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ISOLATION OF AN ACTIVATOR FOR PROSTAGLANDIN HYDROPEROXIDASE FROM BOVINE VESICULAR GLAND CYTOSOL AND ITS IDENTIFICATION AS URIC ACID*

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SUMMARY The enzymatic conversion of prostaglandin G_1 to H_1 was stimulated by an activator present in the cytosol of bovine vesicular gland. The activator was purified by Sephadex G-25 gel filtration and Dowex 1 column chromatography. The purified activator was identified to be uric acid by thin layer chromatography, ultraviolet and infrared absorption spectroscopy and combined gas chromatography-mass spectroscopy. Among various purine compounds tested, only uric acid and 2,8-dihydroxyadenine were active.

Two PG[1/] endoperoxides, PGG and PGH, are produced from polyunsaturated fatty acid by the successive reactions of fatty acid cyclooxygenase (1) and PG hydroperoxidase (2). The cyclooxygenase is a dioxygenase incorporating two molecules of oxygen into PGG as an endoperoxide and a hydroperoxide, respectively. On the other hand, the PG hydroperoxidase is a peroxidase-like enzyme, which converts the 15-hydroperoxide of PGG to a hydroxyl group

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1/ Abbreviation used: PG, prostaglandin.

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and produces PGH (2-4). These two enzymes are still unresolved, and the two reactions are catalyzed by an apparently homogeneous enzyme preparation purified from vesicular gland microsomes (PG endoperoxide synthetase) (2, 3). Heme is required for both reactions (2, 3). In addition, a variety of compounds were shown to stimulate the conversion of PGG to PGH catalyzed by the PG hydroperoxidase; hydroquinone, epinephrine, guaiacol, benzidine, tryptophan and others (2-4). This paper will describe another activator for PG hydroperoxidase, which was isolated from the cytosol of bovine vesicular gland and identified to be uric acid.

MATERIALS AND METHODS

8,11,14-[1-^{14}C]Eicosatrienoic acid (56 mCi/mmol) was purchased from New England Nuclear (Boston). [1-^{14}C]PGG_1 was prepared using manganese protoporphyrin IX by the method described previously (2, 5). [2-^{14}C]Uric acid (51 mCi/mmol) was obtained from the Radiochemical Centre (Amersham). 8,11,14-Eicosatrienoic acid and PGB_1 were kindly donated by the Ono Central Research Institute. Manganese protoporphyrin IX was a gift from Professor T. Yonetani, University of Pennsylvania. Uric acid was obtained from Wako Pure Chemical Industries (Osaka) and 2,8-dihydroxyadenine from Sigma. Silica gel plates 60 F_{254} and cellulose plates for thin layer chromatography were supplied from E. Merck (Darmstadt).

PG endoperoxide synthetase was purified from bovine vesicular gland (3). (A) The PG endoperoxide synthetase activity (from 8,11,14-eicosatrienoic acid to PGH_1), (B) the PG hydroperoxidase activity (from PGG_1 to PGH_1), and (C) the fatty acid cyclooxygenase activity (from 8,11,14-eicosatrienoic acid to PGG_1) were assayed as described previously (2, 3, 5). In all the assays the enzyme activity was corrected for non-enzymatic reactions.

Phosphate was determined by the method of Ames (6), and protein by the method of Lowry *et al.* (7). A Shimadzu automatic recording spectrophotometer model UV-300 was employed in spectrophotometric determination. Infrared absorption spectrum was recorded in a KBr disk using a JASCO spectrophotometer model IRA-1. Combined gas chromatography-mass spectroscopy was performed with a JEOLCO spectrometer model D-300. A column of OV-1 on chromosorb WAW DMCS (2%, 1 m) was used with helium as carrier gas and programmed from 150°C increasing at 10°C/min. The electron impact technique and ionization at 30 eV were employed.

RESULTS

As shown in Fig. 1A, when the enzyme was reacted with 8,11,14-eicosatrienoic acid (Compound I) in the presence of hematin alone, PGG_1 (III) and PGH_1 (IV) were produced each in a small amount. In contrast, the addition of the concentrated activator obtained from the cytosol of bovine vesicular gland (see below) resulted in the accumulation of PGH_1 (IV) in a larger quantity (Fig. 1B). This effect of the activator was similar to the reported stimulation of PGH_1 synthesis by tryptophan (2, 3). Compound II produced in a small quantity remained unidentified.

Bovine vesicular glands (10 g) were homogenized in a Waring blendor with 27 ml of 20 mM potassium phosphate buffer at pH 7.4. The homogenate was centrifuged at 10,800 X g for 10 min. The supernatant fluid (32 ml) was centrifuged at 105,000 X g for 120 min. The supernatant solution (31 ml) was subjected to ultrafiltration using a Diaflo membrane PM-10. The filtrate thus obtained (30 ml) was applied to a Sephadex G-25 column (3.2 X 65 cm) equilibrated with 10 mM potassium phosphate buffer at pH 7.4 (Fig. 2A). Two major peaks appeared as detected by absorption at 280 nm. The activator followed by the PG endoperoxide synthetase assay appeared as the second peak (Fraction Nos. 48-54). Active fractions were combined and applied to a Dowex 1 X 2 column (formate form, 2.1 X 1.5 cm). The column was washed thoroughly with 300 ml of water and then the activator was eluted with 23 mM formic acid (Fig. 2B). Phosphate was not detectable in the eluate containing the activator. Formic acid eluted with the activator was removed by lyophilization. By this procedure, 1.5 mg of the activator as dry powder were obtained.

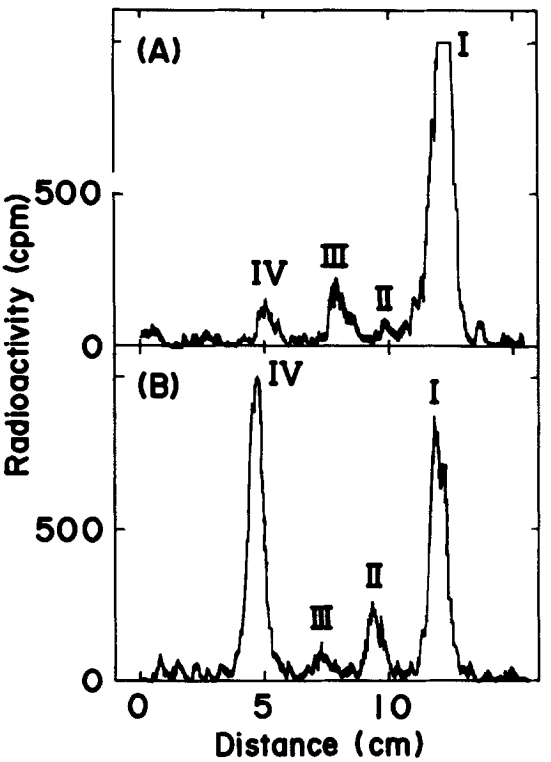

Fig. 1. PG endoperoxide syntheses in the absence and presence of the activator. The enzyme (3.5 μg) was incubated with 8,11,14-[1-^{14}C]eicosatrienoic acid in the standard assay mixture A. (A) 5 mM tryptophan omitted, (B) 5 mM tryptophan replaced by the activator (Fraction No. 51 in Fig. 2A). Monitored by a Packard radiochromatogram scanner model 7201.

Upon gas chromatography the purified activator permethylated by the methyl sulfinyl carbanion technique (8), gave two peaks (retention time, 6.7 and 10.3 min) each having a molecular ion peak at m/e 224. High resolution mass spectrometry of these two peaks (224.0912 and 224.0909) indicated their molecular formulae to be $C_9H_{12}N_4O_3$ (calculated, 224.0909). By perdeuteromethylation these compounds showed molecular ion peaks at m/e 236, suggesting incorporation of four deuteromethyl groups into the activator. Thus, the molecular formula of the activator was presumed to be $C_5H_4N_4O_3$, which was consistent with that of uric acid.

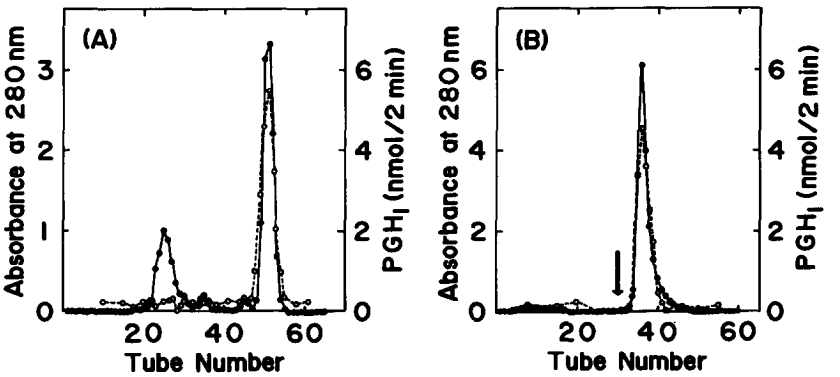

Fig. 2. (A) Sephadex G-25 gel filtration of the activator. Each 19-ml fraction was concentrated to 1 ml by lyophilization, and a 40-µl aliquot was removed for the assay. (B) Dowex 1 column chromatography of the activator. Elution with 23 mM formic acid was performed as indicated by the arrow. Each 15-ml fraction was lyophilized, and the dried material was dissolved in 1 ml of water. A 20-µl aliquot was used for the assay A in which tryptophan was replaced by the activator (o----o). Absorbance at 280 nm of each fraction was measured (●——●).

The purified activator was examined by silica gel thin layer chromatography in a solvent system of n-butyl alcohol/acetic acid/water (12/3/5) and by cellulose thin layer chromatography in n-butyl alcohol/pyridine/water (1/1/1). A single spot comigrating with uric acid (R_F 0.42 and 0.48) was observed in either case, when the plate was exposed in iodine fume. The ultraviolet absorption spectrum of the activator was indistinguishable from that of uric acid with maxima at 205, 234 and 290 nm in 10 mM potassium phosphate buffer at pH 7.4. The infrared absorption spectrum of the activator was essentially identical with that of uric acid. On the basis of the molecular weight of 168.11, the activator and the authentic uric acid at the same molar concentration stimulated the PGH_1 synthesis from 8,11,14-eicosatrienoic acid essentially to the same extent. The extent of the stimulation by the activator was almost identical with that by L-tryptophan (Fig.3A).

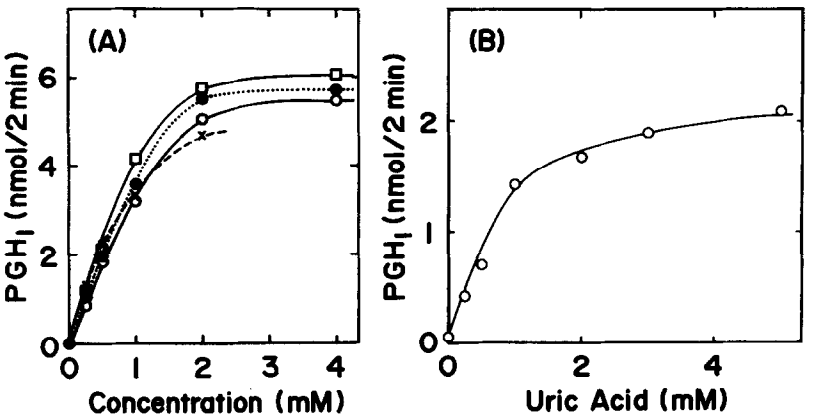

Fig. 3. (A) Stimulatory effect of uric acid and 2,8-dihydroxyadenine as compared with the activator and tryptophan. (A) The PG endoperoxide synthetase activity was determined in the standard assay mixture A in which 5 mM tryptophan was replaced by compounds as indicated. (●), uric acid; (X), 2,8-dihydroxyadenine; (□), tryptophan; (o), the purified activator. (B) Effect of uric acid on the PG hydroperoxidase activity. The enzyme activity was determined by the assay B.

There was no additive stimulatory effect in the presence of both uric acid and tryptophan.

In addition to uric acid, various other purine compounds were tested. As shown in Fig. 3A, 2,8-dihydroxyadenine was as active as uric acid. None of the followings was effective; xanthine, hypoxanthine, guanine, adenine, allantoin, xanthosine, inosine, guanosine, adenosine, xanthosine 5'-monophosphate, inosine 5'-monophosphate, guanosine 5'-monophosphate, adenosine 5'-monophosphate, allopurinol, 3-iso-butyl-1-methylxanthine, allantoic acid and urea. Following pyrimidine compounds were also ineffective; cytosine, uracil, thymine, barbituric acid, isobarbituric acid, dihydrouracil, dihydrothymine, hydroxymethyluracil, orotic acid, dihydroorotic acid and alloxan.

The manganese protoporphyrin-assisted PGG_1 synthesis from 8,11,14-eicosatrienoic acid (the cyclooxygenase activity) showed essentially no requirement for uric acid. In contrast, the con-

version of PGG_1 to PGH_1 in the presence of hematin (the PG hydroperoxidase activity) was markedly stimulated by the addition of uric acid and the saturating concentration was on the order of mM (Fig. 3B). A stoichiometric transformation of [2-^{14}C]uric acid during the PG hydroperoxidase reaction was not clearly demonstrated due to a non-enzymatic degradation of uric acid.

DISCUSSION

It was earlier found that the microsomal conversion of 8,11,14-eicosatrienoic acid to PGE_1 was stimulated by the addition of the cytosol of sheep vesicular gland (9, 10). An attempt to identify this activator in the cytosol revealed that the activation of the PGE_2 synthesis from arachidonic acid was due to the contaminating hemoglobin (11). The over-all PGE synthesis was also found to be stimulated by hydroquinone (12), catecholamines (13) and phenol (14). Our further investigation of the cytosol activator added tryptophan and other indole compounds to the activator group (3, 15). With a purified enzyme preparation it was demonstrated that these activators stimulated the step of PGG to PGH (2-4) and also protected the enzyme from inactivation upon the interaction with heme (5). As described in this paper, our further investigation led to the detection of another activator for the PG hydroperoxidase reaction. This compound was purified and identified to be uric acid. It remains to be clarified, however, whether or not the uric acid is dehydrogenated like epinephrine and guaiacol (2). This is also the case with tryptophan (2). If uric acid works *in vivo* as well as *in vitro* as an activator for the PG hydroperoxidase reaction, this is a hitherto unknown physiological function of uric acid.

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