

Steroid Sulfatase Activities in Human Leukocytes: Biochemical and Clinical Aspects

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Abstract. Steroid sulfatase is a membrane-bound microsomal enzyme, present in various tissues. In this report, data on sulfatase activity in peripheral blood leukocytes isolated from normal women and the characterization of its enzyme are studied. In addition, sulfatase activities in placental sulfatase deficiency (PSD) and ichthyosis patients including ichthyosis vulgaris (IV) and recessive X-linked ichthyosis (RXLI) were analysed and were compared with normal subjects. Steroid sulfatase activity was measured by using tritium labeled steroid sulfate as the reaction substrate. It is demonstrated that human leukocytes contain a sulfatase activity for pregnenolone sulfate (P5-S), dehydroepiandrosterone sulfate (DHA-S) and estrone sulfate (E1-S) respectively. This enzyme has a greatest affinity for P5-S, but the activity for E1-S was the highest among the three substrates. The steroid sulfatase activity in female leukocytes is significantly stronger than that in normal males ($p < 0.001$) as determined by the cleavage of DHA-S. Sulfatase in leukocytes obtained from the PSD babies and RXLI patients had lower sensitivity. In the case of the mother affected with PSD, the activity was less than half of that in normal men ($p < 0.001$) and the levels did not overlap with that in normal women. In patients with IV, the activities were in the normal ranges for both males and females. The measurement of leukocyte sulfatase activity would be a clinically useful tool for the diagnosis of PSD carriers and pedigree analysis.

Key words: Steroid sulfatase, Leukocytes, Dehydroepiandrosterone sulfate, Placental sulfatase deficiency, Recessive X-linked ichthyosis. (Endocrinol Japon 38: 597-602, 1991)

STEROID SULFATASE hydrolyzing a number of 3β -hydroxysteroid sulfates has been demonstrated in placenta, liver, kidney, adrenal, ovary and fibroblast. Placenta is known to possess high sulfatase activity and is an important organ in the conversion of sulfated steroid precursors to estrogens during pregnancy. In the case of placental sulfatase deficiency (PSD), low placental sulfatase activity leads to low levels of urinary estrogens. Since the first description of PSD by France and Liggins in 1969 [1], more than one

hundred cases have been reported. All affected fetuses were males, and it is believed to be X-linked. An interesting relationship between PSD and recessive X-linked ichthyosis (RXLI), a chronic disorder of keratinization, has been disclosed by Shapiro *et al.* [2]. In reviewing cases with proved PSD, it was established that all the boys suffered from ichthyosis. Moreover cultured skin fibroblasts from patients with RXLI were deficient in steroid sulfatase activity. It is now well established that PSD and RXLI are single nosologic entities and are due to microsomal sulfatase deficiency. PSD (RXLI) occurs because the mother has no skin symptoms, but is a carrier of these disease. PSD shows complications such as a high rate of caesarean section in the delayed initiation of labor. For this reason, the prediction of carriers for PSD is

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clinically significant.

In this report, data on sulfatase activity in peripheral blood leukocytes isolated from women and the characterization of its enzyme are presented. In addition, sulfatase activities in PSD and ichthyosis patients were analysed and compared with those of normal subjects.

Materials and Methods

Materials

Blood samples were collected from both normal males and females, PSD patients and their children, and patients suffering from ichthyosis. Human leukocytes were obtained according to the methods of Epstein and Leventhal [3]. Peripheral blood samples were drawn into heparinized plastic syringes. Ten ml of blood was mixed with 2 ml of 5% dextran T-500 and 0.12 M NaCl in a plastic tube, and the erythrocytes were allowed to settle at 22°C for 45 min. The supernatant was removed, and leukocytes were harvested by centrifugation at 22°C at $400 \times g$ for 10 min. The pellet was suspended in 2.0 ml of 0.03 M NaCl to lyse residual erythrocytes, 1.6 ml of 0.31 M NaCl was added to restore isotonicity, and the leukocytes were collected by centrifugation at $400 \times g$ for 10 min. These pellets were kept at -80°C until the incubation were performed.

Leukocytes were homogenized in 1.5 ml of ice cold 0.014 M Tris buffer pH 8.0, using Polytron PT-10 with one or two 10-s bursts at maximal speed. Enzyme assays were performed on the homogenate. DNA was determined by the diphenylamine method of Burton [4] as modified by Giles and Meyers [5].

Isotope labeled steroids, dehydroepiandrosterone sulfate (DHA-S) ammonium salt [$7\text{-}^3\text{H}(\text{N})$] SA 851 GBq/mmol, pregnenolone sulfate (P5-S) ammonium salt [$7\text{-}^3\text{H}(\text{N})$] SA 925 GBq/mmol, estrone sulfate (E1-S) ammonium salt [6, $7\text{-}^3\text{H}(\text{N})$] SA 1.77 TBq/mmol and DHA-S ammonium salt [$4\text{-}^{14}\text{C}$] SA 2.13 GBq/mmol were purchased from New England and Nuclear Corp. For the enzymatic studies, radioactive steroids were purified by thin layer chromatography using a solvent system of ethylacetate: methanol: NH_4OH (75: 25: 2 vol/vol). Ascending chromatography was performed on thin layer plates of silica gel 60F254 (E. Merk, Darmstadt, Germany). Non-radioactive ster-

oids were of reagent grade and obtained commercially.

Quantitative determination of radioactivity was carried out in an Aloka LSC-3500 Liquid Scintillation Counter.

Measurement of steroid sulfatase activity

Enzyme activity was measured by desulfation of ^3H -DHA-S, as suggested first by Burstein and Dorfman [6]. The enzyme suspension (0.15 ml) was mixed with 10 nM DHA-S (0.10 ml) and incubated at 37°C for 1 h. At the end of incubation, the solution was chilled, 1.00 ml benzene was added, and the contents were mixed in a vortex mixer. The phases were separated within 1 min, and 0.50 ml of the upper benzene phase was removed and added to 10 ml of scintillation fluid for determination of the amount of ^3H -DHA extracted. Tritium labeled DHA-S was purified before use to remove ^3H -DHA formed nonenzymatically during storage. All incubations were performed in duplicate. Enzyme activity expressed as femtomoles of ^3H -DHA-S converted to enzyme-soluble ^3H -DHA per hour per microgram DNA. For the kinetic study ^3H -P5-S and ^3H -E1-S were also used for the substrates.

Statistics

The student's *t*-test was used and *p* values of 0.05 or less were considered as statistically significant.

Diagnosis of placental sulfatase deficiency and ichthyosis

Ten cases of placental sulfatase deficiency (PSD) were confirmed in a postnatal *in vitro* incubation study according to the method previously reported [7, 8]. Incubations of the $800 \times g$ supernatant of the placenta were carried out in a total volume of 2 ml Tris buffer with 0.05 μCi ^{14}C -DHA-S to give a final concentration of 10 nmol/flask. Sulfatase activity was expressed as the rate of hydrolysis of DHA-S during incubation. Characteristic absence and or an extremely low level of sulfatase activity was demonstrated in all cases.

Patients with ichthyosis were classified as ichthyosis vulgaris (IV) and recessive X-linked ichthyosis (RXLI) according to the classical diagnostic criteria reported by Wells & Jennings [9]: (1) family history of ichthyosis; (2) age of onset; (3) cutaneous manifestations (colour and size of scales, site of maximal involvement, and presence

of palmoplantar involvement); (4) ocular involvement and (5) histopathology. Each point was classified as IV-like, RXLI-like or uncertain.

Results

Determination of the activities of steroid sulfatase

Steroid sulfatase activity in leukocytes in women incubated with ^3H -DHA-S was demonstrated. Figure 1 shows the sulfatase activities in leukocytes as a function of time, and the DNA concentration. The rate of DHA-S hydrolysis by leukocytes had a linear relationship to incubation time up to 3 h and with a DNA concentration up to 150 μg per incubation tube. From Lineweaver-Burk analysis

the apparent K_m of the enzyme was calculated as 3.10 μM , with a maximum velocity of 0.75 nmol/mg DNA/h (Fig. 2).

To determine the substrate specificity of the enzyme, the Michaelis constant (K_m) of sulfatase for P5-S was also examined with substrate concentrations from 0.25 μM to 8 μM . The K_m value for the enzyme for P5-S was 2.14 μM and V_{max} was 1.10 nmol/mg DNA/h (Fig. 3).

K_m and V_{max} for E1-S were also studied and were found to be 9.74 μM and 11.22 nmol/mgDNA/h, respectively (Fig. 4).

Steroid sulfatase activity in clinical subjects

Leukocyte sulfatase activity in various samples from human subject was measured. Ten ml of the

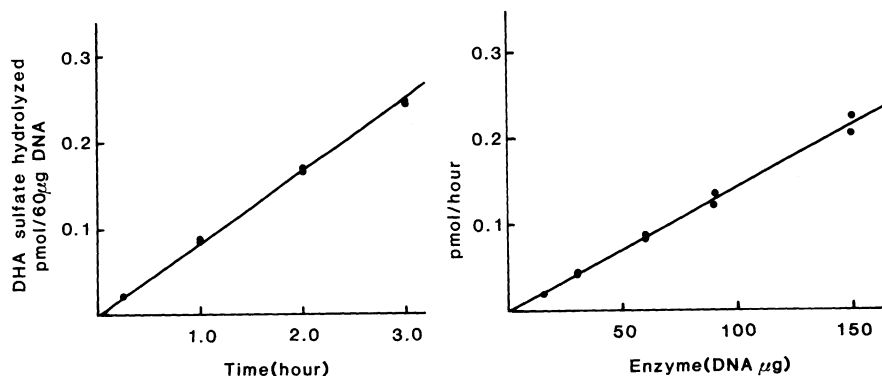


Fig. 1. Kinetics of sulfatase for dehydroepiandrosterone sulfate in leukocytes from normal females. In the left illustration, the incubation time was varied and 60 μg DNA was used in the experiment. In the right illustration, the amount of DNA was varied and incubations were continued for 1 h.

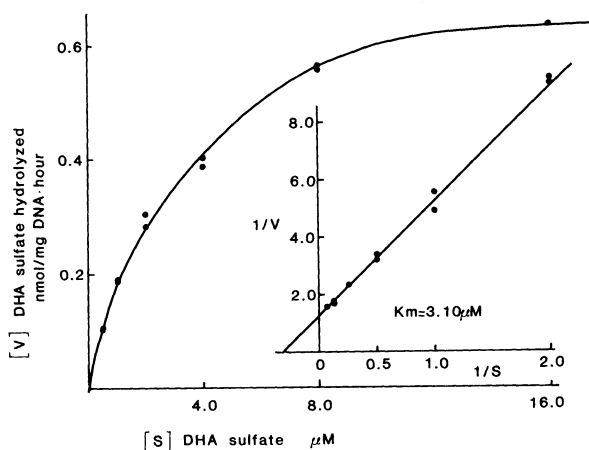


Fig. 2. The effect of the DHA sulfate concentration on sulfatase activity in leukocytes from normal females. 60 μg DNA was used and incubation was continued for 1 h. Inset: Lineweaver-Burk analysis of data.

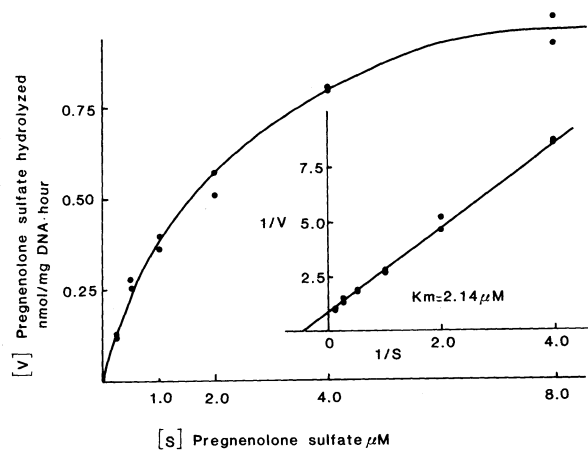


Fig. 3. The effect of the pregnenolone sulfate concentration on sulfatase activity in leukocytes from normal females. 60 μg DNA was used and incubation was continued for 1 h. Inset: Lineweaver-Burk analysis of data.

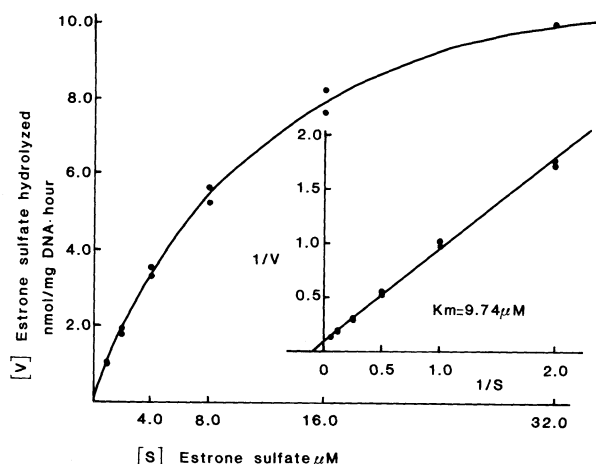


Fig. 4. The effect of the estrone sulfate concentration on sulfatase activity in leukocytes from normal females. 60 μ g DNA was used and incubation was continued for 1 h. Inset: Lineweaver-Burk analysis of data.

blood was used and prepared leukocytes were incubated for 1 h. The DHA-S sulfatase activity of peripheral leukocytes isolated from normal men and women, mothers with PSD and their male babies is shown in Fig. 5. Despite considerable inter individual variation, the mean DHA-S activity in leukocytes from normal women [1.05 ± 0.24 (mean \pm SD) $n=33$ fmol/ μ gDNA/h] was significantly higher than in those from normal men (0.75 ± 0.18 , $n=31$, $p<0.001$), and the activity in men was 72% of that in women.

In ten cases of PSD, sulfatase activities of

peripheral leukocytes obtained from the mother affected with PSD (0.34 ± 0.16 , $n=10$) were lower than half of those in normal men, and the difference was statistically significant ($p<0.001$). The activities in those mothers showed no overlapping levels with those in normal females. In nine cases' samples were obtained from the subjects during the period from one day to one year after delivery. In one case, blood was collected a day before delivery. In four babies at the age of six to twelve months who were born from PSD mothers, the activities of all cases indicated low sensitivity. One of the samples was collected from the cord blood at delivery.

In six male patients who were diagnosed as RXLI, enzyme activity was close to zero. On the other hand, patients suffering from IV had leukocyte sulfatase activity in the normal range.

Discussion

Epstein [3] has reported the K_m value for DHA-S of steroid sulfatase as 6–30 μ M using leukocytes. Then H-D Han [10] has demonstrated that the enzyme for E1-S is 13 μ M in normal female leukocytes. In the present study, additional information was obtained on leukocyte sulfatase activity for P5-S. Using three different substrates, it is demonstrated that human leukocytes contain a sulfatase activity for P5-S, DHA-S and E1-S,

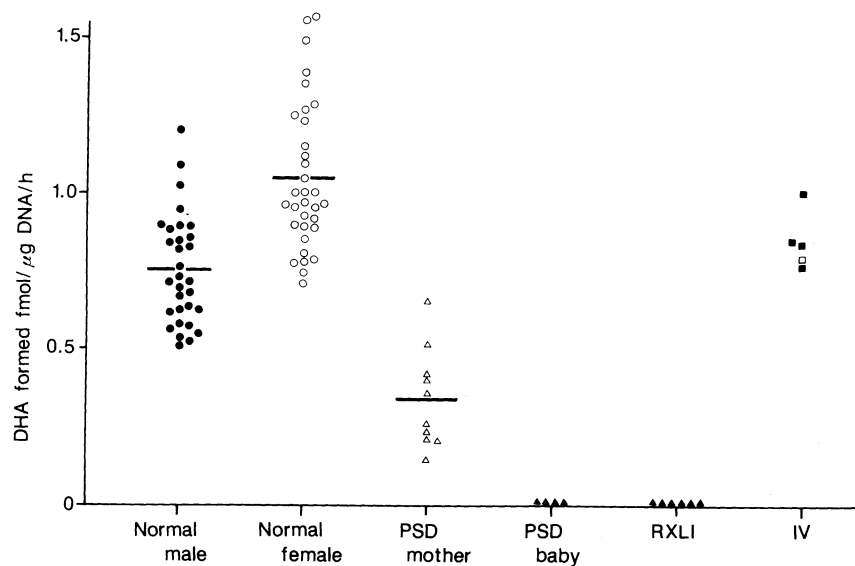


Fig. 5. Steroid sulfatase activity in human leukocytes. PSD, Placental sulfatase deficiency; RXLI, Recessive X-linked ichthyosis; IV, Ichthyosis vulgaris; Closed mark, Male; Open mark, Female. Mean values for normal males and females and PSD mother (carrier) are indicated by horizontal lines.

respectively. Judging from the K_m and V_{max} values obtained from the incubation study on human leukocytes, this enzyme has a greatest affinity for P5-S, but the activity for E1-S was the highest among the substrates used. The characterization of the enzyme showed the same pattern as that reported for human chorion and decidual tissue [11].

The difference between males and females in steroid sulfatase enzyme activities has been demonstrated in various human tissues such as the placenta [12], lymphocytes [3, 12–15] and cultured fibroblast [12, 14, 15] of normal individuals. The placental sulfatase activities in male and female infants were significantly different despite considerable interindividual variability. [12]. In the female leukocytes, the activities were reported to be 27% and 42% higher than that in male leukocytes with DHA-S as the substrate [3, 14]. In the present study, the mean value for the activity in females was 139% of that in males with considerable overlap between the two groups. These results are in good agreement with the hypothesis of a partial inactivation by X chromosome rather than nonactivation of the locus coding for steroid sulfatase, which has been located on the short arm of the X chromosome in Xp 22.3 [2]. A twofold increase in enzyme activity was not observed in females than in males, and the gene dosage effect is no linearly correlated with the number of X chromosomes as observed by Müller *et al* [14].

In the last decade, sixty two cases of placental sulfatase deficiency (PSD) were proven by *in vitro* placental enzyme assay in our laboratory (unpublished data). It was reported that there was only minimal hydrolysis by the affected placental tissue preparation of several sulfate substrates including DHA-S, P5-S and E1-S, demonstrating a generalized deficiency in 3β -yl-steroid sulfatase activity [16]. In PSD cases, in spite of this extremely diminished production of estrogens, both the course of pregnancy and fetal development seemed good except for skin symptoms in newborns. On the other hand, it has been mentioned that PSD adversely affects the normal outcome of delivery, especially in primiparas. In our previous

report [17], a high rate of caesarean sections was recorded because of failure to induce labor. Pregnancy was terminated by caesarean section in 42.9% of the cases reported. In the case of primiparas, this percentage was increased to 60%. Spontaneous labor was observed in only 31.4% of the cases. By obstetrical indications, therefore, the prediction of a carrier for PSD is important.

Our data agree with the findings reported by others [3, 13, 15] indicating that the measurement of leukocyte steroid sulfatase makes possible rapid differentiation of patients with RXLI from those with other types of ichthyosis. They also suggested that this method makes possible the identification of RXLI carriers diagnosed from the clinical appearance and pedigree analysis.

Further information was obtained in the present study indicating that the activities in leukocytes obtained from the confirmed PSD affected mothers were extremely lower than those in normal females. The values that did not overlap indicate that the method of measurement of leukocyte sulfatase activity is very useful in diagnosing PSD carriers. In the present study, steroid sulfatase activity in PSD mothers was measured with DHA-S as the substrate. As the K_m and V_{max} values for DHA-S, P5-S and E1-S in leukocyte from normal females were not equal, the activities in PSD carriers for P5-S and E1-S remain interesting questions which need to be studied in the future. The present data indicated that the leukocyte steroid sulfatase activity in the heterozygote female subjects is considerably less than that in the normal male suggesting that some mechanism other than gene inactivation must affect a compensatory reduction in enzyme activity in women.

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References

1. France JT, Liggins GC (1969) Placental sulfatase deficiency. *J Clin Endocr* 29: 138–141.
2. Shapiro LJ, Weiss R, Webster D, France JT (1978) X-linked ichthyosis due to steroid sulfatase deficiency. *Lancet* 14: 70–72.
3. Epstein EH, Leventhal ME (1981) Steroid sulfatase of human leukocytes and epidermis and the diagnosis of recessive X-linked ichthyosis. *J Clin Invest* 67: 1247–1262.
4. Burton K (1956) A study of the condition and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem J* 62: 315–323.
5. Giles KW, Myers A (1965) An improved diphenylamine method for the estimation of deoxyribonucleic acid. *Nature* 206: 93–93.
6. Burstein S, Dorfman RI (1963) Determination of mammalian steroid sulfatase with 7α - ^3H - 3β -hydroxyandrost-5-en-17-one sulfate. *J Biol Chem* 238: 1656–1660.
7. Nakayama T, Yanaihara T (1982) Placental sulfatase deficiency: Biochemical and clinical aspects. *Contr Gynec Obstet* 9: 145–156.
8. Hirato K, Yanaihara T (1990) Serum steroid hormone levels in neonates born from the mother with placental sulfatase deficiency. *Endocrinol Japon* 37: 731–739.
9. Wells RS, Jennings MC (1967) X-linked ichthyosis and ichthyosis vulgaris. Clinical and genetic distinctions in a second series of families. *J Am Med Assoc* 202: 405–488.
10. Han H-D, Fencle MD, Tulchinsky D (1987) Variation in estrone sulfatase activity in human leukocytes. *J Clin Endocrinol Metab* 65: 1026–1030.
11. Chibbar R, Mitchell BF (1990) Steroid sulfohydrolase in human chorion and decidua: Studies using pregnenolone sulfate and dehydroepiandrosterone sulfate as substrate. *J Clin Endocrinol Metab* 70: 1693–1701.
12. Shinohara M (1989) Study on the steroid sulfatase (STS) activity in normal individuals and patients with abnormal sexual differentiation. *Folia Endocrinol* 65: 161–173 (In Japanese).
13. Lykkesfeldt G, Lykkesfeldt AE, Shakkebek NE (1984) Steroid sulfatase in man: A non inactivated X-locus with partial gene dosage compensation. *Hum Genet* 65: 355–357.
14. Müller CR, Migl B, Traupe H, Ropers HH (1980) X-linked steroid sulfatase: Evidence for different gene-dosage in males and females. *Hum Genet* 54: 197–199.
15. Piraud M, Maire I, Zabot M-T (1989) X-linked recessive ichthyosis: Enzymatic diagnosis of affected males and female carriers. *Enzyme* 41: 227–234.
16. France JT, Seddon RJ, Liggins GC (1973) A study of a pregnancy with low estrogen production due to placental sulfatase deficiency. *J Clin Endocrinol Metab* 36: 1–9.
17. Yanaihara T, Hirato K, Nakayama T (1987) Placental sulfatase deficiency in Japan. *Acta Obst Gynaec Jpn* 39: 861–866 (In Japanese).