Pergamon

0960-0760(94)E0023-E

J. Steroid Biochem. Molec. Biol. Vol. 49, No. 2/3, pp. 195–201, 1994
Copyright © 1994 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0960-0760/94 $7.00 + 0.00

A "Soluble" Form of Sterol Sulphate Sulphohydrolase from Cell Nuclei of Human Placenta Tissue—Examinations with Oestrone Sulphate as Substrate

Jadwiga Gniot-Szulżycka* and Alicja Drywa

Mikołaj Kopernik University, Institute of Biology, Biochemistry Department, 87-100 Toruń, ul.Gagarina 7, Poland

DN-ase digestion of the nuclear envelope–chromatin complex of the cell nuclei preparations from human placenta, released a soluble form of sterolsulphohydrolase. The enzyme revealed three pH optima, at 4.0, 6.2 and 7.4. The K_m value was $4.16 \pm 1.44 \times 10^{-5}$ M. The molecular mass determined by gel filtration on Bio-gel A 15 m was 406 kDa. The enzyme is sensitive to -SH group reacting reagents such as cysteine, p-chloromercuribenzoate and iodoacetamide. Oxidized and reduced forms of NAD, FAD, dithiothreitol and glutathione moderately inhibited enzyme activity. Ascorbic acid (reduced and oxidized) exerted slight activation. The enzyme was insensitive to phosphate ions.

J. Steroid Biochem. Molec. Biol., Vol. 49, No. 2/3, pp. 195–201, 1994

INTRODUCTION

Steroid sulphates are present in human and animal plasma in relatively high concentrations and they serve as precursors in various synthetic processes [1–6]. Although direct pathways by which steroid sulphates are metabolized to other steroid sulphate esters are known, most conversion processes require the action of steroid sulphohydrolase(s).

The first experiments showing steroid sulphate hydrolysis were those of Gibian and Bratfisch [7] and Roy [8]. The steroid sulphohydrolase activity was further proved to be present in various animal and human tissues [9–12]. Human placenta is a very rich source of steroid sulphohydrolase activity [12–18].

The intracellular distribution of the enzyme activity, examined in various laboratories, indicated that steroid sulphatases are microsomal enzymes [13, 14, 17–20], however the enzyme activity was also detected in other cellular subfractions [21–29]. The data from our laboratory established the presence in human placenta tissue of steroid sulphohydrolase activity in microsomes, mitochondria, cell nuclei and nuclear envelopes [17, 20, 21, 27–29].

In this paper, some properties of the enzyme subfraction which was released from the nuclear envelope–chromatin complex by DN-ase are presented.

MATERIALS AND METHODS

Reagents

The reagents were purchased as follows: Triton X-100 and Tris were from Serva (Heidelberg, Germany); reduced and oxidized glutathione, p-chloro-mercuribenzoic acid sodium salt and deoxyribonuclease from bovine pancreas were from Schuchard (Munich, Germany); deoxyribose, oestrone sulphate and p-nitrophenol sulphate, thyreoglobulin and dithiothreitol were from Sigma (St Louis, MO, U.S.A.); Bio-gel A 15 m (100–200 mesh) was from Bio-Rad Labs (Richmond, CA, U.S.A.); β-glicerophosphate was from BDH (Poole, England); ascorbic acid was from Biomed (Kraków, Poland); cytochrome *c*, NAD, NADH, and FAD were from Reanal (Budapest, Hungary); and iodoacetamide was from Koch & Lights (Colnbruck-Bucks, England).

Preparation of cell nuclei

Highly purified nuclei were obtained from human term placenta shortly after delivery by the modified

*Correspondence to J. Gniot-Szulżycka.

Abbreviations: NPS, p-nitrophenyl sulphate; OS, oestrone sulphate, estra-1,3,5 (10)-triene-17-one-3β-yl sulphate; OS-ase, oestrone-sulphate-sulphohydrolase; PCMB, p-chloromercuribenzoate.

Received 13 Aug. 1993; accepted 27 Jan. 1994.

procedure of Philpott and Stanier [30] as described previously [27].

Subfractionation of the cell nuclei

The nuclear fraction was subjected to osmotic swelling, followed by mechanical shrinking, generally according to the Busch and Mauritzen procedure [31], modified as described by ourselves [27, 29]. The nuclei were swollen by resuspending the nuclear pellets in 0.14 M NaCl in 0.05 M Tris–HCl buffer pH 7.6 containing 1 mM $MgCl_2$ and afterwards they underwent a careful shearing in a Potter glass homogenizer. The sheared nuclei were centrifuged at 40,000*g* for 1 h, for the separation of the nuclear sap (fraction I, NS). The debris from the centrifugation was extracted with 2 M NaCl at pH 7.6, kept in an ice bath for 1 h and centrifuged at 16,000*g* for 20 min. This procedure separates most of the histone proteins in the supernatant (fraction II, Ch) and the chromatin–membrane complex in the pellet. The pellet of the chromatin–membrane complex was suspended in 50 mM Tris–HCl buffer pH 7.6 containing 1 mM $MgCl_2$ to a final protein concentration of about 5 mg per ml. Deoxyribonuclease was added (100 g per ml) and the digestion was performed for 16 h at 2°C, followed by centrifugation at 36,000*g* for 15 min to sediment the nuclear envelope–chromatin complex (fraction IV, NE). The supernatant with the deoxyribonuclease solubilized material was designed as fraction III (DN-ase digest). To get nuclear envelope fraction with low content of DNA, the DN-ase digestion procedure should be repeated twice. The flow sheet of the subfractionation procedure of the human placenta nuclei preparations is presented in Scheme 1.

Determination of protein and DNA content

Protein content was determined by the Besadown and Weinstein procedure [32] or by the method of Bradford with Comassie G-250 reagent [33]. DNA content was determined by the procedure of Dische as described by Schneider [34].

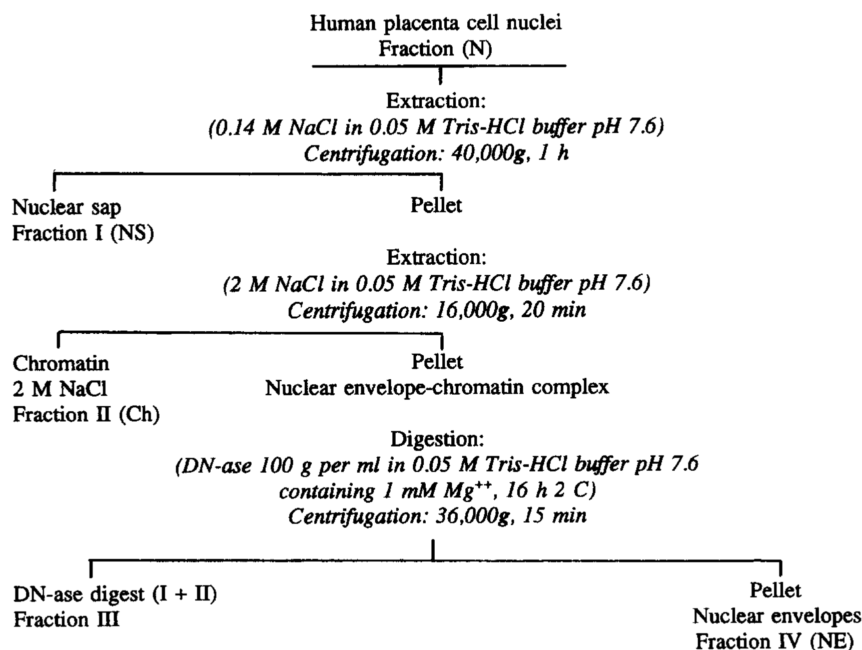
Enzyme activity determination

Arylsulphatase C was determined in the presence of 10 mM *p*-nitrophenol sulphate (NPS) in 0.25 mM Tris–HCl buffer pH 7.5. The reaction was terminated after appropriate time of incubation at 37°C by the addition of 3 ml of 5% NaOH. The released *p*-nitrophenol was measured at 405 nm.

Steroid sulphohydrolase activity was determined at 0.25 mM of oestrone sulphate concentration in 0.25 mM Tris–HCl buffer pH 7.5. The unhydrolysed substrate was measured by the procedure of Roy [35]. The influence of pH on the enzyme activity was tested with the use of acetate buffers at 125 mM concentrations at a pH range of 4.0–6.0 and Tris–HCl buffers at a pH range of 6.0–9.0.

The influence of substrate concentration on the activity was determined in different concentrations of oestrone sulphate in 25 mM Tris–HCl buffer at pH 4.0, 6.2 and 7.4.

The effects of -SH group reacting reagents and reducing and oxidizing substances on enzyme activity were as follows. The reagents under examination, at a concentration four times higher than the intended final one, were mixed with an equal volume of the enzyme preparation (fraction III, DN-ase digest) in 50 mM Tris–HCl buffer pH 7.4. After 10 min at 37°C this mixture was mixed with an equal volume



Scheme 1. Subfractionation of human placenta cell nuclei.

Table 1. The oestrone sulphate sulphohydrolase and arylsulphatase C activities of human placenta cell nuclei and nuclear subfractions

Fraction	Oestrone sulphate sulphohydrolase activity		Arylsulphatase C activity	
	Recovery %	Specific nmol × mg protein^{-1} × min^{-1}	Recovery %	Specific nmol × mg protein^{-1} × min^{-1}
Cell nuclei (N)	100	2.72 ± 0.89	100	4.25 ± 0.88
Nuclear sap (NS) (fraction I)	0	—	27 ± 13	2.41 ± 1.56
Chromatin .2 M NaCl (Ch) (fraction II)	0	—	10 ± 05	11.79 ± 9.43
DN-ase digest (fraction III)	20 ± 8.5	2.90 ± 0.34	37 ± 16	7.08 ± 3.76
Nuclear envelopes (NE) (fraction IV)	30 ± 5.5	7.21 ± 2.60	38 ± 12	16.49 ± 6.90

Data of the recovery calculations are from 5 separate separation procedures; those of specific activities are mean values estimated for 7–12 preparations.

of 0.25 mM oestrone sulphate in 250 mM Tris–HCl buffer pH 7.6. The samples were incubated for 1 h at 37°C and the remaining oestrone sulphate was determined as described above.

Bio-gel chromatography

The DN-ase released subfraction (7.8 mg of protein) was applied to a 2 × 110 cm column of Bio-gel A 15 m equilibrated with 50 mM Tris–HCl buffer pH 7.6 containing 0.1% Triton X-100. The same buffer was used for elution; 4 ml fractions were collected. Fractions 8–12 exhibiting oestrone sulphate sulphohydrolase and arylsulphatase C activities were combined, concentrated and dialysed against 50 mM Tris–HCl buffer pH 7.6 containing 0.05% Triton X-100. Protein content, OS-ase and arylsulphatase C activities were determined in each fraction.

RESULTS

The procedure of cell nuclei subfractionation was generally based on that elaborated by Busch and Mauritzen [31]. The flow sheet of the subfractionation of the cell nuclei preparations into nuclear sap, chromatin, DN-ase digest (fraction III) and nuclear envelopes is presented in Scheme 1.

The data of oestrone sulphatase and arylsulphatase C activities in the whole nuclei preparations, and nuclear subfractions are shown in Table 1. The results of a typical experiment using the subfractionation procedure are shown graphically in Fig. 1.

The molecular sewing on Bio-gel A 15 m of the DN-ase released material from the nuclear envelope–chromatin complex is presented in Fig. 2. The molecular weight value was calculated to be 406 kDa (Fig. 3).

The effect of pH on the enzyme activity, as well as the effect of phosphate ions and calcium ions on the pH optima are shown in Fig. 4. The influence of substrate concentration on the enzyme activity is presented in Fig. 5.

The effect of some -SH group reacting reagents and redox reagents are presented in Table 2.

DISCUSSION

The subfractionation of the human placenta cell nuclei according to the Busch and Mauritzen procedure [31], yielded 100% (100.91 ± 9.0%) protein recovery.

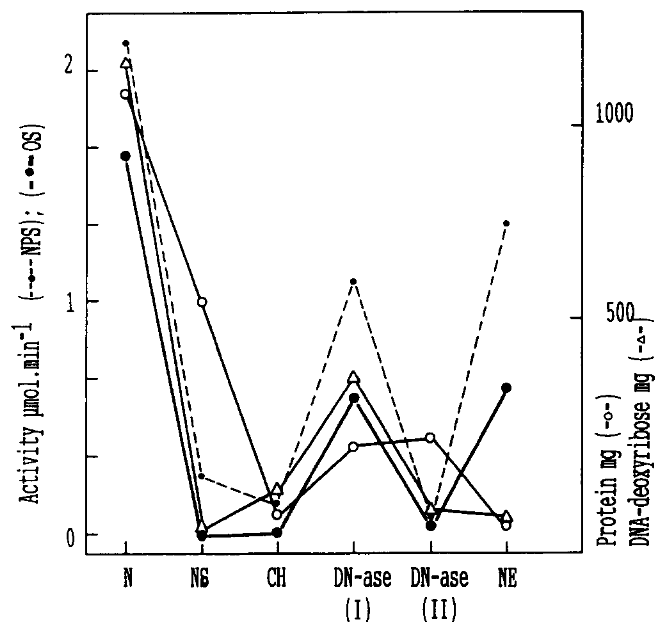


Fig. 1. Subfractionation of human placenta tissue cell nuclei. DNA content (—△—); protein content (—○—); OS-ase activity (—●—) and arylsulphatase C activity (--●--) in N, cell nuclei; NS, nuclear sap; Ch, chromatin fraction (2 M NaCl extract); DN-ase digest I; DN-ase digest II and NE, nuclear envelope fraction.

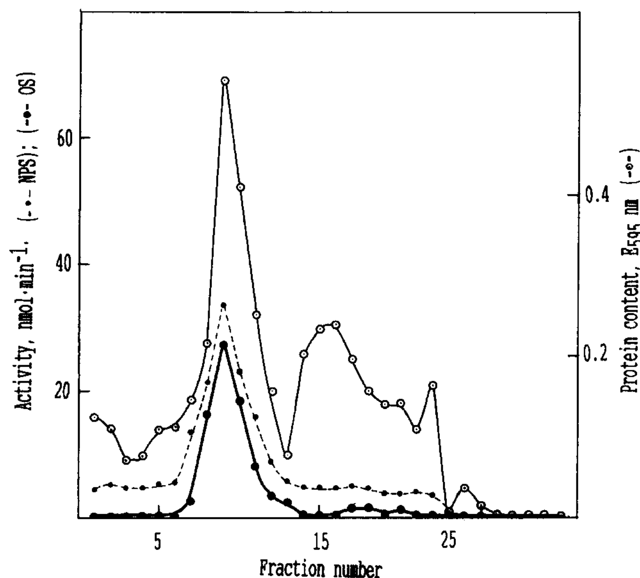


Fig. 2. Bio-gel A 1.5 m chromatography of the soluble form of OS-ase; 7.8 mg of protein was applied on a column (2 × 110 cm) equilibrated with 50 mM Tris–HCl buffer pH 7.6 containing 0.1% Triton X-100. The same buffer was used for elution; 4 ml fractions were collected. The enzymatic activity is expressed in nmol of NPS (--●--); OS (—●—) hydrolysed by 0.1 ml aliquots of the respective fractions after 1 h incubation at 37°C. The protein content is expressed (—○—) as E_{590nm}.

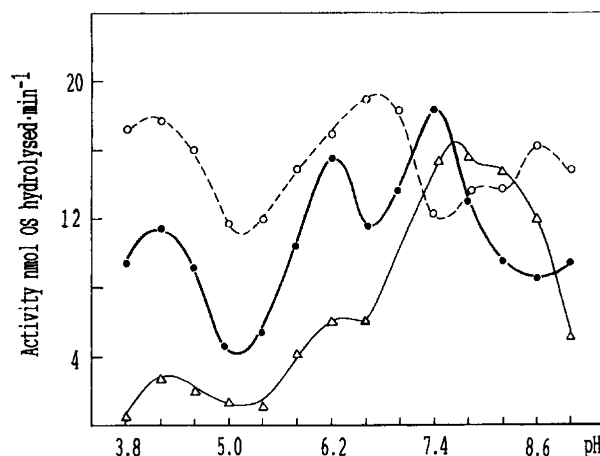


Fig. 4. The effect of pH on the activity of the soluble form of oestrone sulphate sulphohydrolase from cell nuclei of human placenta. The enzyme assays were carried out in the presence of 0.25 mM oestrone sulphate in 125 mM acetate buffer pH 4.0–6.0 and 125 mM Tris–HCl buffer pH 6.0–9.0. The incubation time was 1 h at 37°C. The activity is expressed in nmol of oestrone sulphate hydrolysed. The activity was tested with the DN-ase solubilized enzyme after extensive dialysis against water (—●—); the dialysed enzyme in the presence of 125 mm $CaCl_2$ (—△—); the dialysed enzyme in the presence of 125 mM PO_4 (—○—).

The mean value of DNA recovery was only 46.02%; however if the sum of the deoxyribose in the TCA soluble (nucleotides) and TCA insoluble (high molecular form of DNA) fractions were taken into account, the total recovery of DNA-deoxyribose was 90% (data not presented in this paper).

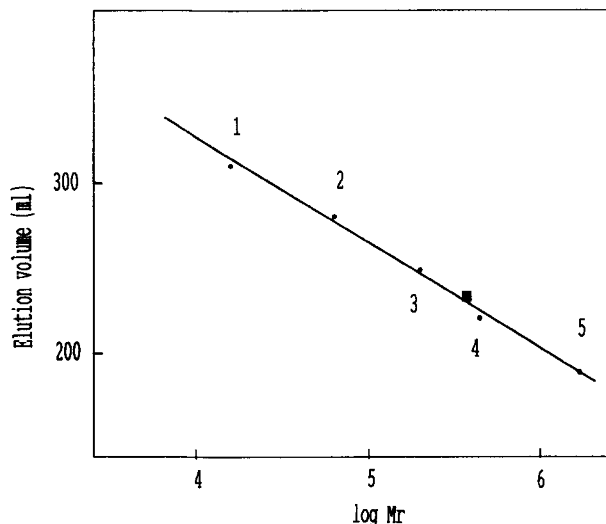


Fig. 3. Molecular weight estimation of the soluble form of oestrone sulphate sulphohydrolase from cell nuclei of human placenta. Bio-gel A 15 m column (2 × 110 cm) equilibrated with 50 mM Tris–HCl buffer pH 7.6 containing 0.1% Triton X-100 was used. Elution was effected with the same buffer; 4 ml fractions were collected. The following proteins were used as molecular weight standards: 1, cytochrome *c*; 2, ovoalbumin; 3, albumin; 4, ferritin; 5, thyreoglobulin; (—■—), oestrone sulphate sulphohydrolase activity.

The subfractionation of the human placenta cell nuclei effected redistribution of arylsulphatase C and oestrone sulphate sulphohydrolase activities. The arylsulphatase C, determined with *p*-nitrophenol sulphate as substrate (NPS) appeared to be present in the nuclear sap (NS, fraction I), chromatin fraction (Ch, fraction II), DN-ase digest (fraction III) and nuclear envelope fraction (NE, fraction IV). The overall recovery of the enzyme activity was 130% (Table 1, Fig. 1).

The oestrone sulphate sulphohydrolase activity was detected only in the DN-ase digest (fraction III), as a "soluble form" and in the nuclear envelope fraction (NE, fraction IV) as a "particulate form". The oestrone sulphate sulphohydrolase recovery varied from 13 to 80% (mean value 43%). The reason for the variability is unclear at present.

Some preliminary data concerning the "particulate form" of steroid sulphohydrolase of the nuclear envelope fraction were described in our previous papers [27, 29].

In this paper some properties of the "soluble form" of steroid sulphohydrolase released from the nuclear envelope–chromatin complex by DN-ase digestion are presented.

The molecular weight value calculated for the most active fraction, which amounted to 90% of the enzymatic activity applied on the Bio-gel A 15 m column, was calculated to be 406 kDa (Figs 2 and 3).

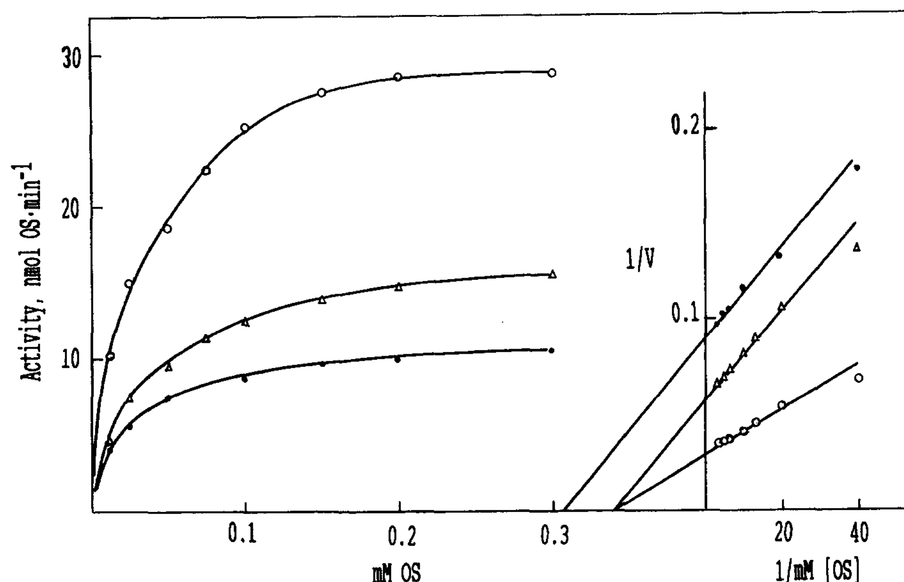


Fig. 5. Influence of oestrone sulphate concentration on the soluble form of oestrone sulphate sulphohydrolase from cell nuclei of human placenta. The assay was carried out in 125 mM acetate buffer at pH 4.2 (——) and 125 mM Tris–HCl buffers at pH 6.2 (—△—) and at pH 7.4 (—○—).

This value appeared to be very similar to the value of 390 kDa established by Dibbelt and Kuss [37] for human placental steroid sulphohydrolase solubilized with cholic acid, as well as to the tetrameric form of arylsulphatase A [38]. The molecular weight values of 260–280, 130, 79, 62 and 58 kDa were established for the particulate forms of steroid sulphohydrolase solubilized with Triton X-100 [39–50]. According to Noel *et al.* [39] the 78.5 kDa monomer polymerized in the absence of Triton X-100. The high molecular mass variants of steroid sulphohydrolase (600–1000 kDa) were reported to be present in rat liver microsomal preparations [40, 41, 42, 17].

Table 2. Effect of compounds reacting with -SH groups and oxidizing and reducing substances on the activity of oestrone sulphate sulphohydrolase "soluble" form, released by DN-ase from the chromatin–nuclear envelope complex

Substance tested	Final concentration in in the incubation (mM)	Activity % of the control
Control	—	100
PCMB	10	44.07 ± 19.45
Iodoacetamide	10	37.96 ± 25.16
Cysteine	10	53.84 ± 0.71
$AgNO_3$	10	25.76 ± 22.55
Ascorbic acid (reduced)	10	109.81 ± 12.34
Ascorbic acid (oxidized)	10	113.02 ± 5.02
$NADH + H^+$	5	94.66 ± 4.76
NAD^+	5	84.31 ± 0.73
FAD^+	5	64.34 ± 13.06
Glutathione (reduced)	10	86.62 ± 15.02
Glutathione (oxidized)	10	86.83 ± 22.86
Dithiothreitol (reduced)	10	84.31 ± 1.46
Dithiothreitol (oxidized)	10	85.09 ± 0.17
β-Mercaptoethanol	10	103.25 ± 5.75

The assay conditions are described under Materials and Methods. The data are mean values for 3–4 series of experiments.

On the other hand the enzyme revealing low molecular weight (26 and 36 kDa) with sterol sulphohydrolase activity, appeared to be present in human placenta microsomes [17], rat liver [43], human testes [44] and human brain [45]; this means that heteropolimeric forms as well as multienzyme complexes of the enzyme may exist.

The molecular mass for the particulate form(s) of enzyme(s) associated with nuclear envelopes were 67 and 130 kDa [53].

The DN-ase released subfraction of oestrone sulphate sulphohydrolase revealed three distinct pH optima. The first one at pH 4.0, the second one at pH 6.2 and the third one at pH 7.4 (Fig. 4). Phosphate ions activated the enzyme at pH 4.0 and pH 6.2, and exerted an inhibitory effect at a pH range of 7.4–8.4 (Fig. 4). In addition to these effects, in the presence of phosphate ions a new activity peak at pH 8.0 arose (Fig. 4). Calcium ions resulted in a pronounced inhibition of the enzyme activity at pH 4.0 to 6.0 (Fig. 4). At pH 8.0, calcium ions exerted an activating effect (Fig. 4).

According to the data reported in literature pH optima for the hydrolysis of steroid sulphate esters with the enzyme preparations from different sources ranged from 6.0 to 8.0 [12, 14, 18–20, 22, 26, 35, 51]. Optimal pH and acidic pH for microsomal sterol sulphohydrolases were reported by Noel *et al.* [19].

The "particulate form" of the enzyme present in the nuclear envelopes exhibit optimal activity at pH 6.6 and 8.6 [53].

The question at present is whether the different pH optima point to the presence of different enzymes or exemplify differences in the rearrangement of the enzyme subunit and regulatory components of the multienzyme complex which participates in sequential reactions of steroids interconversion processes.

The allosteric properties of sterol sulphohydrolases were reported for cholesterol sulphate sulphohydrolase [19], androsterone sulphate sulphohydrolase [44], and oestrone sulphate sulphohydrolase [20, 53].

The allosteric character of the enzyme allowed us to expect a meaningful role of this group of enzymes in the metabolism of steroid hormones.

The influence of substrate concentration on the enzyme activity is presented in Fig. 5. The initial velocity of hydrolysis of oestrone sulphate, determined with the DN-ase released enzyme, at pH 4.0, 6.2 and 7.4 increases with the substrate concentration as should be expected from the Michaelis–Menten theory. The K_m value of the cleavage of oestrone sulphate was in the range of 10^{-5} M ($4.16 \pm 1.44 \times 10^{-5}$ M). This K_m value is in good agreement with the data reported in the literature for the enzymes of microsomal origin [20, 24, 37, 51, 52].

The influence of substrate concentration on the enzyme activity of the "particulate form" of sterol sulphohydrolase appeared to be complex; when determined at pH 6.2 it exhibits sigmoid saturation kinetics, at pH 7.0 or 8.6 it exhibits a nonsigmoidal hyperbolic, Michaelis–Menten kinetics [53].

The "soluble form" of OS-ase from the nuclear–chromatin complex, in contrast to the "particulate form" of the enzyme, which was extracted with Triton X-100 from the nuclear envelopes [29], appeared to be sensitive to the -SH group reacting reagents. The inhibition effected by cysteine, PCMB, $AgNO_3$ and iodoacetamide was respectively: 30, 45, 26 and 55% (Table 2).

Among the redox reagents tested (Table 2), ascorbic acid and β-mercaptoethanol exerted no effect or only slightly activated the enzyme (both in oxidized or reduced forms). The second group of the redox substances tested (NAD, FAD, dithiothreitol and glutathione), exerted an inhibitory effect (both in oxidized and reduced forms) it is worth stressing that the "particulate form" of OS-ase which is associated with the nuclear envelope fraction was inhibited by oxidized forms and activated by reduced forms of the respective redox substances.

The data presented in this paper led us to assume that the "soluble form" of sterolsulphate sulphohydrolase, tested with oestrone sulphate as a substrate, from human placenta cell nuclei and the "particulate form", which is associated with the nuclear envelope fraction are distinct enzymes or enzyme complexes.

Further study is required for characterization of the steroid sulphate hydrolysing enzymes and their function in different compartments of the cell nuclei.

The biochemical functions of both forms of steroid sulphate hydrolysing enzymes, tested here with oestrone sulphate as substrate, are unknown at present.

REFERENCES

1. Siiteri P. K. and MacDonald P. C.: The utilization of circulating dehydroisoandrosterone sulfate for estrogen synthesis during human pregnancy. *Steroids* 2 (1963) 713–730.
2. Jenkin G. and Heap R. B.: Formation of oestradiol-17β from oestrone sulfate by sheep foetal pituitary *in vitro*. *Nature* **259** (1976) 330–331.
3. Rosenthal H. E., Pietrzak E., Slaunwhite W. R. Jr and Sandberg A. A.: Binding of oestrone sulfate in human plasma. *J. Clin. Endocr. Metab.* **34** (1972) 805–813.
4. Schut H. A. J., Pepe G. J., Chez R. A. and Townsley J. D.: Contribution of dehydroepiandrosterone and its sulfate to estriol in baboon pregnancy. *Am. J. Physiol.* **235** (1978) 78–81.
5. Huhtaniemi I. and Vihko R.: Determination of unconjugated and sulfated neutral steroid in human fetal blood of early and mid pregnancy. *Steroids* **16** (1970) 197–200.
6. Archer D. F., Mathur R. S., Wiquvist N. and Diczfalusy: Quantitative assessment of the *de novo* sterol and steroid synthesis in the human foeto-placental unit. 2. Synthesis and secretion of steroids and steroid sulphates by the midgestation foetus. *Acta Endocr.* **66** (1971) 666–678.
7. Gibian H. and Bratfisch G.: Eine steroid sulfatase aus Saugertierleber. *Hoppe-Seyler's Z. Physiol. Chem.* **305** (1956) 265–268.
8. Roy A. B.: The sulphatase of ox liver. 6. Steroid sulphatases. *Biochem. J.* **66** (1957) 700–703.
9. Dolly J. O., Curtis C. G., Dodgson K. S. and Rose F. A.: Metabolism of sodium oestrone/^{35}S/sulphate in the guinea pig. *Biochem. J.* **128** (1972) 347–352.
10. Milewich I., Garcia R. L. and Gerrity L. W.: Steroid sulfatase and 17β-hydroxy steroid oxidoreductase activities in mouse tissues. *J. Steroid Biochem.* **1** (1984) 529–538.
11. Munroe D. G. and Chang P. L.: Tissue specific expression of human aryl sulphatase C isozymes and steroid sulfatase. *Am. J. Human Genet.* **40** (1987) 102–114.
12. Pulkkinen M. O.: Arylsulphatase C and hydrolysis of some steroid sulphate in developing organs and placenta. *Acta Physiol. Scand.* **52** (1961) 1–181.
13. Burnstein S.: Studies on solubilization and purification of rat liver microsomal steroid sulfatase. *Biochim. Biophys. Acta* **146** (1967) 529–534.
14. French A. D. and Warren J. C.: Properties of steroid sulphatase and arylsulphatase activities of human placenta. *Biochem. J.* **105** (1967) 233–241.
15. Gauthier R., Vigneault N., Bleau G., Chapdelaine A. and Roberts K. D.: Solubilization and partial purification of steroid sulphatase of human placenta. *Steroids* **31** (1978) 783–798.
16. France J. T. and Liggins G. C.: Placental sulfatase deficiency. *J. Clin. Endocr. Metab.* **29** (1969) 138–141.
17. Gniot-Szulżycka J.: Arylsulphatase C and sterol sulphatase activities in microsomes from human placenta. *Acta Biochim. Polon.* **29** (1982) 205–212.
18. Dibbelt L. and Kuss E.: Placental steroid sulfatase. *Hoppe-Seyler's Z. Physiol. Chem.* **364** (1983) 187–191.
19. Noel H., Bleau P. G., Chapdelaine A. and Roberts K. D.: Human placental steroid sulfatase: purification and properties. *J. Steroid Biochem.* **19** (1983) 1591–1598.
20. Gniot-Szulżycka J. and Januszewska B.: Purification of steroid sulphohydrolase from human placenta microsomes. *Acta Biochim. Polon.* **33** (1986) 203–215.
21. Gniot-Szulżycka J. and Jakubowska A.: Oestrone sulphate sulphohydrolase in nuclear envelopes from human placenta cell nuclei. *Acta Biochim. Polon.* **38** (1991) 7–16.
22. Kishimoto Y. and Sostek R.: Activity of sterol sulphate sulphohydrolase in rat brain, characterization and change with age. *J. Neurochem.* **19** (1972) 123–130.
23. Dodgson K. S., Spencer B. and Thomas J.: Studies on sulphatases: 6. The localization of arylsulphatases in the liver cell. *Biochem. J.* **56** (1954) 177–181.
24. Dolly J. O., Dodgson K. S. and Rose F. A.: Studies on the oestrone sulphatase and arylsulphatase C activities of rat liver. *Biochem. J.* **128** (1972) 337–345.

25. Perumal A. S. and Robins R.: Regional and subcellular distribution of aryl and steroid sulphatases in rat brain. *Brain Res.* 59 (1973) 349–358.
26. Balasubramanian A. S.: Arylsulphatase C of sheep brain and its relationship to oestrone sulphate and dehydroepiandrosterone sulphatase activities. *Indian J. Biochem. Biophys.* 13 (1976) 325–330.
27. Gniot-Szulżycka J. and Jakubowska A.: Oestrone sulphate sulphohydrolase and arylsulphatase C activities of human placenta cell nuclei and nuclear subfractions. *Acta Univ. Lodz., Folia Biochim. Biophys.* 6 (1988) 39–54.
28. Gniot-Szulżycka J. and Wasilewska W.: Arylsulphatase C and sterol-sulphatase activities of the mitochondria fraction from human placenta. *Acta Univ. N. Copernici* 68 (1988) 35–46.
29. Gniot-Szulżycka J. and Jakubowska A.: Oestrone sulphate sulphohydrolase activity in nuclear envelopes from human placenta cell nuclei. *Acta Biochim. Polon.* 37 (1991) 1–22.
30. Philpot J.-St. L. and Stanier J. B.: The choice of the suspension medium for rat liver cell nuclei. *Biochem. J.* 63 (1956) 214–223.
31. Busch H. and Mauritzen Ch. M.: Nuclear proteins. In *Methods in Cancer Research* (Edited by H. Bush). Academic Press, New York, Vol. 3 (1976) p. 391.
32. Bensadown A. and Weinstein D.: Assay of proteins in the presence of interfering material. *Analyt. Biochem.* 72 (1976) 248–254.
33. Bradford M. M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein binding. *Analyt. Biochem.* 72 (1976) 248–254.
34. Schneider W. C.: Determination of nucleic acids in tissue by pentose analysis. In *Methods of Enzymology* (Edited by S. P. Colowick and N. O. Kaplan). Academic Press, New York, Vol. 3 (1957) pp. 680–684.
35. Roy A. B.: The enzymic synthesis of steroid sulphates. *Biochem. J.* 63 (1956) 294–300.
36. Porath J. and Flodin P.: Gel filtration: A new method for desalting and group separation. *Nature* 183 (1959) 1657–1659.
37. Kawano J., Katani T., Ohtaki S., Minamino N., Matsuo H., Oimuma T. and Aikawa E.: Characterization of rat and human steroid sulfatase. *Biochim. Biophys. Acta* 997 (1989) 199–205.
38. Roy A. B. and Jerfy A.: Sulphatases of ox liver. XIV. The subunit structure of arylsulphatase A. *Biochim. Biophys. Acta* 207 (1970) 156–163.
39. Noel H., Beauregard G., Potier M., Bleau G., Chapdelaine A. and Roberts K. D.: The targed size of membrane bound and Triton X-100 solubilized forms of human placenta steroid sulfatase as measured by radiation inactivation. *Biochim. Biophys. Acta* 758 (1983) 88–90.
40. Burnstein S.: Studies on the solubilization and purification of rat liver microsomal steroid sulfatase. *Biochim. Biophys. Acta* 146 (1967) 529–534.
41. Iwamori M., Moser H. W. and Kishimoto Y.: Solubilization and partial purification of steroid sulfatase from rat liver: characterization of estrone sulfatase. *Biochim. Biophys. Acta* 174 (1967) 199–208.
42. McNaugh R. W. and France J. T.: Studies on the biochemical basis of steroid 3-sulfatase activity. *Endocrinology* 93 (1973) 172–181.
43. Bleau G., Chapdelaine A. and Roberts K. D.: Studies on mammalian and molluscan steroid sulfatase. Solubilization and properties. *Can. J. Biochem.* 49 (1971) 234–242.
44. Payne A. H.: Gonadal steroid sulfates and sulfatase: partial characterization and possible regulation by free steroids. *Biochim. Biophys. Acta* 258 (1972) 443–483.
45. Perumal A. S. and Robins E.: Arylsulphatases in human brain. Purification and characterization of an insoluble arylsulphatases. *J. Neurochem.* 21 (1973) 459–471.
46. Moriyasu M., Ito A. and Omura T.: Purification and properties of arylsulphatase C from rat microsomes. *J. Biochem. (Tokyo)* 92 (1982) 1189–1195.
47. Kawano J., Kotani T., Ohtaki S., Minamino N., Matsuo H., Oimuma T. and Aikawa E.: Characterization of rat and human steroid sulfatase. *Biochim. Biophys. Acta* 997 (1989) 199–205.
48. Van der Loos C. M., Van Breda A. J., Van den Berg F. M., Walboomes J. M. M. and Jbsis A. C.: Human placenta steroid sulphatase-purification and monospecific antibody production in rabbits. *J. Inhert. Metab. Dis.* 7 (1984) 97–103.
49. Vaccaro A. M., Salvioli R., Muscillo M. and Renola L.: Purification and properties of arylsulphatase C from human placenta. *Enzyme* 37 (1987) 115–126.
50. Gauthier R., Vigneault N., Bleau G., Chapdelaine A. and Roberts K. D.: Solubilization and partial purification of steroid sulfatase of human placenta. *Steroids* 31 (1978) 783–798.
51. Zuckerman N. G. and Hagerman D. D.: The hydrolysis of estrone sulfate by rat kidney microsomal sulfatase. *Archs Biochem. Biophys.* 135 (1969) 410–415.
52. Iwamori M., Moser H. W. and Kishimoto Y.: Solubilization and partial purification of steroid sulphatase from rat liver. Characterization of estrone sulphatase. *Archs Biochem. Biophys.* 174 (1976) 159–208.
53. Gniot-Szulżycka J. and Wojczuk B.: Subunits of sterol sulphate sulphohydrolase from human placenta microsomes. *J. Steroid Biochem. Molec. Biol.* 41 (1992) 141–143.
53. Gniot-Szulżycka J. and Jakubowska A.: Studies on oestrone sulphate sulphohydrolase from human placenta nuclear envelopes—solubilization and properties. *Folia Histochem. Cytobiol.* 31 (1993) 221–226.