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SUBUNITS OF STEROL SULPHATE SULPHOHYDROLASE FROM HUMAN PLACENTA MICROSOMES

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Summary—A procedure for separation of the catalytic and regulatory subunits of sterol sulphate sulphohydrolase from human placenta microsomes with the use of Concanavalin A–Sepharose chromatography is presented. The K_m value for the catalytic subunit with oestrone sulphate is 1.2×10^{-5} M. The Hill coefficient value h, for the reconstituted enzyme complex is 3, the $S_{0.5} = 0.68 \times 10^{-3}$ M and the value of K_m is 0.31×10^{-12} M. The regulatory subunit is trypsin sensitive, while the catalytic one is resistant to trypsin digestion.

INTRODUCTION

In a previous paper a method of isolation and partial purification of oestrone sulphate sulphohydrolase from human placenta microsomes was described [1]. The procedure included: separation of microsomes from term placenta; extraction of the enzyme with Triton X-100; ammonium sulphate precipitation; Bio-gel A 15 m, DEAE–cellulose and Concanavalin A–Sepharose chromatography. DEAE–cellulose chromatography allowed separation of the enzyme fraction exhibiting sigmoidal kinetics, which points to the allosteric character of the enzyme.

The present paper deals with a final purification step which is Concanavalin A–Sepharose chromatography; this procedure allows separation of the regulatory and catalytic subunits.

EXPERIMENTAL

Reagents

The reagents were purchased as follows: DEAE–cellulose, Triton X-100 and lectin from Concavalia ensiformes were from Serva (Heidelberg, Fed. Rep. Germany); Bio-gel A 15 m (100–200 mesh) from Bio-Rad Labs (Richmond, CA, U.S.A.); oestrone sulphate sodium salt and α-methyl-D-glucoside were from Sigma Chemical Co. (St Louis, MO, U.S.A.); Sepharose 6B-CNBr-activated was from Pharmacia Fine Chemicals (Uppsala, Sweden); trypsin was from Boehringer (Manheim, Fed. Rep. Germany).

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Enzyme material

As starting material a fraction of steroid sulphohydrolase emerging from DEAE–cellulose (0.12 M NaCl eluate), which exhibits sigmoidal kinetics was used. The isolation of this material has been described previously [1]. Separation of the catalytic subunit from the regulatory one was effected by affinity chromatography on Concanavalin A–Sepharose. Concanavalin A–Sepharose column was prepared by mixing the solution of Concanavalin A (20 mg per 30 ml of the solution containing: 0.01 M $MnCl_2$, 0.01 M $CaCl_2$ and 0.01 M $MgCl_2$) with 1 g Sepharose 6B-CNBr-activated in 1 ml of 1 mM HCl. The pH was adjusted to 8.0 by addition of sodium acetate. A 1.3×10 cm column was packed with a suspension of Concanavalin A–Sepharose at 4°C and equilibrated with 50 mM Tris–HCl buffer, pH 7.6. A 2 ml sample of the enzyme solution (1.5–2.0 mg of protein) from the DEAE–cellulose eluate was applied and fractionated at a flow rate of 2 ml per 30 min. After washing the column with starting buffer solution (fraction I), the elution was continued with 20% α-methyl-D-glucoside (fraction II), 5 mM Tris–HCl buffer pH 7.6 (fraction III), 5 mM Tris–HCl buffer with 0.5% Triton X-100 (fraction IV). Elution of the regulatory subunit was effected with 0.5% Triton X-100 in 50 mM Tris–HCl buffer pH 7.6 (fraction V).

Enzyme activity determinations

The assays of oestrone sulphate sulphohydrolase activities were carried out in 25 mM Tris–HCl buffer pH 7.6 at 37°C. The effect of

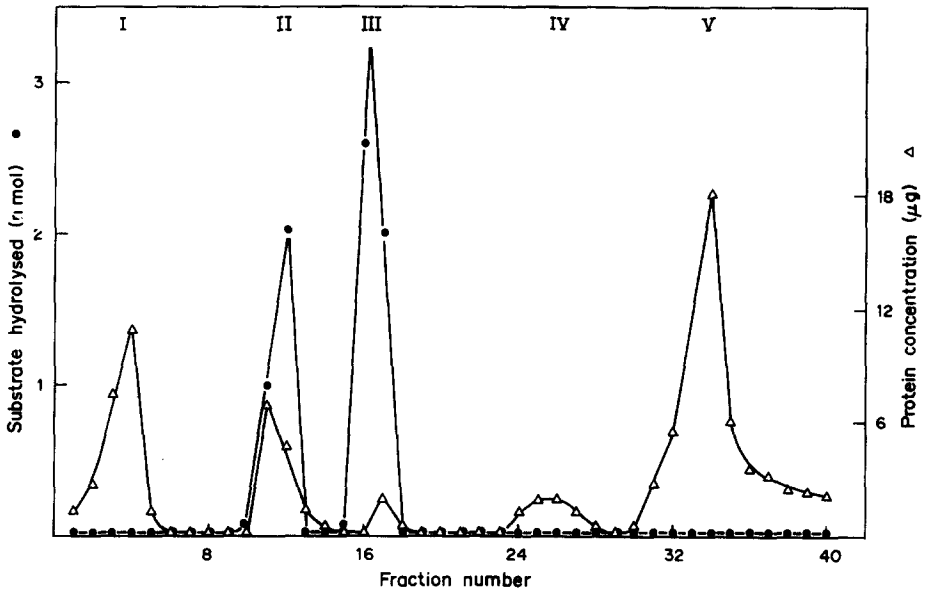

Fig. 1. Concanavalin A–Sepharose 6B (1.3 × 10 cm column) equilibrated with 50 mM Tris–HCl buffer pH 7.6. A 2 ml sample of the enzyme fraction from DEAE–cellulose was applied to the column. After washing the column with starting buffer solution (fraction I) the elution was continued with α-methyl-D-glucoside (fraction II); 5 mM Tris–HCl buffer pH 7.6 (fraction III); 5 mM Tris–HCl buffer pH 7.6 containing 0.5% Triton X-100 (fraction IV) and 0.5% Triton X-100 in 50 mM Tris–HCl buffer pH 7.6 (fraction V). —●— sulphohydrolase activity; —△— protein.

substrate concentration on the initial value (v) of oestrone sulphate hydrolysis was tested at 0.01–0.02 mM concentration of oestrone sulphate.

To test the effect of trypsin, 0.44 mg of protein in 1 ml of 25 mM Tris–HCl buffer pH 7.6 was subjected to trypsin digestion, for 1 h at 37°C. The trypsin concentration was 0.8 mg per ml.

To test the effect of temperature, 0.44 mg of protein in 1 ml of 25 mM Tris–HCl buffer pH 7.6 was heated for 15 min at 100°C.

RESULTS AND DISCUSSION

The evidence for the allosteric character of oestrone sulphate sulphohydrolase from human placenta microsomes, comes from our studies of the enzyme activity as a function of substrate concentration [1]. Further experiments [2–4] showed that heating (15 min at 100°C) effected no change of the enzyme kinetics. Trypsin digestion however, resulted in disappearance of the sigmoidal shape of the substrate dependence activity curves. We conclude therefore that the regulatory and catalytic function of sterol sulphohydrolase from human placenta microsomes could be located on different subunits; trypsin resistant-catalytic, and trypsin sensitive-regulatory.

Affinity chromatography on Concanavalin A–Sepharose, appeared to be an efficient procedure for separation of the two subunits. A typical pattern of separation procedure is presented in Fig. 1. Fraction II, which was eluted with 20% α-methyl-D-glucoside exhibited catalytic activity, while fraction V, which was

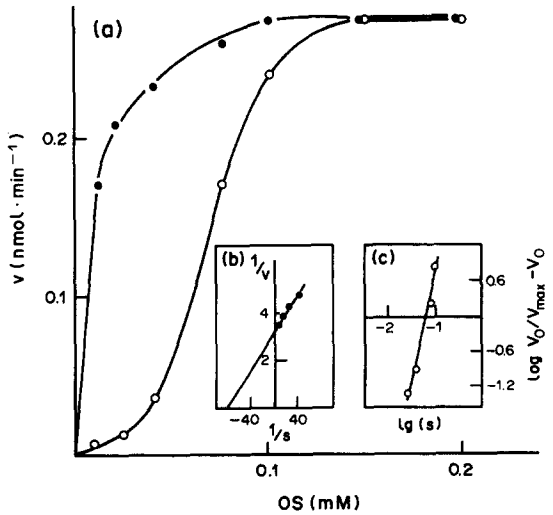

Fig. 2. Effect of substrate concentration on the initial rate (v) of oestrone sulphate hydrolysis by sulphohydrolase from human placenta microsomes after Concanavalin A–Sepharose chromatography: —●— fraction II; —○— fraction II and fraction V. The assays were carried out in 25 mM Tris–HCl buffer pH 7.6 at 37°C (10 μg protein of fraction II and 5.5 μg protein of fraction V was used).

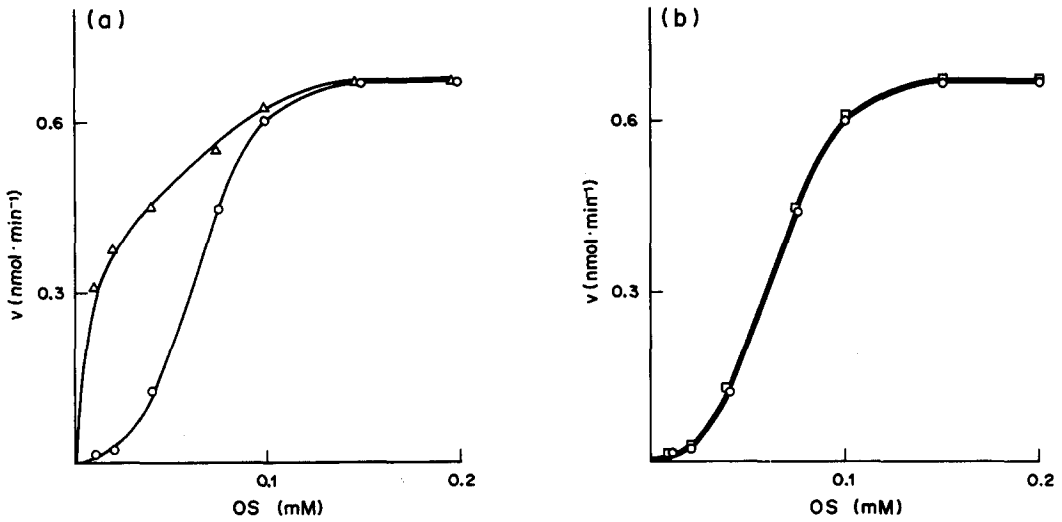

Fig. 3. Effect of substrate concentration on the initial rate (v) of oestrone sulphate hydrolysis by sulphohydrolase from human placenta microsomes after Concanavalin A–Sepharose chromatography: —○— fraction II and fraction V; —△— fraction II and trypsin digested fraction V; —□— fraction II and heated (15 min at 100°C) fraction V. The assays were carried out in 25 mM Tris–HCl buffer pH 7.6 at 37°C. 22 μg protein of fraction II and 5.5 μg protein of fraction V were used.

eluted with 0.5% Triton X-100 in 50 mM Tris–HCl buffer pH 7.6, contained the regulatory subunit.

The initial velocity of the hydrolysis of oestrone sulphate exerted by the catalytic subunit (fraction II) increased with the substrate concentration as should be expected from the Michaelis–Menten theory; the K_m value was established to be 1.2×10^{-5} [Fig. 2(a and b)]; the allosteric behaviour of the enzyme disappeared. Addition of the enzymatically inactive fraction V, to the enzyme bearing fraction II, effected restoration of the sigmoidal kinetics [Fig. 2(a and c)].

The value of h defined as the maximum slope of Hill plot with oestrone sulphate as substrate of the reconstituted complex is 3, the $S_{0.5}$ value is 0.68 and the K_m calculated from the data of h and $S_{0.5}$ is 0.31×10^{-12} M [Fig. 2(a and c)].

No change of inhibitory power was noted after heating of the separated regulatory subunit for 15 min at 100°C. However, digestion with trypsin caused loss of inhibitory effect [Fig. 3(a and b)]. The nature of the subunits of sterol sulphate sulphohydrolase being currently investigated.

Data presented in this paper on the allosteric mechanism of sterol sulphate sulphohydrolase from human placenta microsomes indicate that catalytic and regulatory function of the enzyme are located on different and separable subunits.

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