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THE TARGET SIZES OF THE IN SITU AND SOLUBILIZED FORMS OF HUMAN PLACENTAL STEROID SULFATASE AS MEASURED BY RADIATION INACTIVATION

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Whole microsomal membrane preparations and Triton X-100-solubilized human placental steroid sulfatase were subjected to radiation inactivation analysis using gamma rays from a ^{60}Co irradiator in order to assess the size of the physiological form of the enzyme. The data indicate that the enzyme exists as a monomer of molecular weight 78 600 in Triton-containing buffers and as a polymer of molecular weight 533 000 within the microsomal membrane.

Steroid sulfatase (sterol-sulfate sulfohydrolase, EC 3.1.6.2.) plays an important role in placental estrogen biosynthesis and has been implicated in the capacitation of the mammalian spermatozoon [1]. The enzyme is localized within the membranes of the endoplasmic reticulum and can be quantitatively released from the membrane following treatment with Triton X-100 [2]. Steroid sulfatase that has been isolated in this manner appears to form with Triton a complex of molecular weight 166 000 to which the peptide portion contributes approx. 72 000 [2].

The enzyme readily aggregates below the critical micelle concentration of the detergent and aggregated forms are occasionally observed even in the presence of 0.1% (v/v) Triton. In view of these findings as well as the fact that the activity versus Triton concentration curves suggest that the physiological form might be a polymer, assessment of the molecular weight was sought by radiation inactivation using a ^{60}Co irradiator [3]. The data are compatible with the enzyme existing as a monomer of molecular weight 78600 in Triton-containing buffers and as a polymer of molecular weight 533 000 within the membrane.

Preparation of microsomes was performed as follows. Placentas were obtained from the delivery room of Maisonneuve-Rosemont Hospital and were kept on ice. They were cut into small pieces and washed extensively with 0.15 M NaCl to remove as much of the blood as possible. The minced tissue was homogenized with a motor-driven Teflon homogeneizer in 10 mM Tris-HCl (pH 7.8) containing 0.5 mM EDTA, 1 mM dithiothreitol and 0.25 M sucrose. The microsomal fraction was obtained by differential centrifugation ($3000 \times g$, 15 min; $20\,000 \times g$, 30 min; $105\,000 \times g$, 60 min) and was washed twice, recentrifuged and resuspended in the same buffer. 100 μl aliquots containing 300–500 μg of protein, as measured by the method of Bradford [4], were frozen and lyophilized.

Preparation of the solubilized enzyme was performed as follows. Steroid sulfatase was extracted from the microsomes with Triton X-100 as described elsewhere [2] 5 ml of extract were adsorbed batch-wise onto 2 ml (settled volume) of Con-A-Sepharose (Pharmacia) equilibrated in 10 mM

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Tris-HCl (pH 7.8) containing 0.5 M NaCl, 0.1% (v/v) Triton X-100 and 0.05% (v/v) β-mercaptoethanol for 4 h at room temperature. The gel was pelleted by centrifugation, washed 5 times in 10 ml of buffer and finally resuspended in the same buffer containing 0.5 M α-methylmannopyranoside. The mixture was rotated for 24 h at 4°C. The eluted proteins were dialyzed against 3 × 1 liter changes of 10 mM Tris HCl containing 0.1% (v/v) Triton X-100 and 0.05% (v/v) β-mercaptoethanol over 24 h. Following dialysis, the enzyme preparation was subsequently concentrated approx. 3-fold by ultrafiltration over an Amicon UM2 membrane. An aliquot of 0.5–1.0 ml of this sulfatase preparation containing 60–100 μg of protein was precipitated with 10 volumes of acetone and the precipitate was resuspended in 10 mM Tris (pH 7.8) containing 0.1% (v/v) Triton X-100, 1 mM dithiothreitol and 0.25 M sucrose. Aliquots of 100 μl were frozen and lyophilized.

Radiation inactivation procedure: Aliquots of microsomal membranes and of the partially purified sulfatase preparation were lyophilized in 1.5 ml Eppendorf microcentrifuge tubes. The tubes were flushed with nitrogen and irradiated at room temperature (26 ± 2°C) with gamma rays using a Gammacell Model 220 (Atomic Energy of Canada, Ottawa). The irradiator was calibrated with enzymes of known radiation sensitivities [3]. The tubes were placed in a special support ensuring isodose exposure at approx. 0.1 Mrad/h. Three tubes were irradiated at each dose and the enzyme assays were performed in duplicate. The data were analyzed according to classical target theory [5]. The dose D_{37} (in Mrads), corresponding to the dose where 37% of the initial activity remains, was correlated to the molecular weight using the empirical equation: $M = 6.4 \cdot 10^5 / D_{37}$ [5].

Steroid sulfatase assays: Steroid sulfatase activity was assayed in a 200 μl reaction volume which contained: 0.05 M Tris-HCl (pH 7.8) $5 \cdot 10^{-4}$ M dithiothreitol, $2.1 \cdot 10^{-6}$ M cholesterol sulfate [6], [1,2-^{3}H]cholesterol sulfate (10000 cpm), 0.0225% Triton X-100 (v/v) and enzyme. Following incubation at 37°C, the reaction was stopped by the addition of 1 ml of cold methanol. The liberated radioactive cholesterol was extracted into hexane which was evaporated to dryness under nitrogen and assayed for radioactivity.

The target size of steroid sulfatase was first measured with whole microsomal membranes in order to determine the molecular weight of the in situ form of the enzyme. The irradiation of the microsomal membranes yields a D_{37} of 1.20 Mrad for bound steroid sulfatase, which corresponds to an apparent molecular weight of 533000 (Fig. 1). Irradiation of the enzyme solubilized with, and lyophilized in the presence of Triton X-100 yields a D_{37} of 8.14 Mrad which corresponds to an apparent molecular weight of 78600 (Fig. 2). Thus, it is clear that the solubilization procedure results in the dissociation of a large oligomer.

The molecular weight measured for the in situ form is comparable to estimates obtained by others using gel chromatography. Thus, McNaught and France [6] using 0.02% (v/v) Triton X-100 have reported two peaks of activity with apparent molecular weights of 1000000 and 600000 for the

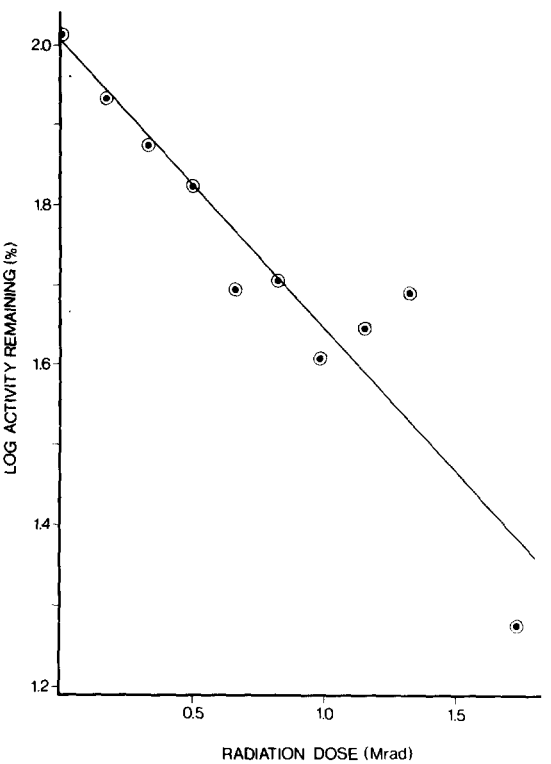

Fig. 1. Radiation inactivation of membrane-bound steroid sulfatase. Samples of microsomal membranes were lyophilized and irradiated in 1.5 ml Eppendorf microcentrifuge tubes as described in the text. The proteins were reconstituted with 0.1% (v/v) Triton X-100 in water and steroid sulfatase was assayed as described in the text.

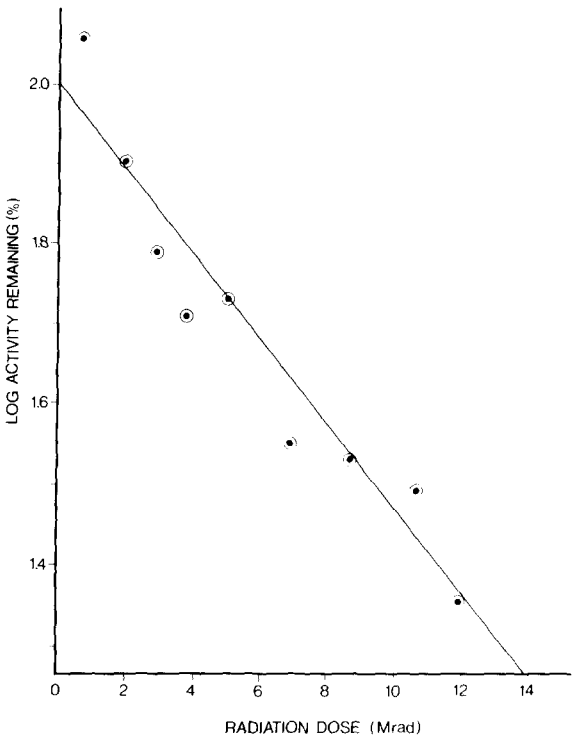

Fig. 2. Radiation inactivation of Triton X-100-solubilized steroid sulfatase. 100 μl samples of partially purified solubilized steroid sulfatase were irradiated as described in the text in the presence of 0.1% (v/v) Triton X-100. The proteins were reconstituted with 100 μl of H_2O and steroid sulfatase was assayed as described in the text.

human placental enzyme; Villaroya and Scholler [7] have calculated a molecular weight of 550000 for this enzyme in the human spermatozoon solubilized with Triton X-100. A somewhat lower molecular weight of 330000 for the placental enzyme has been reported by Gauthier et al. [8] who used Miranol H2M as the solubilizing agent. This could indicate that a different oligomeric structure is obtained in this detergent. The estimate of 78600 obtained for the Triton X-100 solubilized enzyme is quite similar to that calculated from the physiochemical properties and from the measurement of bound Triton which yield a peptide molecular weight of 72000 for the smalles covalent structure [2]. In view of the fact that the molecular weight of the Triton protein complex was calculated to be 166000, the irradiation method appears to yield, in this case, the molecular weight of the polypeptide alone. The discrepancy between the estimated molecular weights obtained with the two methods may be associated with errors in the measurements of the bound Triton in one case as well as the fact that the irradiation method is subject to an error of up to 14% [5,9]. Furthermore, since the target size is proportional to the protein volume [5], the detergent may bring about an increase in the protein volume resulting in the overestimation of its size. This overestimation would range from about 10% if the polypeptide molecular weight is 72000 and up to 25% if it is 62000 as measured by SDS-gel electrophoresis [2]. It is noteworthy that other workers [10] have isolated, from the human placenta, a lipoprotein complex of molecular weight 60000–70000 exhibiting sulfatase activity.

The target sizes of the membrane bound and solubilized forms of cholesterol sulfatase would be compatible with a subunit structure of eight identical subunits (molecular weight 533000). In view of the numerous substrates described for steroid sulfatase, the possible interrelationship between the degree of aggregation and substrate specificity remains to be defined.

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