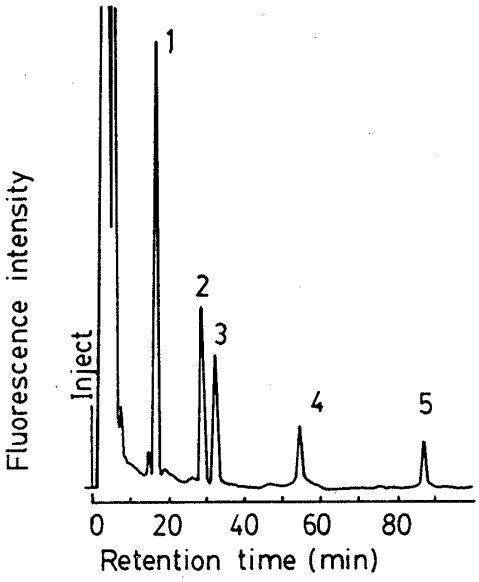
The derivatization procedure was as follows: To 10 μl of the aqueous 90% methanol sample solution containing 8 pmol to 40 nmol of unsaturated disaccharides, 20 μl each of 0.75%(w/v) trichloroacetic acid-ethanol solution and 1.0%(w/v) dansylhydrazine ethanol solution were added. The mixture was incubated at 40°C for 45 min and then cooled to room temperature. An aliquot of the resulting solution was submitted to HPLC. A typical chromatogram of unsaturated disaccharides and the conditions of HPLC are shown in Fig.1.

The calibration curve for ΔDi-0S was linear in the range of 0.8 μM to 4 mM

with a sample size of 10 μl. The curves for ΔDi-6S and ΔDi-4S were 2.8 μM to 4 mM. The detection limits (S/N = 2) were 2-7 pmol.

The retention times of the dansylhydrazine derivatives of unsaturated disaccharides are summarized in Table I.

The proposed method may be useful for the separation and quantification of nmol-pmol levels of the unsaturated disaccharides produced enzymatically from GAGs.



1, ΔDi-0S; 2, ΔDi-6S; 3, ΔDi-4S;
4, ΔDi-diS$_D$; 5, ΔDi-diS$_E$.
Conditions
Column: TSK-gel NH_2-60 (4.6 mm i.d. x 250 mm).
Mobile phase: acetonitrile-0.1 M acetate buffer (pH 5.6)(90/20: v/v).
Flow rate: 1.0 ml/min.
Detector: Shimadzu FLD-1 fluorometric detector (Cut filter EM-5).
Sample size: 10 μl.
Sample: 0.2 nmol each of unsaturated disaccharides.

Fig.1 Chromatogram of Dansylhydrazine Derivatives of Unsaturated Disaccharides

Table I. Retention Times of Dansylhydrazine Derivatives of Unsaturated Disaccharides

Compound	Retention time (min)	Compound	Retention time (min)
ΔDi-HA	14.0	ΔDi-UA2S	25.6
ΔDi-0S	17.0	ΔDi-diS$_B$	79.6
ΔDi-4S	32.0	ΔDi-diS$_D$	54.0
ΔDi-6S	28.4	ΔDi-diS$_E$	87.0

Abbreviations used: ΔDi-HA, 2-acetamido-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-D-glucose; ΔDi-0S, 2-acetamido-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-D-galactose; ΔDi-4S, 2-acetamido-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-4-O-sulfo-D-galactose; ΔDi-6S, 2-acetamido-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-6-O-sulfo-D-galactose; ΔDi-UA2S, 2-acetamido-2-deoxy-3-O-(2-O-sulfo-β-D-gluco-4-enepyranosyluronic acid)-D-galactose; ΔDi-diS$_B$, 2-acetamido-2-deoxy-3-O-(2-O-sulfo-β-D-gluco-4-enepyranosyluronic acid)-4-O-sulfo-D-galactose; ΔDi-diS$_D$, 2-acetamido-2-deoxy-3-O-(2-O-sulfo-β-D-gluco-4-enepyranosyluronic acid)-6-O-sulfo-D-galactose; ΔDi-diS$_E$, 2-acetamido-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-4,6-di-O-sulfo-D-galactose.

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