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Two Types of Microsomal Prostaglandin E Synthase: Glutathione-Dependent and -Independent Prostaglandin E Synthases

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Prostaglandin (PG) E synthase was found to be widely distributed in the microsomal fractions of rat organs. Among them, an extremely high activity was seen in the deferent duct (112 nmol/min · mg) and other genital accessory organs (10-20 nmol/min · mg). In non-genital organs, the kidney had the highest activity (8 nmol/min · mg). Most of the PGE synthase activity in these organs required glutathione (GSH). In contrast, the enzyme activity in the heart, spleen, and uterine microsomes did not require GSH for its catalytic activity. In view of these data and those of other enzymatic parameters (Km values for PGH_2 or pH optima), we suggest that two different types of PGE synthases, GSH-dependent and GSH-independent enzymes, are present in microsomal fractions of rat tissues.

Prostaglandin (PG)E_2 was first discovered in sheep seminal vesicles. PGE_2 is widely distributed in various organs, and exerts control over various biological activities such as smooth muscle dilatation/contraction (cf. ref. 1), Na^+ excretion (2), body temperature (3, 4), and the physiological sleep-wake cycle (4) (cf. ref. 5).

PGE synthase (EC. 5. 3. 99. 3.) catalyzes the conversion of PGH_2 to PGE_2. Several groups tried to purify this enzyme for the last 20 years (6-9). Ogorochi *et al.* (8) and Meyer *et al.* (9) independently purified the enzymes from the cytosol of human brain and *Ascaridia galli*, respectively, and identified them to be glutathione (GSH) *S*-transferases. GSH *S*-transferases catalyze many enzymatic reactions including the synthesis of PGD_2, PGE_2, $PGF_{2\alpha}$, and leukotriene C_4. The membrane-associated enzyme activity may be specific for PGE_2 synthesis because it is topologically coupled to cyclooxygenase in microsomes. However, the distribution and properties of the microsomal PGE synthase activity have not yet been defined. Based on a study of PGE synthase partially purified from bovine vesicular gland, Ogino *et al.* (6) reported that the enzyme required GSH for its activity and an SH-reagent for its stability. Therefore, we examined the tissue distribution of PGE synthase activity in rats both in the presence and absence of GSH, and found that two different types of enzymes are present in rat tissues.

MATERIALS AND METHODS

Materials. [1-^{14}C]PGH_2 (2.07 GBq/mmol) was prepared as described previously (10). Authentic PGs were generous gifts of Ono Pharmaceutical Company, Osaka, Japan. All other chemicals were of reagent grade.

Assay of PGE synthase. The standard reaction mixture contained 0.1 M potassium phosphate buffer (pH 6.0), 40 μM PGH_2 (240,000 dpm), and enzyme in a total volume of 0.05 ml, in the presence or absence of 0.5 mM GSH. Incubation was carried out at 24°C for 1 min. The reaction was started by the addition of [1-^{14}C]PGH_2 and terminated by that of 0.25 ml of diethyl ether/methanol/1 M citric acid (30:4:1 v/v/v). PGE_2 was added to the solution as an authentic marker. The organic phase (0.05 ml) was subjected to thin-layer chromatography in a solvent system of benzene/dioxane/acetic acid (20:20:1 v/v/v). The position of PGE_2 on the silica gel plate was visualized with iodine vapor. Silica gel was scraped off in the sections corresponding to PGE_2 and in other regions, and the radioactivity of each section was measured with a Packard liquid scintillation spectrometer model 2200CA in Scintisol from DOJINDO (Kumamoto, Japan). One unit of enzyme activity was defined as the amount that produced 1 μmol of PGE_2/min at 24°C. Specific activity was expressed as the number of units/mg of protein. Protein was determined after precipitation of protein with trichloroacetic acid according to the method of Lowry *et al.* (11) using bovine serum albumin as a standard.

Preparation of various rat tissues. Eighteen-week-old Sprague-Dawley rats weighing about 200 g (male) and 300 g (female) were sacrificed by perfusion with ice-cold saline under anesthesia with sodium pentobarbital (50 mg/kg of body weight), and tissues were removed quickly. All procedures were carried out at 0-4°C. Tissues were weighed and then cut into small pieces and mixed with 3 volumes of 30 mM potassium phosphate buffer (pH 7.0) containing 0.5 mM dithiothreitol (DTT) and 0.3 M NaCl (Buffer A). Each mixture

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Abbreviations used: PG, prostaglandin; GSH, glutathione; DTT, dithiothreitol.

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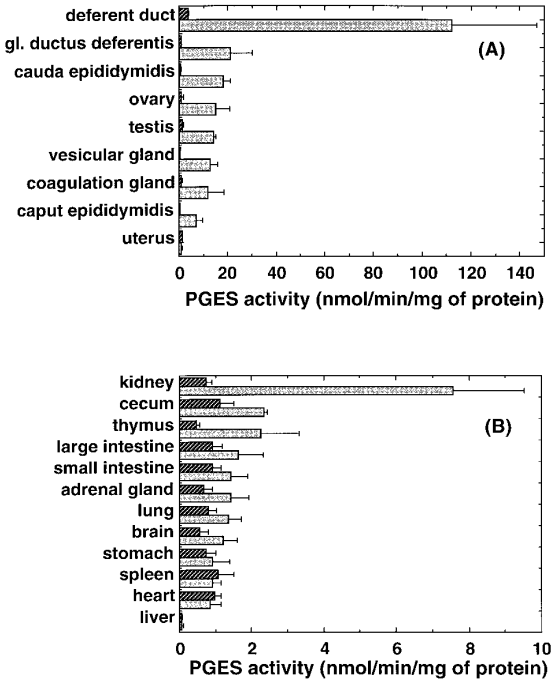

FIG. 1. Distribution of PGE synthase activities of the genital (A) and other (B) tissue microsomes in rats. Male Sprague-Dawley rats weighing about 200 g were sacrificed by perfusion with ice-cold saline. Each weighed tissue was homogenized with 3 volumes of 30 mM potassium phosphate buffer (KPB, pH 7.0) containing 0.5 mM DTT and 0.3 M NaCl (Buffer A). Homogenates were centrifuged at 10,000 × g for 10 min, followed by 105,000 × g for 60 min; and each pellet (microsomes) was rinsed with Buffer A. The rinsed microsomes were used as enzyme sources. The PGE synthase activity was measured by the conversion of [^{14}C]PGH_2 to [^{14}C]PGE_2 in the presence (▨) or in the absence (▨) of 0.5 mM GSH.

was homogenized with a Polytron blender homogenizer, and after centrifugation at 10,000 × g for 10 min, the supernatant was centrifuged at 105,000 × g for 60 min. The precipitate (the microsomal fraction) was rinsed with Buffer A and centrifuged at 105,000 × g for 60 min. The precipitate (the rinsed microsomes) was used as the enzyme source.

RESULTS

Tissue distribution of PGE synthase. The PGE synthase activity of microsomes of various tissues was measured in the presence or absence of GSH. As shown in Fig. 1, the PGE synthase activity in the presence of GSH was high in male reproductive organs (Fig. 1 A): deferent duct (112 nmol/min · mg of protein), glandula ductus deferentis (21.3), cauda epididymidis (18.0), testis (14.2), seminal vesicular gland (13.0), coagulation gland (11.8), and caput epididymidis (7.3). The PGE synthase activity in the presence of GSH was high in the ovary (15.0), but was very low (0.99) in the uterus. In organs besides the genital ones, the GSH-dependent PGE synthase activity was high in kidney (7.6), cecum (2.3), thymus (2.3), and was almost the same in other organs (1-1.6), whereas the activity in liver was negligible (0.06) (Fig. 1 B). The PGE synthase activity in the absence of GSH was lower than that in the presence of GSH in almost all tissues except for heart, spleen, and uterus. The activity in the absence of GSH was the highest in the deferent duct (3.5 nmol/min · mg of protein), which represented 3% of the PGE synthase activity in the presence of GSH. The enzyme activities in the absence of GSH of the genital organs were 2-10% of those in the presence of GSH except in the case of the uterus. These results indicate that genital organs, especially the deferent duct, have mainly PGE synthase activity requiring GSH.

In contrast, PGE synthase activities of heart, spleen, and uterus in the absence of GSH were 112%, 116%, and 123%, respectively, relative to those in the presence of GSH, thus indicating the presence of only the GSH-independent enzyme in these tissues.

Comparison of enzymatic properties of GSH-dependent and GSH-independent PGE synthases. We compared the enzymatic properties of GSH-dependent and GSH-independent PGE synthases using deferent duct and heart microsomes, respectively.

As shown in Fig. 2, deferent duct microsomes catalyzed the conversion of PGH_2 to PGE_2 in the presence of GSH (Fig. 2B), and the activity in the absence of GSH was only 3% of that in the presence of GSH (Fig. 2C). The small peak of Rf value of 0.5 was the same as the Rf value of PGD_2. On the other hand, the conversion of PGH_2 to PGE_2 by heart microsomes in the presence of GSH was to the same extent as that in the absence of GSH (Fig. 2E & F). The PGE synthase activity of the deferent duct was stimulated by GSH and almost saturated at the concentration of 0.2 mM (Fig. 3A). However, the PGE synthase activity of heart was not stimulated at all by GSH (Fig. 3B). These results indicate that PGE synthase of deferent duct microsomes is the GSH-dependent enzyme and that the enzyme is different from PGE synthase of heart microsomes, which is the GSH-independent enzyme.

The Km value of deferent duct microsomal PGE synthase activity for PGH_2 was 25.7 μM (Fig. 4A), and that of heart microsomal PGE synthase activity was 82.6 μM (Fig. 4B). Moreover, the optimum pH of deferent duct microsomal PGE synthase activity was 6-7, and the GSH-dependent PGE synthase activity decreased at above pH 8 (Fig. 4C). The optimum pH of heart microsomal PGE synthase activity was about pH 6-8, and the enzyme activity slightly decreased at pH 9 (Fig. 4D). However, the enzyme activity at pH 9-12 did not decrease, unlike the case for the deferent duct microsomal PGE synthase activity. These results suggest that the GSH-dependent PGE synthase activity decreased at alkaline pH owing to the pKa of 9.1 for GSH.

The above findings indicate that deferent duct microsomal PGE synthase is different from heart microsomal

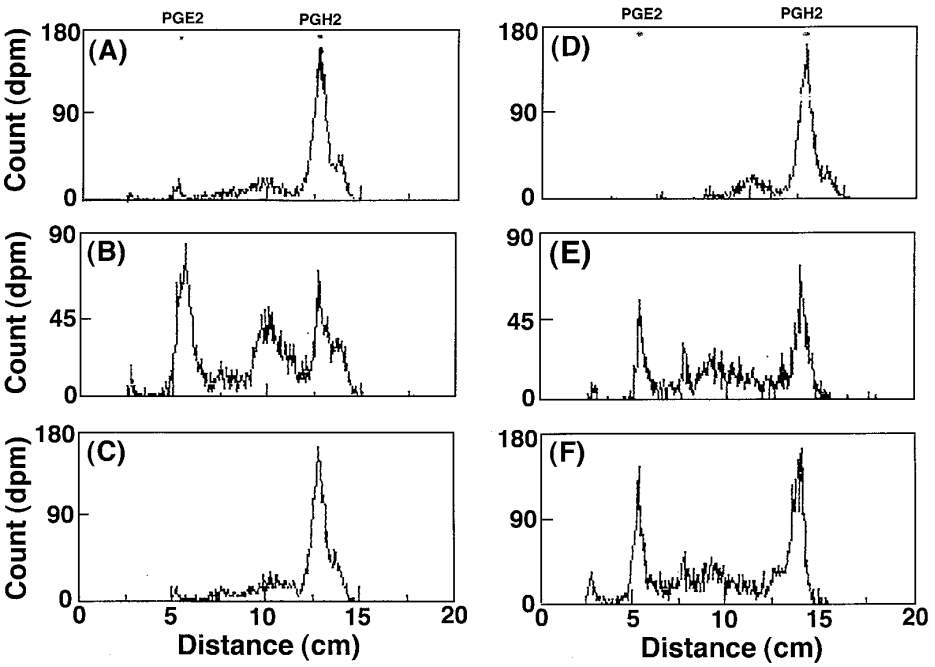

FIG. 2. Enzymatic conversion of PGH_2 by heart and deferent duct microsomes in the presence or absence of GSH. Deferent duct microsomes and 4 μM [1-^{14}C]PGH_2 (24,000 dpm) were incubated in the presence (B) or absence (C) of GSH. Heart microsomes and 4 μM [1-^{14}C]PGH_2 (24,000 dpm) were incubated in the presence (E) or absence (F) of GSH. The control incubations were carried out without the enzymes in the presence of GSH (A) or absence of GSH (D). Thin layer chromatography was carried out with a solvent system of ethyl ether/methanol/glacial acetic acid (90:2:0.1 v/v/v).

PGE synthase. PGE synthases of genital organs except the uterus were mainly the GSH-dependent enzyme, and those of heart, spleen, and uterus were the GSH-independent enzyme.

DISCUSSION

The GSH-dependent PGE synthase was mainly distributed in the genital organs. The highest GSH-dependent PGE synthase activity was observed in the deferent duct, followed by other genital organs of male and female. In rodents, the highest concentration of PGE_2 is found in the deferent duct relative to its level in other reproductive tissues (12-14). The distribution of PGE synthase activity in rats seems to agree with that of PGE_2 in these animals reported by several groups (12-14), who suggested that PGE_2 plays an important role in regulating the contractility of the excurrent

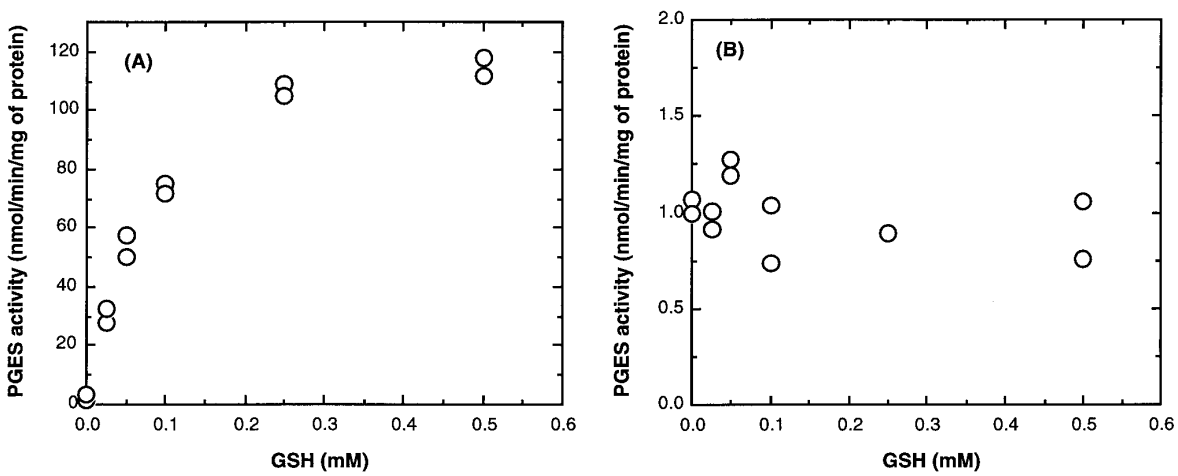

FIG. 3. Effects of GSH on the PGE synthase activities of deferent duct (A) and heart (B) microsomes of rats. The PGE synthase activity was assayed under the standard conditions except that the concentrations of GSH were varied.

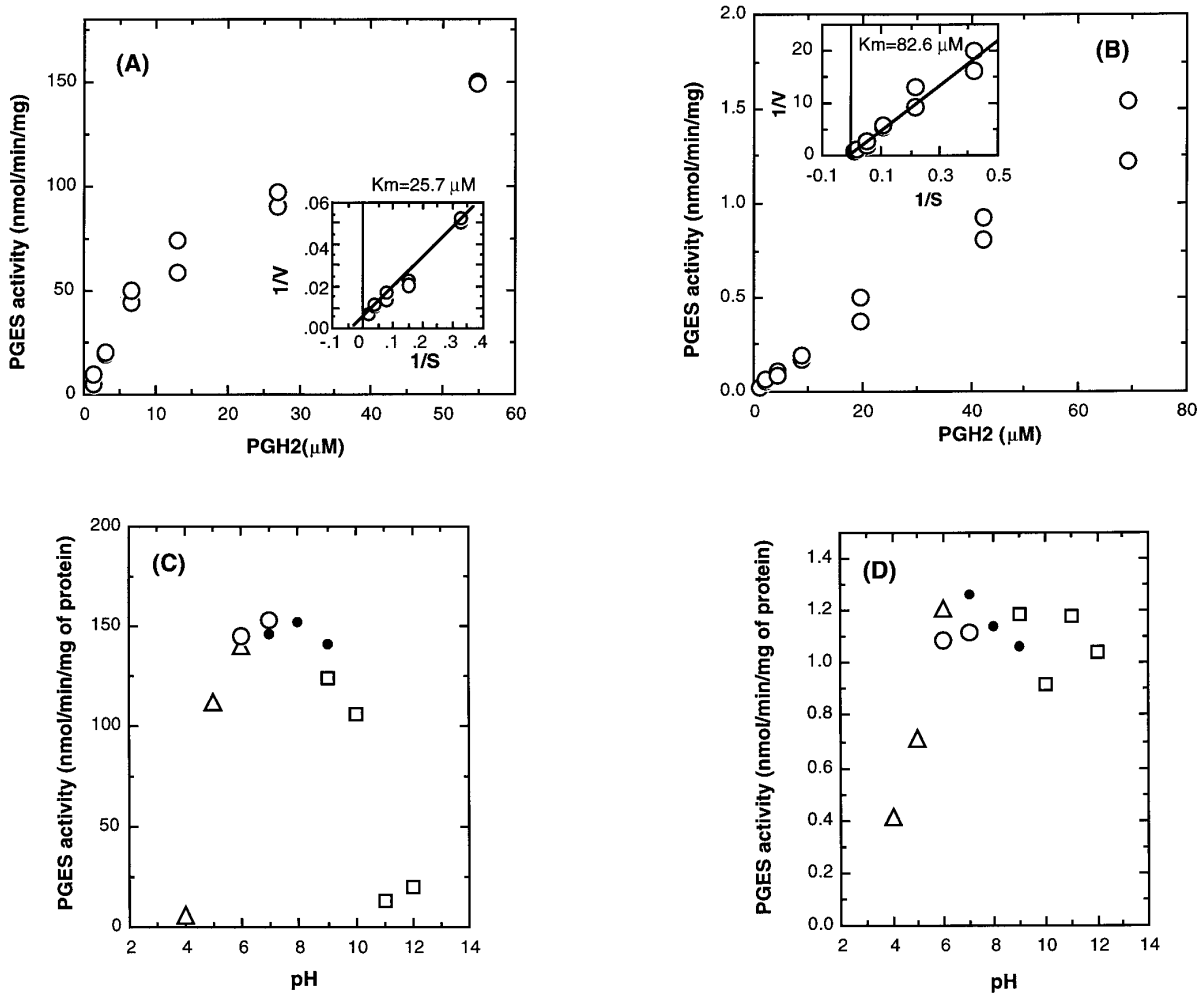

FIG. 4. Substrate dependency of PGE synthases by deferent duct (A) and heart (B) microsomes, and pH dependency of PGE synthesis by deferent duct (C) and heart (D) microsomes. 0.1 M acetate buffer (△); 0.1 M potassium phosphate buffer (○); 0.1 M Tris-HCl buffer (●); and 0.1 M glycine buffer (□).

ducts. Moreover, in the tissues aside from the genital organs, the GSH-dependent PGE synthase showed the highest activity in the kidney. In the kidney, PGE_2 synthesized by collecting tubule epithelia in response to arginine vasopressin (AVP) acts on the parent collecting tubules as well as on the neighboring thick limbs to modulate NaCl and water reabsorption in response to AVP (cf. ref. 15). PGE_2, which exhibits these biological actions in the kidney, may be synthesized by the GSH-dependent PGE synthase.

PGE_2 also shows biological functions in heart, spleen and uterus; for there are PGE receptors on the cardiac sarcolemma (16), and PGE_2 is a suppressor of immune responses (17) and a placental stimulator of ovine placental secretion of progesterone (18). Although PGE synthase activities in microsomes of heart, spleen, and uterus were lower than those in the genital organs, PGE_2 plays an important role in the former. We discovered a novel type of PGE synthase in these tissues: the GSH-independent PGE synthase (Figs. 1-3). In the other tissues, the PGE synthase activities in the absence of GSH were 20-80% compared with those in the presence of GSH, and the synthesis of PGE_2 in the other tissues may be accomplished by both GSH-dependent and -independent PGE synthases.

The PGE synthases purified from the cytosolic fractions of human brain (8) and *Ascardia galli* (9) were identified to be GSH *S*-transferases (sigma-class for that from *Ascardia galli*). Although the sigma-class GSH *S*-transferase shows high specificity for the GSH-dependent isomerization of PGH_2 to PGE_2, this enzyme is located in the cytosolic fraction. However, PGE synthase activity is also located in microsomes of various tissues, and the microsomal PGE synthase seems to be specific for the synthesis of PGE_2, as cyclooxygenase is present in microsomes. The nature of microsomal GSH-independent PGE synthase (a novel-type PGE synthase) as well as that of the microsomal GSH-depen-

dent PGE synthase should become better known following their purification.

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