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THROMBOXANE SYNTHASE FROM BOVINE LUNG - SOLUBILIZATION AND PARTIAL PURIFICATION

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SUMMARY

Prostaglandin endoperoxide synthase and thromboxane synthase were both localized mainly in the microsomal fraction of bovine lung. The capacity to convert prostaglandin H_2 into TXB_2 (thromboxane synthase activity) exceeded the capacity to transform arachidonic acid into products. Thromboxane synthase of lung microsomes was solubilized with Triton X-100 and partially purified by DEAE cellulose chromatography. The preparation thus obtained catalyzed the conversion of PGH_2 to a mixture of TXB_2 and HHT, whereas PGH_1 was predominantly converted to HHD.

INTRODUCTION

Two partial reactions are involved in enzymic transformation of arachidonic acid into PGs and PG-like compounds: 1) initial conversion of arachidonic acid into the unstable intermediates PGG_2 and PGH_2 and 2) further conversion of PGH_2 into final products (1). While the biosynthetic pathway of PGH_2 formation seems to be identical in all tissues investigated, subsequent conversion of the endoperoxide follows a tissue-specific direction and results in formation in different tissues of different end-products (2). Several tissues including blood platelets, lung, spleen and brain, produce potent bioregulators known as thromboxanes (3). The thromboxane-forming enzyme system in human platelets has recently been solubilized and resolved into 2 components, prostaglandin endoperoxide synthase (which catalyzes the reaction 20:4→PGG_2→PGH_2) and thromboxane synthase which isomerizes PGH_2 to

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Abbreviations: PG, prostaglandin; TXA_2, thromboxane A_2; TXB_2, thromboxane B_2; HHD, 12L-hydroxy-8,10-heptadecadienoic acid; HHT, 12L-hydroxy-5,8,10-heptadecatrienoic acid; TLC, thin-layer chromatography.

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TXA_2 (4) and also catalyzes the formation of HHT from PGH_2 (5).

To purify thromboxane synthase a source other than platelets seemed desirable. This paper describes the solubilization and partial purification of an enzyme from bovine lung similar to human platelet thromboxane synthase.

MATERIALS AND METHODS

Prostaglandins E_2, $F_{2\alpha}$, D_2, and thromboxane B_2 were obtained from the Upjohn Company, Kalamazoo, Michigan, U.S.A., arachidonic acid from the Hormel Institute, Austin, Minn., U.S.A., and [1-^{14}C]arachidonic acid (54 Ci/mol) from the Radiochemical Centre, Amersham, U.K. [1-^{14}C]PGH_2 (sp. act. 1 Ci/mol), [1-^{14}C]PGH_1, and 12L-hydroxy-5,8,10-heptadecatrienoic acid (HHT) were prepared according to published methods (6,7). DEAE cellulose (DE-54) was from Whatman and Silica gel G from Merck.

Bovine lungs were obtained from the slaughter house and immediately frozen on dry ice. Portions were dissected, carefully cleaned and homogenized in 3 volumes of 0.1 M potassium phosphate buffer pH 7.4. Subcellular fractions were obtained by differential centrifugation. The 1500 x g (10 min) supernatant obtained after removal of cell debris is referred to below as homogenate. The fraction that sedimented at 9000 x g (15 min) is referred to as mitochondria and the 100 000 x g (60 min) sediment as microsomes. Both fractions were resuspended in the same buffer to give a concentration corresponding to 2 g tissue per ml.

Incubations were performed in 0.1 M phosphate buffer pH 7.4; details are given in appropriate legends. With [1-^{14}C]arachidonic acid as substrate incubations were terminated with 7 volumes chloroform/methanol 1:1 v/v. Precipitated protein was removed by centrifugation and lipids were extracted as elsewhere described (8).

Incubations with [1-^{14}C]PGH_2 and [1-^{14}C]PGH_1 were stopped with ethanol containing 5 mg/ml $SnCl_2$ and products were extracted with ethyl acetate (1). After methylation with diazomethane (15 min at room temperature) they were analyzed by TLC in diethyl ether/methanol 49:1 v/v (solvent I) and ethyl acetate/2,2,4-trimethylpentane/water 5:10:10 (solvent II). For separation of products in the form of free acids the system toluene/dioxane/acetic acid 70:30:2 was used (solvent III).

Thin-layer chromatograms were scanned for radioactivity with a Berthold Dünnschicht Scanner II. The radioactive zones were scraped off and counted in a Packard Tri-Carb liquid scintillation spectrometer model 3375.

The reference compounds were visualized by iodine vapors. DEAE cellulose chromatography was performed as described (4). Protein was determined by Lowry's method (9) with serum albumin as standard.

RESULTS

Incubation of [1-^{14}C]arachidonic acid with subcellular fractions of bovine lung homogenate resulted in formation of 2 predominant products which on thin-layer plates in two different solvent systems (solvents I and III) closely corresponded to TXB_2 and HHT used as references. The microsomal

fraction had the highest capacity to transform the fatty acid into products (Table I). The conversion was, however, low and did not exceed 10% of the substrate. Practically no enzyme activity was present in the 100 000 x g supernatant. On the other hand, when [1-^{14}C]PGH_2 was used as substrate (Table II), rapid conversion into products was observed. Highest enzyme activity appeared to be present in the microsomal fraction which almost quantitatively transformed PGH_2 into TXB_2 and HHT under the assay conditions used (see also Fig. 1, upper panel).

Lung microsomes were solubilized with Triton X-100 as described for platelet microsomes (4). 2 ml of the clear 100 000 x g (1 hr) supernatant corresponding to 4 g lung tissue (about 16 mg protein) was applied to a 5 ml DEAE cellulose column (1 x 5 cm) equilibrated in 10 mM potassium phosphate buffer pH 7.4 - 0.1% Triton X-100. The column was first eluted with 30 ml of the same buffer (Fraction 1), then with 10 ml of 20 mM phosphate buffer - 0.1% Triton X-100 (Fraction 2) and finally with 10 ml of 0.2 M potassium phosphate buffer - 0.1% Triton X-100, pH 7.4 (Fraction 3). The eluates were concentrated to 2 ml each by ultrafiltration. The capacity of the various preparations to transform [1-^{14}C]PGH_2 into products is shown in Table III and Fig. 1. More than 70% of the thromboxane-forming activity appeared in the supernatant after solubilization of the microsomes and about 30% of the activity remained in the 100 000 x g sediment. The solubilized enzyme was quantitatively eluted from the DEAE cellulose column with 0.2 M buffer and practically no activity was found in the two preceding fractions.

Fig. 1 and Table III also show that preparations containing thromboxane synthase activity produced HHT in similar quantities as TXB_2. Solubilized microsomes and column eluates were further incubated with [1-^{14}C]PGH_1 and the products were separated by TLC in solvent systems I and II. No TXB_1 was detected by TLC (Fig. 2). The predominant product formed from PGH_1 by either solubilized microsomes or Fraction 3 co-chromatographed with HHD (72.5 and 73% of substrate converted, respectively). In contrast, the yield of HHD

TABLE I Conversion of [1-^{14}C]arachidonic acid by subcellular fractions of bovine lung

Samples corresponding to 0.5 g lung tissue were incubated at 37°C with 5 µg (16.7 nmoles) of [1-^{14}C]arachidonic acid (100 000 cpm) for 10 min in 0.1 M potassium phosphate buffer pH 7.4 (final volume 1 ml). Products were extracted and analyzed as described in Methods.

	TXB_2 %	HHT %
Homogenate	4.5	5.2
Mitochondrial fraction	3.1	4.1
Microsomal fraction	5.1	5.7
100 000 x g supernatant	0.3	1.8

TABLE II Product formation from [1-^{14}C]PGH_2 by subcellular fractions of bovine lung

Samples corresponding to 0.5 g tissue were incubated with 5 µg (16.7 nmoles) of [1-^{14}C]PGH_2 (30 000 cpm) for 1 min at 37°C in 0.1 M potassium phosphate buffer pH 7.4 (final volume 1 ml). Reaction was stopped with 5 ml ethanol containing 5 mg/ml $SnCl_2$. Products were extracted and analyzed as described in Methods.

	TXB_2 %	HHT %
Homogenate	29.5	34
Mitochondrial fraction	30.5	38
Microsomal fraction	45	44
100 000 x g supernatant	7	21

with Fractions 1 and 2 was only 9 and 12%, respectively. With these fractions, the substrate was converted to PGE_1 and PGD_1 by spontaneous decomposition in aqueous medium (1) and to $PGF_{1\alpha}$ by $SnCl_2$ reduction.

DISCUSSION

The enzymic activity in bovine lung microsomes which catalyzes conversion of PGH_2 into products appeared to possess properties similar to the thromboxane synthase of human blood platelets (4,5). It could be solubilized with Triton X-100 and quantitatively eluted from DEAE cellulose with a buffer

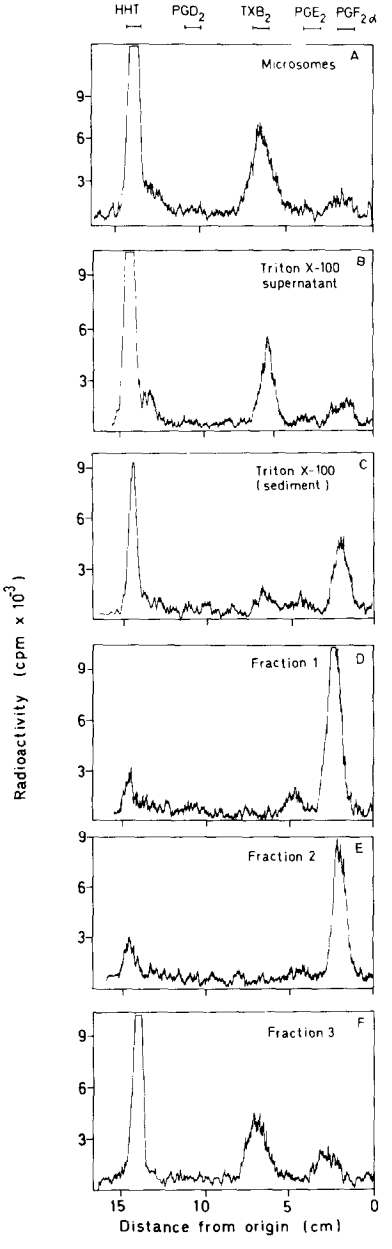

Fig. 1 Thin-layer radiochromatograms of products formed from [1-^{14}C]PGH_2 (12 nmoles, 20 000 cpm) during 1 min incubation at 37°C with A) lung intact microsomes, B) solubilized microsomes (supernatant), C) 100 000 x g sediment of solubilized microsomes, D) Fraction 1, E) Fraction 2, F) Fraction 3. Each preparation contained material corresponding to 0.5 g lung tissue. Excess PGH_2 was reduced to $PGF_{2\alpha}$ with $SnCl_2$ in ethanol at the end of the incubation. Solvent system-diethyl ether/methanol, 49:1 vol/vol. The positions of the reference compounds are indicated at the top of the figure.

TABLE III Product fromation from [1-^{14}C]PGH_2 by preparations of lung microsomes

Portions of lung microsomal fraction, solubilized microsomes, and eluates of DEAE cellulose column, were incubated with 16.7 nmoles of [1-^{14}C]PGH_2 (30 000 cpm) for 2 min at 37°C in 0.1 M potassium phosphate buffer, pH 7.4. Each sample contained material corresponding to 0.5 g original lung tissue and the final volume was 0.5 ml. The reaction was stopped with 3 ml ethanol containing 15 mg $SnCl_2$. Products were extracted and analyzed as described in Methods.

	TXB$_2$		HHT	
	%[x]	rel.[xx]	%[x]	rel.[xx]
Intact microsomes	42	100	44	100
Solubilized microsomes (supernatant)	30	72	37	84
Solubilized microsomes (sediment)	13	31	21	48
Fraction 1	2	5	7	16
Fraction 2	2.5	6	8	18
Fraction 3	29.5	70	38	87

x) of recovered radioactivity in product

xx) relative to the conversion by intact microsomes

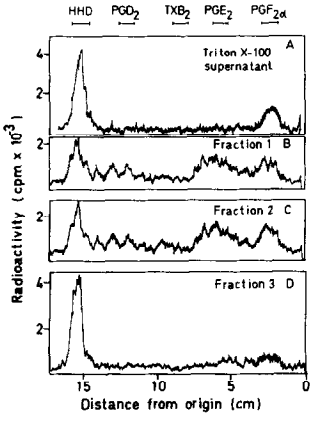

Fig. 2 Thin-layer radiochromatograms of products formed from [1-^{14}C]PGH_1 (10 nmoles, 12 000 cpm) during 2 min incubation at 37°C with A) 100 000 x g supernatant of solubilized lung microsomes, B) Fraction 1, C) Fraction 2, D) Fraction 3 (material corresponding to 0.5 g lung tissue in each preparation). Other details as in Fig. 1.

solution of proper pH and ionic strength. Like the platelet enzyme, thromboxane synthase from bovine lung also converted prostaglandin endoperoxides into C_{17}-hydroxy acids (5). The rather small quantities of HHT formed from PGH_2 in preparations lacking thromboxane synthase activity are likely to be non-enzymatically produced. It was found that native and boiled (7 min at 100°C) sheep vesicular gland microsomes (which lack the thromboxane synthase) produced quite similar quantities of HHT from PGH_2. Also in buffer about 10% of PGH_2 was converted into C_{17}-hydroxy acid (results not shown). On the other hand, both intact and solubilized lung microsomes as well as Fraction 3 produced HHT in yields comparable to those of TXB_2. Moreover, PGH_1 was predominantly transformed into HHD by fractions containing thromboxane synthase, but not by those which did not possess this enzyme activity (Fig. 2). These results suggest that in bovine lung, like in human platelets, TXB_2 and HHT are formed by the same enzyme.

Bovine lung microsomes also possessed prostaglandin endoperoxide synthase activity, as judged by their capacity to transform arachidonic acid into TXB_2 and HHT. The activity of the two enzymes was quite different; while only a small part of arachidonic acid was converted into products (Table I), PGH_2, in the same concentration, was almost quantitatively transformed into TXB_2 and HHT. A similar discrepancy between the enzymic potencies of the 2 components of the "prostaglandin synthase" may exist in some other tissues as well. Thus, microsomes of both cow and guinea-pig uterus have a very low capacity to form prostaglandins from arachidonic acid, but very actively transform PG endoperoxides into $PGF_{2\alpha}$ (10). Blood vessel microsomes might be capable of converting some arachidonic acid into products (11), but in a very low yield, while conversion of PGH_2 into PGI_2 is almost quantitative (12). Thus, the rates of the two component reactions of the "prostaglandin synthase" are in some cases quite different, slow in formation of endoperoxide intermediates and fast and efficient in their further transformation to final products. The second reaction determines the interesting diversifi-

cation of prostaglandin synthase, which results in formation of different end-products in different tissues (2). The products may have special physiological significance for the tissue in question (thromboxanes in platelets and lung, $PGF_{2\alpha}$ in uterus, PGI_2 in blood vessels). One may speculate that, since these end-products are potent bioregulators, finely balanced mechanisms exist which keep their formation in physiologically favorable limits, conceivably by regulating the rate of formation of the intermediate endoperoxide from the fatty acid.

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