BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **246,** 45–49 (1998)
ARTICLE NO. RC988568

Different Biochemical Properties of Nuclear and Microsomal Estrone-3-Sulfatases: Evidence for the Presence of a Nuclear Isozyme[1]

Bao Ting Zhu,*[,2] Jia-Huey Fu,* Shiyao Xu,* Frederick C. Kauffman,† and Allan H. Conney*[,3]
**Laboratory for Cancer Research, Department of Chemical Biology, and †Department of Pharmacology and Toxicology, College of Pharmacy, Rutgers–State University of New Jersey, Piscataway, New Jersey 08854*

Received March 19, 1998

In female rats, *total* estrone-3-sulfatase activity per liver in the nuclear fraction is comparable to the total activity per liver in the microsomal fraction. The combined estrone-3-sulfatase activity in the other fractions (lysosomal, mitochondrial, and cytosolic fractions) is negligible and only accounts for <5% of the total nuclear or microsomal sulfatase activity. Nuclear and microsomal estrone-3-sulfatases have different pH optima (pH 8.0 and 7.2, respectively). The apparent Km values for the nuclear and microsomal estrone-3-sulfatases are 2.5 and 10.1 μM, respectively, suggesting that the nuclear sulfatase has a considerably higher affinity for estrone-3-sulfate than the microsomal sulfatase. Moreover, the nuclear estrone-3-sulfatase is more sensitive to inhibition by several steroids than the microsomal sulfatase. The results suggest that estrone-3-sulfatase in the nuclear fraction is a different isozyme than that in the microsomal fraction.

Key Words: **nuclear estrone-3-sulfatase; nuclear isozyme; subcellular distribution; estrogen sulfate; metabolic desulfonation.**

Sulfonated estrogens are widely used as therapeutic or preventive agents for medical conditions associated with estrogen deficiency such as osteoporosis in elderly women (1, 2). The hormonal activity of sulfonated estrogens results from the release of active estrogens through their enzymatic hydrolysis (desulfonation). Liver contains the largest amounts of arylsulfatase C (EC 3.1.6.2) (4-6), a major isoform of the sulfatase that has high catalytic activity for the desulfonation of estrone-3-sulfate (E_1-3-sulfate). Estrogen target cells (e.g. uterine endometrial cells and mammary cells) also contain significant amounts of estrogen sulfatase activity (5-15), which has been thought to play an important role for the *in situ* formation of active estrogens from estrogen sulfates under certain physiological or pathophysiological conditions.

Because high activity of arylsulfatase C was found in the microsomal fraction of liver, and relatively low activity was observed in other subcellular membrane-containing fractions, this sulfatase has always been considered to be a microsomal enzyme (5, 7, 16). Studies in mice and other rodents suggested that the microsomal arylsulfatase C is genetically and biochemically heterogenous (17). In humans, the microsomal arylsulfatase C was found to have two different isoforms with different electrophoretic mobilities (18), and additional studies showed that these isoforms were encoded by separate genes (19) with a tissue-specific expression pattern (20). In this communication, we report that large amounts of E_1-3-sulfatase are present in the nuclear fraction prepared from female rat liver, and this nuclear E_1-3-sulfatase has different biochemical properties than the microsomal sulfatase, suggesting the presence of a nuclear isozyme.

MATERIALS AND METHODS

Chemicals. E_1-3-sulfate (sodium salt), dehydroepiandrosterone sulfate, androsterone sulfate, and pregnenolone 16α-carbonitrile were purchased from the Sigma Chemical Co. (St. Louis, MO). [6,7-^{3}H]E_1-3-sulfate (specific activity ~57 Ci/mmol) was obtained from Du Pont New England Nuclear Research Products (Boston, MA).

Preparation of hepatic subcellular fractions. Adult (8-week-old) female Long-Evans rats and female Sprague-Dawley rats were purchased from Harlan Sprague-Dawley Laboratory (Indianapolis, IN). The animals were kept on a 12-hr light and 12-hr dark cycle and had free access to Purina Laboratory Chow-5001 (Ralston Purina Co., St. Louis, MO) and water. They were allowed to acclimatize for a week before use in the experiment.

[1] This study was supported in part by a grant (CA 49756) from the National Institutes of Health.
[2] To whom reprint requests should be addressed. Fax: (732) 445-0687. E-mail: baoting@rci.rutgers.edu.
[3] William M. and Myrle W. Garbe Professor for Cancer and Leukemia Research.

0006-291X/98 $25.00

Liver samples from 6 female rats were pooled together and homogenized with a Brinkmann homogenizer in 4 volumes of 20 mM Tris-HCl buffer (ice-cold, pH 7.4) containing 0.25 M sucrose. Hepatic subcellular fractions (nucleus, mitochondria, microsomes, lysosomes, and cytosol) were prepared by multi-step sucrose-gradient centrifugations as described earlier (21). The nuclear fraction prepared according to this procedure also underwent two additional steps of wash by resuspension in the original buffer followed by centrifugation at 1020g for 10 min.

The protein concentration in each subcellular fraction was determined by the Bio-Rad protein assay kit with bovine serum albumin (BSA) as a standard.

Assay of E_1-3-sulfatase activity. E_1-3-sulfatase activity in each of the subcellular fractions (nucleus, mitochondria, microsomes, lysosomes, and cytosol) of female rat liver was determined by measuring the formation of [6,7-^{3}H]E_1 from [6,7-^{3}H]E_1-3-sulfate as described in our recent study (6). The incubation mixture consisted of 0.2-0.5 mg hepatic subcellular protein and 2.5-40 μM E_1-3-sulfate (containing 0.2 μCi [6,7-^{3}H]E_1-3-sulfate) in a final volume of 1.0 ml Tris-HCl buffer (25 mM). The incubation was carried out at 30°C for 15 min. After incubation, the test tubes were immediately placed on ice and were extracted with 5 ml ice-cold toluene. Aliquots of the organic supernatants were removed for radioactivity measurement. Blank values were determined for each individual assay in the absence of hepatic subcellular protein and were subtracted. The E_1-3-sulfatase activity in each of the hepatic subcellular fractions was expressed as "pmol E_1 formed/mg protein/min."

The *total* E_1-3-sulfatase activity per liver in each subcellular fraction was calculated based on the total amount of protein per liver in each of the subcellular fractions that was harvested from a pool of 6 rat livers (average wet weight 12.4 ± 1.1 g).

RESULTS

We determined the E_1-3-sulfatase activity in different hepatic subcellular fractions of adult female Long-Evans rats. The liver microsomal fraction contained the highest specific activity of E_1-3-sulfatase (4732 ± 211 pmol/mg protein/min), followed by the lysosomal fraction (2120 ± 38 pmol/mg protein/min), the nuclear fraction (1345 ± 82 pmol/mg protein/min), and the mitochondrial fraction (1054 ± 53 pmol/mg protein/min) (Fig. 1A). Little or no E_1-3-sulfatase activity was detected in the hepatic cytosolic fraction (Fig. 1A). The E_1-3-sulfatase activity in different hepatic subcellular fractions of female Sprague-Dawley rats showed a very similar pattern as was observed with female Long-Evans rats (data not shown).

Based on the specific activity of E_1-3-sulfatase (pmol/mg protein/min) determined for liver microsomal, lysosomal, nuclear, mitochondrial, and cytosolic fractions, we calculated the *total* E_1-3-sulfatase activity per liver (mean liver wet weight, 12.4 g) that was contributed by each subcellular fraction (Fig. 1B). The *total* E_1-3-sulfatase activity (when assayed with 30 μM E_1-3-sulfate as substrate) per liver in the hepatic nuclear fraction is similar to that in the microsomal fraction, although the latter contains higher specific activity (per mg protein) of E_1-3-sulfatase.

We compared the kinetic parameters of the hepatic nuclear and microsomal E_1-3-sulfatases. The nuclear and microsomal E_1-3-sulfatases from female Long-Evans

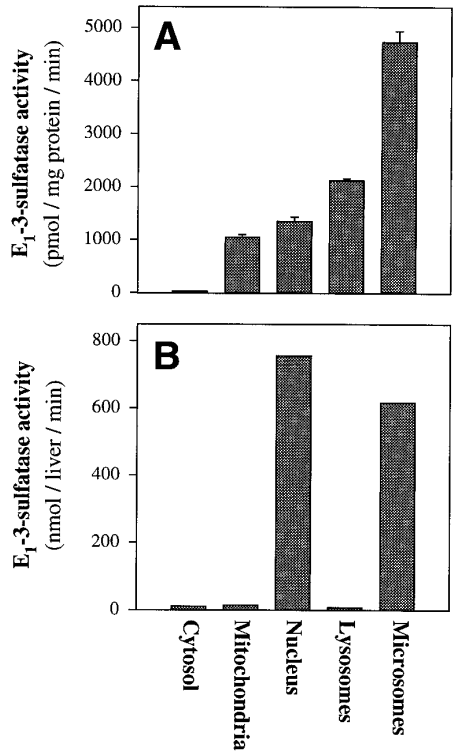

FIG. 1. The specific activity (pmol/mg protein/min; *A*) and *total* activity per liver (*B*) of E_1-3-sulfatase in different hepatic subcellular fractions of female Long-Evan rats. The incubation mixture consisted of 0.2 mg cellular protein and 30 μM E_1-3-sulfate (containing 1.0 μCi [6,7-^{3}H]estrone-3-sulfate) in a final volume of 0.5 ml Tris-HCl buffer (100 mM, pH 7.4). The assay method is described in the Materials and Methods section. Each point represents the mean ± S.D. (*A*) or mean (*B*) of triplicate determinations. The distribution of total E_1-3-sulfatase activity per liver in the microsomal, nuclear, lysosomal, mitochondrial, and cytosolic fractions in female Long-Evans rat liver was calculated based on an average liver weight of 12.4 g. The results shown are representative of two separate experiments that showed similar results.

rats or Sprague-Dawley rats showed slightly different patterns of pH-dependence curves (with pH optima of ~7.2 for the microsomal sulfatase and ~8.0 for the nuclear sulfatase) (Fig. 2). Under optimal conditions for *in vitro* desulfonation, the *nuclear* E_1-3-sulfatase had a Km value of 2.5 μM (at pH 8.0), which was approximately one-fourth of the Km for the *microsomal* E_1-3-sulfatase (Km = 10.1 μM, at pH 7.2) (Fig. 3). Similarly, different Km values were also observed for hepatic nuclear and microsomal E_1-3-sulfatases in female Sprague-Dawley rats (data not shown). These data suggest that the nuclear E_1-3-sulfatase has a substantially higher apparent affinity for E_1-3sulfate than the microsomal sulfatase.

We also compared the sensitivity of hepatic nuclear and microsomal E_1-3-sulfatases to inhibition by two endogenous sulfonated steroids (dehydroepiandro-sterone sulfate and androsterone sulfate) or a synthetic

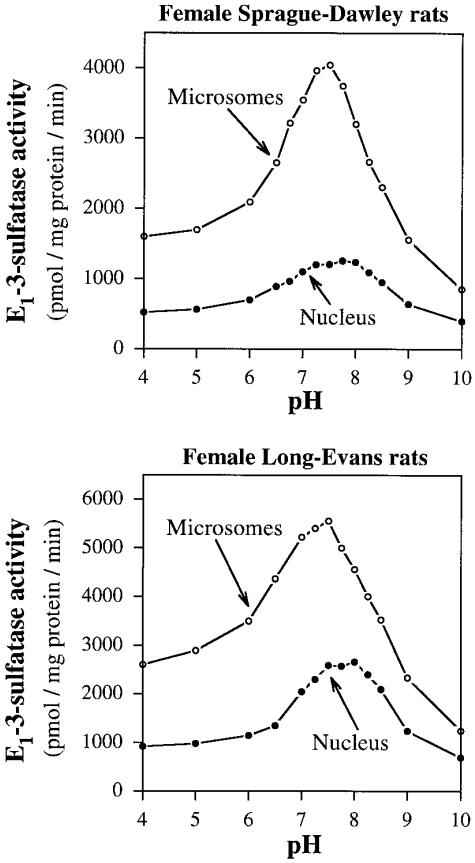

FIG. 2. The effects of different pH values on hepatic microsomal or nuclear E_1-3-sulfatase activity. The incubation mixture consisted of 0.2 mg microsomal or nuclear protein, 40 μM E_1-3-sulfate (containing 0.2 μCi [6,7-^{3}H]E_1-3-sulfate). The assay method is described in the Materials and Methods section. Each point is the mean of duplicate determinations.

nonsulfonated steroid (pregnelonone 16α-carbonitrile) to probe additional biochemical properties of these two sulfatase activities. The IC_{50} values for dehydroepiandrosterone sulfate and androsterone sulfate in inhibiting hepatic nuclear E_1-3-sulfatase (4 and 22 μM, respectively) were approximately one-half of their corresponding IC_{50} values (9 and 42 μM, respectively) for liver microsomal E_1-3-sulfatase (Fig. 4). Similarly, pregnenolone 16α-carbonitrile (a synthetic nonsulfonated steroid) also showed different potencies for inhibiting the nuclear *vs* the microsomal E_1-3-sulfatase (Fig. 5). These data suggest that the nuclear and microsomal E_1-3-sulfatases have different sensitivities to inhibition by certain endogenous or synthetic steroids.

DISCUSSION

The results of the present study showed that E_1-3-sulfatase activity is present in all membrane-containing subcellular fractions (i.e., the microsomal, nuclear, lysosomal, and mitochondrial fractions) prepared from female rat liver. Although the specific activity (per mg protein) of E_1-3-sulfatase in liver is higher in the microsomal fraction than in the nuclear fraction, when calculated on a per liver basis, the total amount of E_1-3-sulfatase activity for the microsomal and nuclear fractions are about equal. The nuclear E_1-3-sulfatase has a substantially higher apparent affinity for E_1-3-sulfate (the substrate) than the microsomal sulfatase. Moreover, the nuclear E_1-3-sulfatase activity is more sensitive to inhibition by dehydroepiandrosterone sulfate, androsterone sulfate or pregnenolone 16α-carbonitrile than the microsomal sulfatase. Altogether, these results suggest that the nuclear and microsomal E_1-3-sulfatases are different. The molecular basis for the observed differences between the nuclear and microsomal E_1-3-sulfatase activities, however, is not known. Although previous studies demonstrated that microsomal arylsulfatase C (or E_1-3-sulfatase) is coded for by more than one gene (19), it is yet to be determined whether the nuclear and microsomal E_1-3-sulfatases are indeed different gene products. It is also possible that nuclear and microsomal sulfatases may be formed from the same gene but undergo different post-transcriptional modifications.

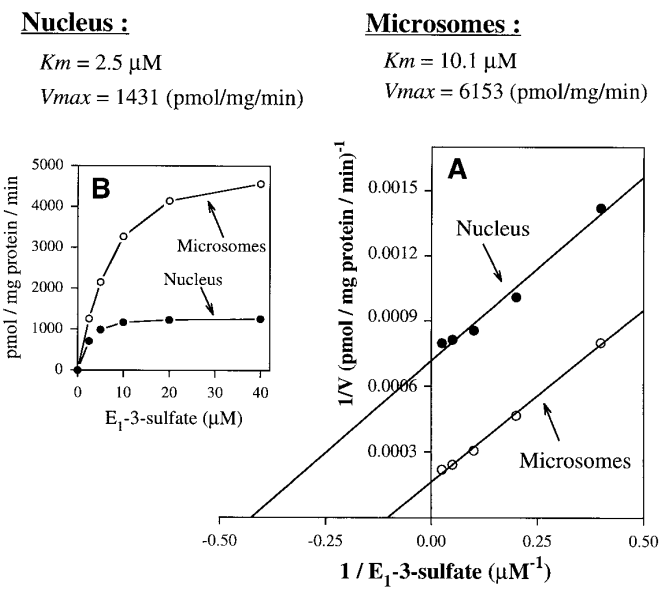

FIG. 3. Studies on the kinetics of E_1-3-sulfate desulfonation catalyzed by the hepatic microsomal or nuclear fraction from female Long-Evans rats. *A* is the Eadie-Hofstee plot of enzymatic desulfonation of E_1-3-sulfate, and *B* shows the rates of desulfonation as a function of increasing E_1-3-sulfate concentrations. The incubation mixture consisted of 0.2 mg microsomal or nuclear protein and 2.5-40 μM E_1-3-sulfate (containing 1.0 μCi [6,7-^{3}H]E_1-3-sulfate) in a final volume of 0.5 ml Tris-HCl buffer (100 mM, pH 7.4). The assay methods are described in the Materials and Methods section. Each point represents the mean of replicate determinations. The results shown are representative of two separate measurements with nuclear and microsomal preparations.

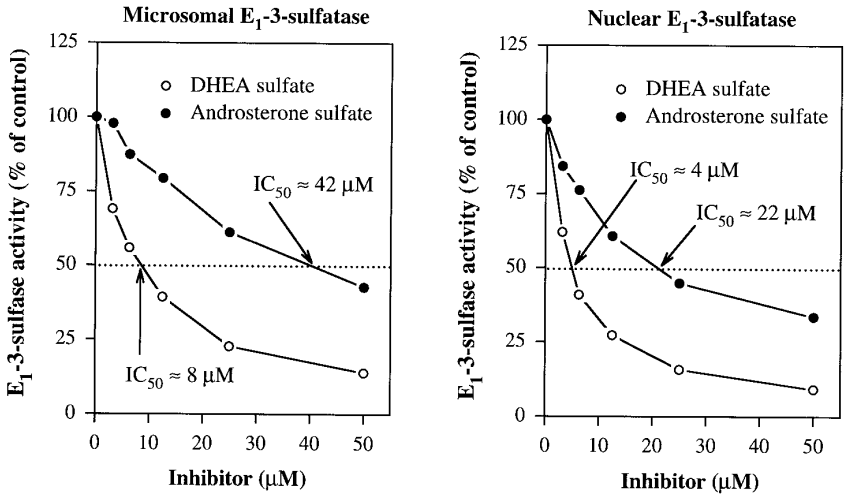

FIG. 4. Differential sensitivity of liver microsomal and nuclear E_1-3-sulfatase to inhibition by dehydroepiandrosterone (DHEA) sulfate or androsterone sulfate *in vitro*. The incubation mixture consisted of 0.2 mg microsomal or nuclear protein, 30 μM E_1-3-sulfate (containing 0.2 μCi [6,7-^{3}H]E_1-3-sulfate) and different concentrations of inhibitor in a final volume of 1.0 ml Tris-HCl buffer (100 mM, pH 7.4). The assay method is described in the Materials and Methods section. Each point is the mean of duplicate determinations. The microsomal and nuclear E_1-3-sulfatase activities in the absence of inhibitor were 4.03 and 1.09 nmol/mg protein/min, respectively.

The presence of large amounts of E_1-3-sulfatase activity in the nuclear fraction, and the substantially higher affinity of this enzyme for E_1-3-sulfate than the microsomal sulfatase, suggest that the formation of active estrogens from estrogen sulfates within the nucleus may be of greater importance than previously realized. The active estrogens formed from estrogen sulfates within the nucleus may have less chances of undergoing metabolic inactivation by the oxidative and/or conjugative enzymes prior to exerting their actions. Since the majority of estrogen receptors are present inside the nucleus, the formation of active estrogens in the nucleus *at or near* estrogen receptors may greatly facilitate the interactions of active estrogens with their receptors, subsequently leading to the expression of hormonal responses.

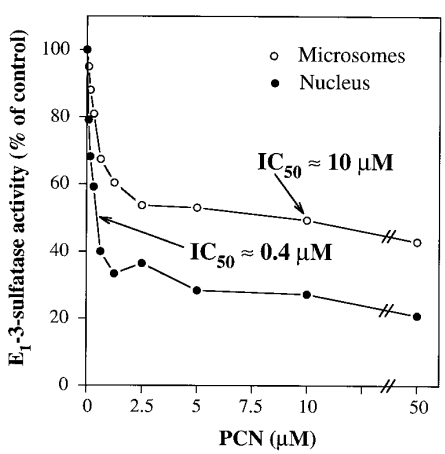

FIG. 5. Differential sensitivity of liver microsomal and nuclear E_1-3-sulfatase to inhibition by pregnelonone 16α-carbonitrile (PCN) *in vitro*. The incubation mixture and the assay method are as described in the legend to Fig. 4. Each point is the mean of duplicate determinations, and the microsomal and nuclear E_1-3-sulfatase activities in the absence of inhibitor were 3.92 and 0.98 nmol/mg protein/min, respectively. The data shown is representative of two separate assays.

In summary, the results of the present study indicate that large amounts of E_1-3-sulfatase are present in the nuclear fraction of adult female rat liver. The nuclear E_1-3-sulfatase is different from the microsomal sulfatase with regard to its pH optimum, its affinity for E_1-3-sulfate, and its sensitivity to inhibition by other steroids. It will be of interest to determine the functional roles of the nuclear *vs* microsomal E_1-3-sulfatase in estrogen target cells.

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