Vol. 162, No. 1, 1989 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 14, 1989 Pages 189-197

THE ENGELBRETH-HOLM-SWARM MOUSE TUMOR PRODUCES UNDERSULFATED HEPARAN SULFATE AND OVERSULFATED GALACTOSAMINOGLYCANS

Kazuyuki Sugahara, Yutaka Okumura and Ikuo Yamashina[1]

Department of Biological Chemistry
Faculty of Pharmaceutical Sciences
Kyoto University, Kyoto 606, JAPAN

Received May 8, 1989

Glycosaminoglycans were prepared from the Engelbreth-Holm-Swarm mouse tumor. Enzymatic analysis demonstrated heparan sulfate (95.8%) and chondroitinase ABC-sensitive galactosaminoglycans (4.2%). HPLC analysis of the disaccharide units showed that heparan sulfate chains were undersulfated on average, comprizing approximately 30% nonsulfated and 60% N-sulfated disaccharide units with small proportions of other monosulfated and disulfated disaccharide units. In contrast, galactosaminoglycan chains were oversulfated, containing an appreciable proportion (15%) of a 4,6-disulfated (so-called E-type) disaccharide unit in addition to 51% of a 4-sulfated, 22% of a 6-sulfated, and 11% of a nonsulfated disaccharide unit. The significance of the oversulfated disaccharide structure is discussed in relation to the possible regulation of functions of hybrid proteoglycans from which the galactosaminoglycan chains are derived.

Recently so-called hybrid proteoglycans (PGs) with two different types of glycosaminoglycans (GAGs) have been reported. Cell surface PG, named syndecan, from mouse mammary epithelial

[1]Present address: Department of Biotechnology, Faculty of Engineering, Kyoto Sangyo University, Kyoto 603, Japan.

Abbreviations used: EHS, Engelbreth-Holm-Swarm; PG, proteoglycan; GAG, glycosaminoglycan; HS, heparan sulfate; CS, chondroitin sulfate; DS, dermatan sulfate; ΔDi-0S, 2-acetamido-2-deoxy-3-O-(4-deoxy-α-L-threo-hex-4-enepyranosyluronic acid)-D-galactose; ΔDi-UA2S, 2-acetamido-2-deoxy-3-O-(4-deoxy-2-O-sulfo-α-L-threo-hex-4-enepyranosyluronic acid)-D-galactose; ΔDi-6S, 2-acetamido-2-deoxy-3-O-(4-deoxy-α-L-threo-hex-4-enepyranosyluronic acid)6-sulfo-D-galactose; ΔDi-4S, 2-acetamido-2-deoxy-3-O-(4-deoxy-α-L-threo-hex-4-enepyranosyluronic acid)-4-O-sulfo-D-galactose; $\Delta Di\text{-}diS_D$, 2-acetamido-2-deoxy-3-O-(4-deoxy-2-O-sulfo-α-L-threo-hex-4-enepyranosyluronic acid)-6-O-sulfo-D-galactose; $\Delta Di\text{-}diS_B$, 2-acetamido-2-deoxy-3-O-(4-deoxy-2-O-sulfo-α-L-threo-hex-4-enepyranosyluronic acid)-4-O-sulfo-D-galactose; $\Delta Di\text{-}diS_E$, 2-acetamido-2-deoxy-3-O-(4-deoxy-α-L-threo-hex-4-enepyranosyluronic acid)4,6-di-O-sulfo-D-galactose; ΔDi-triS, 2-acetamido-2-deoxy-3-O-(4-deoxy-2-O-sulfo-α-L-threo-hex-4-enepyranasyluronic acid)-4,6-di-O-sulfo-D-galactose; ΔUA, 4,5-unsaturated uronic acid.

0006-291X/89 $1.50

cells (1,2) and extracellular PG from the Engelbreth-Holm-Swarm (EHS) mouse tumor (3) bear both heparan sulfate (HS) and chondroitinase ABC-sensitive galactosaminoglycan chains. PG isolated from human placenta contains HS and dermatan sulfate (DS) chains (4). Recent evidence suggests that the HS-PG, or hybrid PG in basement membranes or on cell surfaces stores a growth factor (5-7) or is a growth factor receptor (8). Functions of hybrid PGs seem to be regulated at least in part by the control of GAG biosynthesis (9,10). The structures of GAGs on hybrid PGs, however, are not fully understood. HS chains on hybrid PGs have been studied using the EHS tumor (11), while no detailed structural studies on galactosaminoglycan chains of hybrid PGs have been reported.

We have been working on the biosynthesis of GAGs (12,13) and have recently demonstrated the structure of Gal(4-O-sulfate)β1-3Galβ1-4Xylβ1-O-Ser in the carbohydrate-protein linkage region of chondroitin 4-sulfate PG from Swarm rat chondrosarcoma (13). While investigating the ubiquity of this structure, we isolated from the EHS tumor a peptidoglycan fraction containing undersulfated HS and oversulfated galactosaminoglycan chains.

MATERIALS AND METHODS

Materials. Materials were obtained from the following sources: Actinase E, previously supplied as Pronase P, from Kaken Pharmaceutical Co., Tokyo; bovine pancreas DNase I (EC 3.1.4.5) from Takara Shuzo Co., Kyoto; bovine pancreas RNase A (EC 3.1.27.5) (Type I-A) from Sigma; chondroitinase ABC (EC 4.2.2.4), chondro-4-sulfatase (EC 3.1.6.9), chondro-6-sulfatase (EC 3.1.6.10) and standard unsaturated disaccharides from Seikagaku Kogyo Co., Tokyo. Glycuronate-2-sulfatase was a gift from Dr. K. Yoshida (Seikagaku Kogyo). Glc-6-P from Oriental Yeast Co., Tokyo. DEAE-cellulose, Sephadex and Sephacryl gels from Pharmacia.
Analytical methods. Uronic acid and hexose were determined by the carbazole method (14) and the orcinol method (15), respectively. Unsaturated uronic acid-containing materials were spectrophotometrically quantitated based on an average millimolar absorption coefficient of 5.5 (16). Analysis of amino sugars and amino acids was carried out (12) after hydrolysis in 6M HCl at 100 °C for 3 h and at 110°C for 20 h, respectively.
Preparation of GAGs from the tumor. The EHS tumor was propagated subcutaneously in 5 week-old C57BL mice (17), harvested after 2-3 weeks and stored at -80°C until acetone powder was prepared therefrom. The acetone powder (68 g) was treated in 600 ml of boiling water for 10 min, cooled and digested with 1.38 g of heat-treated (60°C, 30 min) Actinase E (18) in 785 ml of 0.1M Glc-6-P/0.1M borate buffer, pH 8.0, containing 10 mM $CaCl_2$. The incubation was carried out at 60°C for 6 days with 0.7 g of the enzyme being further added every 24 h. Following incubation the sample was adjusted to pH 7.0 with AcOH and heated in boiling water for 10 min. The soluble fraction obtained by centrifugation

was adjusted to pH 4.0 with AcOH and polysaccharides were precipitated by 80% EtOH. The sample was redigested with Actinase E as described above and purified by successive precipitation with cetylpyridinium chloride and EtOH (19). The sample was dissolved in 1.3 l of water and treated with 66 g of activated charcoal for 15 min. A clear solution was recovered by centrifugation and a GAG fraction was obtained therefrom by EtOH precipitation. The crude GAG fraction contained 2.16 mmol of GlcU, 3.94 mmol of hexose, 0.955 mmol of GlcN and 0.212 mmol of GalN.

DEAE-cellulose chromatography. The crude GAG preparation was adjusted to 0.1M LiCl/0.05M acetate, pH 4.0, and applied to a column (5.1 x 13.5 cm) of DEAE-cellulose equilibrated with the same buffer. After washing the column with the starting buffer, elution was performed stepwise with the buffers containing 0.3, 1.0 and 2.0M LiCl. Upon cellulose acetate membrane electrophoresis, GAGs were detected only in 1 and 2M fractions which were designated Fr.A and B, respectively.

Sephacryl S-300 chromatography. Fr.A and B were chromatographed on a column (3.8 x 192 cm) of Sephacryl S-300 equilibrated with 0.5M acetate buffer, pH 7.0. Seventeen ml fractions were collected, monitored by the carbazole and orcinol reactions, and the carbazole-positive fractions were pooled. The materials recovered by EtOH precipitation were treated with DNase I and RNase A (see below) and rechromatographed as above. The carbazole-positive materials were recovered by EtOH precipitation.

Chondroitinase digestion. Chondoritinase ABC digestion was carried out according to Saito et al. (16). The reaction was terminated by boiling for 2 min. After centrifugation the supernatant was chromatographed on Sephadex G-50 using 0.25M NH_4HCO_3/7% PrOH as a solvent. Fractions were monitored by uv-absorption at 232 nm and the carbazole reaction. The GAG and disaccharide fractions were desalted by repeated evaporation and by gel filtration on Sephadex G-15, respectively.

Sulfatase digestion. The disulfated disaccharide fraction (20 nmol) obtained by chondroitinase digestion was treated with 100 munits of glycuronate-2-sulfatase (20) in 30 µl of 10 mM imidazole buffer, pH 6.5 at 37 °C. After a 10 min incubation, the mixture was boiled for 30 s and subjected to HPLC analysis.

Another aliquot (20 nmol) of the chondroitinase-produced disaccharides was incubated with 70 munits of chondro-6-sulfatase in 63 µl of 33 mM Tris/HCl, pH 7.5, containing 33 mM AcONa at 37 °C. An aliquot was withdrawn after a 10 min incubation, boiled for 30 s and subjected to HPLC analysis. Another aliquot (45 µl) of the digest was mixed with 5 µl (50 munits) of chondro-4-sulfatase, incubated for another 10 min, boiled for 30 s and subjected to HPLC analysis.

Heparitinase digestion. Chondroitinase-resistant materials corresponding to 1 µmol GlcU were digested with a mixture of heparitinases I (90 munits) and II (70 munits) in 140 µl of 20mM acetate buffer, pH 7.0, containing 2mM $Ca(AcO)_2$ at 37 °C for 2 h. The reaction was terminated by boiling for 20 s. Aliquots were analysed by HPLC to assess depolymerization and to quantitate unsaturated disaccharides, respectively (see below).

Nuclease digestion. Fr.A and B were digested with a mixture of DNase I and RNase A (1/50 each of the substrate, w/w) in 50 mM Tris/HCl buffer, pH 7.4, containing 10 mM $MgSO_4$ at 37 °C for 20 h. The sample was adjusted to pH 7.0 with AcOH and treated at 80 °C for 10 min. After centrifugation the supernatant was chromatographed on Sephacryl S-300 as described above.

HPLC. Unsaturated disaccharides produced by digestion with heparitinases were analyzed by HPLC according to Yoshida et al. (21). CS-disaccharides produced by digestions with chondroitin-

ase or sulfatases were analyzed with slight modifications of the reported method (21). Chromatography was performed on a 4.6 x 250 mm amine-bound silica PA03 column (YMC Co., Kyoto) in an LC6A HPLC system (Shimadzu Corp., Kyoto) using a linear gradient from 16 to 530 mM NaH_2PO_4 over a 60 min period at a flow rate of 1.0 ml/min at room temperature. Eluates were monitored by uv-absorption at 232 nm. Samples were diluted so as to contain 5-10 nmol of disaccharides in 100-400 µl of 16 mM NaH_2PO_4, treated with a C3HV membrane filter (Millipore) and an aliquot (2-5 nmol) was injected.

Gel permeation HPLC was carried out to assess depolymerization of GAGs using a TSK-gel $G2500PW_{XL}$ column (Tosoh, Tokyo) monitored by refractive index.

RESULTS

GAGs were extracted by exhaustive protease digestion from the acetone powder (68 g) of the EHS tumor (650 g) and were recovered by successive precipitation with cetylpyridinium chloride and EtOH. Fractionation of the GAGs by DEAE-cellulose chromatography yielded two GAG fractions: a major (Fr.A) and a minor (Fr. B) fraction eluted with 1 and 2M LiCl, respectively. These GAG fractions contained oligosaccharides and nucleic acids, and were purified by nuclease digestion and gel filtration on Sephacryl S-300. The purified Fr.A and B contained GAGs corresponding to 969 and 47.1 µmol of uronic acid, and 5.17 and 1.32 µmol of Ser, respectively. A ratio of the other amino acids to Ser was 12.0 in Fr.A and 5.8 in Fr.B, indicating that they contained peptidoglycans with small peptide moieties.

GAG analysis was carried out by HPLC determination of disaccharides produced by GAG-degrading enzymes. Fr.B (15.7 µmol as GlcU) was digested exhaustively with chondroitinase ABC and subjected to Sephadex G-50 column chromatography. Approximately half (57%) of the uronic acid-containing materials were recovered in the void fraction while the rest were in the column volume fraction (not shown). The chondroitinase-resistant materials were extensively depolymerized by a mixture of heparitinases I and II to disaccharides (92%) and partially to oligosaccharides (8%) as judged by gel permeation HPLC (not shown), indicating that these materials consisted of HS. Thus Fr.B contained 43% of CS/DS and 57% of HS.

Likewise, Fr.A was sequentially digested by chondroitinase ABC and heparitinases I/II. A small proportion (2.3%) of the uronic acid-containing materials in Fr.A were sensitive to chondroitinase ABC while the rest were depolymerized by heparitinases I/II. Thus Fr.A contained 2.3% of CS/DS and 97.7% of HS. Since

Table 1. Unsaturated disaccharide composition of heparitinase digest

Disaccharides	Fr.A	Fr.B
	(%)	
ΔUAα1-4GlcNAc	32.6	31.4
ΔUA(2-OSO_3)α1-4GlcNAc	0.6	--
ΔUAα1-4GlcNSO_3	62.5	60.3
disulfated HS-disaccharides	2.5	6.8
(HS-tetrasaccharides)	1.8	1.5

Recoveries were over 93% based on uv-absorption at 232 nm.

Fr.A and B represent 95.4 and 4.6% of the total uronic acid isolated from the tumor, respectively, the overall ratio of HS to CS/DS in the total isolated GAGs was calculated to be 95.8 : 4.2.

Aliquots (2-10 nmol as GlcU) of the chondroitinase or heparitinase digests of Fr.A or B were subjected to disaccharide analysis by HPLC. Results from analysis of the heparitinase digests are summarized in Table 1. Standard unsaturated HS-disaccharides were well separated as reported (21) with quantitative recoveries except that ΔUAα1-4GlcNAc(6-OSO_3) and ΔUA(2-OSO_3)α1-4 GlcNAc were eluted at the same position. Approximately 60 and 30% of the uronic acid was recovered in ΔUAα1-4GlcNSO_3 and ΔUAα1-4GlcNAc, respectively, for both Fr.A and B. Small proportions of disulfated disaccharides were also found. ΔUA(2-OSO_3)α1-4GlcNAc in Fr.A was identified by its sensitivity to glycuronate-2-sulfatase (not shown). Peaks eluted after the authentic trisulfated HS-disaccharide were also observed and were assumed to be tetrasaccharides based on their sizes using gel filtration (not shown). The results altogether indicate that HS chains in Fr.A and B have similar disaccharide compositions and are undersulfated, which are consistent with the previous findings (11). The proportion of disulfated disaccharides is slightly higher in Fr.B than in Fr.A, but not enough to account for the stronger adsorption of the former to DEAE-cellulose.

Results from analysis of the disaccharide fractions of the chondroitinase ABC digests are shown in Fig.1. Standard unsaturated CS-disaccharides were well separated with quantitative recoveries except that ΔDi-diS_E (Peak 5) and ΔDi-diS_B (Peak 8) were eluted at the same position. The samples from both Fr.A and B showed qualitatively similar elution patterns (Fig.1B & 1C). ΔDi-4S was a major and ΔDi-0S, ΔDi-6S and ΔDi-diS_E (or ΔDi-diS_B)

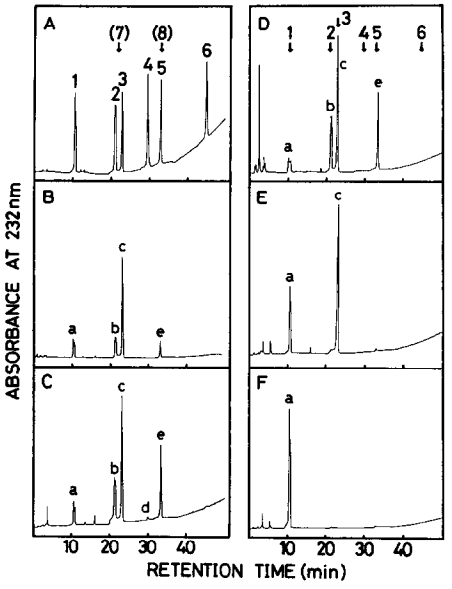

Figure 1. HPLC analysis of the oligosaccharides produced by chondroitinase ABC digestion. Panel A, standard CS-derived unsaturated disaccharides (0.5 nmol each). 1, ΔDi-0S. 2, ΔDi-6S. 3, ΔDi-4S. 4, ΔDi-diS$_D$. 5, ΔDi-diS$_E$. 6, ΔDi-triS. 7, ΔDi-UA2S. 8, ΔDi-diS$_B$. Panel B, CS-derived oligosaccharides from Fr.A. Panel C, CS-derived oligosaccharides from Fr.B. Panel D, The chondroitinase ABC/glycuronate-2-sulfatase digest from Fr.B. Panel E, The chondroitinase ABC/chondro-6-sulfatase digest from Fr.B. Panel F, The chondroitinase ABC/chondro-6-sulfatase/chondro-4-sulfatase digest from Fr.B (see "Methods"). Chromatographies were performed for 60 min as described in "Methods" and the chromatograms of the first 50 min are shown.

were minor components of both fractions although a small proportion of ΔDi-diS$_D$ was additionally found in the latter.

In order to identify Peak e eluted at the position of ΔDi-diS$_E$/ΔDi-diS$_B$, the chondroitinase digest of Fr.B was digested with various sulfatases followed by disaccharide analysis with HPLC. The results are shown in Fig.1D-F. After the glycuronate-2-sulfatase digestion ΔDi-diS$_D$ disappeared, but Peak e remained unchanged (Fig.1D), suggesting that it was not ΔDi-diS$_B$ which was sensitive to the enzyme. Peak e disappeared after the chondro-6-sulfatase digestion and a corresponding increase in ΔDi-4S was observed (Fig.1E). Disappearance of ΔDi-6S and a corresponding increase in ΔDi-0S were also observed. Upon chondro-4-sulfatase digestion of the chondro-6-sulfatase digest, ΔDi-4S was quantitatively converted to ΔDi-0S (Fig.1F). These results clearly indicate that Peak e is ΔDi-diS$_E$. The corresponding peak derived from Fr.A was also identified as ΔDi-diS$_E$ in the same manner. These results are summarized in Table 2. Disaccharide compositions showed remarkable differences on a quantitative basis bet-

Table 2. Unsaturated disaccharide composition of chondroitinase ABC digest

Disaccharides	Fr.A	Fr.B
	(%)	
ΔDi-0S	13.9	8.7
ΔDi-6S	18.7	24.8
ΔDi-4S	58.7	41.7
$\Delta Di\text{-}diS_D$	--	1.8
$\Delta Di\text{-}diS_E$	8.7	23.0

Recoveries were quantitative based on uv-absorption at 232 nm.

ween Fr.A and B. Fr.B, as compared with Fr.A, yielded lower amounts of ΔDi-0S (5.2% lower) and ΔDi-4S (17% lower) and higher proportions of ΔDi-6S (6.1% higher) and $\Delta Di\text{-}diS_E$ (14.3% higher), which should account for the stronger adsorption of Fr.B to DEAE-cellulose. Since CS/DS in Fr.A and B occupies 2.2 and 2.0% of the total isolated GAGs, respectively, the overall proportions of ΔDi-0S, ΔDi-4S, ΔDi-6S, $\Delta Di\text{-}diS_D$ and $\Delta Di\text{-}diS_E$ of the total CS/DS were calculated to be 11 : 54 : 22 : 1 : 15.

DISCUSSION

Chondroitin sulfate E was first isolated from squid cranial cartilage (22). PG possessing CS chains of an E-type structure has also been demonstrated in T cell-dependent, presumably mucosal, rodent mast cells (23,24) while in connective-tissue-type mast cells heparin PG as well as CS-PG have been found (25,26).

In the present study we demonstrated an E-type structure in chondroitinase ABC-sensitive CS/DS chains isolated from EHS tumors which contain a hybrid PG bearing both HS and CS/DS chains (3). The oversulfated CS/DS chains were most likely derived from the hybrid PG produced by the tumor. The E-type-rich CS/DS chains in Fr.B were co-purified by ion exchange chromatography with HS chains which were indistinguishable based on disaccharide compositions from those in Fr.A containing less negatively charged GAGs. It seems likely that Fr.B contained peptidoglycans bearing both HS and CS/DS chains even after exhaustive protease digestion (unpublished observation), suggesting that they were indeed derived from the hybrid PG.

The characteristic of oversulfated CS/DS chains may contribute to the function of the parent hybrid PG in mediator storage including the retention of cationic growth factors. Recently it

has been reported that the basement membrane component, presumably HS, produced by cornea and vascular endothelial cells can store a basic fibroblast growth factor (5). More recently it has been demonstrated that HS of mouse bone marrow stroma possesses the ability to adsorb growth factors such as granulocyte/macrophage colony stimulating factor and the multilineage haemopoietic growth factor which, once bound, can be presented in the biologically active form to haemopoietic cells (6,7). Although chondroitin sulfate E itself may not bind growth factors, oversulfation of CS/DS chains may change the binding activity of the neighbouring HS chains of the hybrid PG.

Recently Segarini and Seyedin (8) reported that one form of the transforming growth factor-β receptors *per se* is a cell surface hybrid PG bearing HS and CS chains in mouse 3T3 cells. The structure of syndecan of mouse mammary epithelial cells has been investigated in detail. This hybrid PG is expressed on the cell surface and exhibits molecular polymorphism, showing a distinct length and number of CS/DS and HS chains on simple and stratified epithelia (9). It has recently been suggested that this PG shows an alteration in the proportion of CS/DS to HS chains attached to the core protein in response to transforming growth factor-β (10). Although the structural relationship of hybrid PGs from cell surfaces to those from basement membranes is not well understood, it is likely that potential binding activity of the hybrid PGs can be regulated at least through the control of the number and length of GAG chains. It is interesting to investigate the ubiquity of oversulfated CS/DS in various hybrid PGs. Alternatively, if oversulfation is unique to EHS PGs, it may be involved in the uncontrollable growth of the tumor.

Acknowledgments. This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education and Culture of Japan. We thank Drs. K. Kimata (Aichi Medical University, Nagoya) and G.R. Martin (National Institutes of Health) for providing us with the EHS tumor. We are grateful to Dr. K. Yoshida (Seikagaku Kogyo) for performing the HS-disaccharide analysis and providing us with glycuronate-2-sulfatase.

REFERENCES

1. David,G., and Van den Verghe,H.(1985)J. Biol. Chem. 260, 11067-11074.
2. Rapraeger,A., Jalkanen,M., Endo,E., Koda,J., and Bernfield,M.(1985)J. Biol. Chem. 260, 11046-11052.
3. Kato,M., Koike,Y., Itoh,Y., Suzuki,S., and Kimata,K.(1987) J. Biol. Chem. 262, 7180-7188.

4. Isemura,M., Sato,N., Yamaguchi,Y., Aikawa,J., Munakata,H., Hayashi,N., Yoshizawa,Z., Nakamura,T., Kubota,A., Arakawa,M., and Hsu,C-C.(1987)J. Biol. Chem. 262, 8926-8933.
5. Vlodavsky,I., Folkman,J., Sullivan,R., Fridman,R., Ishai-Michaeli,R., Sasse,J., and Klagsbrun,M.(1987)Proc. Natl. Acad. Sci. USA 84, 2292-2296.
6. Gordon,M.Y., Riley,G.P., Watt,S.M., and Greaves,M.F.(1987) Nature 326, 403-405.
7. Roberts,R., Gallagher,J., Spooncer,E., Allen,T.D., Bloomfield,F., and Dexter,T.M.(1988)Nature 332, 376-378.
8. Segarini,P.R., and Seyedin,S.M.(1988)J. Biol. Chem. 263, 8366-8370.
9. Sanderson,R.D., and Bernfield,M.(1988)Proc. Natl. Acad. Sci. USA 85, 9562-9566.
10. Rasmussen,S., and Rapraeger,A.(1988)J. Cell Biol. 107, 1959-1967.
11. Pejler,G., Backstrom,G., Lindahl,U., Paulsson,M., Dziadek,M., Fujiwara,S., and Timple,R.(1987)J. Biol. Chem. 262, 5036-5043.
12. Sugahara,K., Okamoto,H., Nakamura,M., Shibamoto,S., and Yamashina,I.(1987)Arch. Biochem. Biophys. 258, 391-403.
13. Sugahara,K., Yamashina,I., De Waard,P., Van Halbeek,H., and Vliegenthart,J.F.G.(1988)J. Biol. Chem. 263, 10168-10174.
14. Bitter,M., and Muir,H.M.(1962)Anal. Biochem. 4, 330-334.
15. Hewitt,L.F.(1937)Biochem. J. 31, 360-366.
16. Saito,H., Yamagata,T., and Suzuki,S.(1968)J. Biol. Chem. 243, 1536-1542.
17. Orkin,R.W., Gehron,P., McGoodwin,E.B., Martin,G.R., Valentine,T., and Swarm,R.(1977)J. Exp. Med. 145, 204-220.
18. Freeze,H.H., Yeh,R., Miller,A.L., and Kornfeld,S.(1983) J. Biol. Chem. 258, 14874-14879.
19. Rodén,L., Baker,J.R., Cifonelli,J.A., and Mathews,M.B.(1972) Methods Enzymol. 28, 73-140.
20. Watanabe,K., Yoshida,K., Kikuchi,H., Maeyama,K., Tawada,A., and Tokuyasu,K.(1986)Seikagaku(in Japanese) 58, 780.
21. Yoshida,K., Miyauchi,S., Kikuchi,H., Tawada,A., and Tokuyasu,K.(1989)Anal. Biochem. 177, 327-332.
22. Suzuki,S., Saito,H., Yamagata,T., Anno,K., Seno,N., Kawai,Y., and Furuhashi,T.(1968)J. Biol. Chem. 243, 1542-1550.
23. Razin,E., Stevens,R.L., Akiyama,F., Schmid,K., and Austen,K.F. (1982)J. Biol. Chem. 257, 7229-7236.
24. Kusche,M., Lindahl,U., Enerbäck,L., and Rodén,L.(1988) Biochem. J. 253, 885-893.
25. Yurt,R.W., Leid,R.W., and Austen,K.F.(1977)J. Biol. Chem. 252, 518-521.
26. Katz,H.R., Austen,K.F., Caterson,B., and Stevens,R.L.(1986) J. Biol. Chem. 261, 13393-13396.