Biochimica et Biophysica Acta, 1152 (1993) 328–334

BBAMEM 76113

Glucuronidation of all-*trans*-retinoic acid in liposomal membranes

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(Received 8 June 1993)

Key words: UDP-glucuronosyltransferase; Kinetics; Retinoic acid; Glucuronidation; Retinoyl β-D-glucuronide; Liposome; Microsome

Retinoyl β-D-glucuronide is a biologically active metabolite of retinoic acid. The kinetics of UDP-glucuronosyltransferase-catalyzed biosynthesis of retinoyl β-D-glucuronide was examined in rat liver and intestinal native microsomes incubated with [^{3}H]retinoic acid incorporated into liposomes. The product was identified by cochromatography with authentic all-*trans* retinoyl β-D-glucuronide, by hydrolysis with β-D-glucuronidase, and by mass spectrometry. In vitamin A-sufficient rats the apparent K_m values for all-*trans*-retinoic acid were 173 μM and 125 μM, and the apparent V_{max}, 62 and 41 pmol/min per mg, for small intestinal and liver microsomes, respectively. In vitamin A-deficient rats repleted with all-*trans*-retinyl acetate, the apparent K_m (91 μM) and V_{max} (53 pmol/min per mg) for intestinal microsomes were in range of those of vitamin A-sufficient rats. The similarities in the kinetic parameters for UDP-glucuronosyltransferase in small intestinal mucosa and liver suggest that the reactions are catalyzed by the same enzyme. In vitamin A-deficient rats given a large amount all-*trans*-retinoic acid (1.2 mmol/day for 3 days) the apparent K_m was 105 μM and V_{max}, 127 pmol/min per mg of intestinal microsomal protein. We conclude that the kinetics of intestinal retinoic acid glucuronidation are not characteristic of simple detoxification reactions. Retinoyl glucuronide may be important in mediating retinoic acid metabolism and function.

Introduction

UDP-glucuronosyltransferase (UDPGT, EC 2.4.1.17) is a membrane-bound family of isoenzyme found in eukaryotes; it catalyzes glucuronidation of many structurally diverse aglycones [1–3]. The acceptor substrates are xenobiotics and various endogenous compounds including retinoids. Although previously researchers have restricted themselves to the thinking that glucuronidation serves only elimination, recent studies demonstrate that glucuronides are significant components of nonexcretory tissue pools of important physiological regulators such as neurotransmitters [4,5], estradiol [6], and retinoids [7–14]. Retinoyl glucuronide, originally thought to be the major excretory metabolite of retinoic acid [15–18], has been subsequently demonstrated to be only a minor biliary metabolite of retinoic acid [19–21] and to be a major metabolite of retinoic acid in the small intestinal mucosa of bile duct-cannulated [7,8] as well as of intact rats [7–9]. These observations suggest that retinoyl glucuronide may have a functionally significant role in metabolism of retinoic acid. In support of this hypothesis is the finding that retinoyl glucuronide is more active than retinoic acid or retinol in modulating differentiation in keratinizing vaginal epithelium [11], and is as active as retinoic acid in inducing differentiation of HL-60 cells [12,13] without being hydrolyzed to retinoic acid [12].

Glucuronidation of vitamin A compounds has been reported in several tissues [7,9,16,17]; in the liver it occurs to the same extent whether physiological or pharmacological levels are administered [16]. There is only a 2-fold increase in the retinoyl glucuronide formed as a result of a 600–1000-fold increase in retinoic acid dose [9,21]. Similarly, others have reported that a 333-fold increase in retinoic acid dose

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Abbreviations: UDPGT, UDP-glucuronosyltransferase; RBG, all-*trans*-retinoyl β-D-glucuronide; ^{3}H-RBG, tritiated all-*trans*-retinoyl β-D-glucuronide; HPLC, high pressure liquid chromatography; UDPGA, uridine 5′-diphosphoglucuronic acid; UDP, uridine 5′-diphosphate; PC, L-phosphatidylcholine; BHT, butylated hydroxytoluene; BSA, bovine serum albumin.

results only in a 4-fold increase in retinoyl glucuronide [20]. Furthermore, the specific activity of UDPGT in liver is increased in vitamin A deficiency [10], an adaptation not typical of enzymes involved in catabolic functions.

However, little is known about the kinetics of retinoid glucuronidation. Glucuronidation of retinoic acid has been examined in solubilized microsomal systems from intestine [22] and liver [10,22]. Of importance is the characterization of the biosynthesis of retinoyl glucuronide in small intestinal epithelium, a target site for vitamin A, where it is the major metabolite of retinoic acid [7,8] and forms within minutes after administration of all-*trans*-retinoic acid to vitamin A-depleted rats [8]. These observations reinforce our hypothesis that glucuronidation of retinoic acid is not merely a mechanism of elimination but that it may be a part of certain intermediate processes linked to vitamin A metabolism and function at molecular level.

In order to obtain an insight into the possible physiological significance of retinoyl glucuronide, we examined retinoic acid glucuronidation. In the present paper we report the kinetics of UDPGT-catalyzed glucuronidation of all-*trans*-retinoic acid in the microsomes of rat small intestinal mucosa and liver, to which we provide the substrate in a liposomal system, so as to more closely replicate the native environment of microsomal UDPGT.

Materials and Methods

Retinoids

All-*trans*-retinoic acid was purchased from Sigma, St. Louis, MO. Additional all-*trans*-retinoic acid, all-*trans*-retinyl acetate, 13-*cis*-retinoic acid, 4-oxoretinoic acid and 5,6-epoxyretinoic acid were generous gifts of Hoffmann-LaRoche, Nutley, NJ. All-*trans*-[11-^{3}H]retinoic acid, 39 Ci/mmol, was purchased from Amersham International, Arlington Heights, IL. All-*trans*-retinoyl β-D-glucuronide (RBG) and tritiated all-*trans*-retinoyl β-D-glucuronide (^{3}H-RBG) were either gifts from Dr. A. Barua or were synthesized in vitro. All retinoids were checked for purity and, if necessary, purified by high pressure liquid chromatography (HPLC) [8,23]. A stock solution of retinoic acid (in methanol) for use as substrate was prepared by combining all-*trans*-[11-^{3}H]retinoic acid with unlabeled crystalline all-*trans*-retinoic acid to a desired specific activity. Retinoids were stored under N_2 at −70°C.

Chemicals; enzymes

β-Glucuronidase (bovine type B-10), uridine 5′-diphosphoglucuronic acid sodium salt (UDPGA), uridine 5′-diphosphate sodium salt (UDP), L-phosphatidylcholine (from egg yolk type 1X-E) (PC), Brilliant blue R (Coomassie blue), sodium dodecyl sulfate, butylated hydroxytoluene (BHT), saccharo-1,4-lactone monohydrate, sodium acetate, tris(hydroxymethyl)aminomethane (Tris) and bovine serum albumin (BSA) were all obtained from Sigma. Calcium chloride, disodium ethylenediaminetetraacetic acid (Na_2EDTA), and glycerol were purchased from Mallinckrodt, Paris, KY. Sepharose 4B was obtained from Pharmacia, Piscataway, NJ. Other chemicals and solvents were of HPLC or analytical reagent grade.

Animals; preparation of microsomes

Weanling female Sprague-Dawley rats (Holtzmann, Madison, WI) were housed separately in suspended cages in a room at 20–25°C at 40% humidity, and a 12 h dark and 12 h light cycle. A group of 24 rats were fed ad libitum the AIN-76 semipurified diet (Teklad, Madison, WI) [24] lacking in vitamin A and supplemented orally with 30 μg of retinyl acetate/day until they were killed at the age of 10–12 weeks (vitamin A-sufficient rats). A group of 30 rats were fed the AIN-76 vitamin A-deficient diet until their weight reached the plateau stage at 10–12 weeks of age. The animals which weighed 190 ± 30 g were then dosed either with all-*trans*-retinoic acid, 350 mg/day for three days to induce UDPGT or with all-*trans*-retinyl acetate, 30 μg/day for three days to replete the vitamin A stores and to serve as normal controls for retinoic acid-treated rats. Prior to killing the rats were fasted for 18 h. Livers were immediately removed and perfused with cold 0.9% NaCl; small intestine was flushed with cold 0.9% NaCl, mucosal epithelium removed from the muscle layer and pooled. Since glucuronidation capacity varies in various parts of the small intestine [25], microsomes were prepared from the mucosa of the entire small intestine. Individual livers and pooled small intestinal mucosa were homogenized in cold 0.25 M sucrose to give a 25% homogenate. Microsomes were prepared by aggregation with calcium [26]; aliquots were stored in 50% glycerol under N_2 at −70°C and used within 8 weeks. Immediately prior to use microsomes were thawed on ice, resuspended in 10 volumes of 25 mM Tris (pH 7.4), centrifuged at 105 000 × *g* for 90 min, and resuspended in 1 ml of Tris (pH 7.4). Microsomal supernatant did not contain detectable UDPGT activity. Protein was determined by a modified Lowry method [27].

Preparation and characterization of liposomes

Small unilamellar vesicles (liposomes) with the substrate, ^{3}H-labeled all-*trans*-retinoic acid incorporated into the bilayer, were prepared as described by Finkelstein and Weissman [28]. Liposomal components, given in final concentrations were prepared as follows: crude L-phosphatidylcholine (PC), 50 mM and ^{3}H-labeled all-*trans*-retinoic acid, 5–1000 μM (spec. act. 80 μCi/μmol, 5–25 μM; 16 μCi/μmol, 50–100 μM),

were dissolved in chloroform containing 10 mM BHT and the solvent removed by evaporation under reduced pressure, leaving in the flask a thin layer of PC-^{3}H-labeled all-*trans*-retinoic acid; this layer was emulsified at 30°C with Tris buffer, 25 mM (pH 7.4) containing 10 mM UDPGA. Each emulsion was sonicated with a microtip probe at 50 watts for 15 min on ice, and centrifuged at 105 000 × *g* for 90 min to remove titanium probe particles and large multilamellar vesicles. The supernate obtained by this procedure contained liposomes approx. 300 Å in diameter. The radiolabeled liposomes were stored at 4°C in the dark, or under yellow light, and used within 6 h. Liposomes and microsomes were characterized by gel exclusion chromatography using 25 mM Tris (pH 7.4) and by electronic particle sizing (Coulter Electronics, Hialeah, FL).

Enzyme assays

UDPGT was assayed in a liposomal system using retinoic acid as substrate. Each reaction mixture consisted of the liposomal preparation, Tris buffer, 25 mM (pH 7.4 at 37°C) and microsomes. Liposomes and buffer were preincubated for 15 min in a shaking water bath at 37°C. The reaction was initiated by addition of microsomes, and the reaction mixtures incubated for 0–60 min in a shaking water bath at 37°C.

Hydrolysis of RBG by UDPGT was examined in a native microsome-liposome system from small intestinal mucosa of vitamin A-sufficient rats. Microsomes were incubated with tritium-labeled RBG incorporated into liposomes, as described above. The reaction was carried out for 1 h at 37°C in 40 mM sodium acetate (pH 5.1), containing 30 mM UDP, 30 mM glucose, 100 mM EDTA and 40 mM ATP.

Hydrolysis of RBG by β-glucuronidase was determined in reaction mixtures containing 20 μg of BSA, 25 000 units of β-glucuronidase, and 3.0 μg of ^{3}H-labeled RBG, all in 50 mM sodium acetate buffer (pH 4.5) [19], final volume, 1 ml. In control reactions β-glucuronidase was denatured by boiling for 30 min. Reactions were initiated by adding the enzyme, then incubated for 1 h at 37°C in a shaking water bath.

The above reactions were terminated by the addition of methanol, the reaction vials flushed with N_2, stored at −20°C in dark and analyzed within 4 days. Boiled enzymes served as controls.

Extraction of retinoids; analysis by HPLC and radioactivity

Retinoids were extracted and assessed by a modification of the HPLC methods previously established in our laboratory [8,23]. Recovery from extraction and HPLC was > 85%. HPLC was accomplished on a C_{18} reversed phase column (Whatman ODS-3 10/25, 25 × 0.46 cm) with a precolumn (7 × 0.46 cm) containing Whatman Co:Pell ODS pellicular support (Whatman, Clifton, NJ). Retinoids were eluted with 75% methanol, containing 0.01 M ammonium acetate. Fig. 1 illustrates the separation of retinoid standards and experimentally derived retinoids. HPLC eluate was collected and radioactivity determined in a liquid scintillation spectrometer (Packard 4430, Packard Instrument). Identity of radioactive metabolites was established by coelution with authentic retinoids.

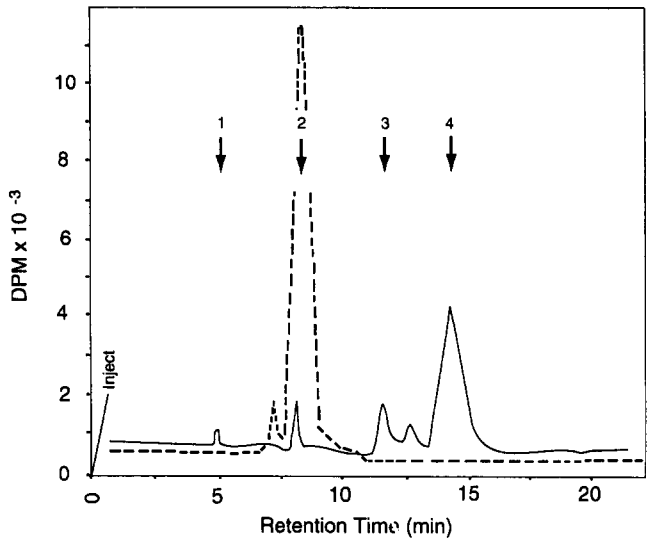

Fig. 1. Chromatography of retinoids. Retinoyl β-D-glucuronide generated in the assay of UDPGT in a liposomal system using retinoic acid as substrate, and retinoic acids liberated in β-glucuronidase assay were chromatographed by HPLC. Radioactivity in control reaction, dashed lines; radioactivity after incubation with 25 000 units of β-D-glucuronidase, solid lines. Arrows indicate positions of authentic standards verified by UV at 340 nm. All-*trans*-4-oxoretinoic acid (1); retinoyl β-D-glucuronide (2); 13-*cis*-retinoic acid (3); all-*trans*-retinoic acid (4).

Kinetic analysis of UDPGT activity toward the substrate all-trans-retinoic acid in the liposomal system

Initial reaction rates for UDPGT of small intestinal mucosal and liver microsomes were determined using [^{3}H]retinoic acid-containing liposomes at ten different substrate concentrations in each of three separate experiments, with 0.6 mg of microsomal protein/ml of reaction mixture and at a constant cosubstrate (UDPGA) concentration of 10 mM, incubated for 30 min at 37°C in 25 mM Tris (pH 7.4). The initial reaction rates were determined at [^{3}H]retinoic acid concentrations of 5–100 μM and 5–250 μM with intestinal and liver microsomes, respectively. Analysis of statistical significance between K_m and V_{max} values of small intestinal and liver microsomal enzymes was performed using Student's *t*-test [29]. The error was expressed as mean ± S.D. Double-reciprocal plot analysis of the kinetic data was according to Lineweaver and Burk [30]. Apparent kinetic parameters were determined by linear regression analysis (Cricket Graph Pro, Computer Associates, Malvern, PA).

Mass spectral analysis of all-trans-retinoyl β-D-glucuronide

Mass spectral analysis of the reaction product RBG was performed on a JEOL HX110 mass spectrometer (Michigan State University - National Institute of Health Mass Spectral Facilities) with triethanolamine as the matrix for the negative ion fast atom bombardment spectral analysis.

Results

Characterization of phosphatidylcholine-[^{3}H]retinoic acid liposomes

Fig. 2 illustrates chromatography of liposomes and microsomes on Sepharose 4B equilibrated and eluted with 25 mM Tris (pH 7.4 at 22°C). When [^{3}H]retinoic acid, 100 μM, was incorporated into liposomes containing Coomassie blue, [^{3}H]retinoic acid and dye entrapped in the liposomes coeluted in a single narrow peak at approx. 40% of the bed volume (Fig. 2A, V_e). Fig. 2B shows the elution of liposomes after an incubation with microsomes. Half of the radioactivity eluted in the void volume, V_o, together with the microsomes; 15–25% of the radioactivity eluted at V_e with unilamellar liposomes; 25% of the radioactivity interacted with the column matrix and chromatographed beyond V_t. Liposome and microsome diameters were determined to be < 1 μm and 3–4 μm, respectively.

Glucuronidation of retinoic acid by native microsomes in liposomal system

The percent conversion of ^{3}H-labeled all-*trans*-retinoic acid to ^{3}H-labeled RBG as a function of protein concentration and time is shown in Fig. 3. The reaction was linear with protein concentrations up to 0.6 mg/ml; at the protein concentration of 0.6 mg/ml the reaction was linear for the first 30 min. The reaction rate was studied in the pH range of 7.0 to 7.8; optimal pH for the reaction was 7.4 (not shown). The above conditions were also found to be applicable to the glucuronidation of all-*trans*-retinoic acid in liver microsomes. β-D-Glucuronidase inhibitor, 1,4-saccharolactone was not added to the liposomal system because it inhibited product formation (not shown).

Identification of all-trans-retinoyl β-glucuronide (RBG)

Fig. 1 illustrates the reaction with retinoids isolated from reactions catalyzed by intestinal microsomal UDPGT. Radioactivity that coeluted with authentic RBG was incubated with bovine liver β-D-glucuronidase and the products isolated and cochromatographed with authentic standards and assessed for radioactivity. In the absence of enzyme or in the presence of boiled enzyme all of the radioactivity was associated with the RBG peak. After an incubation of the putative RBG with β-D-glucuronidase, the radioactivity in both the

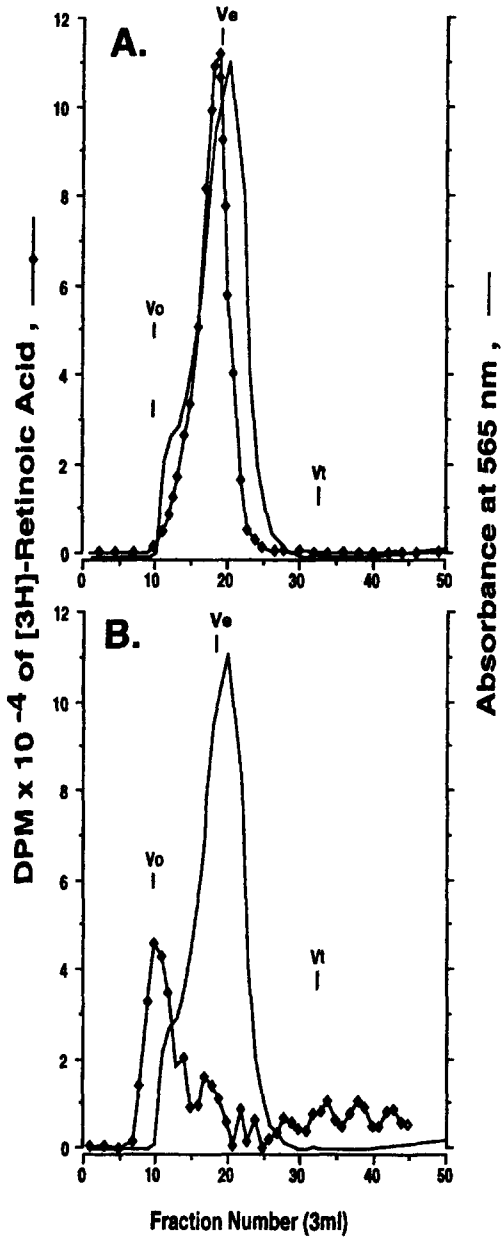

Fig. 2. Chromatography of retinoic acid-liposome complex and retinoic acid-liposome-microsome complex on Sepharose 4B. Crude L-phosphatidylcholine-^{3}H-all-*trans*-retinoic acid liposomes were labeled with Coomassie blue (3 mg/ml) in 25 mM Tris (pH 7.4) containing 10 mM UDPGA; homogeneity of the preparation was assessed by chromatography on a 56×1.5 cm Sepharose 4B column, equilibrated and eluted with 25 μM Tris (pH 7.4). (A) Absorbance representing Coomassie blue-labeled liposomes, solid line; radioactivity from ^{3}H-labeled retinoic acid in dye-containing liposomes, line with diamonds. (B) Liposome-microsome reaction mixture (microsomal protein, 0.6 mg/ml) after 3 h incubation at 37°C. Absorbance representing Coomassie blue labeled liposomes, solid line; radioactivity from [^{3}H]retinoic acid, line with diamonds. V_o is the void volume of the column, V_e is the elution volume of liposomes prepared by sonication-centrifugation, and V_t is the total volume of solute plus gel matrix of column bed. Additional details are described in Materials and Methods.

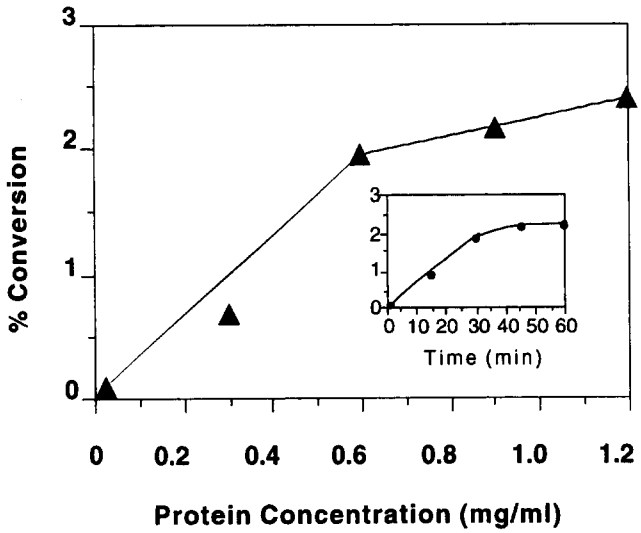

Fig. 3. Determination of optimal incubation conditions. The conversion of liposomal [^{3}H]retinoic acid to [^{3}H]retinoyl glucuronide by native intestinal microsomes was measured at 37°C as a function of microsomal protein concentration and the length of incubation. Three separate experiments were conducted, each at four different protein concentrations and four different time points. The substrate concentration was held constant at 100 μM; time linearity was at 0.6 mg/ml of microsomal protein. Other details of the reaction are described in Materials and Methods.

liver and intestinal microsomal preparations eluted with authentic retinoic acid. A similar chromatographic profile was obtained with the products isolated from the reactions catalyzed by liver microsomal UDPGT (not shown). These results provide indirect evidence that the product from the incubation of retinoic acid with UDPGT is a retinoic acid conjugate with a β-D-glycosidic linkage. Direct proof that RBG was the product was obtained by mass spectral analysis of pooled and purified RBG peaks from intestinal UDPGT-catalyzed reactions (Fig. 4). The negative ion fast atom bombardment mass spectrum in triethylamine had a prominent ion at $[m/z - H]^- = 475$ of 20% relative intensity, corresponding to the deprotonated molecular ion characteristic of RBG [31]. An identical mass spectrum profile was obtained with pooled RBG fractions from reactions with liver microsomes.

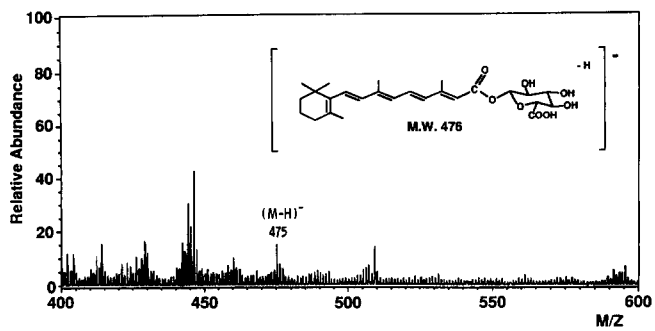

Fig. 4. Mass spectrometry of retinoyl β-D-glucuronide. Mass spectrometry was performed on a JEOL HX 110 mass spectrometer with triethanolamine as the matrix.

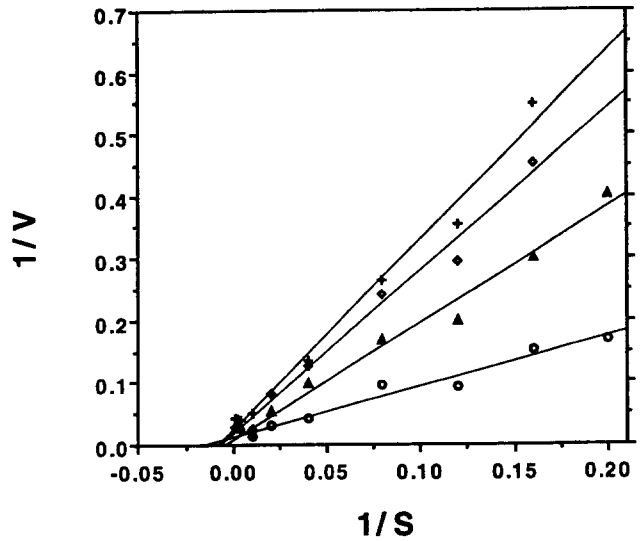

Fig. 5. Double-reciprocal plots of UDP-glucuronosyltransferase activity for the conversion of retinoic acid to retinoyl glucuronide (RBG) in small intestinal and liver microsomes. Liposome-bound retinoic acid was incubated with native microsomes as described in Materials and Methods. Each data point represents the initial velocity of RBG synthesis for three separate experiments. Only the substrate concentrations that gave first order reaction rates are plotted. From top line to bottom line: plus signs, small intestinal microsomes from vitamin A-sufficient rats, apparent $K_m = 173$ μM, apparent $V_{max} = 62$ pmol/min per mg protein; diamonds, liver microsomes from vitamin A-sufficient rats, apparent $K_m = 125$ μM, apparent $V_{max} = 41$ pmol/min per mg protein; triangles, small intestinal microsomes from vitamin A-deficient rats supplemented with 30 μg of retinyl acetate/day for 3 days, apparent $K_m = 91$ μM, apparent $V_{max} = 53$ pmol/min per mg protein; circles, small intestinal microsomes from vitamin A-deficient rats supplemented with 350 mg of all-*trans*-retinoic acid/day for 3 days, apparent $K_m = 105$ μM, apparent $V_{max} = 127$ pmol/min per mg protein. Straight lines were drawn from linear regression values generated by computer from experimentally derived values.

Kinetic analysis of UDPGT activity

The double-reciprocal plots of the initial reaction rates for biosynthesis of all-*trans*-retinoyl β-glucuronide (RBG) from liposome-bound retinoic acid by native microsomes from small intestinal mucosa and liver are shown in Fig. 5. The apparent K_m and V_{max} values for the reactions in small intestinal mucosa and liver with the substrate all-*trans*-retinoic acid are shown in Table I.

Hydrolysis of retinoyl glucuronide catalyzed by microsomal UDPGT

Hydrolysis of radiolabeled RBG was studied with the liposomal system in small intestinal microsomes isolated from vitamin A-sufficient rats. The reaction was optimal at pH 5.1. There was 0.9–1.3% hydrolysis at the concentration of 40 μM all-*trans*-RBG by rat small intestinal microsomes (2 mg of protein/ml) at an

TABLE I

Kinetic values [a] of UDPGT for retinoic acid glucuronidation in rat small intestinal mucosa and liver

Treatment of rats	Liver		Small intestinal mucosa	
	K_m (μM)	V_{max} (pmol/min per mg protein)	K_m (μM)	V_{max} (pmol/min per mg protein)
Vitamin A-sufficient	125±11	41±1	173±17	62± 3
Vitamin A-deficient given retinyl acetate [b]	n.d.	n.d.	91±20	53±10
Vitamin A-deficient given retinoic acid [c]	n.d.	n.d.	105±25	127±16

[a] Values are means±S.D.; $n = 3$. n.d., not determined.
[b] Retinyl acetate, 30 μg/day, was administered to rats for 3 days prior to analysis.
[c] All-*trans*-retinoic acid, 350 mg/day, was administered to rats 3 days prior to analysis.

apparent velocity of 0.4–1.2 pmol/min per mg of protein. Using conditions of constant substrate, co-substrate and protein concentrations, the rate of hydrolysis was decreased 67% in the presence of 7 mM saccharolactone.

Discussion

The focus of this work concerns the question of biological significance of retinoyl β-D-glucuronide (RBG) in the intestinal epithelium, a target site for vitamin A action where RBG is the major metabolite of retinoic acid [7–9,32]. In order to understand the biological significance of RBG we examined the kinetics of retinoic acid glucuronidation using liposomes as a vehicle for retinoic acid so as to provide a glucuronidation environment more similar to that within the cell. In the liposomal system with all-*trans*-retinoic acid as a membrane-associated substrate, liver microsomal UDPGT was four times more active in synthesizing all-*trans*-retinoyl β-D-glucuronide than previously reported [10], and small intestinal microsomal UDPGT was 30-times more active than in a soluble system [22] which suggests that RBG biosynthesis in vivo occurs via a membrane-membrane transfer [33]. However, the mechanism of the enhanced glucuronidation of retinoic acid in the liposomal system is not known. The liposome-bound retinoic acid glucuronidation by native microsomes appears to obey Michaelis-Menten kinetics similar to that shown for bilirubin [34] and for the glucuronidation by small intestinal and liver microsomes was found to be 173 μM and 125 μM, respectively; the specific activities were 62 and 41 pmol/min per mg of protein, respectively. Although the K_m for intestinal glucuronidation of retinoic acid in this system was 33% higher than that for liver, and statistical significance at $P < 0.05$ exists between liver and small intestine and among small intestinal mucosal treatments, the differences are not of a magnitude that warrants an interpretation that the enzymes are different isoenzymes. Similar results have been reported previously [35].

It was shown earlier that in vitamin A deficiency the RBG pool in intestinal epithelium expands [7,8] and that the specific activity of retinoic acid UDPGT in liver is enhanced [10]; these responses are clearly conservation and function related and would not be anticipated if RBG were merely an excretory form of retinoic acid. Our studies presented here provide additional support for a non-excretory physiological role of retinoyl glucuronide in the small intestine. Although the K_m values of the liposome-bound retinoic acid glucuronidation by UDPGT appear to be typical of detoxification reactions, the reaction rates are much lower than those for endogenous substrates or xenobiotics undergoing detoxification (reviewed in Refs. 1–3) and having a planar structure [3,35]. In the liposomal environment the UDPGT demonstrated properties characteristic of both endogenous substrate activation and xenobiotic detoxification enzymes.

In attributing a non-excretory physiological significance to the glucuronidation of retinoic acid in small intestinal mucosa, the high K_m values cannot be explained at this time. Since nanomolar quantities of retinoic acid exist in circulation and tissues [8,31,36], a low-affinity enzyme would seem to have limited usefulness unless retinoic acid concentration is high in a subcompartment of the endoplasmic reticulum of some tissues. It is also likely that an isozyme of UDPGT is allosteric [37] with respect to high- and low-affinity binding and catalytic sites for retinoic acid and UDPGA, and that only the low affinity sites for retinoic acid have been examined in the present work.

In an effort to obtain additional insights into the significance of the above findings, UDPGT activity was examined in microsomes from vitamin A-deficient retinoic acid-induced rats using the liposomal system. While the K_m (105 μM) was similar to that of the UDPGT of vitamin A-sufficient rats, the V_{max} was 3-fold higher, and in the range for inducible enzymes [3] suggesting retinoic acid receptor-mediated regulation of the UDPGT gene.

Our observation of some (0.9–1.3%) hydrolysis of retinoyl glucuronide by UDPGT at pH 5.1 suggests the potential of RBG to serve as a source of the free ligand retinoic acid for binding to nuclear receptors in an environment such as the nuclear membrane. However, contamination of the microsomal preparation by lysosomal membranes, a source of β-D-glucuronidase, can

not be excluded, particularly since β-saccharolactone, a glucuronidase inhibitor, partially (67%) inhibited the UDPGT-catalyzed hydrolysis of RBG. It is possible both UDPGT and β-glucuronidase compete for the substrate retinoyl glucuronide.

In conclusion, our findings suggest that in normal vitamin A nutritional states retinoic acid glucuronidation both in liver and small intestine is a slow, relatively low affinity event, not typical of detoxification. Although biologically not more active than retinoic acid [12,13,38], retinoyl glucuronide may serve as a temporary reserve form of retinoic acid [7,8] or function to protect cells from toxicity of free retinoic acid. More work must be conducted to characterize retinoic acid glucuronidation and to elucidate its physiological significance. Of particular importance will be the study of this reaction in the nuclear envelope since it is known to have UDPGT [39]. Retinoic acid glucuronidation-deglucuronidation may be an important component in the mediation of nuclear action of vitamin A.

Acknowledgements

This work was supported by USDA grants 84-CRCR-1-1377 and 87-CRCR-1-2449 and NIH R29 CA45860. Acknowledgement for their support of this research is made to the Michigan Agricultural Experiment Station. We thank Dr. D. Romsos for a critical review of the manuscript.

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