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A Hydroxysteroid Sulfotransferase, St2b2, Is a Skin Cholesterol Sulfotransferase in Mice[1]

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The mRNA of a sulfotransferase (St2b2) mediating cholesterol sulfation was detected in mouse skin. Recombinant St2b2 also mediated the sulfation of pregnenolone, 3β-hydroxy-5-cholen-24-oic acid, and dehydroepiandrosterone. St2b2 protein was detected in skin cytosols on Western blotting. The addition of 10 nM TPA to skin epidermal cells from newborn mice resulted in a twofold increase in cholesterol sulfation and concomitantly enhanced the St2b2 content after 40 h. Other candidate cholesterol sulfotransferases, St2a4 and St2a9, were not detected in skin by RT-PCR. These results indicate that St2b2 is a cholesterol sulfotransferase in mouse skin.

Key words: cholesterol sulfation, epidermal cell, hydroxysteroid sulfotransferase, mouse skin, phorbol ester.

Cholesterol sulfate is suggested to be involved in epidermal differentiation (*1, 2*). The activity of cholesterol sulfotransferase is increased during squamous differentiation *in vitro* and is believed to be a marker enzyme for terminal differentiation (*3–5*).

In the present study, the cholesterol-sulfating activities of skin from 8-week-old male and female mice were determined using 5 μM ^{35}S-PAPS and 2 mM cholesterol at 0.63 ± 0.10 and 0.75 ± 0.28 pmol/mg of cytosolic protein/min, respectively. The sulfotransferase mediating cholesterol sulfation, however, has not yet been identified.

Recently, a cDNA of a novel hydroxysteroid sulfotransferase, St2b2, has been isolated from mouse I.M.A.G.E. consortium cDNA clones (ID. 679153) (*6*). We also isolated a DNA fragment including the open reading frame of St2b2 from brain by RT-PCR and ligated it into the T vector (Promega). The nucleotide sequence of the PCR product matched that reported previously except for ^{516}C/T (silent mutation). To subclone the cDNA for the expression, the following primers were used for RT-PCR. St2b2-5′ primer has a *Sph*I restriction site, a sequence encoding 6 histidine residues (6xHis), and an enterokinase cleavage site in the 5′-upper region of the initiation codon. St2b2-3′ primer has a *Hin*dIII restriction site at the 3′-terminal. The PCR product was digested with *Sph*I and *Hin*dIII, and then ligated into a bacterial expression vector, pQE30 (Qiagen). Recombinant St2b2 was purified on a Nickel-chelated column as previously reported (*7, 8*). The recombinant protein supported the sulfating activity towards cholesterol, pregnenolone, 3β-hydroxy-5-cholen-24-oic acid, and dehydroepiandrosterone, but no activity towards typical ST1 substrates, *p*-nitrophenol, dopamine, triiodothyronine, and estradiol (Table I).

On Western blotting, a band corresponding to about 41 kDa was detected for skin cytosols from mice of both sexes using anti-St2b2 antisera raised against mouse recombinant St2b2 (Fig. 1A), but not with preimmune serum (Fig. 1B). The skin St2b2 content was determined using a standard St2b2 (recombinant renaturated after 6xHis cleavage at the N-terminus) as a standard. Skin cytosols from male and female mice had specific contents of 0.63 ± 0.07 and 0.72 ± 0.15 pmol St2b2/mg of cytosolic protein, respectively. An anti-St2b2 serum available for immunoblotting did not inhibit cholesterol sulfation mediated by recombinant St2b2 (data not shown). Specific cholesterol sulfating activity was calculated to be 1.0 nmol/nmol St2b2/min from skin cytosol data, whereas recombinant St2b2 showed specific activity of 0.05 nmol/nmol St2b2/min under the conditions of 2 mM cholesterol, 5 μM ^{35}S-PAPS, and 0.3% Triton X-100. Although the reason for the discrepancy is unclear, the specific activity of recombinant St2b2 increased more than 3-fold after the addition of heat-inactivated skin cytosolic protein (15 μg) to the incubation mixture (data not shown). These results suggest an additional requirement of a certain environment for the maximal reconstitution of St2b2. The addition of membrane components such as lipid (60 μg of dilauroylphosphatidylcholine) or ceramide (6 μg) to the incubation mixture increased St2b2-mediated cholesterol sulfation 3.3- and 5.4-fold, respectively, although the possible involvement of another unknown factor can not be excluded at this time.

A potent tumor promoter, TPA, was reported to increase the synthesis of skin epidermal cholesterol sulfate *in vivo* and *in vitro* (*9, 10*). Skin epidermal cells were thus pre-

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Abbreviations: St or ST, sulfotransferase; RT-PCR, reverse transcription–polymerase chain reaction; TPA, 12-*O*-tetradecanoyl phorbol 13-acetate.

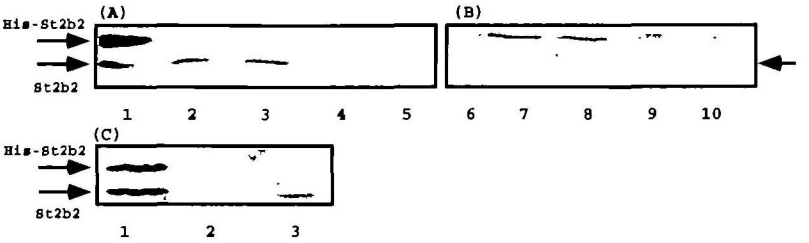

Fig. 1. **Detection of St2b2 protein in skin and epidermal cells.** Cytosolic proteins were subjected to SDS-PAGE on a 10.5% gel and then electrically transferred to a nitrocellulose membrane. The membrane was stained with anti-His-St2b2 serum (A and C) or pre-immune serum (B). The lanes in A and B contained the following. Lanes 1 and 6, His-St2b2 and St2b2 (total 200 ng); lanes 2 and 7, male skin (100 μg); lanes 3 and 8, female skin (100 μg); lanes 4 and 9, male liver (100 μg); lanes 5 and 10, female liver (100 μg). The lanes in C contained the following. Lane 1, His-St2b2 and St2b2 (total 200 ng); lane 2, non–TPA-treated (10 μg); lane 3, 10 nM TPA-treated (10 μg) newborn skin epidermal cells.

TABLE I. **Sulfating activities of recombinant St2b2.**

Substrate	Sulfating activity (nmol/mg protein/min)
p-Nitrophenol	N.D.[a]
Dopamine	N.D.[a]
3,3′,5-Triiodothyronine	N.D.[a]
Estrone	N.D.[a]
β-Estradiol	N.D.[a]
Dehydroepiandrosterone	30.3 ± 2.2
Cholesterol	0.62 ± 0.07
Pregnenolone	68.4 ± 2.0
Cortisol	N.D.[a]
Lithocholic acid	N.D.[a]
3β-Hydroxy-5-cholen-24-oic acid	39.0 ± 3.0
Desipramine	N.D.[a]
4-Phenyl-1,2,3,6-tetrahydropyridine	N.D.[a]
Minoxidil	N.D.[a]
1-Pyrenemethanol	N.D.[a]

[a]N.D. <0.15 nmol/mg protein/min. The incubation mixture (10 μl) contained 10 μM substrates, except in the case of desipramine (100 μM), 4-phenyl-1,2,3,6-tetrahydropyridine (500 μM), 125 μM ^{35}S-PAPS, and purified His-St2b2 (50 ng). After incubation at 37°C for 20 min, the reaction mixture was subjected to TLC, and then the radioactivities of the sulfates were calculated with a BAS 1000 image analyzer (Fuji Film). All data are the means ± SD of three determinations.

pared from newborn mice by the method previously reported (*11, 12*). The skin epidermal cells (3×10^6 cells) were plated on a 100-mm culture dish (Falcon Scientific Co., Oxnard, CA) with 8 ml of an MEM medium containing 10% fetal calf sera. The medium was replaced 4 h later with medium containing 10 nM TPA, followed by incubation for 40 h. A cytosolic fraction of epidermal cells was prepared by ultracentrifugation. The addition of TPA caused a twofold increase in cholesterol sulfating activity as compared to the vehicle (2.13 *vs.* 1.04 pmol/mg of cytosolic protein/min). A concomitant increase (1.95-fold) in the St2b2 content was observed in TPA-treated epidermal cells (Fig. 1C).

Another subfamily member, the ST2A form, was reported to catalyze cholesterol sulfation (*13*). St2a4 and St2a9 were the known candidate sulfotransferases mediating cholesterol sulfation in mice (*14, 15*). To confirm the expression of these forms, RT-PCR with specific primers was performed (Fig. 2). St2b2 mRNA was detected in skin, but scarcely in liver. In contrast, St2a4 and St2a9 mRNAs were detected in liver, but not skin.

These results strongly suggest that St2b2 is a sulfotransferase mediating skin cholesterol sulfation in mice.

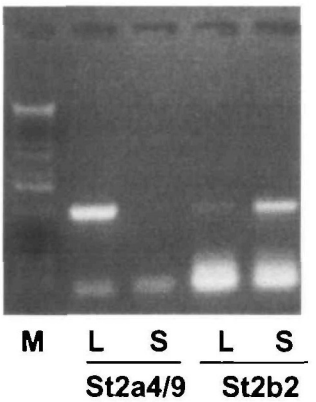

Fig. 2. **Detection of hydroxysteroid sulfotransferase mRNA in mouse skin and liver on RT-PCR.** To detect St2b2 and St2a4/9 mRNAs individually, the following primers were designed: 5′-CGGCATGCGATGACGATGACAAAATGGACGGGCCGCAGCCCCGC-3′ (5′-primer for St2b2), 5′-GCGAAGCTTTTATTGGTGAGGATCCTGGGTTGGGGTC-3′ (3′-primer for St2b2), 5′-TACAAGGAGTTATTCATGGATG-3′ (5′-primer for St2a4/9), and 5′-CCACAACAGGTTTTATTCTATGACC-3′ (3′-primer for St2a4/9). M, molecular marker; L, male liver; S, male skin, respectively. First-strand cDNA, as a template for PCR, was synthesized from total RNA of rat skin or liver, using Ready-to-Go You-prime First-Strand Beads (Pharmacia Bio Tech). RT-PCR amplification was performed using recombinant Taq polymerase (TAKARA SHUZO). The amplification reactions included initial denaturation for 3 min at 94°C, 35 cycles of 1 min at 94°C, 30 s at 55°C, and 2 min at 72°C, final extension for 7 min at 72°C, and then a 4°C soak. The reaction products were resolved by 1% agarose gel electrophoresis and stained with ethidium bromide.

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