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COMMUNICATION

NADP-Linked 15-Hydroxyprostaglandin Dehydrogenase for Prostaglandin D_2 in Human Blood Platelets[1]

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Two forms of NADP-linked 15-hydroxyprostaglandin dehydrogenase for prostaglandin D_2 were found in the cytosol fraction of human blood platelets. These enzymes were purified by ammonium sulfate fractionation, Blue Sepharose, and Sephadex G-100 column chromatography. The two enzymes differed in molecular weights (65,000 for peak I enzyme and 31,000 for peak II as estimated by gel filtration) and their substrate specificities. The relative rates for reaction with peak I enzyme were: prostaglandin D_2, 100(%); E_2, 14; $F_{2\alpha}$, 2; I_2, 29; and B_2, 0, whereas for peak II enzyme, D_2, 100; E_2, 23; $F_{2\alpha}$, 61; I_2, 29; and B_2, 131. Prostaglandin D_2 was converted to 15-ketoprostaglandin D_2 and then 13,14-dihydro-15-ketoprostaglandin D_2, which were identified by spectrophotometry and gas chromatography/mass spectrometry, respectively. These metabolites were three orders of magnitude less potent in inhibiting human platelet aggregation than prostaglandin D_2. The results indicated that NADP-linked dehydrogenases participated in the metabolic inactivation of prostaglandin D_2 in the platelets. Furthermore, the dehydrogenase activity for prostaglandin D_2 was high in monkey (0.128 nmol/min · mg at 24°C) and human platelets (0.066), but was not detectable (less than 0.007) in the rabbit, rat, and chicken. Because prostaglandin D_2, which was demonstrated by several authors to be synthesized in platelet-rich plasma during platelet aggregation, exhibited significant antiaggregatory activity only in human and monkey platelets, these prostaglandin dehydrogenases appear to play a physiological role in the circulatory system.

In addition to various functions in the central nervous system (1-9), prostaglandin (PG)D_2[3] stimulates the adenylate cyclase system of human blood platelets and inhibits platelet aggregation (10-14). A specific receptor for this PG, distinct from that of prostacyclin (PGI_2) has been found in human blood platelets (15-18). It was also demonstrated that PGD_2 was synthesized during platelet aggregation (19-22). However, the metabolic fate of the compound in the circulatory system has yet to be demonstrated.

The first step in the metabolic inactivation of PGs of E, F, and I types was reported to be the oxidation of the 15-hydroxy group by two types of 15-hydroxy-PG dehydrogenase (15-hydroxyprostanoate oxidoreductase, EC 1.1.1.141); NAD-linked (so-called type I) and NADP-linked (type II) PG dehydrogenase (23-31). NAD-linked PG dehydrogenase specific for PGA was also reported in the rabbit kidney (32). Recently,

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[3] Abbreviations used: PG, prostaglandin; GC/MS, gas chromatography/mass spectrometry; PRP, platelet-rich plasma; PPP, platelet-poor plasma.

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another type of NADP-linked PG dehydrogenase specific for PGD_2 (PGD dehydrogenase) was isolated in our laboratory from swine brain (33). However, the presence of PG dehydrogenase in the platelets and the antiaggregatory potency of the metabolites derived from PGD_2 have not been clarified.

In the present investigations, we detected and purified two forms of NADP-linked PG dehydrogenase for PGD_2 from human blood platelets and these dehydrogenases were shown to be responsible for the conversion of PGD_2 to 15-keto derivatives, almost devoid of the antiaggregatory activity. The physiological significance of the enzyme in the platelets is also discussed. Some of these results were reported in a preliminary form (22).

MATERIALS AND METHODS

Materials. [1-^{14}C]Arachidonic acid (53.9 mCi/mmol) was purchased from the Radiochemical Centre. [5,6,8,11,12,14,15-^{3}H]PGE_2 (165 Ci/mmol) and [5,6,8,9,12,14,15-^{3}H]PGD_2 (100 Ci/mmol) were obtained from New England Nuclear. [1-^{14}C]PGH_2 was prepared as described previously (34). [1-^{14}C]PGD_2 was synthesized by the incubation of [1-^{14}C]PGH_2 with purified human serum albumin and identified as PGD_2 by GC/MS. Arachidonic acid was purchased from P-L Biochemicals. 13,14-Dihydro-15-keto-PGD_2 and 15-keto-PGD_2 were synthesized by Dr. D. Fukushima of Ono Central Research Institute. 15-Keto-PGB_2 was synthesized from PGB_2 by the oxidation with MnO_2 (Mangan(IV)-oxid auf Träger aktiv gekörnt zur Elementaranalyse, Merck). The molecular extinction coefficients of 15-keto-PGB_2 at 302 (absorbtion maximum) and 340 nm were 21,700 and 2900 M^{-1} cm^{-1} at pH 9, respectively. Other unlabeled PGs were gifts from Ono Central Research Institute. Bovine serum albumin, NAD (grade III), NADP, D-glucose-6-phosphate, glucose-6-phosphate dehydrogenase from Baker's yeast (type XV), and ADP (grade I) were obtained from Sigma. Collagen Reagent Horm (from horse tendon) was from Hormon-Chemie (München). Precoated silica gel glass plates G60 (F254) were from Merck. Blue Sepharose 6B and Sephadex G-100 were products of Pharmacia. All other chemicals were of reagent grade and were used without further purifications.

Assay of NADP-linked PG dehydrogenase in blood platelets. Human blood was obtained from healthy volunteers by venipuncture into a 230-ml Teflon bag containing 30 ml ACD (acid citrate dextrose)[4] as an anticoagulant. Blood samples from crab-eating monkeys (body wt 2–4 kg) were taken from the femoral artery and placed in plastic tubes containing 1/10 vol of 3.8% sodium citrate. Rat and chicken blood was collected by cardiac puncture and rabbit blood was obtained by catheterization of the carotid artery. All blood samples were centrifuged at 300*g* for 7 min at room temperature to remove red and white blood cells. The upper layer, platelet-rich plasma (PRP), was further centrifuged at 1000*g* for 15 min. All the following manipulations were performed at 0–4°C. The pellets were washed three times with 0.15 M NaCl solution containing 10 mM EDTA (pH 6.5). The washed pellets almost free from red or white blood cells, as determined microscopically, were suspended in 5 vol of 10 mM potassium phosphate buffer at pH 7.0 containing 0.5 mM dithiothreitol (Buffer A), and sonicated four times at 20,000 Hz for 15 s with a Branson Sonifier Model W185D. The sonicates were centrifuged at 105,000*g* for 60 min. The resultant supernatant solution (cytosol fraction) was used as the enzyme source in the standard assay as described previously (33). The standard reaction mixture contained 0.1 M Tris-HCl buffer at pH 9.0, 200 μM NADP, 200 μM PGD_2 (10 nmol dissolved in 5 μl of ethanol), 1 mM dithiothreitol, and enzyme in a total volume of 0.5 ml. The increase in absorbance at 415 nm was continuously followed by using a Shimadzu spectrophotometer Model uv 300 in a 1-ml cuvette with a 1-cm light path. The enzyme activity was calculated from the initial velocity of increase in absorbance at 415 nm based on the molecular extinction coefficient (35,000 M^{-1} cm^{-1}) of 15-keto-PGD_2 at pH 9.0 (33, 35). One unit of the enzyme activity was defined as the amount which produced 1 μmol of 15-keto-PGD_2 per minute at 24°C. Specific activity was expressed as the number of units per milligram of protein. The assay with other substrates was performed under the same conditions except that the absorbance was followed at 340 nm instead of 415 nm. When assayed with PGB_2 as a substrate, the ϵ value of 9100 M^{-1} cm^{-1} (6200 plus 2900, an ϵ value of 15-keto-PGB_2 at 340 nm at pH 9) was used. The protein concentration was determined according to Lowry *et al.* (36) with bovine serum albumin as a standard.

Purification of the PG dehydrogenases from human platelets. The cytosol fraction of human platelets (28.5 ml) from 2.4 liters of blood was subjected to ammonium sulfate fractionation. The precipitate between 40 and 70% saturation was dissolved in 2 ml of Buffer A. The solution was dialyzed overnight against three changes of 100 vol of Buffer A. After removal of insoluble materials by centrifugation at 10,000*g* for 10 min, the sample was applied to a column of Blue Sepharose 6B (2.5 × 10 cm) previously equilibrated with Buffer A. The column was washed with Buffer A and then Buffer A containing 0.1 M KCl until the absorbance of the eluate at 280 nm decreased to less than 0.05. The enzyme was eluted with Buffer A containing 1 M KCl. The eluate (48 ml) was concentrated with the aid of a Diaflo membrane PM-

[4] ACD, acid citrate dextrose, contains 2.2 g of sodium citrate, 0.8 g of citric acid, and 2.2 g of D-glucose/100 ml water.

10 to 3.8 ml and applied to a column of Sephadex G-100 (2 × 63 cm) previously equilibrated with Buffer A. Elution was carried out with Buffer A at a flow rate of about 10 ml/h and 1.3-ml fractions were collected. The dehydrogenase activities for PGD_2 and PGB_2 and the absorbance at 280 nm were measured in every second fraction.

Identification of the reaction product by GC/MS. The reaction mixture contained 0.1 M Tris-HCl buffer at pH 9.0, 27 μM [1-^{14}C]PGD_2 (540,000 cpm), 200 μM NADP, 1 mM dithiothreitol, and enzyme (1.05 mg of protein with the specific activity of 0.139 munit/mg of protein) in a total volume of 2 ml. Incubation was carried out at 24°C for 150 min and the reaction was terminated by the addition of 0.5 ml of 0.5 M citric acid. The mixture was extracted three times with 10 ml of diethyl ether. The organic phase was evaporated under reduced pressure and subjected to thin-layer chromatography in a solvent system of benzene/dioxane/methanol (20:20:1). The major product with an R_f value of 0.33 was extracted three times from the plate each with 5 ml of a mixture of diethyl ether/methanol (9:1). The product was converted to methyl ester using diazomethane prepared from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide. The methyl ester was converted to a methoxime methyl ester form by a pyridine solution of methoxyamine·HCl. The compound was further converted to a trimethylsilyl derivative and examined using a JEOL combined gas chromatograph Model 20K/mass spectrometer Model JMS D-100. The operating conditions were as follows: column, 1 m SE-30 (100–120 mesh); column temperature, 210°C; injection and detector temperature, 300°C; ionization current, 300 μA; ionization voltage, 20 eV; and ion multifier voltage, 2 kV.

Platelet aggregometry. A mixture of nine parts of blood and one part of 3.8% trisodium citrate was centrifuged at 300*g* for 7 min and the upper layer (PRP) was obtained. A portion of PRP (1 ml) was further centrifuged at 1000*g* for 15 min to obtain platelet-poor plasma (PPP). The aggregation of platelets was monitored according to the method of Born (37). PRP was placed and stirred at 37°C in a volume of 0.25 ml in an aggregometer Hema Tracer 1 Model PAT-2M (Niko Bioscience, Tokyo). The light transmission of PRP and that of PPP were taken as 0 and 100%, respectively. Platelet aggregation was initiated by the addition of 5 μl of 250 μM ADP, 100 μg/ml collagen in 50 mM Tris-HCl buffer at pH 7.4, or 50 mM arachidonic acid in 0.1 M Na_2CO_3. Various compounds dissolved in ethanol (20 mM PGD_2, 100 mM 13,14-dihydro-15-keto-PGD_2, and 100–150 mM 15-keto-PGD_2) were diluted to appropriate concentrations with 50 mM Tris-HCl buffer at pH 7.4 and an aliquot (5 μl) of each solution was added 1 min prior to the addition of an aggregating agent. The final concentration of ethanol was below 0.1% and alone had no effect on the aggregation of platelets. ID_{50} of each compound for platelet aggregation was defined as the dose required to inhibit the aggregation by 50% from the amplitude without PGD_2 or its metabolites (13).

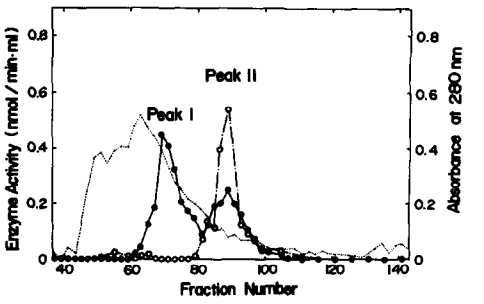

FIG. 1. Purification of two forms of NADP-linked PG dehydrogenase by Sephadex G-100. About 4.4 mg of protein were applied to a column (2 × 63 cm) of Sephadex G-100. Elution was carried out with Buffer A at a flow rate of 10 ml/h, and 1.3-ml fractions were collected. Absorbance at 280 nm (●···●) was measured in every second fraction. Enzyme activities were measured spectrophotometrically by following the increase in absorbance at 415 nm (PGD_2 as a substrate, ● —— ●) and 340 nm (PGB_2 as a substrate, O—·—O).

Assay of PGD 11-ketoreductase and PGE 9-ketoreductase. PGD 11-ketoreductase activity was assayed using 5 mM [^{3}H]PGD_2 (946,000 cpm) as a substrate, according to the method of Watanabe *et al.* (38). PGE 9-ketoreductase activity (26) was assayed under the same conditions as PGD 11-ketoreductase except that 200 μM [^{3}H]PGE_2 (960,000 cpm) was used as a substrate in place of PGD_2.

Molecular weight determinations by gel filtration. The molecular weights of the two dehydrogenases were estimated by gel filtration, according to the method of Laurent and Killander (39) using bovine serum albumin (M_r 68,000), ovalbumin (43,000), chymotrypsinogen A (25,000), and ribonuclease A (13,700) as standard proteins.

RESULTS

Purification and properties of PG dehydrogenases for PGD_2 from human blood platelets. PG dehydrogenase was purified from the cytosol fraction of human blood platelets, as described under Materials and Methods. The dehydrogenase activity for PGD_2 was eluted in two peaks (peak I and peak II) on Sephadex G-100 column chromatography (Fig. 1). The peak II fractions contained more catabolizing activity for PGB_2 than for PGD_2 (Fig. 1). A typical result of purification is summarized in Table I. Overall purifications for peaks I and II were 22- and 77-fold, respectively. The combined fractions (fractions 65–75

TABLE I

PURIFICATION OF TWO FORMS OF PG DEHYDROGENASE FROM HUMAN BLOOD PLATELETS[a]

Step	Volume (ml)	Total protein (mg)	Total activity (munits)[b]	sp act (munits/mg of protein)	Yield (%)	Purification (-fold)
1. High-speed supernatant	28.5	223.7	8.64	0.039	100	1
2. Ammonium sulfate	5.5	66.0	6.63	0.100	77	2.6
3. Blue Sepharose	3.9	16.9	6.44	0.380	75	9.7
4. Sephadex G-100						
Peak I	5.1	2.5	2.15	0.847	25	21.7
Peak II	4.5	0.6	1.68	3.006	19	77.1

[a] Starting materials were 2.4 liters of blood from drug-free Japanese males.
[b] One milliunit was defined as the amount of enzyme which produced 1 nmol of 15-keto-PGD_2/min at 24°C.

for peak I and 85–95 for peak II) were concentrated with the aid of Diaflo membrane PM-10 and used for further analyses. Molecular weights of peak I and peak II were estimated to be approximately 65,000 and 31,000, respectively. The formation of 15-keto-PGD_2 was proportional to the enzyme amount up to 100 μg with peak I enzyme (Fig. 2A). The K_m values for PGD_2 and NADP were 35 and 0.6 μM, respectively (Figs. 2B and C). The enzyme fraction in peak II exhibited essentially similar K_m values (65 μM for PGD_2 and 0.4 μM for NADP) as those observed in peak I. The formation of 15-keto-PGD_2 could not be detected with either enzyme when NAD (2 mM) was used instead of NADP. The optimal pH for reaction with both enzymes was around 9. However, there was a striking difference in substrate specificities between the two enzymes, as is shown in Table II. PGD_2 was the best substrate for peak I enzyme, whereas PGB_2 was the best substrate for peak II enzyme, followed by PGD_2. The enzyme in peak II but not peak I exhibited PGE 9-ketoreductase activity (5.28 nmol/min · mg of protein at 24°C). Both enzymes were free of NADPH-linked PGD 11-ketoreductase activity (38, 40, 41).

Identification of the reaction products. When incubation was carried out under the standard assay conditions, a time-dependent increase in absorbance was observed with a peak at 415 nm (Fig. 3). The spectral changes were completely dependent upon the presence of PGD_2, enzyme, and NADP. The absorbance at 415 nm disappeared by acidification of the reaction mixture to pH 3 with 1 N HCl and reappeared at alkaline pH values (33, 35, 42). These spectral properties suggested its identity with 15-keto-PGD_2. Further identification of 15-keto-PGD_2 using GC/MS procedure was hampered because of its instability.

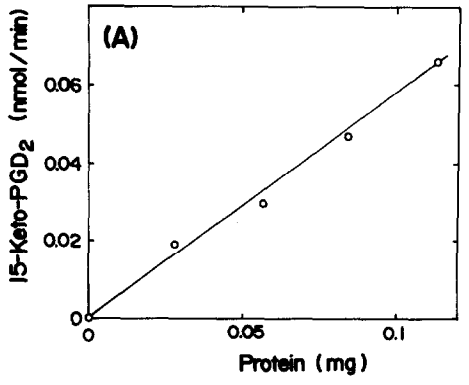

FIG. 2A. Effect of the enzyme amount (peak I) on the rate of the formation of 15-keto-PGD_2 at 24°C. The reaction was carried out under the standard assay conditions with varying concentrations of enzyme of peak I.

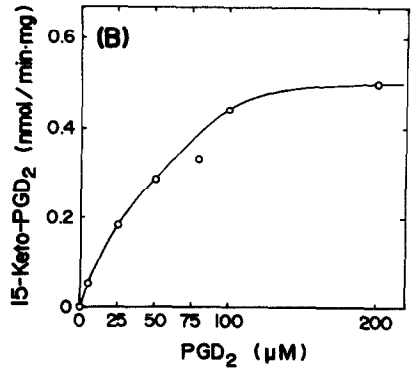

FIG. 2B. Effect of varying concentrations of PGD_2. The incubation was carried out with 0.085 mg of peak I enzyme (sp act, 0.427 munit/mg of protein).

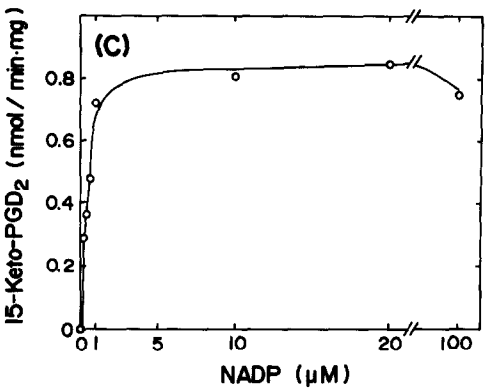

FIG. 2C. Effect of varying concentrations of NADP. The incubation was performed using 0.049 mg of peak I enzyme (sp act, 0.847 munit/mg of protein). NAD at 2 mM was completely inactive in replacing NADP.

As described under Materials and Methods, [1-^{14}C]PGD_2 (27 μM) was incubated with the enzyme in the presence of 200 μM NADP and 1 mM dithiothreitol for 150 min, the mixture was extracted with diethyl ether, and the organic phase was subjected to thin-layer chromatography. The major product formed from PGD_2 migrated with an R_f value of 0.33 which was an isographic value with the authentic 13,14-dihydro-15-keto-PGD_2 in a solvent system of benzene/dioxane/acetic acid (20:20:1). The extracted material was analyzed by GC/MS under the conditions described under Materials and Methods. A peak with a retention time of 6 min gave a mass spectrum essentially identical with that obtained with the corresponding derivative of the authentic 13,14-dihydro-15-keto-PGD_2. The following ions were observed at *m/e* 465 (M-31), 435, 375, 343, 265, 234, 200 (base peak), 188, and 156. These results suggested that PGD_2 was first oxidized at the 15-hydroxy group by an NADP-linked PG dehydrogenase, followed by the reduction of C13-14 double bond, as was found in the swine brain (33).

Antiaggregatory activities of PGD_2 and its metabolites. Studies of the antiaggregatory activities of PGD_2 and its metabolites showed that ID_{50} values for ADP-induced human platelet aggregation of PGD_2, 15-keto-PGD_2, and 13,14-dihydro-15-keto-PGD_2 were 10, 61,000, and 72,000 nM, respectively. Similar results were obtained when human platelets were stimulated by collagen (2 μg/ml) or arachidonic acid (1 mM) (Table III). Two metabolites of PGD_2, 15-keto-PGD_2 and 13,14-dihydro-15-keto-PGD_2, were by three orders of magnitude less potent in their antiaggregatory activities than PGD_2.

PG dehydrogenase activity in platelets of various species. The PG dehydrogenase activities were determined in the cytosol fractions of various species using 200 μM PGD_2 as a substrate (Table IV). Monkey and human platelets showed high specific activities (0.066-0.128 munit/mg of protein). A weak but significant activity (0.007 munit/mg of protein) was found in rabbit platelets and the activity was not detected in rat and chicken platelets. In the monkey platelets, NAD-linked dehydrogenation of PGD_2 was observed, the rate being approximately 10% of that with NADP. PG dehydrogenase for PGD_2 was not detectable in human plasma when assayed with 3-9 mg of protein.

TABLE II

SUBSTRATE SPECIFICITY OF TWO FORMS OF PG DEHYDROGENASE

Substrates	Percentage[a]	
	Peak I	Peak II[b]
PGD_2	100	100
PGE_2	14	23
$PGF_{2\alpha}$	2	61
PGI_2	29	29
PGB_2	0	131

[a] The reaction was carried out under the standard assay conditions with partially purified enzyme (0.13 mg of protein for peak I and 0.03 mg of protein for peak II), and the relative rates for reaction were determined. The rate with 200 μM PGD_2 (0.827 and 2.870 nmol/min · mg at 24°C for peak I and peak II, respectively) was defined as 100%.

[b] Peak II enzyme had the PGE 9-ketoreductase activity (5.28 nmol/min · mg of protein at 24°C) but not peak I. Therefore, the products from $PGF_{2\alpha}$ were both 15-keto-$PGF_{2\alpha}$ and PGE_2.

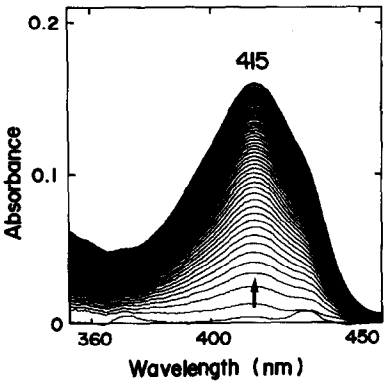

FIG. 3. Spectral changes associated with enzyme reaction. The reaction was carried out under the standard assay conditions with 0.339 mg of peak I enzyme (sp act, 0.427 munit/mg of protein). The spectra were taken at 1-min intervals.

TABLE III

ID_{50} VALUES OF PGD_2 AND TWO METABOLITES ON THE HUMAN PLATELET AGGREGATION INDUCED BY ADP, COLLAGEN, AND ARACHIDONIC ACID

Stimulants	nM		
	PGD_2	15-Keto-PGD_2	13,14-Dihydro-15-keto-PGD_2
ADP (5 μM)	10	61,000	72,000
Collagen (2 μg/ml)	24	54,000	67,000
Arachidonic acid[a] (1 mM)	8	490	40,000

[a] The concentrations were expressed as the minimum amounts of PGs that caused complete inhibition of platelet aggregation, since it is difficult to obtain an accurate concentration of ID_{50} vlaues in case of arachidonic acid-induced aggregation.

When the effects of PGD_2 on ADP (5 μM)-, or collagen (10 μg/ml)-induced platelet aggregation with the sample obtained from various animals were investigated, PGD_2 was active in human and monkey platelets, but not in platelets of other species (Table IV), as reported by other investigators (12-14).

DISCUSSION

Recent studies in several laboratories including our own have demonstrated that PGD_2 is actively synthesized and metabolized in the brain and acts as a neuromodulator in the nervous system (1-9). Prior to the discovery of the action of PGD_2 in the central nervous system, the compound was known to be a potent antiaggregatory substance (10-14). It was also demonstrated that this active compound was synthesized in PRP during aggregation, up to levels sufficient to inhibit platelet aggregation *in vitro* (19-22). Thus, it can reasonably be assumed that PGD_2 in the circulatory system may function as a modulator in the feedback inhibition of platelet aggregation *in vivo*. This interpretation was further supported by the observations of Cooper *et al.* (15-16) that in some of the patients with acute thrombosis and myeloproliferative disorders, the platelet receptor for PGD_2 was reduced in number, whereas that for PGI_2 remained normal. Although it was reported that PGI_2, another potent antiaggregatory compound, was converted to 15-keto-PGI_2 by PG dehydrogenase in the vascular wall (28) or rapidly decomposed to 6-keto-$PGF_{1\alpha}$ in the blood, little attention has been given to the metabolic fate of PGD_2 in the circulatory system. In our present work, we discovered the NADP-linked 15-hydroxy-PG dehydrogenase activity for PGD_2 in the platelets of primates and partially purified this dehydrogenase from human platelets. By Sephadex G-100 column chromatography (Fig. 1) at least two forms of PG dehydrogenase for PGD_2 were observed. The peak I enzyme is similar to PGD dehydrogenase isolated from swine brain in terms of molecular and catalytic properties (33, 43). For the catalytic activity of peak II enzyme, PGB_2 was the best substrate, followed by PGD_2 and $PGF_{2\alpha}$. It exhibited the NADPH-linked PGE 9-ketoreductase activity. The molecular weight of 31,000 was estimated by gel filtration. According to these results, peak II enzyme resembles the so-called "type II" enzyme as first reported in monkey brain (25), human erythrocytes (30), and swine kidney (31) by Lee and Levine and further characterized with human placenta by Jarabak and Fried (27). Although it remains to be clarified how these two enzymes play differential roles in the inactivation of PGD_2 in the platelets *in vivo*, the peak I enzyme (PGD dehydrogenase) appears to play a major role in the metabolism of PGD_2, since it acted on PGD_2 with relatively high specificity. The physiological role of peak II enzyme in the platelets and the role of PGB_2 itself in the hemostasis remain to be elucidated. It should be also noted that the previously reported velocity with PGB_2 (26, 27) was overestimated because they neglected the significant absorbance of 15-keto-PGB_2 (2900 M^{-1} cm^{-1}) at 340 nm.

The reaction products by the two enzymes were identified as 15-keto-PGD_2 and 13,14-dihydro-15-keto-PGD_2 on the basis of spectrophotometric evidence and GC/MS analyses, respectively. The antiaggregatory potency of these two metabolites for human platelet aggregation was much less than that of PGD_2

TABLE IV

THE PG DEHYDROGENASE ACTIVITY FOR PGD_2 IN THE PLATELETS OF VARIOUS ANIMALS AND ID_{50} VALUES OF PGD_2 FOR PLATELET AGGREGATION

Species	PG dehydrogenase activity (munits/mg of protein)		ID_{50} values of PGD_2 for platelet aggregation[a] (nM)
	NADP	NAD	
Monkey	0.128	0.015	13
Human	0.066	0	10
Rabbit	0.007	0	355
Rat[b]	0	0	>40,000
Chicken[b]	0	0	>40,000

[a] Platelets were (except from chickens) aggregated with 5 μM ADP. Since the aggregation of chicken platelets could not be observed with ADP in the present study, collagen (10 μg/ml) was used as a stimulant.

[b] The enzyme activity could not be detected with 0.69 mg of protein (rat) and 0.5 mg of protein (chicken) in the standard assay mixture.

(Table III). Since 15-keto-PGD_2 was not available in the past, this is the first report confirming that the oxidation of the 15-hydroxy group of PGD_2 results in a substantial loss of the bioactivity, as already reported in the case of other PGs including E, F, and I (23, 24, 28).

The physiological significance of the enzyme activity was further supported by the finding of the species difference of the PG dehydrogenase activity and the antiaggregatory activity of PGD_2. It should be emphasized that the dehydrogenase activity for PGD_2 could be detected only in the platelets which were sensitive to PGD_2 and thus appear to have a specific receptor for this particular PG.

These results, taken together, indicate that PGD dehydrogenase which was recently found in the brain tissue (33) is also present in the cytosol fraction of the human blood platelets and is responsible for the inactivation of PGD_2 in the circulatory system, and that the enzyme is found only in the platelets of the primates, which are sensitive to the antiaggregatory action of PGD_2.

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