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学位論文　1　部

題　名　Comparative Studies on Proteodermatan Sulfate of Bovine Gastrointestinal Tract
（牛消化管プロテオデルマタン硫酸の比較検討）

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参考論文　6　部

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学位請求論文の内容の要旨

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（論文題目）

Comparative Studies on Proteodermatan Sulfate of Bovine Gastrointestinal Tract

（牛消化管プロテオデルマタン硫酸の比較検討）

（内容の要旨）

【研究目的】

プロテオグリカン（以下PGと略）は細胞間マトリックスの主要な構成成分であり、細胞接着や細胞認識機構において重要な役割を担っていることが知られている。また、組織の腫瘍化によってPGが質的および量的に変化することが報告されている。最近、この腫瘍や炎症との関わりにおいて、消化管各部位のPGが注目されるようになってきたが、その構造や機能の詳細は不明である。そこで著者は、消化管におけるPGの構造や機能と疾患との関係を明らかにする目的で、その基礎的研究を行った。即ち、牛の消化管各部位よりPGを分離し、その全体像を明らかにするとともに、その主成分であったプロテオデルマタン硫酸（以下proteo-DSと略）の構造を臓器ごとに比較した。

【方法】

1）　PGの分離：脂肪や周囲の結合組織を取り除いた消化管各部位（食道、胃、小腸、大腸）100gを液体窒素下で細粉化し、これを出発標品とした。プロテアーゼ阻害剤を含む4Mグアニジン塩酸／0.05M酢酸ナトリウム緩衝液（pH 5.8）でPGを抽出後、DEAE-セルロースイオン交換クロマトグラフィーとセファロースCL-6Bゲルク

ロマトグラフィーを行いproteo-DSを単離精製した。

２）　グリコサミノグリカン（以下GAGと略）糖鎖の調製：PGを構成しているGAG糖鎖はPGをプロナーゼで消化し、続いてエンド-β-キシロシダーゼで消化することにより調製した。（この方法で得られたGAG糖鎖はペプチドの結合していない完全なデルマタン硫酸（以下DSと略）糖鎖であった。）

３）　GAG糖鎖の同定：ｉ）セルロースアセテート膜二次元電気泳動法；一次元目の泳動は0.1 M ピリジン/0.47 M ギ酸緩衝液（pH 3.0）で１時間行い、二次元目の泳動は 0.1 M 酢酸バリウム溶液（pH 8.0）で４時間行ない、アルシアンブルーで染色後、GAGを標準品との比較により同定した。ii）各種酵素に対する感受性；各種GAG分解酵素（ヒアルロニダーゼ、コンドロイチナーゼ ABC、AC-II、B、ヘパリチナーゼ、ヘパリナーゼ）で消化後、セルロースアセテート膜電気泳動を行いそれぞれの酵素に対する感受性を調べた。

４）　分子サイズの比較：Proteo-DS全体の分子サイズはセファロースCL-6Bゲルクロマトグラフィーで調べ、DS糖鎖としての分子サイズはShodex OHpak KB-802とOHpak KB-803カラムを用いた高速液体クロマトグラフィー(以下HPLC)で調べた。

５）　DS糖鎖の硫酸化の比較：各DS糖鎖をコンドロイチナーゼABCで消化し、得られた消化産物(不飽和二糖)をShodex RS（TypeDC－613）カラムを用いたHPLCで分析し、DS糖鎖への硫酸の結合様式を比較した。

【結果】

１）　各臓器の全体としてのPGはproteo-DSを主体として、他に、食道と胃ではヒアルロン酸が、小腸と大腸ではヒアルロン酸、プロテオコンドロイチン硫酸とプロテオヘパラン硫酸が少量ずつ存在していた。また、小腸でのみプロテオヘパリンが認められた。

２）　精製されたproteo-DSの全体としての分子サイズは、食道と

胃では100万以上、小腸と大腸ではそれよりもやや小さく80～90万であった。すなわち、上部消化管由来のものの分子サイズは下部消化管由来のものより大きい傾向を示した。

３）　DS糖鎖としてのサイズは食道、胃、小腸、大腸でそれぞれ、27,000、24,500、21,000、21,500であった。また、胃と小腸と大腸では、DS糖鎖を構成するN－アセチルガラクトサミンの４位に硫酸が結合しているものが89～93%であった。しかし、食道ではN－アセチルガラクトサミンの65%しか４位に硫酸が結合しておらず、他に比べて硫酸化の程度の低いことが知られた。

４）　組織より最終的に精製されたproteo-DSはウロン酸値としてみると、食道、胃、小腸、大腸でそれぞれ組織湿重量100gあたり、1.76、1.30、5.36、2.79 mg であり小腸におけるproteo-DSの収量が最も高かった。

【考察および結語】

以上の結果より、消化管のproteo-DSについて次のような特徴が明らかになった。１）他の組織に見いだされるproteo-DSの分子サイズ（80,000～285,000）に比べ、消化管のproteo-DSは80万以上で極めて大きかった。２）消化管各部位相互の比較では、各部位ともproteo-DSは全体としての分子サイズや、それを構成しているGAG糖鎖において相互に異なっているが、特に食道はproteo-DSのサイズ、DS糖鎖のサイズとも大きく、またDS糖鎖の硫酸化の程度が低く、他の３つの臓器のproteo-DSとは著しく異なっていることが明らかになった。この違いは消化管各部位の臓器特異性の表われであると考えられる。消化管のPGの詳しい分析、臓器ごとの比較は本研究が初めてであり、このような基礎データーは、各疾患とPGとの関連を調べる上でも非常に重要であると考えられる。

Comparative Studies on Proteodermatan Sulfate of Bovine Gastrointestinal Tract

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Abbreviations used in this paper: GAG, glycosaminoglycan; Ch4S, chondroitin 4-sulfate; Ch6S, chondroitin 6-sulfate; DS, dermatan sulfate; HA, hyaluronic acid; Hep, heparin; HS, heparan sulfate; HPLC, high-performance liquid chromatography; 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; PG, proteoglycan; proteo-DS, proteodermatan sulfate; Gu-HCl, guanidine-HCl; Kav, distribution coefficient average.

Abstract

Proteodermatan sulfate (proteo-DS) was extracted from four areas of the bovine gastrointestinal tract (esophagus, stomach, small intestine and colon) with 4 M guanidine-HCl and then purified by ion-exchange and gel filtration chromatography. Dermatan sulfate (DS) chains composed of proteo-DS from each area were separately prepared by pronase and endo-β-xylosidase digestion. The properties of the proteo-DS and DS chains were compared using electrophoresis and high-performance liquid chromatography. The molecular size of proteo-DS from all areas was estimated to be more than 8 x 10^5, and the molecular sizes of DS chains from esophagus, stomach, small intestine and colon were 27,000, 24,500, 21,000 and 21,500, respectively. The DS chains from the esophagus were slightly undersulfated compared with the others. These results show that the molecular sizes of proteo-DS from different gastrointestinal tract regions are similar to each other, but are larger than those of other tissues. DS chains differed to a slight degree with respect to chain length and sulfation, that from the esophagus being the largest. It seems that these differences in proteo-DS structure reflect the organ specificities of the gastrointestinal tract.

Introduction

Proteoglycans (PGs) are important structural macromolecules in the extracellular matrix (1, 2). Interest in PGs of the gastrointestinal tract has increased considerably in recent years, because of their relationship to physiological functions such as cell adhesion and recognition (3, 4) and to several diseases including cancers (5-10). The main component of PGs in the gastrointestinal tract is proteodermatan sulfate (Proteo-DS) (7, 8, 11, 12). However, only limited details of the chemical nature and physiological function of proteo-DS in the gastrointestinal tract are available (11, 12).

In order to elucidate the organ specificities of proteo-DS in each area of the gastrointestinal tract, it is necessary to purify the dermatan sulfate (DS) chains composed of proteo-DS. Recently, endo-β-xylosidase, which specifically cleaves the xylosylserine linkage of PGs including proteo-DS, was isolated from the mollusc Patinopecten in our laboratory (13, 14, 15). As this enzyme would be an excellent tool for isolating the native glycosaminoglycan (GAG) chains from PG, the author attempted to elucidate the organ specificities of proteo-DS from the bovine gastrointestinal tract, comparing the DS chains liberated from PG using endo-β-xylosidase. This paper describes now the molecules of proteo-DS from each area of bovine gastrointestinal tract differ slightly from each other.

Materials and Methods

Chemicals

Sepharose CL-6B was purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. DEAE-cellulose (DE-32) was obtained from Whatman Chemical Separation Ltd., Maidstone, U.K., and Diaflow PM-10 membrane for ultrafiltration was purchased from Amicon Corp., Lexington, MA, U.S.A. The following materials and enzymes were obtained from Seikagaku Kogyo Co., Tokyo: Hyaluronidase from *Streptomyces hyaluro-lyticus*, heparitinase from *Flavobacterium heparinum*, chondroitinase ABC from *Proteus vulgaris*, chondroitinase AC-II from *Arthrobacter aurescens*, chondroitinase B from *Flavobacterium heparinum*, chondroitin 6-sulfate (Ch6S, mol wt=43,000) from shark cartilage, chondroitin 4-sulfate (Ch4S, mol wt=31,000) from whale cartilage, dermatan sulfate (DS, mol wt=45,000) from pig skin, hyaluronic acid (HA, mol wt=230,000) from human umbilical cord, heparan sulfate (HS, mol wt=15,000) from bovine kidney and 4,5-unsaturated disaccharides (ΔDi-0S, ΔDi-4S and ΔDi-6S).

Pronase P (from *Streptomyces griseus*) was obtained from Kaken Kagaku Co., Tokyo. Endo-β-xylosidase was prepared by the method described in a previous paper (15). Guanidine-HCl (Gu-HCl) and urea were purchased from Wako Pure Chemical Ind. Co., Osaka. Other reagents were of analytical grade and were also obtained from commercial sources.

Isolation of Proteo-DS

Fresh bovine gastrointestinal tracts were obtained from a local abbatoir in Hirosaki. The tissues used were the oral end of the esophagus, the first stomach as representative of the stomach, the jejunum as representative of the small intestine, and the anal end of the colon. The extraction of PG was carried out according to the method described by Heinegård et al. (16). The gastrointestinal tract tissue was immediately dissected free from surrounding tissue and perichondrium, cut into small pieces, frozen in liquid nitrogen, powdered with a Wiley Mill using a 20-mesh screen, and stored at -20°C.

The powder of each tissue (50 g) was extracted with 200 ml of 4 M Gu-HCl in 0.05 M sodium acetate buffer (pH 5.8), containing protease inhibitors (10 mM EDTA, 0.1 M ε-aminocaproic acid, 10 mM N-ethylmaleimide, 5 mM benzamidine-HCl, and 1 mM phenylmethanesulfonyl fluoride) at 4°C with gentle shaking for 24 h. The suspension was centrifuged at 15,000 x g for 30 min at 4°C. The supernatant was concentrated by an ultrafiltration system with a PM 10 filter, dialyzed against 7 M urea in 0.05 M Tris-HCl buffer (pH 6.8) containing protease inhibitors, and passed through a DEAE-cellulose column (3.0 x 30 cm) equilibrated with the same buffer. The column was washed with the same buffer, then proteoglycans were eluted with a linear gradient of 0-2 M NaCl in the same buffer at a flow rate of 40 ml/h. The fractions, which were positive in the carbazole-H_2SO_4 method (18),

were collected and concentrated to 1/10 volume by ultrafiltration, and applied to a column (0.9 x 94 cm) of Sepharose CL-6B equilibrated with 7 M urea in 0.05 M Tris-HCl buffer (pH 6.8). Elution was carried out with the same buffer at a flow rate of 10 ml/h. Proteo-DS prepared as above as dialyzed against distilled water and lyophilized. The purified proteo-DS was stored at 4°C until use.

Isolation of Dermatan Sulfate Chains (DS Chains)

GAG chains were prepared by the method described in an earlier paper (15). In brief, proteo-DS fractions were digested with pronase in 0.1 M Tris-HCl buffer (pH 8.0) containing 10 mM $CaCl_2$ at 50°C for 24 h. The obtained peptido-DS was digested with endo-β-xylosidase in 0.1 M sodium acetate buffer (pH 4.0). The reaction mixture was then treated with 10% trichloroacetic acid. After centrifugation, four volumes of ethanol saturated with NaCl were added to the supernatant. The resulting precipitates were washed with ethanol and ether, dried *in vacuo* and used as DS chains.

Cellulose Acetate Membrane Electrophoresis

One-dimensional electrophoresis on cellulose acetate membrane was carried out using Separax (6 x 22 cm, Jookoo Co., Tokyo) in 0.47 M formic acid/0.1 M pyridine buffer (pH

3.0) at 1 mA/cm for 40 min.

Two-dimensional electrophoresis on cellulose acetate membrane was carried out using Separax (10 x 10 cm) as reported previously (18). The conditions were as follows: first dimension electrophoresis in 0.47 M formic acid/0.1 M pyridine buffer (pH 3.0) at 1 mA/cm for 60 min, and second dimension electrophoresis in 0.1 M barium acetate (pH 8.0) at 1 mA/cm for 4 h. The resulting electrophoretograms were visualized with 0.1% alcian blue in 70% ethanol.

Enzymatic Digestion

GAGs were treated with hyaluronidase, chondroitinase AC-II, chondroitinase ABC, chondroitinase B, and heparitinase. The reactions with hyaluronidase were performed in 0.1 M acetate buffer (pH 8.0) (19), those with chondroitinase AC-II, chondroitinase ABC (20) and chondroitinase B (21) in 0.1 M Tris-HCl buffer (pH 8.0), and those with heparitinase were performed in 0.1 M Tris-HCl buffer (pH 7.0) (22). The reaction mixture was treated with four volumes of ethanol saturated with NaCl. The resulting precipitates were washed with ethanol and ether and dried in vacuo.

For unsaturated disaccharide analysis, samples were prepared as shown below. Each DS chain was digested with chondroitinase ABC, four volumes of ethanol were added and the sample was centrifuged. The resulting supernatant was used for analysis.

High-performance Liquid Chromatography (HPLC)

A liquid chromatograph (Hitachi L-6200) equipped with a refractive index monitor (Hitachi L-3300) and a UV monitor (Hitachi L-4200) was used.

Gel filtration of GAG was carried out with a Shodex OHpaK KB-802 column (0.8 x 30 cm, Shoko, Tokyo) and a Shodex OHpak KB-803 column (0.8 x 30 cm) using 0.2 M NaCl. The flow rate was 0.5 ml/min and the column temperature was 30°C. The eluate was monitored by measurement of refractive index.

Analysis of unsaturated disaccharides (ΔDi-0S, ΔDi-4S and ΔDi-6S), which were obtained from GAG chains by chondroitinase ABC digestion, was performed with two columns connected end to end with Shodex RS (Type DC-613, 0.6 x 15 cm, Shoko, Tokyo) using acetonitrile/methanol/0.5 M ammonium formate buffer (pH 4.5) (13 : 3 : 4, v/v/v) (23). The flow rate was 0.9 ml/min and the column temperature was 70°C. The eluate was monitored at 232 nm.

Chemical Analysis

Uronic acid was determined by the carbazole-H_2SO_4 method of Bitter and Muir (17). Protein concentration was determined from the absorbance at 280 nm (24).

Results

Properties of Proteo-DS from the Gastrointestinal Tract

PGs were extracted from whole layers of each part of the gastrointestinal tract with 4 M Gu-HCl. In this step, the amounts of GAG in each area (100 g) were compared in terms of uronic acid content (Table 1). The uronic acid content in each area ranged from 33 to 84 mg per 100 g wet tissue, the value for the small intestine being the highest.

To characterize GAG components of PGs from the gastrointestinal tract, a part of the proteoglycans from the crude extract step was digested successively with pronase and endo-β-xylosidase, and subjected to two-dimensional electrophoresis on a cellulose acetate membrane (Figure 1).

HA and DS accounted for a major component of GAGs in the gastrointestinal tract. Hep was detected specifically in the small intestine. Ch4S or Ch6S, and HS were detected in the small intestine and colon. Each GAG was identified by sequential enzymatic digestion (hyaluronidase, chondroitinase ABC, chondroitinase AC-II, chondroitinase B, and heparitinase; data not shown).

Comparative Studies of Proteo-DS

Proteo-DSs of the gastrointestinal tract were initially purified by ion-exchange chromatography on a DEAE-cellulose

column in 7 M urea with NaCl gradient elution (Figures 2-5). PGs (uronic acid-containing fraction) from the esophagus and small intestine were eluted as a single peak, whereas those from the stomach and colon eluted as multiple peaks. Therefore, after digestion with pronase and endo-β-xylosidase, the GAG components of each fraction were checked using electrophoresis on cellulose acetate membrane. As shown in Figures 2-5, the fractions which contained a large amount of DS were pooled, and then further purified by chromatography on Sepharose CL-6B (Figure 6). It was found that proteo-DSs from the esophagus and stomach were eluted in the void volume, whereas those from the small intestine and colon were eluted with Kav values of 0.05 and 0.12, respectively. These results indicated that the molecular size of proteo-DS from esophagus and stomach was greater than 1×10^6, and those of the small intestine and colon were estimated at about $8\text{-}9 \times 10^5$. Thus, the molecular size of proteo-DS from the esophagus and stomach was larger than that from the small intestine and colon.

As shown in Figure 6, fractions were pooled as indicated by horizontal bars, dialyzed against distilled water and lyophilized. The preparations were used as purified proteo-DS. As shown in Table 1, the yield of purified proteo-DS ranged from 0.0013% to 0.005% of wet tissue weight.

Comparative Studies of DS Chains

To obtain the native DS chains, each proteo-DS was digested successively with pronase and endo-β-xylosidase. The GAG components were identified using two-dimensional electrophoresis on cellulose acetate membrane (Figure 7). All the GAG fractions of proteo-DS were composed of DS chains, and it was also confirmed that the purified PGs contained only DS chains. The molecular size of DS chains was compared by HPLC using a Shodex OHpak KB-802 column and a OHpak KB-803 column for gel filtration (Figure 8). The results indicated that the molecular sizes of DS chains from the esophagus, stomach, small intestine and colon were 27,000, 24,500, 21,000, and 21,500, respectively. Thus, the molecular size of DS chains from upper gastrointestinal tract regions (esophagus and stomach) was larger than that from lower tract (small intestine and colon).

In order to determine the degree of sulfation of DS chains were digested with chondroitinase ABC and the unsaturated disaccharides formed by the digestion were analyzed using HPLC on Shodex RS (Type DC-613). As shown in Figure 9, complete separation and accurate quantitation of ΔDi-OS, ΔDi-4S and ΔDi-6S were accomplished. The results are summarized in Table 2. For all samples, ΔDi-4S was demonstrated to be the major product which deriving from DS chains. Also a significant amount of ΔDi-OS was found, deriving from under-sulfated DS chains. However, the percentage of ΔDi-OS from esophagus preparations was higher than that from

other areas. This indicated that the DS chain from esophagus was undersulfated. No appreciable amount of $^{\Delta}$Di-6S was detected. Thus, none of the DS chains of the gastrointestinal tract appeared to contain chondroitin sulfate hybrid structures.

Discussion

The present study involved purification of proteo-DS obtained from four different areas of the bovine gastrointestinal tract (esophagus, stomach, small intestine and colon), and comparison of the native DS chains which were prepared by endo-β-xylosidase digestion. Endo-β-xylosidase specifically acts only on the xylosylserine linkage between GAG chains and the protein moiety of PG. Thus, using this enzyme, it was possible to analyze native GAG chains by HPLC or electrophoresis, and to make comparisons of those with similar characteristics. The following results were obtained.

1) The major component of PGs from each area of the bovine gastrointestinal tract was proteo-DS. Small amounts of proteo-Hep were detected specifically only in the small intestine, and a small amount of proteo-ChS and proteo-HS was detected in the small intestine and colon. A small amount of HA was detected in four areas of the gastrointestinal tract.

2) The molecular size of esophagus and stomach proteo-DS,

as estimated by gel filtration on Sepharose CL-6B, as greater than 1×10^6, and that of small intestine and colon proteo-DS as about $8\text{-}9 \times 10^5$. Thus, the molecular size of proteo-DS from the upper gastrointestinal tract (esophagus and stomach) was larger than that of the lower tract (small intestine and colon).

3) The molecular sizes of native DS chains from esophagus, stomach, small intestine and colon were 27,000, 24,500, 21,000 and 21,500, respectively. The molecular size of DS chains of the upper gastrointestinal tract was therefore also larger than that of the lower tract.

4) The degrees of sulfation of DS chains from stomach, small intestine and colon were similar to each other. However, the DS chain from the esophagus was slightly undersulfated compared with the others.

Proteo-DS has been isolated and characterized from connective tissue with elastic properties such as skin (25, 26), aorta (27), sclera (28), articular cartilage (28) and tendon (29). These proteo-DSs ranged in molecular size from 80,000 to 285,000, and were classified as small PGs. However, there have been few studies on proteo-DS from gastrointestinal tract regions possessing elasticity (11, 12). In this study, the molecular size of proteo-DS from the gastrointestinal tract was estimated to be more than 8×10^5. This is larger than most other proteo-DS, but similar to PG from porcine ovarian follicular fluid (30).

These results show that the proteo-DS of esophagus is different from that of other parts of the gastrointestinal

tract, in terms of molecular size and degree of sulfation of the DS chain. The esophagus does not perform the function of digestion and absorption, but plays a role in transferring food through its tract by peristaltic movement. The main histological differences between the esophagus and other gastrointestinal tract regions are that the inner surface of the esophagus is mostly covered with squamous epithelium and that only a small number of glands are contained in the esophageal wall (27). Therefore, the proteo-DS of esophagus might be different from those of other gastrointestinal tract regions. Specific diseases of the esophagus, such as progressive systemic sclerosis (31), have been observed. This specific disturbance may be related to the constituents of the connective tissue including proteo-DS.

It seems that PGs may play a role not only in connective tissue but also in tumor tissue. Several investigations have reported the presence of PGs in animal tumors (9) or in certain specific human gastrointestinal cancers (7, 8, 10). However, organ specificities of PG including proteo-DS of the gastrointestinal tract remain vague. The organ specificities of PG described above may reflect physiological function. As reports on PGs including proteo- DS concerned with cancer of the gastrointestinal tract are increasing, the importance of PGs in the gastrointestinal tract will become on important research topic.

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Legend

Figure 1. Two-dimensional electrophoretograms on cellulose acetate membrane of proteoglycan fractions derived from the bovine gastrointestinal tract. Electrophoresis was carried out on Separax (10 x 10 cm) under the following conditions: 0.47 M formic acid/0.1 M pyridine buffer (pH 3.0) at 1 mA/cm for 60 min in the first dimension and 0.1 M barium acetate (pH 8.0) at 1 mA/cm for 4 h in the second dimension. The electrophoretogram was stained with 0.1% alcian blue in 70% ethanol. A, esophagus; B, stomach; C, small intestine; D, colon. Reference standards used are: chondroitin 6-sulfate (C6S), dermatan sulfate (DS), hyaluronic acid (HA), heparin (Hep) and heparan sulfate (HS).

Figure 2. Ion-exchange chromatography on DEAE-cellulose of proteoglycans from esophagus. The column (3.0 x 30 cm) was eluted with a NaCl gradient in 7 M urea/0.05 M Tris-HCl buffer (pH 6.8) at 40 ml/h, and 15-ml fractions were collected. The fractions were determined for uronic acid (●) and absorbance at 280 nm (○) and each fraction was analyzed by one-dimensional electrophoresis. Fractions were pooled as indicated (▬). ChS, chondroitin 6-sulfate; DS, dermatan sulfate; HA, hyaluronic acid.

Figure 3. Ion-exchange chromatography on DEAE-cellulose of PG from stomach. Conditions are described in the legend of Figure 2.

Figure 4. Ion-exchange chromatography on DEAE-cellulose of PG from small intestine. Conditions are described in the legend of Figure 2.

Figure 5. Ion-exchange chromatography on DEAE-cellulose of PG from colon. Conditions are described in the legend of Figure 2.

Figure 6. Gel filtration chromatography on Sepharose CL-6B of proteo-DS from regions of the bovine gastrointestinal tract. The columns (0.9 x 94 cm) were equilibrated and eluted with 7 M urea/0.05 M Tris-HCl buffer (pH 6.8) at 10 ml/h, and 1.2-ml fractions were collected. The fractions were determined for uronic acid. Fractions were pooled as indicated (▬). A, esophagus; B, stomach; C, small intestine; D, colon.

Figure 7. Two-dimensional electrophoretograms on cellulose acetate membrane of GAG obtained by pronase and endo-β-xylosidase digestion from purified proteo-DS. A, esophagus; B, stomach; C, small intestine; D, colon. Conditions are described in the legend of Figure 1.

Figure 8. Determination of molecular size of DS chains from the bovine gastrointestinal tract. The molecular size was estimated by gel HPLC on gel filtration with Shodex OHpak KB-802 and KB-803 columns using 0.2 M NaCl. The flow rate was 0.5 ml/min and the column temperature was 30°C. The eluate was monitored by measurement of refractive index. The following standard glycosaminoglycans were used for comparison, hyaluronic acid (mol wt=230,000), chondroitin 6-sulfate (mol wt=43,000), chondroitin 4-sulfate (mol wt=31,000), dermatan sulfate (mol wt=45,000), and heparan sulfate (mol wt=15,000). Arrows 1, 2, 3 and 4 indicate DSs from esophagus, stomach, small intestine and colon, respectively.

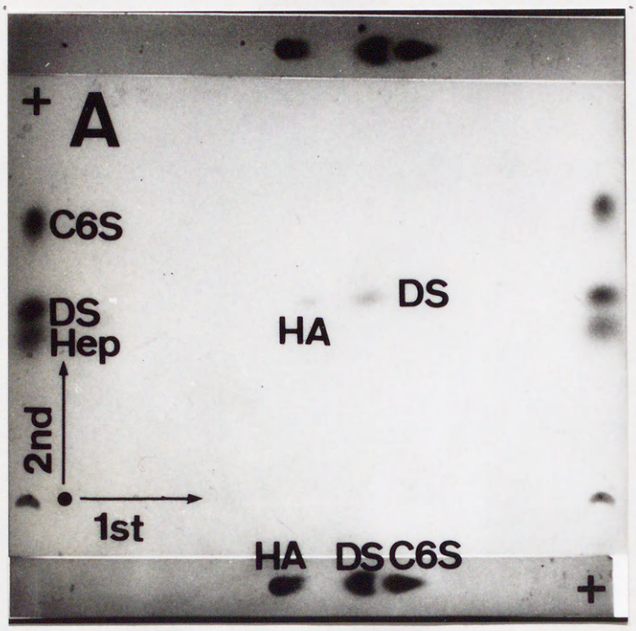
Figure 9. HPLC of unsaturated disaccharides derived from DS by chondroitinase ABC digestion. Two Shodex RS (Type DC-613, 0.6 x 15 cm) columns connected end to end were used, and elution was carried out with acetonitrile/methanol/0.5 M ammonium formate buffer (pH 4.5) (13 : 3 : 4, v/v/v) at a flow rate of 0.9 ml/min and a column temperature of 70°C. A, esophagus; B, stomach; C, small intestine; D, colon. Arrows 1, 2 and 3 indicate ΔDi-6S, ΔDi-0S and ΔDi-4S, respectively.

Table 1. Hexouronic acid contents of crude extract and purified proteodermatan sulfates of various regions of bovine gastrointestinal tract

	Tissues			
	Esophagus	Stomach	Small intestine	Colon
	mg/100 g wet tissue			
Crude extract	50	33	84	51
Purified proteo-DS	1.76	1.30	5.36	2.79

Table 2. Unsaturated disaccharide analysis of chondroitinase ABC-digested DS chains

	ΔDi-0S	ΔDi-4S	ΔDi-6S
		%	
Esophagus	35	65	trace
Stomach	11	89	trace
Small intestine	7	93	trace
Colon	7	93	trace


+
A
C6S
DS
Hep
DS
HA
2nd
1st
HA
DS
C6S
+

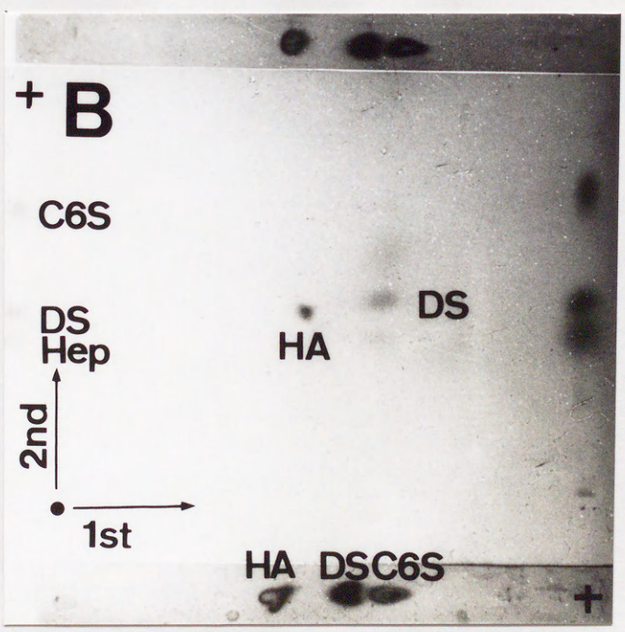
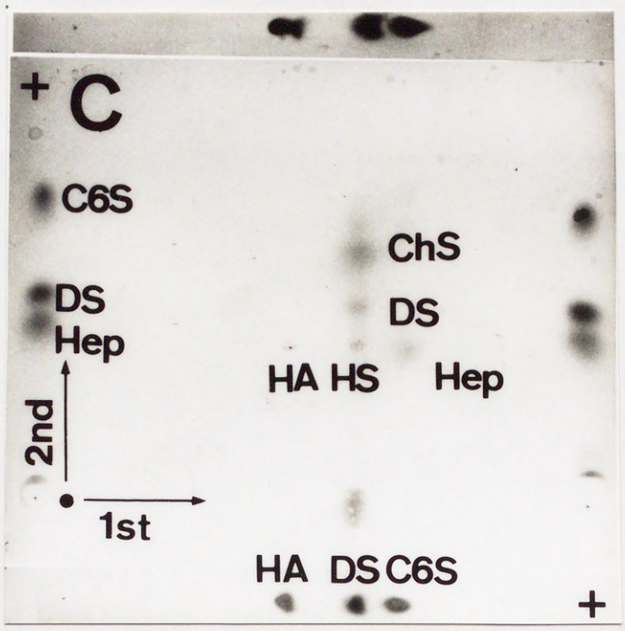

+
B
C6S
DS
Hep
DS
HA
2nd
1st
HA
DS
C6S
+

Figure 1


+
C
C6S
ChS
DS
DS
Hep
HA HS Hep
2nd
1st
HA DS C6S
+

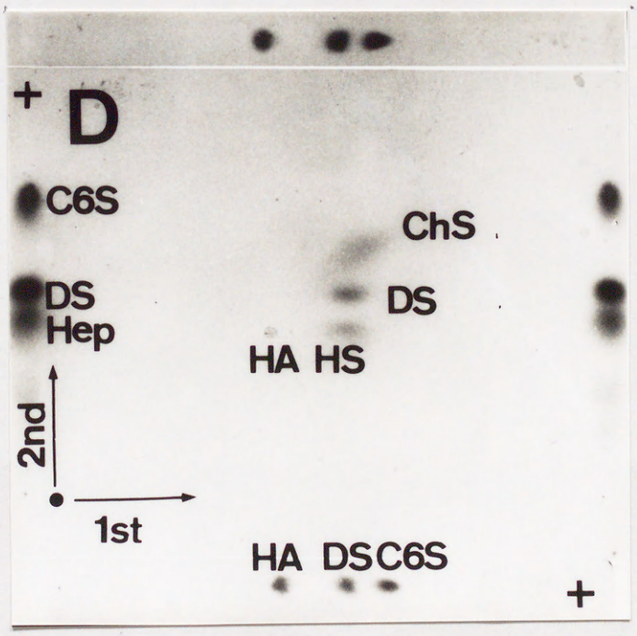

+
D
C6S
ChS
DS
DS
Hep
HA HS
2nd
1st
HA DS C6S
+

Figure 1

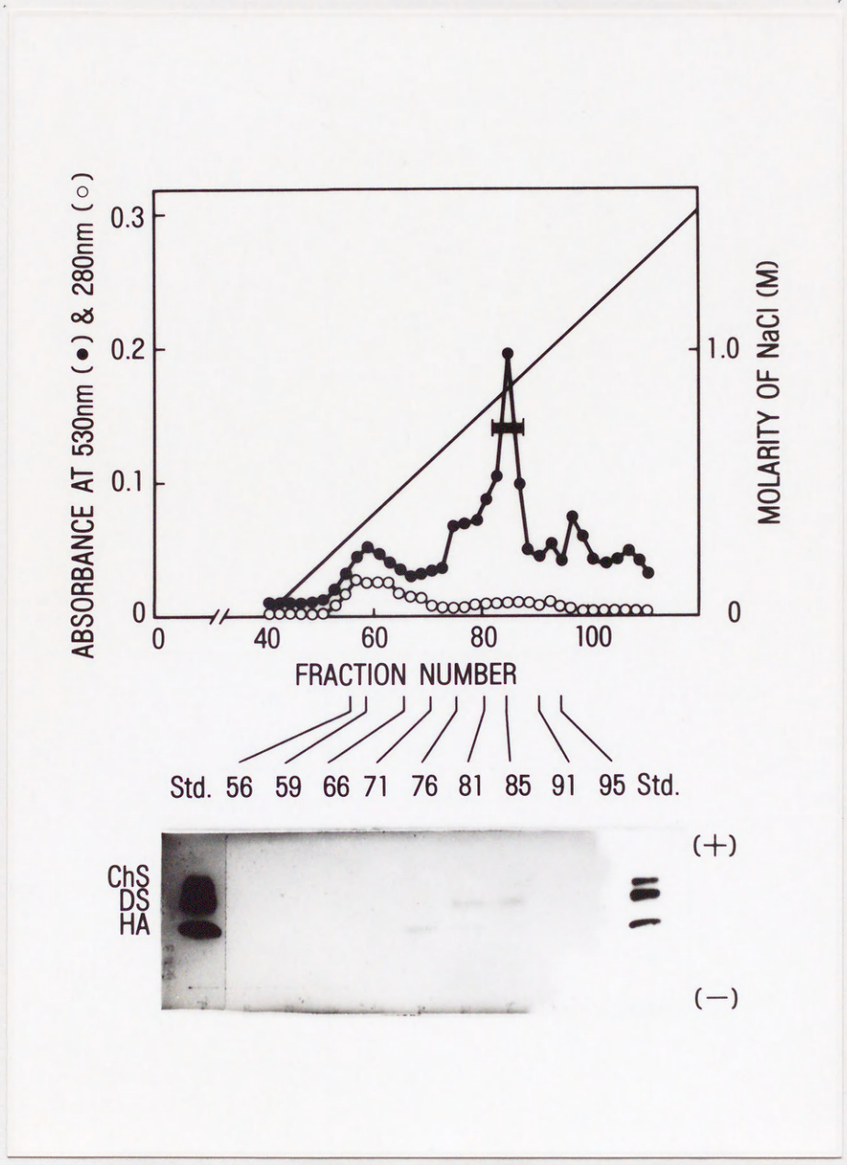

ABSORBANCE AT 530nm (●) & 280nm (○)
0.3
0.2
0.1
0
0
40
60
80
100
1.0
0
MOLARITY OF NaCl (M)
FRACTION NUMBER
Std. 56 59 66 71 76 81 85 91 95 Std.
ChS
DS
HA
(+)
(−)

Figure 2

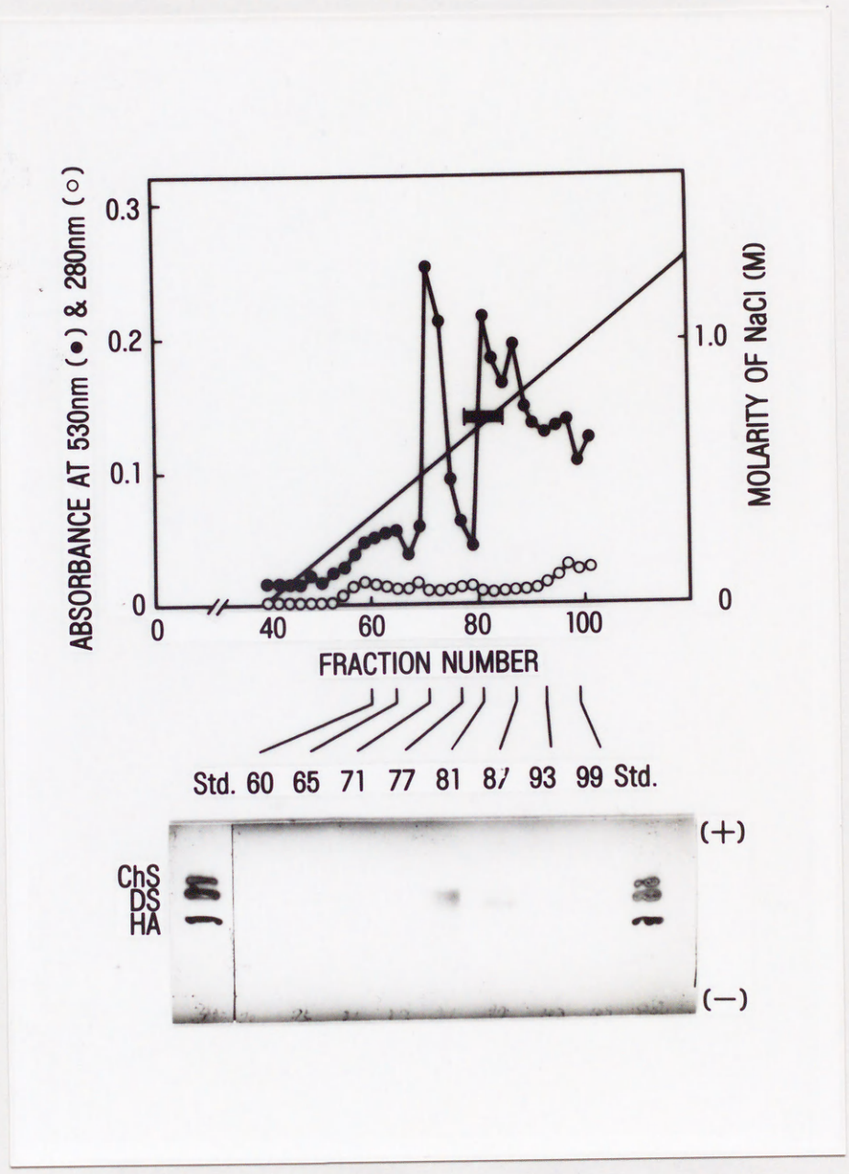

ABSORBANCE AT 530nm (●) & 280nm (○)
0.3
0.2
0.1
0
0
40
60
80
100
1.0
0
MOLARITY OF NaCl (M)
FRACTION NUMBER
Std. 60 65 71 77 81 87 93 99 Std.
ChS
DS
HA
(+)
(−)

Figure 3

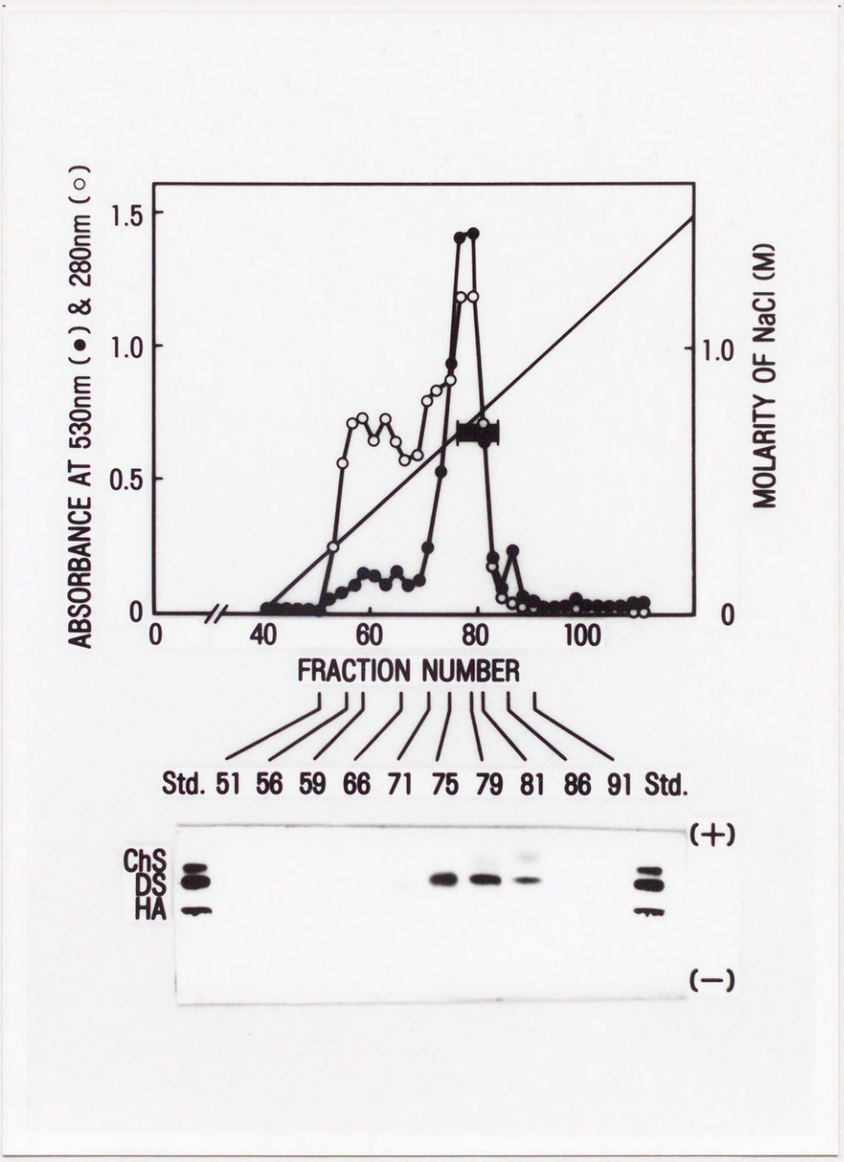

ABSORBANCE AT 530nm (●) & 280nm (○)
1.5
1.0
0.5
0
0
40
60
80
100
1.0
0
MOLARITY OF NaCl (M)
FRACTION NUMBER
Std. 51 56 59 66 71 75 79 81 86 91 Std.
ChS
DS
HA
(+)
(−)

Figure 4

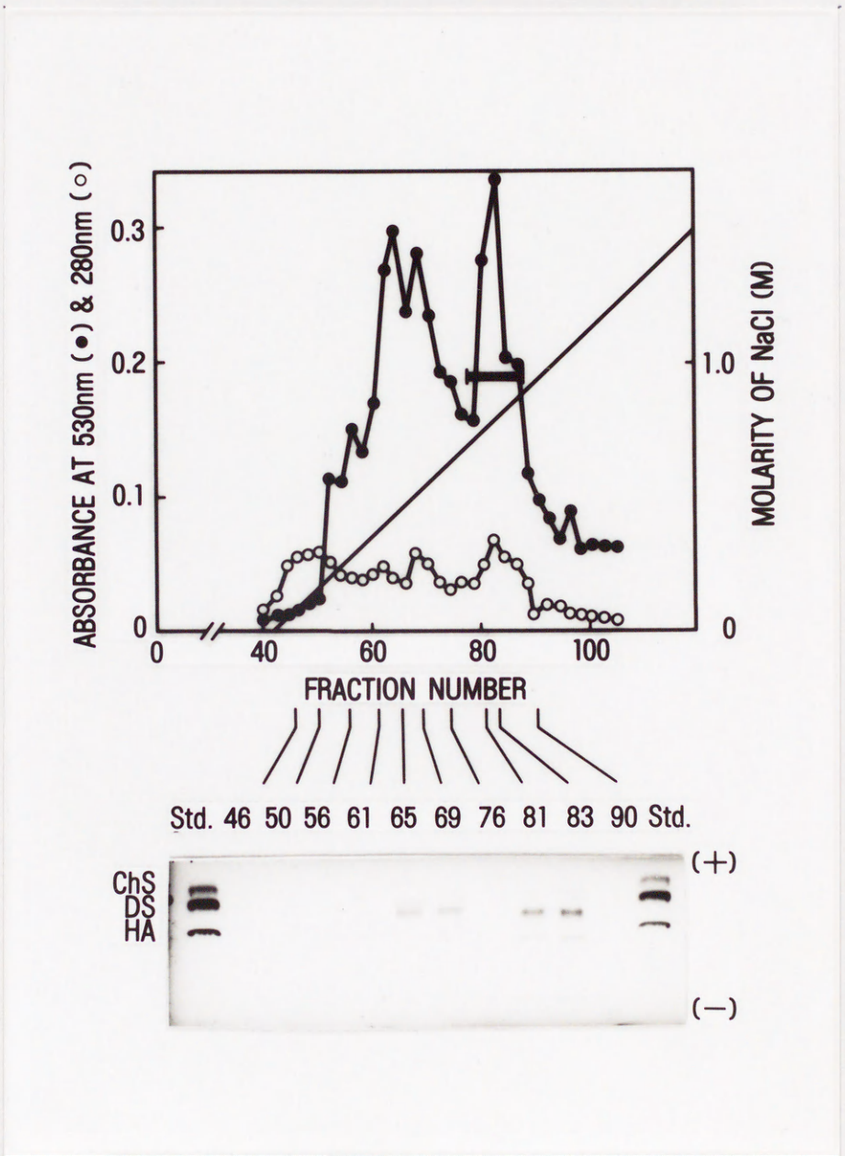

ABSORBANCE AT 530nm (●) & 280nm (○)
0.3
0.2
0.1
0
0
40
60
80
100
1.0
0
MOLARITY OF NaCl (M)
FRACTION NUMBER
Std. 46 50 56 61 65 69 76 81 83 90 Std.
ChS
DS
HA
(+)
(−)

Figure 5

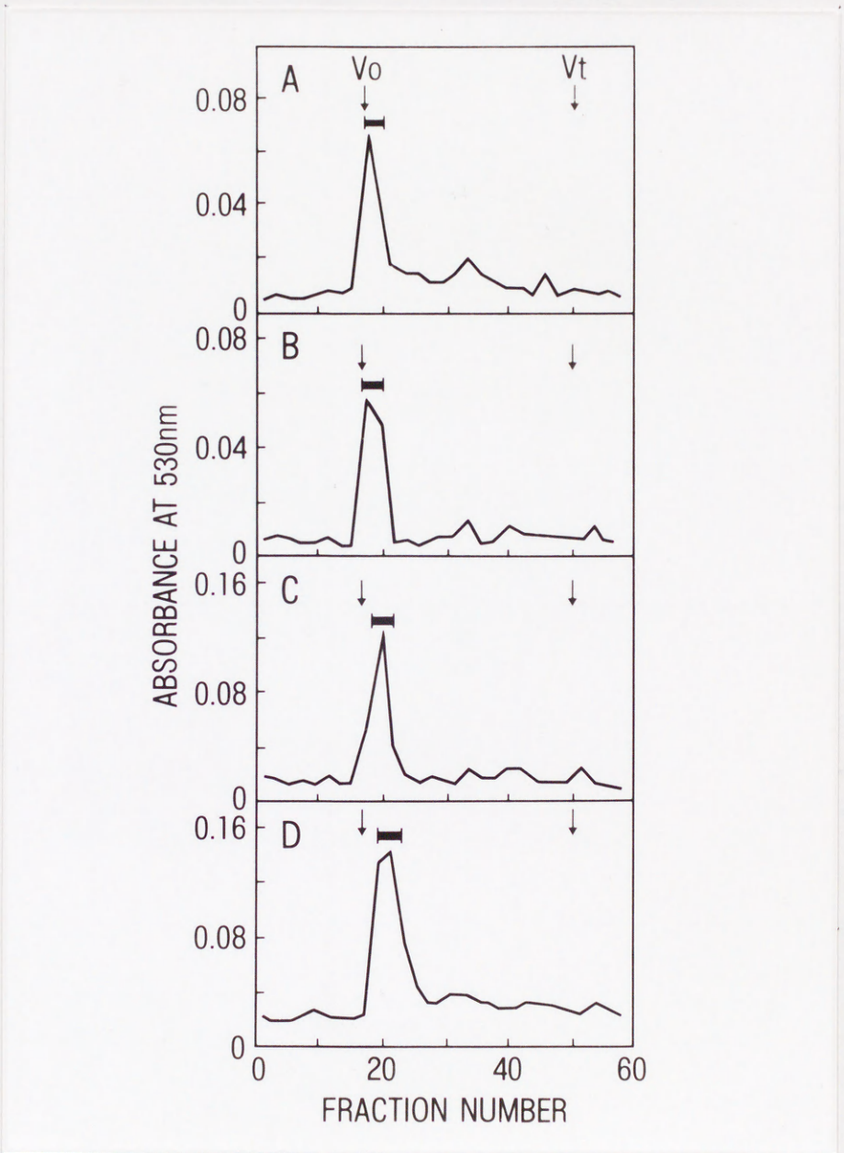
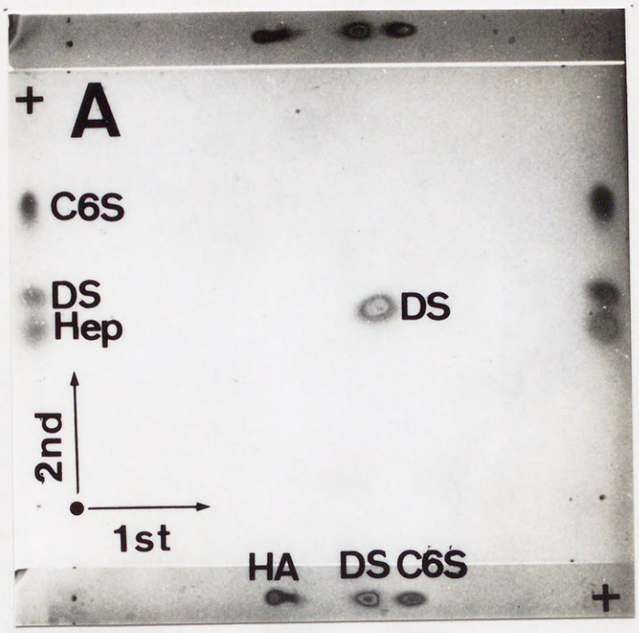

A
Vo
Vt
0.08
0.04
0
B
0.08
0.04
0
C
0.16
0.08
0
D
0.16
0.08
0
ABSORBANCE AT 530nm
0
20
40
60
FRACTION NUMBER

Figure 6



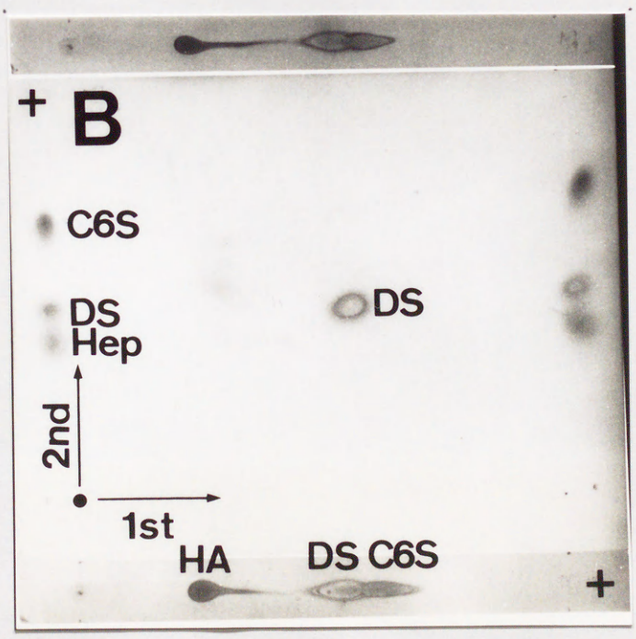
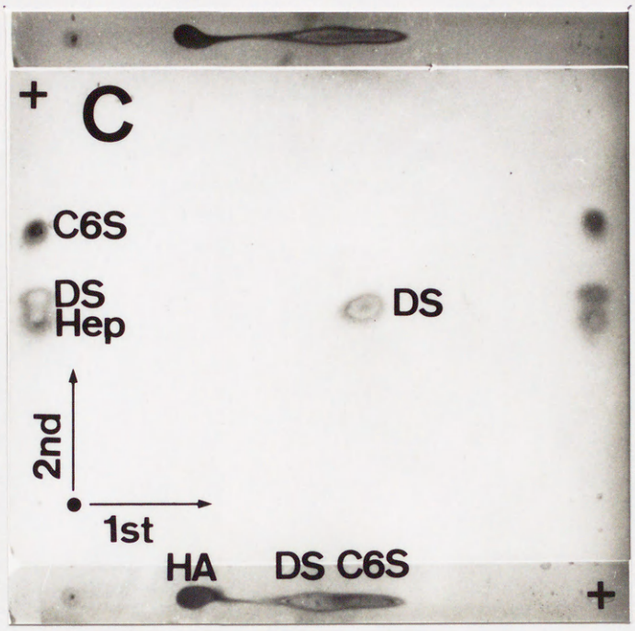


Figure 7



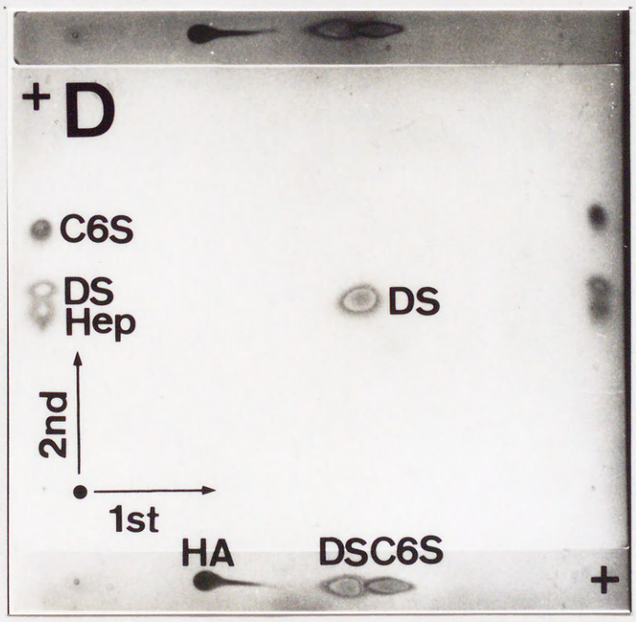


Figure 7

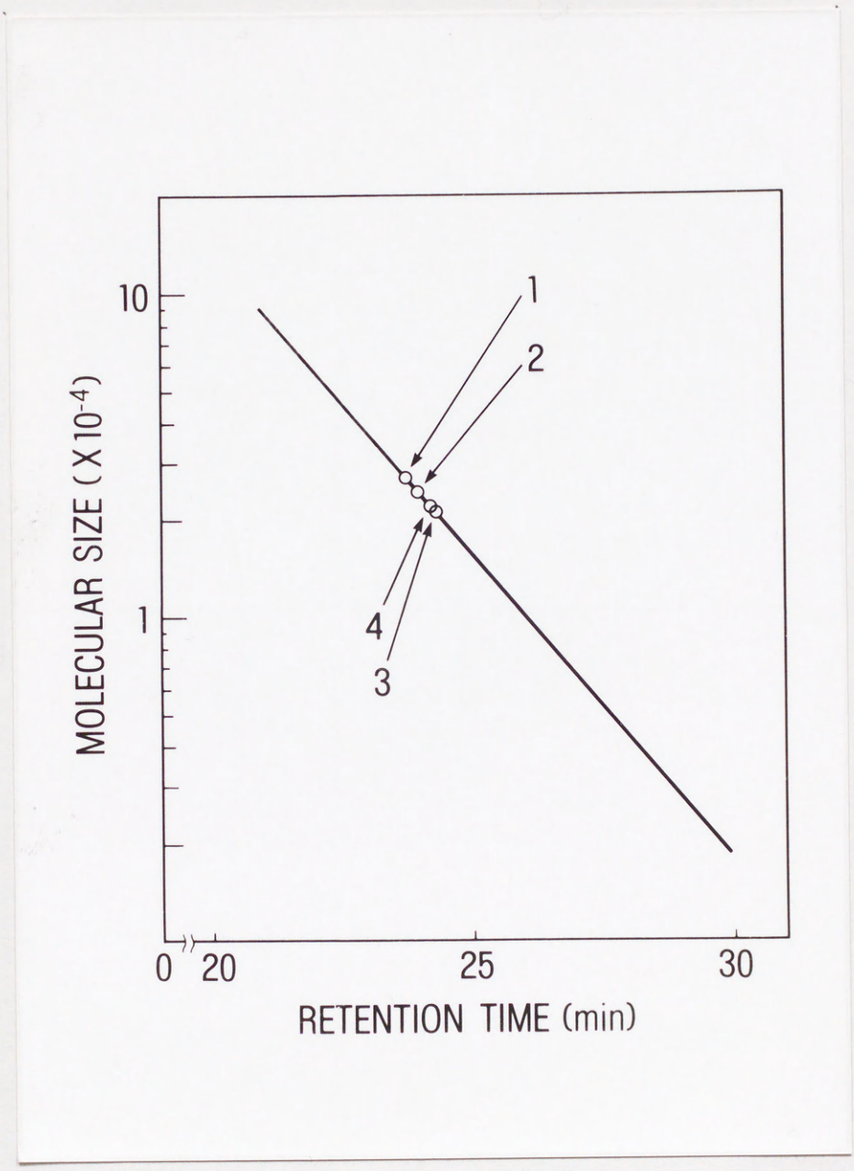


Figure 8

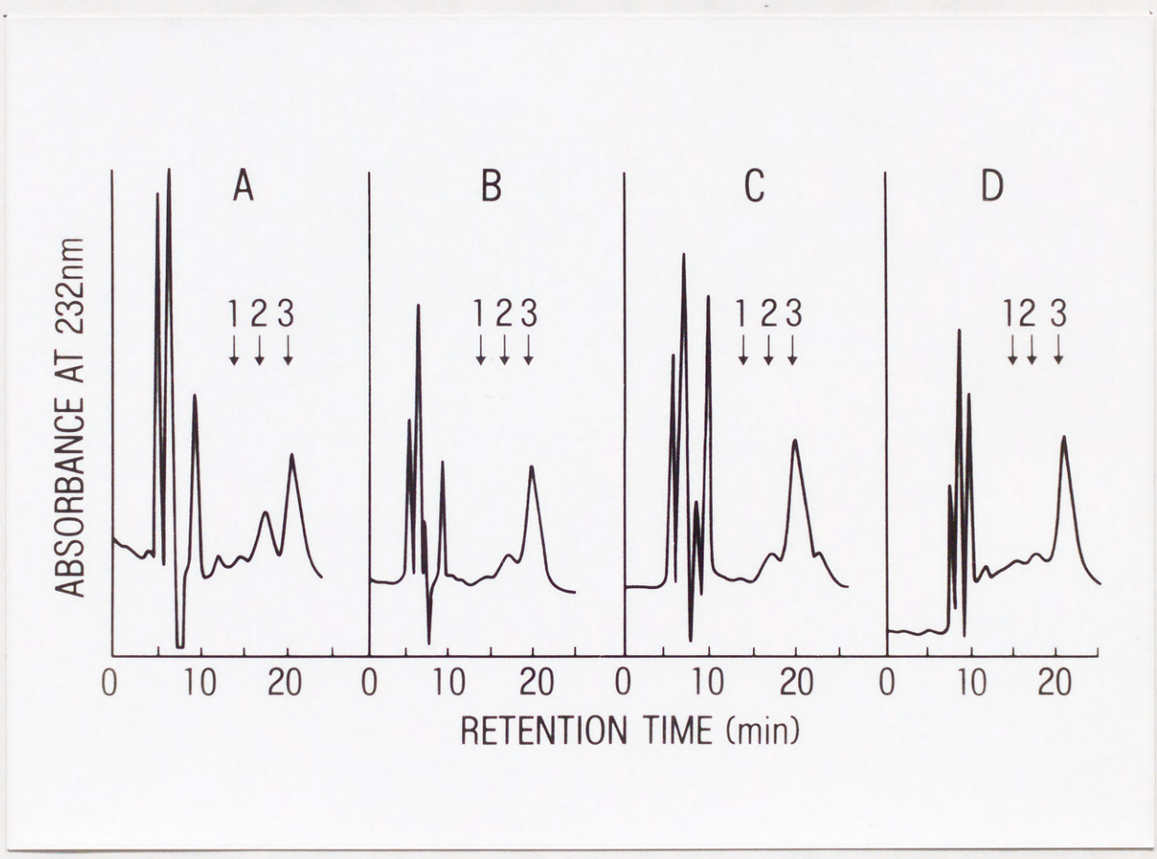

A
B
C
D
1 2 3
1 2 3
1 2 3
12 3
ABSORBANCE AT 232nm
0
10
20
RETENTION TIME (min)

Figure 9