Sulfation of Chondroitin Sulfate and Related Sugar Chains

コンドロイチン硫酸および関連糖鎖の硫酸化

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Abstract

Sulfotransferases so far cloned which are involved in the synthesis of chondroitin sulfate are classified into three gene families: chondroitin 6-sulfotransferase (C6ST) family, chondroitin 4-sulfotransferase (C4ST) family and uronosyl 2-O-sulfotransferase (UA2OST) family. The C6ST family includes sulfotransferases which catalyze the transfer of sulfate to position 6 of Gal residue of keratan sulfate and sialyllactosamine oligosaccharide, and to position 6 of nonreducing terminal GlcNAc residue. In the C4ST family, sulfotransferases which catalyze the transfer of sulfate to position 4 of nonreducing terminal GalNAc residue and to position 3 of nonreducing terminal GlcA residue are present. UA2OST was cloned as a cDNA showing homology with heparan sulfate 2-sulfotransferase that transfers sulfate to position 2 of Ido A residue in heparan sulfate. It became evident that, in some cases, sulfotransferases involved in the sulfation of glycosaminoglycans and sulfotransferases involved in the sulfation of oligosaccharides of glycoproteins are included in a common gene family. A sulfotransferase with a novel specificity may be found in these families, or a new sulfotransferase other than known families may be revealed in the future. Findings of a new sulfotransferase will offer important information about the biological function of sulfated sugar chains.

要 約

今までにクローニングされたコンドロイチン硫酸の合成に関わる硫酸転移酵素は、コンドロイチン6 硫酸転移酵素(C6ST)、コンドロイチン4 硫酸転移酵素(C4ST)、ウロノシル 2-O-硫酸転移酵素(UA2OST)の3つの遺伝子ファミリーに分類される。C6STのファミリーには、ケラタン硫酸やシアリルラクトサミンのGal 残基の6 位、非還元末端のGlcNAc残基の6位を硫酸化する硫酸転移酵素が含まれる。C4STファミリーには、非還元末端のGalNAc残基の4 位、非還元末端GlcAの3 位を硫酸化する硫酸転移酵素が含まれる。UA2OSTは、ヘパラン硫酸のIdoAの2 位を硫酸化する硫酸転移酵素との相同性により得られた。硫酸転移酵素のクローニングにより、グリコサミノグリカンと糖タンパク質糖鎖の硫酸化は、同じ遺伝子ファミリーに属する硫酸転移酵素により行われることが明らかになった。これらの遺伝子ファミリーからさらに新たな硫酸転移酵素が見いだされるかもしれない。またこれら以外の新たな遺伝子ファミリーに属する硫酸転移酵素が今後発見されるかもしれない。新たな硫酸転移酵素の発見は、硫酸化糖鎖の生物学的機能を明らかにするのに重要な情報を提供するであろう。

A. Introduction

There is significant structural diversity in chondroitin sulfate isomers (including dermatan sulfate that is related to chondroitin sulfate in its biosynthesis and structure). The position and number of sulfate residue attached to the repeating disaccharide units as well as the kind of component sugars contribute to the formation of the divergent structures. The structural diversity is thought to reflect the divergent biological functions. Recently, marked advances in the cloning and characterization of the sulfotransferases involved in the synthesis of chondroitin sulfate and related sugar chains have been achieved. An intimate relationship between the sulfation of chondroitin sulfate and the sulfation of glycoprotein oligosaccharides became apparent from studies on the molecular nature of each

A. はじめに

コンドロイチン硫酸(生合成的、構造的に関連を持つデルマタン硫酸を含める)は、二糖繰返し構造に含まれる構成糖の種類、硫酸基の結合位置の組み合わせにより、構造多様性を示す。コンドロイチン硫酸の多様構造は生物学的な機能の多様性と深い関連があると考えられる。コンドロイチン硫酸の多様構造の形成に関わる硫酸転移酵素遺伝子のクローニングはこの数年の間に大きく進展した。さらに、硫酸転移酵素遺伝子のクローニングを通して、コンドロイチン硫酸の硫酸化と糖タンパク質糖鎖の硫酸化の密接な関係が明らかになってきた。本稿で

Fig. 1. Substrate specificity of sulfotransferases involved in the sulfation of chondroitin sulfate and related sugar chains. For the abbreviation of each sulfotransferase, see the text.

sulfotransferase. In this review, I have summarized the properties of the sulfotransferases involved in the synthesis of chondroitin sulfate and the related sugar chains. I also describe the uptake of sulfate ion and synthesis of 3'-phosphoadenosine 5'-phosphosulfate (PAPS) that occur before the transfer of sulfate by the sulfotransferases. Sulfotransferases involved in the synthesis of heparan sulfate have been reviewed previously (1, 2). Figure 1 shows the acceptor substrate specificity of each sulfotransferase.

B. Transport of Sulfate Ion

Every sulfotransferase transfers sulfate from PAPS to the defined position of the sugar residues. Sulfate ion, which is one of the source materials for the production of PAPS, is transported from the extracellular milieu or supplied from sulfur-containing amino acids. Diastrophic dysplasia (DTD) is autosomal recessive osteochondrodysplasia with clinical features including dwarfism, spinal deformation and specific joint abnormalities. DTD gene was identified as a gene encoding sulfate/chlo-

はコンドロイチン硫酸と関連糖鎖の硫酸化に関わる硫酸転移酵素、および硫酸基転移反応の前段階である硫酸イオンの輸送とPAPS合成についてまとめた。ヘパラン硫酸合成に関する硫酸転移酵素については他の文献(1、2)を参照されたい。コンドロイチン硫酸および関連糖鎖の硫酸化に関わる硫酸転移酵素の特異性を図1に示した。

B. 硫酸イオンの膜透過

糖鎖の特定の位置に結合した硫酸基は、特異的な硫酸転移酵素の働きでPAPSから転移される。PAPSの原料の一つ硫酸イオンは、硫酸イオントランスポーターにより細胞外から輸送されるか、細胞内の含硫アミノ酸の代謝により供給される。矮小発育、脊椎変形、関節異常を示す常染色体劣性の遺伝疾患di-

ride antiporter (DTDST) (3). Mutations in DTDST gene were also found in the lethal chondrodysplasia, achondrogenesis type 1B (4) and McAlister dysplasia (5). Fibroblasts with the most severe clinical phenotype showed the lowest sulfate uptake (5). Undersulfation of proteoglycan synthesized by chondrocytes from achondrogenesis type 1B (6) and McAlister dysplasia (5) was evident. A unique characteristic of high endothelial venules (HEV) endothelium is the capacity of HEV endothelial cells (HEVEC) to incorporate large amounts of inorganic sulfate. Large amounts of sulfate ion may be required for the synthesis of L-selectin ligand, sulfated sialyl Lewis X. Uptake of sulfate by HEVEC was inhibited by 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS), an inhibitor of sulfate/chloride antiport, but about 50% of the uptake was resistant to DIDS, indicating the presence of another sulfate transport in these cells. From HEVEC cDNA library, SUT-1 gene, which is a homologue of a rat Na⁺-dependent sulfate transporter, was cloned (7). When SUT-1 cDNA was injected to *Xenopus laevis* oocytes, >30-fold stimulation of Na⁺-dependent sulfate uptake was observed. DTDST gene was expressed in all tissues examined (3), whereas, SUT-1 gene was expressed in the placenta and HEV (7), suggesting the specific function of SUT-1.

C. Synthesis of PAPS and the Nuclear Localization of PAPS Synthetase

PAPS, one of the substrates for sulfotransferases, is a universal donor of the sulfate group in every organism. Sulfation of chondroitin sulfate is largely regulated by the level of PAPS synthesis. Murine brachymorphism is characterized by a dome-shaped skull, short thick tail, and shortened limbs. The phenotype is inherited as an autosomal recessive. Since chondrocytes of brachymorphic mouse synthesized undersulfated proteoglycan, and showed decreased PAPS synthetase activity, this mutant was assumed to have defective PAPS synthetase (8-10). PAPS is synthesized through two successive enzyme reactions as follow:

Reaction 1 is catalyzed by ATP-sulfurylase and reaction 2 by APS-kinase. These enzymes are different proteins in bacteria, fungi and yeast. On the other hand, in higher organisms, these two enzymes were assumed to be contained in a bifunctional protein, because the purified PAPS synthetase from chondrosarcoma showed two activities (11). This assumption was confirmed by cloning PAPS synthetase (12). Mouse PAPS synthetase 1 deduced from the cDNA contains APS-kinase domain with 199 amino acids and ATP-sulfurylase domain with 389 amino acid. A 37-amino acid stretch is present between the two domains. Human PAPS synthetase 1 was cloned from HEV cDNA library (13). The introduction of human PAPS synthetase 1 cDNA into CHO cells resulted in a high level of PAPS pro-

astrophic dysplasia(捻曲性骨異形成症)の原因遺伝子が、SO₄²⁻/Cl⁻ アンチポートを行うトランスポーター(DTDST)と同定された(3)。致死性の遺伝疾患であるachondrogenesis type 1B (4)やMcAlister dysplasia (5)もこの遺伝子に変異を持つことが明らかにされた。症状の重さはDTDSTの残存活性によることが皮膚線維芽細胞の硫酸イオンの取り込みにより示された(5)。achondrogenesis type 1B (6)、McAlister dysplasia (5)では軟骨で合成されるコンドロイチン硫酸の低硫酸化が起きていた。高内皮細静脈の内皮細胞(HEVEC)は硫酸イオンを取り込む活性が高いことが知られていた。これはL-セレクトリンリガンドである硫酸化糖鎖を合成するために必要と考えられる。HEVECによる硫酸イオンの取り込みは、SO₄²⁻/Cl⁻アンチポートの阻害剤である4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS)により阻害されるが、約50%の活性がDIDS抵抗性を示すので、HEVECにはDTDST以外に別の硫酸イオントランスポーターが存在することが考えられ、ラットのNa⁺依存性SO₄²⁻トランスポーター遺伝子と相同性を示す遺伝子(SUT-1遺伝子)がヒトHEVECのライブラリーからクローニングされた(7)。この遺伝子産物がトランスポーター活性を持つことは、cDNAをアフリカツメガエル卵母細胞に注入するとNa⁺依存性の硫酸イオンの取り込みが30倍以上に増加することにより示された。DTDST遺伝子が多くの組織で発現しているのに対し、SUT-1遺伝子は胎盤とHEVで選択的に発現しており、特異的な機能を担っていることが考えられる。

C. PAPSの合成およびPAPS合成酵素の核への局在

PAPSは硫酸転移酵素の基質であり、生物界での硫酸基の普遍的な供与体である。コンドロイチン硫酸の硫酸化はPAPSの合成によって大きく調節されている。Brachymorphic (bm) mouse (短型マウス)はドーム型の頭蓋、太くて短い尾、短肢を特徴とし、その形質は常染色体劣性の遺伝子により伝えられる。bm mouseでは軟骨のPAPS合成酵素活性が減少していることから、PAPS合成酵素に変異があると推定された(8-10)。PAPSは硫酸イオンとATPから次の2段階の反応で合成される。

1の反応を行うのがATP-スルフィラーゼ、2の反応を行うのがAPS-キナーゼである。細菌、カビ、酵母などではこれらの酵素は独立した別のタンパク質として存在している。一方軟骨肉腫細胞から精製されたPAPS合成酵素は両方の酵素活性を持つことから、高等生物では2機能を持つ単一タンパク質として存在すると考えられていたが(11)、PAPS合成酵素の遺伝子のクローニングにより証明された(12)。マウスからクローニングされたPAPS合成酵素1は、199残基のアミノ酸からなるAPS-キナーゼドメイン、389アミノ酸残基からなるATP-スルフィラーゼドメイン、およびそれらをつなぐ37アミノ酸残基の部分から構成される。ヒトのPAPS合成酵素1が高内皮細静脈内皮細胞のライブラリーからクローニングされた(13)。ヒトPAPS合成酵素1を導

duction. Chlorate treatment of the transfected CHO cell inhibited PAPS synthetase activity. Mouse PAPS synthetase 1 gene was located to chromosome 3, while *bm* gene was located to chromosome 19, indicating that PAPS synthetase 1 gene is not responsible for the phenotype of *bm*. PAPS synthetase 2, which is a homologue of PAPS synthetase 1, was cloned (14). PAPS synthetase 2 gene was demonstrated to be responsible for the expression of *bm* phenotype because (a) PAPS synthetase 2 gene was located at chromosome 19, (b) PAPS synthetase gene from *bm* mouse showed mutation in the kinase domain (Gly79 to Arg), (c) the APS-kinase activity of the mutated PAPS synthetase 1, which was expressed in *E. coli* and affinity purified, showed less than one-tenth activity of normal PAPS synthetase 1, although ATP-sulfurylase activity of the mutant protein was normal. Missense and nonsense mutations in the APS-kinase domain of PAPS synthetase 2 were demonstrated in the human inherited disorder, spondyloepimetaphyseal dysplasia (15).

While PAPS synthesis has been assumed to occur in the cytosol, human PAPS synthetase 1 was shown to accumulate in the nucleus of mammalian cells, suggesting that PAPS may be synthesized in the nucleus as well (16). When PAPS synthetase 1 cDNA was transfected in CHO cells or COS-7 cells, the expressed protein was located in the nucleus. The nuclear localization of PAPS synthetase 1 was shown in the endogenous enzyme of ECV endothelial cells. When transfected in yeast with methionine auxotrophy caused by a deficient ATP-sulfurylase or APS-kinase, PAPS synthetase 1 was localized in the nucleus and rescued the methionine auxotrophy of the strain, indicating that PAPS synthetase was fully functional when localized to the nucleus. Unlike PAPS synthetase 1, ectopically expressed PAPS synthetase 2 in COS-7 cells or CHO cells was found to accumulate in the cytoplasm. However, cotransfection of PAPS synthetase 1 and PAPS synthetase 2 shifted the latter from a cytosolic to a nuclear localization. The APS-kinase domain of PAPS synthetase 1 was shown to be necessary and sufficient for the nuclear localization.

Transporters of nucleotide sugars and nucleotide sulfate, which are involved in the transport of these materials into the Golgi apparatus or ER, have been found (17). PAPS transporter has been purified from rat liver Golgi fraction (18, 19), but cloning of the transporter has not been reported.

D. Purification, Substrate Specificity and Cloning of the Sulfotransferases Involved in the Synthesis of Chondroitin Sulfate

Among the sulfotransferases involved in the synthesis of chondroitin sulfate, chondroitin 6-sulfotransferase (C6ST), chondroitin 4-sulfotransferase (C4ST) and uronosyl 2-O-sulfotransferase have been cloned. GalNAc 4-sulfate 6-O-sulfotransferase (GalNAc4S-6ST) involved in the synthesis of chondroitin sulfate E has been purified from squid cartilage and

入した CHO 細胞では PAPS 合成活性が増加したが、塩素酸イオンの添加により PAPS 合成は阻害された。PAPS 合成酵素 1 遺伝子はマウスでは第 3 染色体にあるが、*bm* 遺伝子は第 19 染色体にあるので、PAPS 合成酵素 1 の変異が *bm* の原因とは考えられない。PAPS 合成酵素 1 の相同遺伝子の、PAPS 合成酵素 2 がクローニングされた (14)。この遺伝子は次の結果から *bm* の原因と考えられた。(a) PAPS 合成酵素 2 遺伝子が第 19 染色体にあること、(b) *bm* mouse の持つ PAPS 合成酵素 2 遺伝子には APS-キナーゼドメインに含まれる 79 番目の Gly が Arg に変異していること、(c) 変異した遺伝子を大腸菌に導入し、発現したタンパク質をアフィニティー精製したところ、ATP-スルフィラーゼ活性は対照と同じであるのに対し、APS-キナーゼ活性は対照の 10 分の 1 以下となった。ヒトの遺伝疾患 spondyloepimetaphyseal dysplasia (脊椎・骨端・骨幹端ジストロフィー) では PAPS 合成酵素 2 の APS-キナーゼドメインにナンセンス変異、ミスセンス変異が検出された (15)。

PAPS 合成はサイトゾルで起きると考えられてきたが、PAPS 合成酵素 1 が核に局在することから、核での PAPS 合成の可能性が示された (16)。CHO 細胞や COS-7 細胞に PAPS 合成酵素 1 の遺伝子を導入すると、発現したタンパク質は核に局在した。核への局在はヒトの ECV 内皮細胞が持っている内在性の PAPS 合成酵素 1 でも示された。APS スルフィラーゼまたは APS-キナーゼの変異により PAPS 合成活性が欠損し、Met 要求性となった酵母に、PAPS 合成酵素 1 遺伝子を導入すると、発現した PAPS 合成酵素 1 は核に局在し、酵母は Met 要求性を示さなくなった。このことは核に局在した PAPS 合成酵素 1 が機能を持つことを示している。PAPS 合成酵素 2 の遺伝子を COS-7 細胞や CHO 細胞に導入すると、発現したタンパク質は核ではなくて細胞質に分布する。しかし PAPS 合成酵素 1 の遺伝子を同時に導入すると、PAPS 合成酵素 2 も核に局在するようになる。核に局在するシグナルは、PAPS 合成酵素 1 の APS-キナーゼドメインにあることが示された。

ゴルジ膜や ER 膜にはサイトゾルから糖ヌクレオチドや PAPS を輸送する選択的なトランスポーターが存在している (17)。PAPS のトランスポーター活性を持つタンパク質が精製されているが (18, 19)、遺伝子クローニングはまだ報告されていない。

D. コンドロイチン硫酸合成に関与する硫酸転移酵素の精製、特異性、クローニング

コンドロイチン硫酸合成に関与すると考えられる硫酸転移酵素のうち、今までにコンドロイチン 6 硫酸転移酵素 (C6ST)、コンドロイチン 4 硫酸転移酵素 (C4ST)、ウロノシル 2-O-硫酸転移酵素 (UA2OST) がクローニングされた。コンドロイチン硫酸 E の合成に働く GalNAc4 硫酸 6 硫酸転移酵素 (GalNAc4S-6ST) がイ

characterized.

D-1. Chondroitin 6-Sulfotransferase (C6ST)

A sulfotransferase of 75 kDa, which transfers sulfate to position 6 of GalNAc residue, was purified to homogeneity from the serum-free culture medium of chick embryo chondrocytes (20). Since the same size of C6ST was obtained from chicken serum, secretion of C6ST into the extracellular space seems to occur not only in the culture condition but also in the physiological state (21). Based on the amino acid sequence, cDNA of C6ST was cloned from chick embryo chondrocyte library (22). The deduced protein had a type II transmembrane topology. C6ST catalyzed transfer of sulfate to position 6 of GalNAc residues of chondroitin, position 6 of Gal residue of keratan sulfate (23) and sialyl lactosamine oligosaccharides (24). C6ST-2 that catalyzed sulfation of position 6 of GalNAc residue of chondroitin was cloned (25). The same enzyme was cloned independently by other groups as an enzyme that catalyses sulfation of position 6 of nonreducing terminal GlcNAc residue (see below).

D-2. Chondroitin 4-Sulfotransferase (C4ST)

C4ST, which catalyzes transfer of sulfate to position 4 of GalNAc residue of chondroitin, was purified from the serum free culture medium of rat chondrosarcoma cells (26). The purified C4ST catalyzed transfer of sulfate to not only chondroitin but also desulfated dermatan sulfate. When the sulfated products formed from desulfated dermatan sulfate was digested with chondroitinase ACII, about half of the products were converted to Δ Di-4S and small amounts were recovered in monosulfated tetrasaccharide and monosulfated hexasaccharide fractions. On the other hand, the desulfated dermatan sulfate was hardly depolymerized by digestion with chondroitinase ACII. These results suggest that C4ST preferentially transferred sulfate to GlcA-rich domain contained in the desulfated dermatan sulfate. When the tetrasaccharide obtained from the ^{35}S -labeled desulfated dermatan sulfate after digestion with chondroitinase ACII was treated with mercuric acetate, ^{35}S -radioactivity was recovered exclusively in GalNAc4-sulfate (our unpublished data). C4ST thus appears to transfer sulfate to GalNAc residue adjacent to reducing side of GlcA, but not to GalNAc residue adjacent to reducing side of IdoA. These results indicate that C4ST recognizes not only position of GalNAc residue but also structure of the neighbouring sugar residue.

On the basis of the amino acid sequence of the purified C4ST, mouse C4ST cDNA was cloned (27). The deduced protein from the nucleotide sequence of the cDNA was a type II transmembrane protein with N-linked oligosaccharides as observed in glycosaminoglycan sulfotransferases so far cloned. C4ST did not show any significant homology to other glycosaminoglycan sulfotransferases so far cloned, but 29% identity of amino acid sequence was found between C4ST and HNK-1 sulfotransferase. HNK-1 sulfotransferase transfers sulfate to position 3 of Glc A to yield $\text{GlcA}(3\text{SO}_4)\beta 1-3\text{Gal}\beta 1-4\text{GlcNAc}\beta 1-$

カの軟骨から均一に精製され、性質が調べられた。

D-1. コンドロイチン 6 硫酸転移酵素(C6ST)

コンドロイチン硫酸のGalNAc 残基の6位を硫酸化する75 kDaの酵素(C6ST)がニワトリ培養軟骨細胞の無血清培養液から均一に精製された(20)。同じ分子量の酵素がニワトリ血清からも得られているので(21)、硫酸転移酵素の細胞外への分泌が培養という環境だけでなく、生理的条件下でも起きていると考えられる。精製C6STのアミノ酸配列に基づきcDNAがクローニングされた(22)。cDNAから推定されるタンパク質はII型膜タンパク質であった。C6STはコンドロイチンのGalNAc残基の6位だけでなく、ケラタン硫酸のGal残基の6位(23)、シアリルラクトサミン構造を持つオリゴ糖のGal残基の6位を硫酸化した(24)。コンドロイチンのGalNAc残基に硫酸基を転移するC6ST-2がクローニングされた(25)。この酵素はGlcNAc-6-硫酸転移酵素活性を持つ酵素として別のグループがクローニングした酵素と同一のタンパク質であった(後述)。

D-2. コンドロイチン 4 硫酸転移酵素(C4ST)

コンドロイチンのGalNAc残基の4位を硫酸化するC4STは、ラットコンドロザルコーマ細胞の培養液から均一に精製された(26)。精製C4STはコンドロイチン、脱硫酸化デルマタン硫酸に硫酸基を転移した。硫酸基を転移された脱硫酸化デルマタン硫酸をコンドロイチナーゼACIIで消化すると、約50%の放射能が Δ Di-4Sに回収され、1硫酸化4糖、1硫酸化6糖の位置にも少し回収されたが、高分子部分には殆ど放射能は残らなかった。受容体に用いた脱硫酸化デルマタン硫酸はコンドロイチナーゼACIIでほとんど低分子化しないことを考慮すると、C4STが脱硫酸化デルマタン硫酸を受容体とするときは、GlcAを含む部分に選択的に硫酸基を転移することが考えられる。コンドロイチナーゼACII消化で生じる4糖を単離し、酢酸水銀との反応で3糖とし、コンドロイチナーゼABCで分解すると、GalNAc(4SO₄)のみが生じたので(筆者ら未発表)、C4STはGlcAの還元側に隣接するGalNAc残基に硫酸基を転移するが、IdoAの還元側に隣接するGalNAc残基には転移しないと考えられる。これらの結果は、C4STはGalNAc残基の位置に関して特異的であるだけでなく、隣接する糖鎖の構造も認識することを示している。

精製したC4STのアミノ酸配列に基づき、C4STのcDNAがクローニングされた(27)。cDNAの塩基配列からアミノ酸配列を推定すると、C4STは今までにクローニングされたグリコサミノグリカン硫酸転移酵素と同じく、N-結合糖鎖を持つII型膜糖タンパク質であった。C4STはその他のグリコサミノグリカン硫酸転移酵素のいずれとも有意な相同性を示さなかったが、意外にもHNK-1硫酸転移酵素とアミノ酸配列で29%一致した。HNK-

R (HNK-1 epitope) (28, 29). Although acceptor substrate specificities are quite different from each other, C4ST and HNK-1 sulfotransferase seem to belong to a common sulfotransferase family. Mouse C4ST was expressed in brain and kidney (27). In contrast, human C4ST was expressed in peripheral leukocytes and colorectal adenocarcinoma cell (30, 31). Human C4ST (C4ST-1) and the second member of C4ST, C4ST-2 were cloned independently from the EST clones showing homology to HNK-1 sulfotransferase (30). Sulfotransferase activity of the expressed C4ST-2 was much lower than that of C4ST. Human C4ST gene was mapped to 12q23.2-q23.3 (31).

D-3. Uronosyl 2-O-Sulfotransferase (UA2OST)

UA2OST was cloned from human lymphoma Raji cell as a cDNA showing homology to heparan sulfate 2-sulfotransferase (33), which transfers sulfate to position 2 of IdoA of heparan sulfate and involved in the formation of bFGF-binding domain (32). When this cDNA was transfected in Sf9 insect cells, activity of sulfotransferase, which catalyzed transfer sulfate to dermatan sulfate and chondroitin sulfate, was overexpressed. Sulfation of dermatan sulfate occurred at position 2 of IdoA adjacent to GalNAc(4SO₄), whereas sulfation of chondroitin sulfate occurred at position 2 of IdoA adjacent to GalNAc(6SO₄).

D-4. GalNAc 4-Sulfate 6-O-Sulfotransferase (GalNAc4S-6ST)

GalNAc4S-6ST transfers sulfate to position 6 of GalNAc(4SO₄) residues of chondroitin sulfate to yield disaccharide unit of chondroitin sulfate E. This sulfotransferase was purified to homogeneity from squid cartilage containing large amounts of chondroitin sulfate E (34). The purified enzyme showed a broad band of 63 kDa on SDS-PAGE. The protein bands of 63 kDa disappeared after *N*-glycosidase F digestion, and protein bands of 38 and 40 kDa appeared, indicating that the purified protein contained *N*-linked oligosaccharides as observed in C6ST and C4ST. The purified enzyme transferred sulfate to chondroitin sulfate A, chondroitin sulfate C and dermatan sulfate. A low activity was observed toward squid skin chondroitin. The transfer of sulfate to chondroitin sulfate A and dermatan sulfate occurred at position 6 of the internal GalNAc(4SO₄) residues. Sulfation of chondroitin occurred at position 6 of GalNAc(4SO₄) residues but not at position 6 of GalNAc residues, which are contained in abundance in chondroitin. When a 4-sulfated trisaccharide and pentasaccharide, which bear GalNAc(4SO₄) residue at the nonreducing terminal, were used as acceptors, 27 and 40%, respectively, of the total incorporation were found in the nonreducing terminal GalNAc(4,6-bis SO₄), indicating that GalNAc4S-6ST is able to transfer sulfate not only to the internal GalNAc(4SO₄) but also to the nonreducing terminal GalNAc(4SO₄). The *K_m* for chondroitin sulfate A was about ten times as large as the *K_m* for

1硫酸転移酵素はGlcA β 1-3Gal β 1-4GlcNAc β 1-Rの非還元末端のGlcAの3位を硫酸化する(28, 29)。C4STとHNK-1硫酸転移酵素は、糖残基と転移される位置に関しては全く特異性が異なるにもかかわらず、配列に相同性を示すことから、共通の硫酸転移酵素遺伝子ファミリーに属すると考えられる。マウスC4STは脳と腎臓で比較的強い発現がみられた(27)。一方ヒトC4STは末梢白血球、大腸ガン由来の細胞で高い発現が見られた(30, 31)。C4STおよびC4STと相同性を示しC4ST活性を持つ第二の遺伝子C4ST-2がHNK-1硫酸転移酵素と相同性を示すタンパク質として独自にクローニングされた(30)。C4ST-2の特異性はC4STと似ていると考えられるが、発現した酵素の活性はC4STに比べて低かった。ヒトC4ST遺伝子は12q23.2-q23.3にマップされた(31)。

D-3. ウロノシル2-O-硫酸転移酵素(UA2OST)

ヘパラン硫酸のIdoA-GlcN(SO₃)二糖単位のIdoAの2位に硫酸基を転移し、bFGF認識ドメインの合成に関与するヘパラン硫酸2硫酸転移酵素(32)と相同性を示す遺伝子の中からUA2OSTがクローニングされた(33)。このcDNAをSf9昆虫細胞にトランスフェクトすると、デルマタン硫酸とコンドロイチン硫酸に硫酸基を転移する活性が発現した。デルマタン硫酸の硫酸化はGalNAc(4SO₄)の非還元側に隣接するIdoAの2位で起きることが示された。コンドロイチン硫酸の硫酸化活性はデルマタン硫酸よりも低いが、GalNAc(6SO₄)の非還元側に隣接するGlcAの2位で起きることが示された。

D-4. GalNAc4硫酸6-O-硫酸転移酵素(GalNAc4S-6ST)

コンドロイチン硫酸Eを大量に含むイカの軟骨から、GalNAc(4SO₄)残基の6位に硫酸基を転移してコンドロイチン硫酸Eの2糖単位を合成する硫酸転移酵素、GalNAc4硫酸6-O-硫酸転移酵素(GalNAc4S-6ST)が均一に精製された(34)。精製酵素はSDS-PAGEで63 kDaの幅広いバンドを示し、*N*-グリカナーゼ消化により、38と40kDaに変化したことからGalNAc4S-6STはC6STやC4STと同じく、*N*結合糖鎖を含む糖タンパク質と考えられる。精製酵素はコンドロイチン硫酸A、C、デルマタン硫酸に硫酸基を転移した。コンドロイチンも受容体活性を示した。コンドロイチン硫酸A、デルマタン硫酸の硫酸化は、繰り返し2糖部分のGalNAc(4SO₄)残基の6位に起きた。コンドロイチンの硫酸化も、主要な構成糖であるGalNAcではなく、やはりGalNAc(4SO₄)残基の6位に起きた。非還元末端にGalNAc(4SO₄)残基を持つコンドロイチン硫酸A由来の3糖、5糖を基質としたときは、転移された硫酸基のそれぞれ27%、40%が非還元末端のGalNAc(4SO₄)に取り込まれることから、この酵素は糖鎖内部の繰り返し二糖部分だけでなく、非還元末端のGalNAc(4SO₄)残基にも硫酸基を転移することが示された。コンドロイチン硫酸Aとデルマタン硫酸を基質にしたときの*K_m*を比較すると、デルマ

dermatan sulfate.

E. Sulfotransferases That Belong to C6ST Gene Family

E-1. Nervous System Involved Sulfotransferase (NSIST)

A mAb 3B3, which recognized agrin-binding proteins, was raised using the synaptic membrane proteins as the antigen (35). The reactivity of the mAb was decreased by the digestion with neuraminidase but not affected by digestion with chondroitinase or keratanase. This suggests that the epitope for the mAb may be oligosaccharides with sialic acid. NSIST cDNA was cloned from Torpedo electric organ by expression cloning using mAb 3B3 (36). Amino acid sequence of NSIST showed 56% identity with that of chick C6ST, suggesting that NSIST may be a sulfotransferase having similar specificity to C6ST. We found that transfection of NSIST cDNA in COS-7 cells resulted in the overexpression of C6ST activity (unpublished data). However, it is not obvious how C6ST activity of NSIST is involved in the synthesis of the 3B3 epitope. One of the epitope-bearing proteins was found to be dystroglycan. The structure of major O-linked oligosaccharide of dystroglycan was reported to be NeuAc α 1-3Gal β 1-4GlcNAc β 1-2Man-O- (37). NSIST might be involved in the sulfation of such an oligosaccharide chain. It remains to be studied to clear the natural substrate for NSIST.

E-2. Keratan Sulfate Gal-6-Sulfotransferase (KSGal6ST)

Keratan sulfate bears sulfate at position 6 of Gal residue and position 6 of GlcNAc residue. KSGal6ST was cloned from human fetal brain library by cross hybridization with chick C6ST cDNA (38). KSGal6ST expressed in COS-7 cells catalyzed the transfer of sulfate to position 6 of Gal residue contained in keratan sulfate, sialyl lactosamine oligosaccharides and fetuin oligosaccharides (39). Although the rate of sulfation of sialyl lactosamine oligosaccharide by C6ST was much lower than the rate of sulfation of keratan sulfate by C6ST, the sulfation of sialyl lactosamine oligosaccharide by KSGal6ST proceeded at nearly the same rate as the sulfation of keratan sulfate by KSGal6ST. KSGal6ST may function in the reconstitution of ligand for L-selectin because when KSGal6ST was introduced into CHO cells, which had been transfected with fucosyl transferase VII and core 2 β 1-6GlcNAc transferase, enhanced binding activity to L-selectin/IgM chimera was detected (40). Northern blot analysis showed that human KSGal6ST was expressed in the brain (38) and various immunologically relevant tissues (39).

E-3. GlcNAc 6-O-Sulfotransferase Family

Sulfation of position 6 of GlcNAc residue of glycoprotein oligosaccharides was reported to occur when the GlcNAc residue is located at the nonreducing terminal (41-43). Several kinds of GlcNAc 6-O-sulfotransferase, which transfer sulfate to position 6 of the nonreducing terminal GlcNAc, have been cloned on the basis of homology with C6ST, KSGal6ST or cloned

タン硫酸の方が約1桁小さかった。

E. C6STファミリーに属する硫酸転移酵素

E-1. 神経系硫酸転移酵素(Nervous system involved sulfotransferase, NSIST)

アグリンと結合するタンパク質を認識する単クローン抗体3B3は、シビレエイのシナプス膜タンパク質を抗原として作製された(35)。3B3のエピトープはノイラミナーゼ消化により3B3との反応性が減少すること、コンドロイチナーゼやケラタナーゼ消化では変化しないことから、シアル酸を持つオリゴ糖と考えられた。3B3抗体を用いる発現クローニングにより、シビレエイの発電器官からNSIST遺伝子がクローニングされた(36)。NSISTはニワトリC6STとアミノ酸配列が56%一致することから、C6STと似た特異性の硫酸転移酵素と考えられたが、実際にCOS-7細胞に導入するとC6ST活性が発現した(筆者ら未発表)。しかし、NSISTがC6ST活性を示すことと、3B3と反応するエピトープとの関係は明らかではない。3B3と反応するエピトープを持つタンパク質の一つにジストログリカンがある。ジストログリカンにはNeuAc α 1-3Gal β 1-4GlcNAc β 1-2Man-O-という糖鎖が含まれることが知られている(37)。このような糖鎖の硫酸化とNSISTが関連を持つことも考えられるが、NSISTの基質の確定には今後の研究が必要である。

E-2. ケラタン硫酸Gal-6-硫酸転移酵素(KSGal6ST)

ケラタン硫酸はGlcNAcの6位とGalの6位が硫酸化されている。C6STの相同遺伝子としてKSGal6STがクローニングされた(38)。COS-7細胞で発現したタンパク質は、ケラタン硫酸、シアリルラクトサミンオリゴ糖、フェツインに含まれるGal残基の6位を硫酸化する活性を示した(39)。C6STによるシアリルラクトサミンオリゴ糖の硫酸化はコンドロイチンやケラタン硫酸の硫酸化に比べてずっと遅い速度でしか進まないのに対し、KSGal6STによるシアリルラクトサミンオリゴ糖の硫酸化はケラタン硫酸の硫酸化と同じ程度で起きた。フコース転移酵素VIIとコア2 β 1-6GlcNAc転移酵素とともにKSGal6ST遺伝子を導入したCHO細胞では、Lセレクトインとの結合活性が上昇したことから、KSGal6STはLセレクトインリガンドの合成に機能していると考えられる(40)。ヒト組織での発現をノーザンブロットで調べると、KSGal6STは脳(38)や種々の免疫組織(39)で発現していた。

E-3. GlcNAc 6-O-硫酸転移酵素群

糖タンパク質の糖鎖のGlcNAc残基の6位の硫酸化は、GlcNAcが非還元末端にある時にのみ起こることが報告されている(41-43)。非還元末端のGlcNAc残基の6位を硫酸化する硫酸転移酵素(GlcNAc 6-O-硫酸転移酵素)が何種類かクローニングされた。これらの遺伝子のクローニングでは、C6ST、KSGal6STあるいはクローニングされたGlcNAc 6-O-硫酸転移酵素と相同性を示す遺伝子をESTデータベースで検索する方法が基礎となっている。記述を簡略化するため、GlcNAc 6-O-硫酸転移酵素群に

GlcNAc 6-O-sulfotransferase. To simplify the description, I have used abbreviations GN6ST-1, GN6ST-2, GN6ST-3 and GN6ST-4 for the members of GlcNAc 6-O-sulfotransferase family in the following sections.

Macular corneal dystrophy (MCD) is an autosomal recessive inheritance disorder which accompanies corneal opacity. The corneal explants of MCD has been reported to synthesize low sulfated keratan sulfate, suggesting that sulfotransferase involved in the synthesis of keratan sulfate may be mutated (44-46). When sulfotransferase activities contained in the extracts from the cornea of patients with MCD were determined, we found that GlcNAc6ST activity decreased in MCD patients, although Gal 6-O-sulfotransferase activity of MCD was nearly the same as the control (47). MCD gene was mapped to 16q22 (48). Among GlcNAc 6-O-sulfotransferase so far cloned, GN6ST-2 and GN6ST-3 were mapped to near the MCD locus, and are possibly related with the expression of MCD phenotype. Alternatively, unidentified GlcNAc 6-O-sulfotransferase might be present in the cornea.

E-3-1. GN6ST-1 (GlcNAc 6-O-sulfotransferase)

The initially cloned GlcNAc 6-O-sulfotransferase, GN6ST-1, transferred sulfate to position 6 of nonreducing terminal GlcNAc residues of GlcNAc β 1-3Gal β 1-4GlcNAc or GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc (49, 50). When GN6ST-1 cDNA was singly transfected in COS-7 cells, 6-sulfo sialyllactosamine was expressed. When GN6ST-1 and fucosyltransferase VII cDNA were cotransfected in COS-7 cells, 6-sulfo sialyl Lewis x was expressed. Human endothelial cell line, ECV304, which was cotransfected with GN6ST-1 and fucosyltransferase VII cDNA, expressed 6-sulfo sialyl Lewis X and showed significant adhesion activity to L-selectin-expressing cells (51). mRNA of GN6ST-1 was ubiquitously expressed in various human tissues (50). Benzylglycosides having GlcNAc residue at the nonreducing terminal, GlcNAc β 1-OBz and GlcNAc β 1-6GlcNAc α 1-OBz served as acceptors for GN6ST-1; GlcNAc β 1-6GlcNAc α 1-OBz was a better acceptor. GN6ST-1 was shown to transfer sulfate, albeit at a lower rate, to a trisaccharide glycolipid containing internal GlcNAc residue, Gal β 1-4GlcNAc α 1-6Gal β 1-(CH₂)₂CONHC₈H₁₇, suggesting that GN6ST-1 appears to be able to transfer sulfate to GlcNAc residue located not only at the nonreducing terminal but also at the internal position (52). Human GN6ST-1 gene was mapped to 7q31 (50) or 3q24 (53).

E-3-2. GN6ST-2 (HEC-GlcNAc-6-O-Sulfotransferase, L-Selectin Ligand Sulfotransferase (LSST))

GN6ST-2 was cloned as a GlcNAc 6-O-sulfotransferase whose expression was highly restricted to HEV (40, 54). Transfection of cDNA of GN6ST-2, KSGal6ST and CD34 in CHO/FTVII/C2GnT, which had been stably transfected with fucosyltransferase VII and core 2 β 1-6GlcNAc transferase cell, resulted in the appearance of L-selectin binding activity. The

属する酵素の略称として、GN6ST-1、GN6ST-2、GN6ST-3、GN6ST-4を以下の記述で用いた。

斑状角膜変成症(MCD)は角膜の濁りを生じる常染色体劣性の遺伝疾患である。MCD患者の角膜ではケラタン硫酸の低硫酸化が起きており(44-46)、ケラタン硫酸合成に関わる硫酸転移酵素に変異がある可能性が考えられた。MCD患者の角膜の抽出液に含まれるケラタン硫酸合成に関連する硫酸転移酵素活性を測定したところ、Gal 残基の 6 位を硫酸化する活性は対照と変わらないのに対し、非還元末端の GlcNAc 残基の 6 位を硫酸化する活性が選択的に減少していた(47)。MCD 遺伝子は 16q22 にマップされている(48)。今までにクローニングされた GlcNAc 6-O-硫酸転移酵素のうち、GN6ST-2 と GN6ST-3 は MCD 遺伝子の近くにマップされているので、MCD との関連があるかもしれない。あるいはまだ知られていない角膜で選択的に発現する GlcNAc 6-O-硫酸転移酵素が存在することも考えられる。

E-3-1. GN6ST-1(GlcNAc 6-O-硫酸転移酵素)

GlcNAc 6-O-硫酸転移酵素活性を持つ酵素として最初にクローニングされたGN6ST-1は、GlcNAc β 1-3Gal β 1-4GlcNAc、GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAcを基質にしたとき末端のGlcNAc残基の 6 位に硫酸基を転移した(49, 50)。この遺伝子を単独でCOS-7細胞に導入すると 6スルホシアリルラクトサミンが、フコース転移酵素VIIと同時に導入すると 6スルホシアリルルイス x が合成された。ヒト内皮細胞の培養細胞 ECV304にフコース転移酵素VII cDNA と同時にGN6ST-1 cDNA を導入すると、6スルホシアリルルイスxが発現し、Lセレクトインを発現する細胞との接着活性が著明に増加した(51)。GN6ST-1の mRNAはヒトでは調べた限りほとんどの組織で普遍的に発現していた(50)。非還元末端にGlcNAc残基を持ち、アグリコンとしてベンジル基を持つグリコシド、GlcNAc α 1-OBz、GlcNAc β 1-OBz、GlcNAc β 1-6GlcNAc α 1-OBzがGN6ST-1の基質となった。そのうちGlcNAc β 1-6GlcNAc α 1-OBzが最も高い活性を示した。内部にGlcNAc残基を持つ3糖誘導体Gal β 1-4GlcNAc α 1-6Gal β 1-(CH₂)₂CONHC₈H₁₇がGN6ST-1の基質となることから、GN6ST-1は非還元末端だけでなく内部にあるGlcNAc残基にも硫酸基を転移すると思われる(52)。GN6ST-1遺伝子はヒト染色体の 7q31 (50)または3q24 (53)にマップされた。

E-3-2. GN6ST-2(HEC-GlcNAc 6-O-硫酸転移酵素, L-セレクトインリガンド硫酸転移酵素 (LSST))

高内皮細静脈に特異的に発現している遺伝子としてGN6ST-2がクローニングされた(40, 54)。フコース転移酵素VIIとコア 2 β 1-6 GlcNAc転移酵素を導入したCHO細胞に、GN6ST-2、KSGal6ST、CD34遺伝子を導入すると、Lセレクトインとの結合活性が出現した。GN6ST-2とKSGal6STはそれぞれ単独で導入した

combination of GN6ST-2 and KSGal6ST produced strongly enhanced binding of L-selectin, which greatly exceeded the sum of the signals from the individual sulfotransferases (40). GN6ST-2 was shown to exhibit striking catalytic preference for core 2-branched mucin-type O-glycans as found natural L-selectin counter-receptor (54). Human GN6ST-2 and GN6ST-3 genes were reported to be clustered in band 16q23.1-23.2 (40). GN6ST-2 transferred sulfate *in vitro* to GlcNAc β 1-OBz, GlcNAc α 1-OBz, GlcNAc β 1-6GlcNAc α 1-OBz, GlcNAc β 1-O(CH₂)₇CH₃ and GlcNAc β 1-6Gal α 1-(CH₂)₂CONHC₈H₁₇ (40).

E-3-3. GN6ST-3 (I-GlcNAc-6-O-sulfotransferase)

When COS-7 cells were transfected with GN6ST-3 cDNA together with GlyCAM-1 cDNA and metabolically labeled with ³⁵SO₄, ³⁵SO₄ was incorporated into position 6 of GlcNAc residue contained in GlyCAM-1 oligosaccharides. Human GN6ST-3 mRNA was mainly expressed in colon and small intestine (55).

E-3-4. GN6ST-4 (Chondroitin 6-Sulfotransferase-2 (C6ST-2), GlcNAc-6-O-Sulfotransferase 4, GST-5)

A soluble form of recombinant GN6ST-4 transferred sulfate to position 6 of GalNAc residue of chondroitin. Unlike C6ST, GN6ST-4 did not transfer sulfate to keratan sulfate (25). A microsomal fraction prepared from CHO cells that had been transfected with GN6ST-4 transferred sulfate to position 6 of GlcNAc residue of GlcNAc β 1-6Man-OMe, GlcNAc β 1-2Man, GlcNAc β 1-6[Gal β 1-3]GalNAc-pNP and GlcNAc β 1-3Gal β 1-4Glc, but not Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc, also served as acceptors. Activity toward chondroitin was 2% of the activity toward GlcNAc β 1-6Man-OMe (55). A soluble form of recombinant GN6ST-4 transferred sulfate to GlcNAc β 1-OBz, GlcNAc β 1-6GlcNAc α 1-OBz, GlcNAc β 1-O(CH₂)₇CH₃ and GlcNAc β 1-6Gal α 1-(CH₂)₂CONHC₈H₁₇. Human GN6ST-4 was mapped to Xp11.

F. Sulfotransferases That Belong to C4ST Gene Family

F-1. HNK-1 Sulfotransferase (HNK-1ST)

HNK-1 epitope has been demonstrated to be expressed specifically in the neural tissues and implicated in the movement of neurons and the formation of neural network. The HNK-1 epitope contained GlcA(3SO₄) residue at the nonreducing terminal of N-linked oligosaccharides or glycolipids. A sulfotransferase (HNK-1 sulfotransferase) responsible for the formation of GlcA(3SO₄) was cloned by the expression cloning using an antibody for HNK-1 antigen (57, 58). When C4ST cDNA was cloned on the basis of the amino acid sequence, a significant homology was observed between C4ST and HNK-1 sulfotransferase (27).

F-2. GalNAc 4-Sulfotransferase (GalNAc4ST)

Nonreducing terminal GalNAc(4SO₄) residue is present in oligosaccharides attached to pituitary glycoprotein hormones, and was shown to play an important role in a pulsatile appear-

ance. GN6ST-2 and KSGal6ST produced strongly enhanced binding of L-selectin, which greatly exceeded the sum of the signals from the individual sulfotransferases (40). GN6ST-2 was shown to exhibit striking catalytic preference for core 2-branched mucin-type O-glycans as found natural L-selectin counter-receptor (54). Human GN6ST-2 and GN6ST-3 genes were reported to be clustered in band 16q23.1-23.2 (40). GN6ST-2 transferred sulfate *in vitro* to GlcNAc β 1-OBz, GlcNAc α 1-OBz, GlcNAc β 1-6GlcNAc α 1-OBz, GlcNAc β 1-O(CH₂)₇CH₃, GlcNAc β 1-6Gal α 1-(CH₂)₂CONHC₈H₁₇ that was GN6ST-2's substrate (40).

E-3-3. GN6ST-3 (I-GlcNAc 6-O-sulfotransferase)

GN6ST-3 cDNA was transfected with GlyCAM-1 cDNA and simultaneously COS-7 cells were transfected, metabolically labeled with ³⁵SO₄ and then analyzed. GlyCAM-1 oligosaccharides contained GlcNAc residue at position 6 of which sulfate was transferred. GN6ST-3 mRNA was mainly expressed in colon and small intestine (55).

E-3-4. GN6ST-4 (Chondroitin 6-Sulfotransferase-2 (C6ST-2), GlcNAc 6-O-Sulfotransferase-4, GST-5)

A soluble form of recombinant GN6ST-4 transferred sulfate to position 6 of GalNAc residue of chondroitin. Unlike C6ST, GN6ST-4 did not transfer sulfate to keratan sulfate (25). A microsomal fraction prepared from CHO cells that had been transfected with GN6ST-4 transferred sulfate to position 6 of GlcNAc residue of GlcNAc β 1-6Man-OMe, GlcNAc β 1-2Man, GlcNAc β 1-6[Gal β 1-3]GalNAc-pNP and GlcNAc β 1-3Gal β 1-4Glc, but not Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc, also served as acceptors. Activity toward chondroitin was 2% of the activity toward GlcNAc β 1-6Man-OMe (55). A soluble form of recombinant GN6ST-4 transferred sulfate to GlcNAc β 1-OBz, GlcNAc β 1-6GlcNAc α 1-OBz, GlcNAc β 1-O(CH₂)₇CH₃, GlcNAc β 1-6Gal α 1-(CH₂)₂CONHC₈H₁₇ that was GN6ST-4's substrate (52). GN6ST-4 cDNA was mapped to Xp11.

F. C4ST Family Members Sulfotransferases

F-1. HNK-1 Sulfotransferase (HNK-1ST)

HNK-1 epitope has been demonstrated to be expressed specifically in the neural tissues and implicated in the movement of neurons and the formation of neural network. The HNK-1 epitope contained GlcA(3SO₄) residue at the nonreducing terminal of N-linked oligosaccharides or glycolipids. A sulfotransferase (HNK-1 sulfotransferase) responsible for the formation of GlcA(3SO₄) was cloned by the expression cloning using an antibody for HNK-1 antigen (57, 58). When C4ST cDNA was cloned on the basis of the amino acid sequence, a significant homology was observed between C4ST and HNK-1 sulfotransferase (27).

F-2. GalNAc 4-Sulfotransferase

Nonreducing terminal GalNAc(4SO₄) residue is present in oligosaccharides attached to pituitary glycoprotein hormones, and was shown to play an important role in a pulsatile appearance. GN6ST-2 and KSGal6ST produced strongly enhanced binding of L-selectin, which greatly exceeded the sum of the signals from the individual sulfotransferases (40). GN6ST-2 was shown to exhibit striking catalytic preference for core 2-branched mucin-type O-glycans as found natural L-selectin counter-receptor (54). Human GN6ST-2 and GN6ST-3 genes were reported to be clustered in band 16q23.1-23.2 (40). GN6ST-2 transferred sulfate *in vitro* to GlcNAc β 1-OBz, GlcNAc α 1-OBz, GlcNAc β 1-6GlcNAc α 1-OBz, GlcNAc β 1-O(CH₂)₇CH₃, GlcNAc β 1-6Gal α 1-(CH₂)₂CONHC₈H₁₇ that was GN6ST-2's substrate (40).

Fig. 2. Sequence-based evolutionary tree of the various glycosaminoglycan sulfotransferases.

In the dendrogram obtained with the program Clustal W, branch length (*horizontal lines*) is approximately proportional to evolutionary distances. For the abbreviation of each sulfotransferase, see the text. Sulfotransferases described in this review are shown in **bold letters**. Letters in parentheses represent the species: *m*, mouse; *h*, human; *r*, rat; *t*, Torpedo; *c*, chick; *d*, Drosophila.

ance of lutropin in the blood through the binding to the hepatic receptor for the sulfated GalNAc residue (59, 60). GalNAc4ST, which transfers sulfate to position 4 of nonreducing terminal GalNAc residue, was cloned by two groups independently (61, 62). Identity of the amino acid sequence between GalNAc4ST and human C4ST was 30 %. The recombinant GalNAc4ST did not transfer sulfate to chondroitin or desulfated dermatan sulfate that served as acceptors for C4ST, but transferred to car-

あると考えられている(59、60)。非還元末端GalNAc4-硫酸転移酵素の遺伝子がC4STと相同性を示す遺伝子として同時に2カ所の研究室でクローニングされた(61、62)。GalNAc4STはC4STとアミノ酸配列が30%一致した。発現した酵素はC4STの基質であるコンドロイチン、脱硫酸化デルマタン硫酸を硫酸化せず、カルボニックアンヒドラーゼVI、GalNAc残基を非還元末端に持つ合成糖鎖を硫酸化した。非還元末端GalNAc残基の4位が選択的に硫酸化された。GalNAc4ST遺伝子は脳下垂体で最も強

bonic anhydrase VI and synthetic glycosides having GalNAc residue at the nonreducing terminal. Sulfation occurred at position 4 of the nonreducing terminal GalNAc residue. The message of GalNAc4ST was expressed strongly in the human pituitary, suggesting that GalNAc4ST may be involved in the synthesis of the nonreducing terminal GalNAc(4SO₄) residues found in the N-linked oligosaccharides of pituitary glycoprotein hormones. By searching the human genome data base, GalNAc4ST gene was found to be mapped to 19q13.1, and to be composed of at least four exons. As observed in C4ST, GalNAc4ST also contains Cys in 5'-PSB domain, which has not been found in glycosaminoglycan sulfotransferases so far cloned. Although the functional role of this Cys residue is not clear at present, Cys in 5'-PSB domain may be relevant to the stimulation of GalNAc4ST and C4ST by sulfhydryl compounds.

G. Future Prospects for the Study of Sulfotransferases

Marked progress in the cloning of the sulfotransferases involved in the synthesis of glycosaminoglycans has been achieved recently. Among the sulfotransferases involved in the synthesis of chondroitin sulfate, GalNAc4S-6ST has not been cloned yet. If cloning of GalNAc4S-6ST is attained, nearly all the genes of sulfotransferases involved in the synthesis of chondroitin sulfate isomers (chondroitin sulfate A, C, D and E) will become available. As shown in Fig. 2, divergent glycosaminoglycan sulfotransferases are thought to have evolved from a small number of common ancestral genes. During their evolution, sulfotransferases with a unique substrate specificity may appear and they may support the higher biological phenomena such as immunological cellular interaction and neural function. From the gene families showing homology with sulfotransferases so far cloned, a unique sulfotransferase with important biological functions may be found. Studies on the substrate specificity and regulation of gene expression of sulfotransferases will offer important information to elucidate the biological function of sulfated sugar in the cellular interactions in multicellular organisms.

く発現しており、脳下垂体ホルモンのN結合糖鎖の非還元末端にあるGalNAc(4SO₄)残基の合成に関わっていることが強く示唆された。この遺伝子を持つヒトゲノムクローンの検索から、GalNAc4STは19q13.1にマップされ、少なくとも4個のエキソンを含むことが分かった。C4STと同様にGalNAc4STはPAPS結合ドメインと推定される5'-PSBドメインにCysを含んでいる。今までにクローニングされたグリコサミノグリカン硫酸転移酵素の中には5'-PSBドメインにCysを含んでいる硫酸転移酵素は他には知られていない。このCysの機能は明らかではないが、C4STとGalNAc4STがどちらもSH化合物により活性化されることと関連があるかもしれない。

G. 硫酸転移酵素研究の今後の展望

コンドロイチン硫酸異性体合成に関わる硫酸転移酵素の遺伝子クローニングは、この数年の間に大きな進展があった。コンドロイチン硫酸の合成に関与する硫酸転移酵素のうち、GalNAc4S-6STはまだクローニングされていない。GalNAc4S-6STがクローニングされれば、コンドロイチン硫酸A、C、D、Eという異性体合成に関わるほぼすべての硫酸転移酵素の遺伝子操作が可能となるであろう。図2に示すように、糖鎖硫酸化反応を行う多様な硫酸転移酵素は、少数の共通の祖先から進化してきたと考えられる。この進化の過程で、特異な基質特異性をもった硫酸転移酵素が出現し、それらが免疫現象や神経機能などの高度な生物学的現象を支えてきたかもしれない。今までにクローニングされた硫酸転移酵素と相同性を示す遺伝子ファミリーの中から、重要な生物学的機能を持った硫酸転移酵素が見いだされるかもしれない。硫酸転移酵素の特異性と遺伝子発現の調節の研究は、多細胞生物の細胞間相互作用における糖鎖硫酸化の生物学的役割を明らかにするための重要な情報を提供するであろう。

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Since 1972 his professional activity has developed at Aichi University of Education. From 1983 to 1984, he studied sulfation of chondroitin sulfate using cultured chick embryo chondrocytes at Dr. H. E. Conrad's laboratory in the University of Illinois. His group purified and cloned chondroitin 6-sulfotransferase and chondroitin 4-sulfotransferase. Recently, his group purified GalNAc 4-sulfate 6-O-sulfotransferase that is involved in the synthesis of chondroitin sulfate E. He is now interested in the specificity, molecular nature and biological significance of the sulfotransferases involved in the synthesis of chondroitin sulfate and related sugar chains.