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Expression and activity of dehydroepiandrosterone sulfotransferase in human gastric mucosa

A. Tashiro[a,*], H. Sasano[b], T. Nishikawa[c], N. Yabuki[b], Y. Muramatsu[a], M.W.H. Coughtrie[d], H. Nagura[b], M. Hongo[e]

[a]*Department of Psychosomatic Medicine, Tohoku University School of Medicine, 1-1 Seiryo-machi, Aoba, Sendai 980 8574, Japan*
[b]*Department of Pathology, Tohoku University School of Medicine, 1-1 Seiryo-machi, Aoba, Sendai 980 8574, Japan*
[c]*Department of Medicine, Yokohama Rosai Hospital, Yokohama, Japan*
[d]*Department of Biochemical Medicine, University of Dundee Ninewells Hospital and Medical School, Dundee, UK*
[e]*Department of Comprehensive Medicine, Tohoku University School of Medicine, 1-1 Seiryo-machi, Aoba, Sendai, 980 8574, Japan*

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Abstract

Dehydroepiandrosterone sulfotransferase (DHEA-ST) is a key enzyme in the formation of Dehydroepiandrosterone sulfate (DHEAS) and is thought to be involved in the conversion of various substances such as bile acids and cholesterol. The existence of DHEA-ST in the small intestine in addition to the adrenal gland and liver in adult humans was recently reported. As the sulfotransferases can act on toxic or potentially toxic substances to reduce their biological activity, we attempted to clarify the significance of DHEA-ST in gastrointestinal tract. We examined surgically resected human stomach for the presence of DHEA-ST and attempted to determine its possible biological significance. DHEA-ST activity ranged widely from 6 to 84 pmoles/mg protein/90 min in 7 cases. Immunoblotting revealed one single band of a 35-kDa protein corresponding to the moleculr weight of DHEA-ST. Both DHEA-ST immunoreactivity and mRNA hybridization signals were localized in parietal cells of the gastric glands. The results of our present study demonstrated that the sulfation of DHEA by DHEA-ST occurs in the gastric glands. The localization of DHEA-ST in parietal cells suggests that this enzyme is correlated to mucosal function in the human stomach in addition to detoxification of exogenous substances.

1. Introduction

Sulfation has been recognized as an important steps in the synthesis, transport, and metabolism of steroids in human tissue [1]. Sulfate conjugation also plays an important role in modulating the biological activity of steroids because steroid sulfates are not capable of binding and activating the appropriate steroid receptors. That is, steroid sulfonation indirectly influence the action of steroid hormones [2]. On the other hand sulfated steroids can act directly cellular membrane [3,4]. These findings indicate that sulfation has two pathway to influence transcriptional regulation [2]. Dehydroepiandrosterone sulfotransferase (DHEA-ST) is a key enzyme in the formation of DHEAS [1,5,6]. The existence of DHEA-ST has been previously reported only in the liver [5] and small intestine [7], except for the adrenal gland, in adult humans. This enzyme is also considered to be involved in the conversion of various substances such as bile acids and cholesterol [5,6], but the precise physiological role of extra-adrenal DHEA-ST has not been understood. As the sulfotransferases can act on toxic or potentially toxic substances to reduce their biological activity [8], we attempted to clarify the significance of DHEA-ST in gastrointestinal tract. In this study, we first examined whether functional DHEA-ST is present in the human gastric mucosa. We determined the activity of DHEA-ST and its expression by immunoblotting in

* Corresponding author. Fax: +81-22-717-7330.
E-mail address: tashiro@mail.cc.tohoku.ac.jp (A. Tashiro).

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the human gastric mucosa obtained from surgery, then studied the localization of DHEA-ST with immunohistochemistry and correlated the expression of H^+K^+-ATPase in order to understand the biological significance of its expression. Finally, we performed mRNA in situ hybridization to examine the biosynthesis of DHEA-ST in the gastric mucosa.

2. Experimental

2.1. Tissue samples

Twenty-three cases of surgically resected human stomach were examined; among these samples, 7 cases were available for enzyme activity and an immunoblotting study. The age and sex of these examined patients are summarized in Table 1. All specimens were obtained from total or subtotal gastrectomy due to adenocarcinoma. Light microscopic examination of these specimens revealed no evidence of neoplastic changes. One case of adrenal gland obtained from nephrectomy due to renal cell carcinoma was also examined as a positive control. Portions of gastric mucosa were immediately frozen in liquid nitrogen and stored at −70°C for the measurement of enzyme activity and immunoblotting analysis. Other portions were fixed in 8% paraformaldehyde at pH 7.4 for 24 h at 4°C and embedded in paraffin for immunohistochemistry and mRNA in situ hybridization.

2.2. Assay of enzyme activity

The activity of DHEA-ST was determined as previously reported [9] with some modification. The incubation mixture contained 0.1 mM PAPS, 50 mM Tris-HCl (pH 7.4), 1.5 mM EDTA, 25 μM trilostane for inhibiting 3β-hydroxysteroid dehydrogenase, which is able to metabolize DHEA, [^{3}H]-DHEA (0.125 μCi/tube) with an appropriate amount of non-radioactive DHEA, and an aliquot of the cytosolic fraction prepared from gastric tissues. The mixture was incubated at 37°C for 90 min, and the reaction was stopped by the addition of dichloromethane. After the tubes were vortexed and centrifuged, the aqueous phase was transferred and washed with dichrolomethane. The sulfated steroid was extracted from the aqueous phase with 2 ml of ethyl acetate after adding 1 ml of a saturated NaCl solution. The labeled DHEAS was purified by thin-layer chromatography using a system consisting of ethyl acetate:ethanol:NH_4OH, according to the methods as reported previously [9]. The radioactivity of DHEAS which was counted directly from the aqueous phase was always the same as that in purified samples by the TLC system described above. Then the aqueous phase was directly used for estimating the radioactivity of the formed DHEAS without further purification.

2.3. Primary antibodies

The antibody used in this study was a rabbit anti-rat liver DHEA-ST IgG that had been absorbed with human serum proteins. This rabbit IgG preparation has been reported to cross-react with DHEA-ST in human adrenal gland and liver tissues on immunoblot [6] and immunohistochemistry [10,11]. A strong correlation was also reported between DHEA-ST enzyme activity and enzyme protein levels quantitated by immunoblot analysis in human liver cytosol using this antibody [3]. In order to characterize the immunoreactivity of DHEA-ST, we employed rabbit polyclonal antibody against H^+K^+-ATPase or proton pump for the immunostaining of parietal cells in serial tissue sections. This antibody was kindly donated by Dr. Ohmori (Kansai Medical University, Osaka, Japan).

2.4. Immunoblotting

Specimens were homogenized in the solution containing 0.25 M sucrose at 4°C and centrifuged at 3000 rpm for 15 min. The supernatants were then recentrifuged at 38,000 rpm for 60 min at 4°C, and these supernatants were used as cytosol fractions. Each cytosol fraction from the human adrenal gland and stomach was mixed with Laemmli sample buffer containing 5′2-mercaptoehanol and then electrophoresed on SDS-10% polyacrylamide gels. Resolved proteins

Table 1
Summary of DHEA-ST activity in 7 cases of the gastric mucosa

Sl. No.	Age	Sex	Region	DHEA-ST activity (pmol/mg protein/90 min)
1	56	M	Body	58.48
2	64	M	Body	52.91
3	67	F	Body	55.87
4	74	F	Body	84.03
5	82	F	Body	34.83
6	55	M	Lower body to antrum	6.02
7	56	M	Lower body to antrum	17.33

were subsequently electrotransferred to nitrocellulose. The blots were subsequently incubated with rabbit anti DHEA-ST IgG as described above after blocking non-specific sites with 5% powdered low fat milk. The reacted blots were then incubated with goat anti-rabbit IgG conjugated to horseradish peroxidase (Bio-Rad, Richmond, CA), and visualized using an ECL detection system (Amersham Life Sciences, Aylesbury, UK) after washing.

2.5. Immunohistochemistry

Deparaffinized sections were placed in methanol containing 0.3% hydrogen peroxide for 30 min to inhibit endogenous peroxidase activity. The sections were washed with 0.01 mol/l phosphate-buffered saline (PBS) three times and incubated with normal goat serum for 20 min at room temperature and with the primary antibodies for 18 h at 4°C. Immunohistochemical staining was carried out by a biotin-streptavidin-amplified method using the Histofine immunostaining system (Nichirei, Tokyo, Japan). No immunoreactivity was observed in control sections incubated with normal rabbit IgG or 0.01 mol/l PBS instead of the primary antibodies. Optimal dilution of the primary antibodies was as follows: 1:500 for anti DHEA-ST and 1:8000 for anti H^+K^+-ATPase antibodies.

2.6. DNA probes

Oligonucleotide probes corresponding to the position from 200 to 210 of the amino acid sequence at exon 5 of human DHEA-ST gene were synthesized based on the reported sequence of the gene [12]. The sequence of the 30-base oligonucleotide probe used for mRNA in situ hybridization analysis was as follows; CAA TTC CTG GGA AAG ACG TTA GAA CCC GAA. Sense oligonucleotide probes were used as negative controls. The probes were labeled with a 3′biotinylated tail (Brigati tail; 5′-probe-biotin-biotin-biotin-TAG-TAG-biotin-biotin-biotin-3′) [13].

2.7. In situ hybridization

In situ hybridization was performed with the Micro ProbeTM staining system (Fisher Scientific, Pittsburgh, PA); details of the procedure have been reported previously [14].

3. Results

3.1. Enzyme activity

We first examined the effects of incubation time and protein concentration of the gastric mucosa tissues on sulfotransferase activity. Sulfate conjugate formation proceeded at a completely linear rate for the time periods up to 120 min (Fig. 1). Using a 90-min incubation, sulfotrasferase activity was proportional to protein concentration in the range of 20–500 μg protein (data not shown).

DHEA-ST activity determined by the assay varied from 6 to 84 pmoles/mg protein/90 min among the patients (Table 1). However, gastric mucosa obtained from the body of the stomach tended to demonstrate higher DHEA-ST activity than that from the lower body to the antrum.

3.2. Immunoblotting

The immunoblot demonstrated the presence of one single band of a 35 kDa protein corresponding to the molecular size of DHEA-ST in the adult human adrenal gland and stomach in all cases (Fig. 2). The relative immunointensity of DHEA-ST in the gastric mucosa was lower than that of the adrenal gland.

3.3. Immunohistochemistry

DHEA-ST immunoreactivity was detected in the non-atrophic gastric glands in all cases (Fig. 3) except for those undergoing intestinal metaplasia. The immunoreactivity was not detected in the foveola epithelium, chief cells, the pyloric glands, or the non-epithelial cells of the gastric mucosa. The immunohistochemistry of H^+K^+-ATPase in serial tissue sections revealed that the great majority of DHEA-ST positive cells in the gastric glands were also positive for H^+K^+-ATPase or proton-pump (Fig. 3).

3.4. mRNA in situ hybridization

There was a heterogeneity in the distribution of

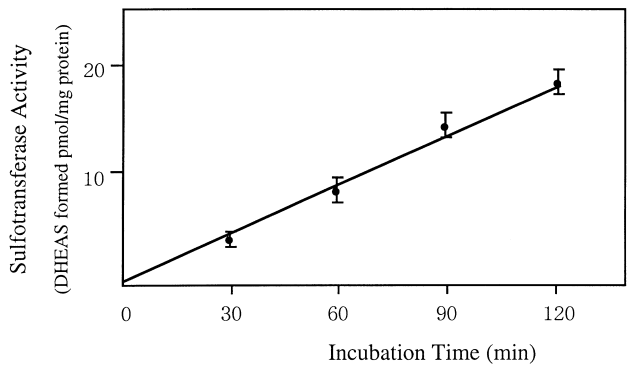

Fig. 1. Time course of sulfate conjugate formation. Experimental procedures are described in assay of enzyme activity. The cytosolic fraction of one gastric mucosa tissue (100 μg protein/assay) was used.

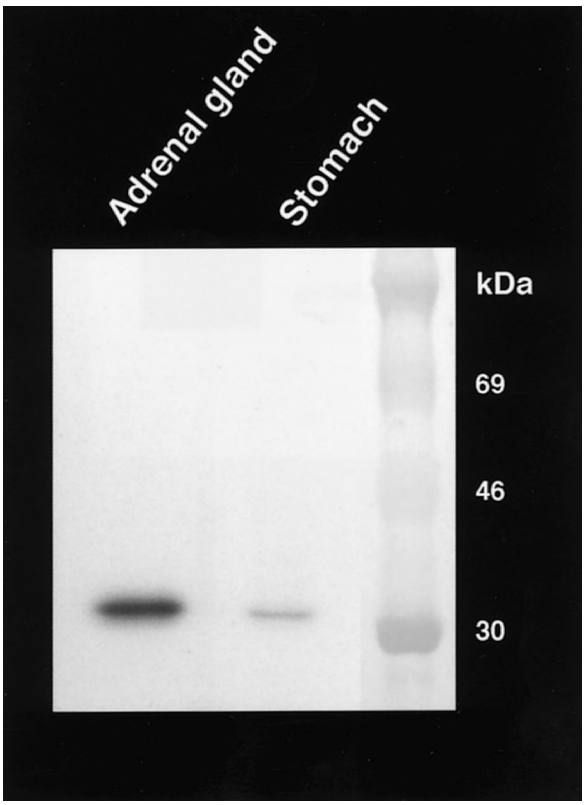

Fig. 2. Results of immunoblotting of DHEA-ST in the adrenal and stomach.

mRNA hybridization signals, but the accumulation of DHEA-ST mRNA hybridization signals was primarily identified in the parietal cells positive for H^+K^+-ATPase in serial tissue sections (Fig. 4). None of the sections hybridized with a sense oligonucleotide probe displayed any signals (Fig. 4).

4. Discussion

Steroid sulfation plays an important role in the synthesis and metabolism of both adrenal and sex steroids in humans [1]. DHEA-ST is a family of cytosolic sulfotransferases that catalyze the 3′-phosphoadenosine 5′-phosphosulfate-dependent sulfation of a wide variety of steroids including DHEA [9]. High levels of DHEA-ST expression have been detected in both fetal and neocortical zones of the fetal adrenal [10,15] and in the zona reticularis of the adult adrenal cortex after adrenarche [16,17]. The existence of extra-adrenal DHEA-ST has been reported only in the liver [5] and small intestine [7] in adult humans. Although this enzyme is considered to be involved in the conversion of various endogenous and exogenous substances to more hydrophilic sulfate conjugates in these sites [4,6,9], the precise physiological role of DHEA-ST in the liver and small intestine has not yet been established. In the liver, DHEA and DHEAS are considered to play an important role in regulating peroxisomal activity through stimulatory peroxisomal gene induction [18,19], which suggests a possible involvement of DHEA-ST in hepatic DHEAS actions as peroxisome proliferation.

In the gastrointestinal tract, Her et al. have recently demonstrated DHEA-ST immunoreactivity in the

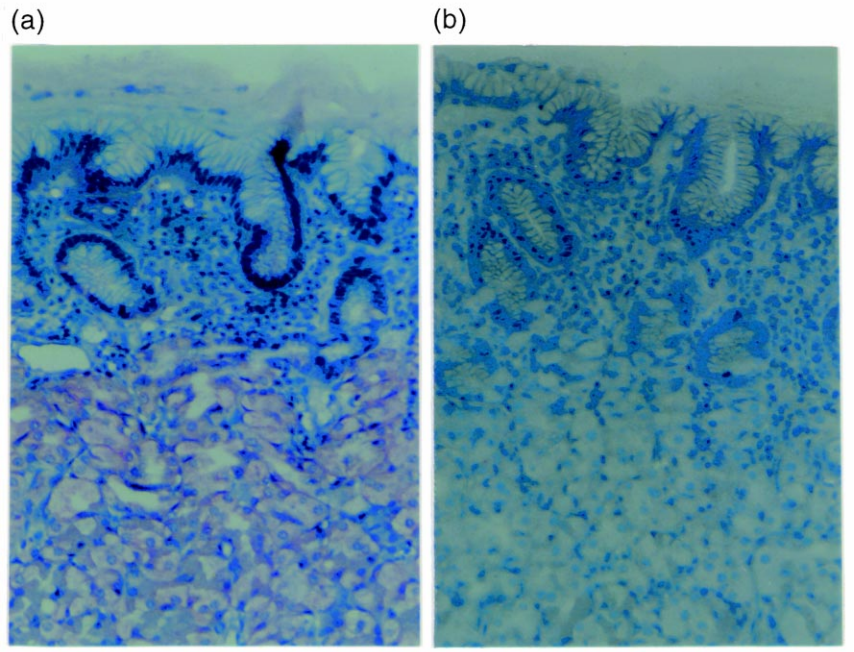

Fig. 3. (a) Immunohistochemistry of DHEA-ST and (b) H^+K^+-ATPase in the gastric mucosa obtained from the body of stomach.

human jejunum, although its localization was not examined [7]. There was no discussion of the role of DHEA-ST in the human jejunum in this report, but orally administered steroids are well known to undergo sulfate conjugations [5], and DHEA-ST expressed in the gastrointestinal tract is considered to be involved in these sulfate-conjugation pathways. In addition, Uehara et al. have reported that the exogenous administration of DHEAS results in mucosal protective effects against the induction of ulcers in the rat stomach by various methods [20]. As the protective effects of DHEAS are not related to the prostaglandin system, another undefined action of DHEAS was suspected as being responsible for this protective effect. The presence of DHEA-ST in parietal cells may suggest that DHEA and/or DHEAS is involved in exerting possible antiulcerogenic effects through the regulation of parietal cell function in addition to detoxification of exogenous substances.

Results of our present studies have demonstrated that functional DHEA-ST is expressed in the parietal cells of human gastric mucosa and is actively synthesized there. However, to establish the biological significance of DHEA-ST expression in the

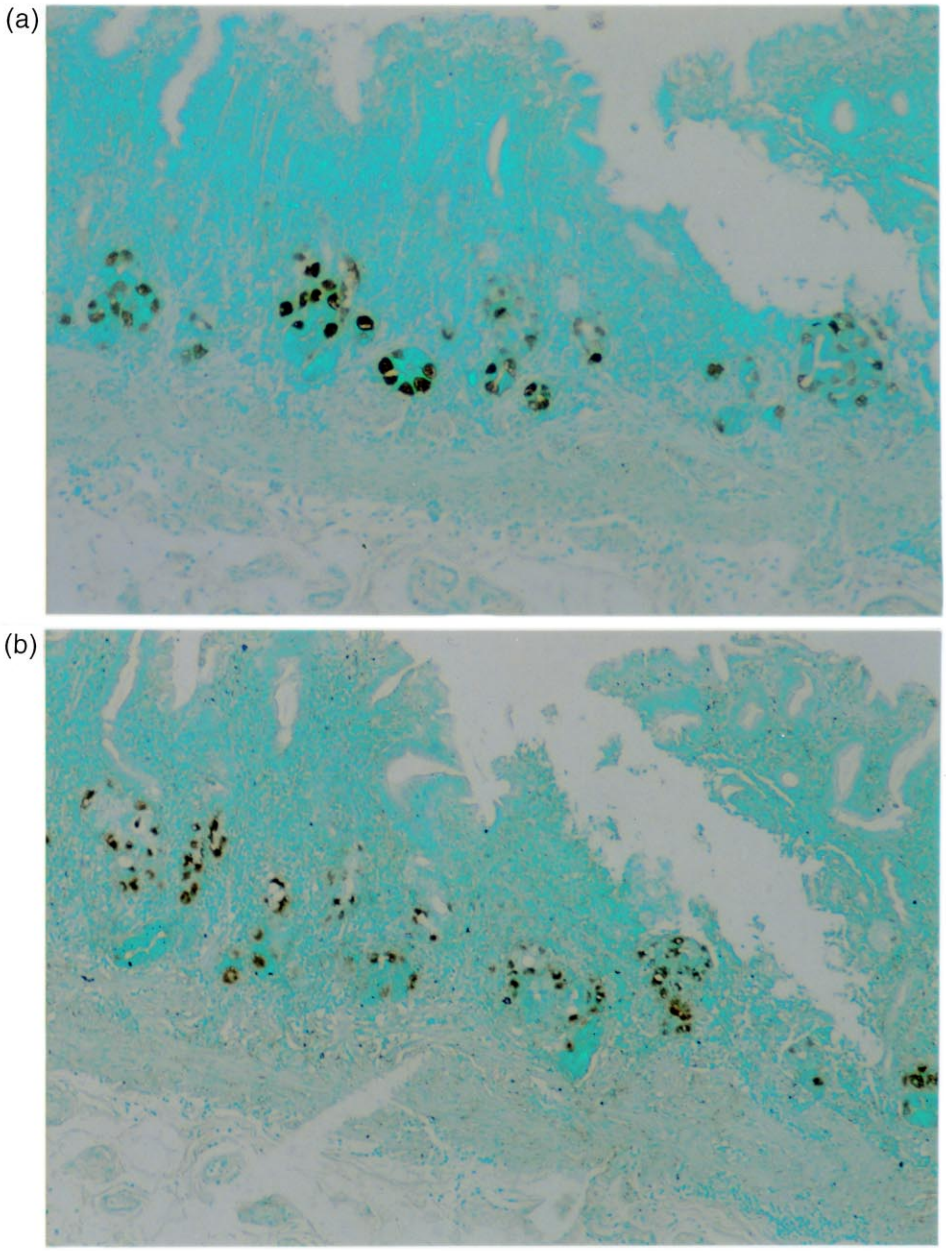

Fig. 4. (a) In situ hybridization of DHEA-ST mRNA in the gastric mucosa DHEA-ST mRNA hybridization signals appearing as a fast red reaction were detected in parietal cells. (b) Negative control with a sense oligonucleotide probe.

gastrointestinal tract, especially in the stomach, further investigations are required.

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