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Cholesterol sulphate sulphohydrolase of human placenta lysosomal membrane

Katarzyna Roszek *, Jadwiga Gniot-Szulżycka

Nicolaus Copernicus University, Institute of General and Molecular Biology, Biochemistry Department, ul. Gagarina 7, 87-100 Toruń, Poland

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Abstract

In this paper we report that the activity of cholesterol sulphate sulphohydrolase (CHS-ase) is associated with the lysosomal membranes. The procedure of purification of CHS-ase from human placenta lysosomes was elaborated. The purified enzyme is highly specific to cholesterol sulphate (specific activity 2126.60 ± 940.90 nmol min^{-1} mg protein^{-1}) and acts optimally at pH 3.4. The K_M value for the hydrolysis of cholesterol sulphate is $3.6 \pm 0.95 \times 10^{-5}$ mol/l. The isoelectric point (p*I*) has the value 5.7, molecular weight estimated by SDS-PAGE electrophoresis is 38 kDa. The described enzyme may be involved in a regulation of cholesterol and cholesterol sulphate levels in the lysosomal membrane.

Keywords: Steroid sulphatase; Cholesterol sulphate sulphohydrolase; Lysosomal membrane

1. Introduction

Steroid sulphohydrolase (STS), EC 3.1.6.2, is ubiquitously expressed in numerous animal and human tissues. Human placenta's tissue is the richest source of steroid sulphohydrolase [1].

There is only one gene coding STS in human, located on the distal short-arm of the X-chromosome [2]. Nevertheless, it is evident that isoenzymes of steroid sulphohydrolase with more selective or absolute substrate preference exist [3–9]. Alternative expression of distinct exons of the gene or diverse posttranslational modifications may explain the existence of distinct forms of STS with different biological activity (substrate specificity) and subcellular localization.

Biochemical and immunocytochemical methods allowed to determine the subcellular localization of STS. This enzyme is primarily localized in microsomal membranes [10–14]. Relatively high activity of STS was also described in mitochondria [15], cell nuclei [16], endosomes and lysosomes [2,17]. Although microsomal steroid sulphohydrolase from various tissues including human placenta, was extensively studied, there is little information about the presence and properties of steroid sulphohydrolase in lysosomes.

Lysosomes are responsible for hydrolysing the molecules destinated for degradation owing to the fact that they contain almost 50 different hydrolases [18–20]. Recently, numerous publications appeared concerning the novel functions of well-known organelles: in chaperone-mediated autophagy [21], repairing the cell membrane [22], in the immune response [23] and aging [24].

Lysosomes actively participate in circulation and distribution of cholesterol inside the cell [25–27]. Free cholesterol liberated from its esters inside the lysosome, becomes incorporated in the lysosomal membrane and thereof reaches the proper subcellular compartment [25,28]. However, the participation of lysosomes in the metabolic pathways of cholesterol sulphate is still obscure.

Cholesterol sulphate sulphohydrolase (CHS-ase) cleaves the cholesterol sulphate to free cholesterol and sulphate ion. Up to now, there are no data demonstrating the lysosomal localization and properties of the CHS-ase. Therefore, the aim of this work became the purification and examining the molecular and kinetic properties of lysosomal CHS-ase.

Abbreviations: AS, androsterone sulphate, 5α-androstan-3α-ol-17-one sulphate; CHS, cholesterol sulphate, 5-cholesten-3β-ol sulphate; CHS-ase, cholesterol sulphate sulphohydrolase; *p*-CMB, *p*-chloromercuribenzoate; DHEAS, dehydroepiandrosterone sulphate, 5-androsten-3β-ol-17-one sulphate; NCS, 4-nitrocatechol sulphate; OS, oestrone sulphate, 1,3,5(10)-estratrien-17-one sulphate; PS, pregnenolone sulphate, 5-pregnen-3β-ol-20-one sulphate; STS, steroid sulphohydrolase, steroid sulphatase.

* Corresponding author. Fax: +48 56 6114772.
E-mail address: kroszek@biol.uni.torun.pl (K. Roszek).

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2. Materials and methods

2.1. Reagents

We used the following reagents: acetonitrile, acrylamide, androsterone sulphate (sodium salt), bovine albumin, cholesterol, cholesterol sulphate (sodium salt), dehydroepiandrosterone sulphate (sodium salt), DEAE cellulose, *N*,*N*′-methylenebisacrylamide, oestrone sulphate (sodium salt), ovoalbumin, percoll, pregnenolone, pregnenolone sulphate (sodium salt), Sigma IEF Mix (3.6–9.3), Sigma Marker (6500–205,000 Da), sucrose, sodium dodecyl sulphate (SDS), trichloroacetic acid (TCA) and tris(hydroksymethyl)aminomethane from Sigma Chemical Co. (USA); glutathione oxidized, glutathione reduced (Fluka Chemie GmbH, Switzerland); Servalyt 2-11 (Serva, Germany); β-mercaptoethanol, Triton X-100 (Windsor Laboratories Ltd., Great Britain); sodium taurocholate (BDH Chemicals Ltd., Great Britain); dehydroepiandrosterone, progesterone (Koch-Light Laboratories Ltd., Great Britain); dithiotreitol (Calbiochem, USA); methanol HPLC grade (J.T. Baker, Holland); ammonium molybdate, copper(II) chloride, glycine, hydrochloric acid, phosphoric acid, potassium phosphate monobasic, salicylic acid, silver nitrate, sodium chloride, sodium hydroxide, sodium sulphate, sodium sulphite, tartaric acid and vanadium (V) oxide (PPH POCh, Poland).

2.2. Enzyme material

The human placenta lysosomes were prepared as described previously [29]. The lysosomal membranes were isolated from human placenta lysosomes according to Diettrich et al. [30].

The enzymes were solubilized from the crude lysosomal fraction with 2% Triton X-100 and the resulting extract was used in further experiments.

2.3. Filtration

The extract was filtered three times through the Amicon Centriprep YM-30 filter (Milipore, Canada). The resulting filtrate was collected and then applied on DEAE-cellulose column.

2.4. DEAE-cellulose chromatography

Approximately 1 mg of protein from the previous step was applied to the DEAE-cellulose column (Ø1.5 cm × 9 cm) equilibrated with 50 mM Tris–HCl buffer pH 7.6. The proteins were eluted using following solutions: 50 mM Tris–HCl buffer pH 7.6, then 0.15 M NaCl, 0.3 M NaCl and 1.0 M NaCl in 50 mM Tris–HCl buffer pH 7.6. Finally column was washed with 0.5% Triton X-100 in the above buffer. Two milliliters fractions were collected.

Fractions 23–26 eluted with 0.3 M NaCl were combined (approximately 0.08 mg of protein), dialysed against 50 mM Tris–HCl buffer pH 7.6 and concentrated. This enzyme preparation was stored at −20 °C and used in further experiments.

2.5. Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis was performed according to Ogita and Markert [31]. Gel slabs (8 cm × 6 cm × 0.75 mm) contained 4% acrylamide in stacking gel and 10% acrylamide in running gel. The separations in non-denaturing conditions were performed by 1.5 h under voltage 150 V (about 20 mA) in 12.5 mM Tris–glycine buffer pH 8.3.

The separations in denaturing conditions were performed by 2 h under voltage 150 V (about 60 mA) in 25 mM Tris–glycine buffer pH 8.3 containing 0.1% SDS. The Sigma Marker (6500–205,000 Da) was used as the molecular weight standard.

The protein bands after separation were stained with silver according to the method of McVeigh et al. [32].

2.6. Isoelectric focusing

Isoelectric focusing was performed according to Bollag and Edelstein [33]. Gel slabs (8 cm × 6 cm × 0.75 mm) contained 7% acrylamide and 1.6% Servalyt (pH range 2–11). The Sigma IEF Mix (3.6–9.3) was used as isoelectric point (p*I*) marker. During separation we used 20 mM sodium hydroxide as catholyte and 10 mM phosphoric acid as anolyte. The separation was performed by 1.5 h under constant voltage 200 V and then for 2 h under 400 V. After that, gels were placed for 30 min in 10% TCA and soaked overnight in 1% TCA in order to remove ampholytes. The separated proteins were stained with silver according to the method of McVeigh et al. [32].

2.7. Enzyme activity determination

The assay of steroid sulphohydrolase activity was performed by the procedure described by Roy [34]. CHS, OS, DHEAS, PS and AS at final concentration of 0.2 mM in 250 mM acetate (pH 3.4) or Tris–HCl (pH 7.6 and 9.4) buffer were used as substrates for steroid sulphohydrolases.

The CHS-ase activity determination as a function of pH was performed in 250 mM buffers of pH 3.0–5.8 and 250 mM Tris–HCl buffers of pH 6.0–10.0.

The effect of substrate concentration on the rate of hydrolysis of cholesterol sulphate was tested within the range of 0.01–0.15 mM concentration of substrate in 250 mM acetate buffer pH 3.4.

The effects of some sulphohydrolase and phosphatase inhibitors, steroids, compounds reacting with –SH groups and inhibitors of anion transport on the purified cholesterol sulphate sulphohydrolase were tested in 250 mM acetate buffer pH 3.4.

2.8. Determination of the protein concentration

Protein concentration in crude lysosomal fraction was determined by the method of Bradford [35]. Protein concentration in other fractions was determined by the procedure of Bensadoun and Weinstein [36] with the bovine serum albumin as a standard.