Correction. In the article "A 15-hydroxyprostaglandin dehydrogenase specific for prostaglandin A in rabbit kidney" by H. G. Oien, E. A. Ham, M. E. Zanetti, E. H. Ulm, and F. A. Kuehl, Jr., which appeared in the April issue of Proc. Natl. Acad. Sci. USA 73, 1107–1111, an error was made by the PROCEEDINGS Office. Fig. 1B was shown as Fig. 2B and Fig. 2B was presented as Fig. 1B. The correct sequence is given below.

FIG 1. Time and protein dependence of rabbit kidney papilla PGDH. (A) Dependence on incubation time using 30 μM PGA₁ and 13 μg of protein per 0.2 ml of incubation volume. (B) Dependence on enzyme protein using 30 μM PGA₁ and 20 min incubation time.

FIG 2. Comparison of NAD dependence of PGDH of rabbit kidney papilla and bovine lung. (A) NAD dependence of rabbit kidney papilla PGDH using 30 μM PGA₁ and 13 μg of protein per 0.2 ml of incubation volume. (B) NAD dependence of bovine lung PGDH using either 2.8 μM PGE₁ or 3.0 μM PGA₁ and 2.3 μg of protein per 0.2 ml of incubation volume.
**A 15-hydroxyprostaglandin dehydrogenase specific for prostaglandin A in rabbit kidney**

(prostaglandin A dehydrogenase/rabbit kidney papilla/diuretic agents/hypertension/prostaglandin metabolism)


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Communicated by Josef Fried, January 26, 1976

**ABSTRACT** Examination of a soluble fraction derived from homogenates of rabbit kidney papilla revealed the existence of a 15-hydroxyprostaglandin dehydrogenase specific for A-type prostaglandins. Prostaglandins of the E- and F-series were not substrates for this enzyme. In agreement with published data, the 15-hydroxyprostaglandin dehydrogenase(s) derived from the kidney cortex were found to degrade all prostaglandins examined (PGE, PGF, PGA) in the presence of added cofactor NAD. Thus it is evident that in this species the kidney 15-hydroxyprostaglandin dehydrogenases are anatomically compartmentalized so that the papilla is able to metabolize only A-prostaglandins in contrast to the cortex which is capable of degrading E-, F-, and A-type prostaglandins by this metabolic pathway.

Although there are many catabolic pathways for the prostaglandins (PGs), conversion of the 15(S)-hydroxy group to a keto-function by 15-hydroxyprostaglandin dehydrogenase (PGDH) is considered to be both the initial and major route for their transformation to inactive metabolites (1). This enzyme(s) has been shown to be present in most tissues examined, although it is particularly abundant in the lung, the organ most thoroughly studied in this regard (2, 3). Studies revealed that prostaglandins of both the E-type (PGEs) and the F-type (PGFs) are almost completely inactivated by this enzyme during a single passage through the lung. On the other hand, prostaglandins of the A-type (PGAs) survive circulation through the lung, a fact leading to the suggestion that PGAs, as opposed to other prostaglandins, may be circulating hormones (4, 5). Nevertheless, upon in vitro exposure to the action of PGDH isolated from the lung, PGAs are almost as readily converted to their 15-keto metabolites as are other prostaglandins (6). Although the reason for this is not clear, the recent reports that tritiated PGA1 has an affinity for blood proteins, exceeding that of PGEs and PGFs, may relate to this phenomenon (7, 8). The affinity of PGA1 for plasma proteins may not permit its exposure to, and degradation by, the lung PGDH. Despite the failure of tritiated PGAs to be significantly metabolized when perfused through the lung, PGA2 has been reported to be rapidly converted to more polar metabolites when perfused through the rabbit kidney (9). Thus, if the ability of PGA2 to survive circulation through the lung is attributable to the affinity of PGA2 for plasma proteins, then this suggests the existence of a new enzyme that undergoes an unique metabolic interaction with the PGAs in the kidney. The partial purification and characterization of a kidney PGDH, specific for PGAs, is the substance of this report.

**MATERIALS AND METHODS**

Tritiated prostaglandins, specific activity of >60 Ci/mmol, were obtained from New England Nuclear Corp., Boston, Mass. 15-Keto-PGA1 was prepared by acid treatment of 15-keto-PGE1 or enzymatically using rabbit papilla PGDH. The use of either method produced a product that was identical by mass spectrometry.

15-Keto-PGE1 and 13,14-dihydro-PGE1 (PGE6) were prepared by Dr. D. Taub and associates of Merck & Co., Rahway, N.J., according to the procedure of Anggård and Samuelsson (1). PGB1 was prepared by the method of Anderson (10). Other prostaglandins were purchased from Ono Pharmaceuticals, Osaka, Japan.

Ethacrynic acid, dihydroethacrynic acid, dihydroethacrynic acid, 2,3-dichloro-4-(2,2-diacetylviny1)phenoxycetic acid, mersalyl acid, and furosemide were obtained from Dr. E. J. Cracoe of Merck Sharp and Dohme, West Point, Pa. NAD, NADP, NADH, and NADPH were purchased from P-L Biochemicals, Inc., Milwaukee, Wisc.

Frozen rabbit cortex and papilla tissue was obtained from Pell-Freeze Biologicals, Inc., Rogers, Ark.

Preparation of PGDH from Rabbit Kidney Papilla or Cortex. All procedures were carried out at 4° unless otherwise stated. Rabbit kidney cortex and papilla tissue, dissected from fresh kidneys for immediate use or obtained commercially in frozen form, were used in these experiments with indistinguishable results. Tissue was mixed with an equal volume of 0.05 M sodium phosphate, 1 mM EDTA, pH 7.4 buffer, and homogenized with a Polytron PT-10ST. Centrifugation of the homogenate at 10,000 X g for 30 min was followed by centrifugation of the resultant supernatant fraction at 100,000 X g for 60 min. Solid ammonium sulfate was added to the 100,000 X g supernatant fraction to 30% saturation and stirred for 1 hr. Centrifugation at 16,000 X g for 30 min gave a supernatant fraction which was brought to 70% saturation by the addition of more ammonium sulfate, care being taken to maintain the pH at 7.4. The suspension was stirred overnight and the precipitate obtained by centrifugation at 16,000 X g for 30 min. The precipitate was dissolved in phosphate-EDTA buffer, dialyzed against the buffer, subdivided, and stored at -90°. It was found that 1 g of either cortex or papilla tissue provided about 10 mg of enzyme-active protein in the final preparation. The papilla preparation was found to be free of NADH oxidase.

Bovine Lung Prostaglandin Dehydrogenase. This enzyme was prepared according to the method of Saeed and Roy (11) without the acetone precipitation step and stored at -90° until used. One gram of lung tissue was found to produce 10 mg of final protein.

Dehydrogenase Assay (Isotope Method). The dehydrogenations were carried out in a final volume of 0.2 ml of 0.05 M sodium phosphate, 1 mM EDTA, pH 7.4 buffer, and 4 µl of methanol. Tritiated prostaglandin (about 30,000

Abbreviations: PGs, prostaglandins of type A, B, E, or F; PGDH, 15-hydroxyprostaglandin dehydrogenase.
Table 1. A comparison of the properties of the PGDHs derived from rabbit kidney and bovine lung

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rabbit kidney papilla</th>
<th>Rabbit kidney cortex</th>
<th>Bovine lung</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_m$ (µM)</td>
<td>$K_i$ (µM)*</td>
<td>Inhibitor type</td>
</tr>
<tr>
<td>PGA₁</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGA₂</td>
<td>6</td>
<td>28</td>
<td>C</td>
</tr>
<tr>
<td>PGE₁</td>
<td>n.s.</td>
<td>58</td>
<td>C</td>
</tr>
<tr>
<td>PGE₂</td>
<td>n.s.</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>PGE₆</td>
<td>n.d.</td>
<td>11</td>
<td>C</td>
</tr>
<tr>
<td>PGE₁₄</td>
<td>n.s.</td>
<td>490</td>
<td>C</td>
</tr>
<tr>
<td>PGE₂₄</td>
<td>n.s.</td>
<td>340</td>
<td>C</td>
</tr>
<tr>
<td>PGB₁</td>
<td>n.s.</td>
<td>3</td>
<td>C</td>
</tr>
<tr>
<td>15-keto-PGA₁</td>
<td>n.s.</td>
<td>11</td>
<td>C</td>
</tr>
</tbody>
</table>

Assays were performed as described in Materials and Methods (isotope method) with 20 min incubation times. Incubations with papilla PGDH contained 4.2 mM NAD and 15 µg of protein per 0.2 ml; $V_{max}$ = 2.0 µg of 15-keto-PGA₁/mg of protein per min. Incubations with cortex PGDH contained 0.42 mM NAD and 25 µg of protein per 0.2 ml; $V_{max}$ = 0.60 µg of 15-keto-PGA₁/mg of protein per min. Incubations with lung PGDH contained 4.2 mM NAD and 5–10 µg of protein per 0.2 ml; $V_{max}$ = 0.15 µg of 15-keto-PGA₁/mg of protein per min. n.s. = not a substrate. n.d. = not determined. C = competitive inhibitor.

* Values as determined by Matschinski et al. (ref. 18).
† Using PGA₁ as a substrate.

RESULTS

Substrate specificity and kinetic properties

As shown in Table 1, for the prostaglandins examined, only those of the A-series were metabolized by the rabbit papilla enzyme preparation; PGE₅s and PGF₂αs were completely resistant to the action of the papilla enzyme. This metabolism of cpm), NAD, and enzyme were added separately in buffer solutions while unlabeled prostaglandins (for amounts see legends to figures) and inhibitors were added separately in methanol solutions. The reactions were initiated by the addition of the enzyme and continued at 37°C, usually for 20 min. The incubations were terminated by the addition of 0.2 ml of 0.035 M citric acid, to adjust the pH to 4, with 10 µg of carrier substrate and 2 µg of carrier product dissolved in 50 µl of methanol. The incubations were extracted once with 4 ml of ethyl acetate and the extracts were washed twice with 0.5 ml of water and taken to dryness with a stream of nitrogen. The residues were analyzed by thin-layer chromatography on Analtech silica-gel GF plates (250 µ) using ethyl acetate-isooctane-acetic acid (50:50:0.5) when [³H]PGAs and [³H]PGB₁ were substrates, and system F-VI of Andersen (12) when [³H]PGEs and [³H]PGFs were substrates. The carrier substrates and products were visualized with ultraviolet light and/or iodine vapor and these zones were counted in ethyl alcohol-toluene (30:70) phosphor using a Packard Liquid Scintillation counter. Recoveries were of the order of 85%, correcting for silica-gel blanks, and a reproducibility of ±2% was obtained.

Prostaglandin Dehydrogenase Assay (500 nm A Method). This assay was performed by a modification of the method of Vonkeman et al. (13). Incubations were carried out in 0.5 ml of 0.25 M Tris-HCl at pH 9.0 with 118 µM PGA₁ and 1 mM of NAD, when required. Reactions were initiated by the addition of papilla enzyme. After 20 or 60 min at 37°C, 0.05 ml of 2 M NaOH was added, and the maximum absorbance at 500 nm was recorded using a Beckman K25 spectrometer. Maximum absorbance was reached within 15 sec of mixing, and then rapidly declined.

![Figure 1](image-url)
FIG. 2. Comparison of NAD dependence of PGDH of rabbit kidney papilla and bovine lung. (A) NAD dependence of rabbit kidney papilla PGDH using 30 μM PGA₁ and 13 μg of protein per 0.2 ml of incubation volume. (B) NAD dependence of bovine lung PGDH using either 2.8 μM PGE₁ or 3.0 μM PGA₁ and 2.3 μg of protein per 0.2 ml of incubation volume.

PGA₁ was dependent upon both time and protein concentration as shown in Fig. 1 and did not take place in the presence of heat-inactivated enzyme. The enzyme exhibited a pH and temperature optimum of 7.5 and 37°C, respectively. A comparison of properties of the papilla enzyme with the PGDHs prepared similarly from the rabbit kidney cortex and bovine lung revealed some striking differences as shown in Table 1. The apparent Michaelis constant (Kₘ) for PGA₁ with the PGDH from the rabbit kidney papilla (15 μM) was the same as that derived from the kidney cortex enzyme (17 μM). This value contrasts with the Kₘ for PGA₁ using the bovine lung enzyme which is 1 μM. Interestingly, the Kₘ value for PGE₁ is about the same for the kidney cortex PGDH (2 μM) as observed for this prostaglandin with the bovine lung enzyme (1 μM).

Cofactor requirements

Added NAD did not enhance the conversion of PGA₁ (30 μM) to 15-keto PGA₁ by rabbit kidney papilla PGDH using the isotope method. Similarly, the oxidation of PGA₁ by the rabbit kidney cortex PGDH did not require added NAD; however, conversion of PGE₁ to 15-keto-PGE₁ by this enzyme did require added NAD. In contrast, PGDH of bovine lung origin required NAD for metabolism of PGA₁ as well as PGE₁ (Fig. 2). An effect of added NAD on papilla PGDH was observed at a higher level of PGA₁ (118 μM) employing the alkaline A method of measurement at 500 nm. An obvious enhancement of the initial rate of formation of 15-keto-PGA₁, as well as the amount formed in 60 min, is evident with added NAD (Table 2).

NADP did not enhance the metabolism of PGA₁ by the papilla or cortex enzyme nor did it allow the metabolism of PGE₁ by the papilla. The reduced cofactors, NADH and NADPH did not inhibit PGA₁ metabolism by either papilla or cortex PGDH; however, NADH inhibited the NAD-dependent conversion of PGA₁ to 15-keto-PGA₁ by bovine lung PGDH.

Effect of inhibitors

The data in Table 1 reveal that despite the inability of prostaglandins other than those of the A-type to act as substrates for the papilla enzyme, they are without exception competitive inhibitors of the metabolism of PGA₁; PGB₁ is particularly effective in this regard. In addition, the ability of a number of diuretic agents to inhibit the metabolism of PGA₁, with the papilla enzyme compared to that derived from the bovine lung (Table 3), reveals an interesting difference. Although the order of potency of the individual compounds is seen to follow the same pattern in both instances, the nature of the inhibition against the papilla enzyme is seen to be consistently competitive whereas that against the lung enzyme is noncompetitive in nature. The fact that mercuric chloride is noncompetitive in both instances may relate to the avidity of heavy metal compounds for sulphydryl groups.

DISCUSSION

Although there are some differences in the Kₘ and Vₘₐₓ values for the individual prostaglandins when they serve as

Table 2. NAD stimulation of PGDH activity at a higher level of substrate PGA₁

<table>
<thead>
<tr>
<th>Enzyme added (μg of protein)</th>
<th>0–20 min</th>
<th>20–60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-NAD</td>
<td>+NAD</td>
</tr>
<tr>
<td>300</td>
<td>0.083</td>
<td>0.104</td>
</tr>
<tr>
<td>600</td>
<td>0.158</td>
<td>0.211</td>
</tr>
<tr>
<td>900</td>
<td>0.210</td>
<td>(0.270)*</td>
</tr>
<tr>
<td>1200</td>
<td>(0.141)*</td>
<td>0.338</td>
</tr>
<tr>
<td>1500</td>
<td>0.264</td>
<td>0.370</td>
</tr>
</tbody>
</table>

Determinations were performed as described in Materials and Methods (500 nm A method). A zero reaction blank (60 min incubation without PGA₁) was subtracted from all values. All values are averages of duplicate determinations except for ( )* which are single determinations.

substrates for PGDH isolated from various tissues, no unique preference for individual PGs was initially reported for any of these enzyme preparations. The recent report, however, that some mammalian tissues contain both NAD (type I) and NADP (type II)-dependent PGDHs which have some preference for PGEs and PGFs, respectively (14), indicates that differences in enzyme characteristics can be of a more fundamental nature than originally envisioned. The findings reported here, describing the existence of a PGDH with a unique specificity for PGAs, further serve to emphasize the fact that PGDH can play a key role in regulating tissue levels of specific prostaglandins, rather than simply serving to catabolize prostaglandins as a group. Thus, depending on the organ involved, and the physiological function of individual PGs, PGDHs could play key regulatory roles in controlling tissue levels of specific prostaglandins. This concept is consistent with the observation, noted earlier, that PGAs survive circulation through the bovine lung but not through the rabbit kidney.

The precise mechanism by which the rabbit papilla PGDH exerts a high degree of specificity for PGAs cannot be established conclusively in the absence of a purified preparation of this enzyme. However, it is reasonable to conclude that the property of the PGAs that distinguishes them from other prostaglandins, namely, the presence of an unsaturated carbonyl function in the cyclopentane ring, is central to their interaction with the papilla PGDH. Recent evidence has been provided to show that unlike other prostaglandins PGAs, like ethacrynic acid, react covalently with the sulfhydryl group of cysteine and the cysteine-containing peptide glutathione (15). The binding of PGAs and its displacement by ethacrynic acid, in a cytosol preparation of the papilla,
has been suggested to involve the sulfhydryl groups of PGDH. This concept is supported by the fact that dihydroethacrynic acid, which is incapable of interacting with sulfhydryl groups, has only 1/2 of the activity of the parent compound as a PGDH inhibitor (Table 3). Thus, it is tempting to speculate that an interaction of the unsaturated carbonyl function of PGAs with an active sulfhydryl group in the papilla enzyme is central to the substrate specificity of this enzyme. However, such a suggestion remains to be confirmed by studies with the purified enzyme. Nevertheless, it is clear that PGA-PGDH interaction may involve components of the overall PG structure in addition to the cyclopentenoone ring. This is suggested by the fact that PGFs and PGFs, although not substrates, are competitive inhibitors of the oxidation of PGAs by this enzyme. Furthermore, the fact that 15-keto-PGA is released from the enzyme indicates that despite the unsaturated ring carbonyl group, common to PGA and its 15-keto derivative, the 15-hydroxyl group is an important factor in the affinity of this enzyme for substrates.

In addition to its substrate specificity, other features of the PGDH obtained from the rabbit papilla distinguish it from that derived from the bovine lung, as well as from the cortex of the rabbit kidney. The apparent high degree of affinity of the enzyme for NAD makes it possible to demonstrate an NAD requirement only after reduction of bound NAD by addition of a large excess of substrate. Such tenacious binding of NAD by dehydrogenases is not without precedent (16). However, the precise cofactor requirement of this PGA PGDH needs further study on a more purified preparation before its exact nature can be determined. Unlike the type I and type II enzymes described by Lee and Levine (14), no inhibitory action of NADH or NADPH can be noted for this enzyme. Most striking, however, is the difference in the kinetic nature of the inhibition of this enzyme by a number of diuretic agents, including ethacrynic acid and its analogs, when compared to the bovine lung enzyme. Although inhibition of the lung PGDH by these substances is noncompetitive, inhibition against the papilla enzyme is consistently competitive. The exception, mersalyl acid, a mercurial compound, was noncompetitive in both instances, a property likely attributable to the irreversible reaction of the heavy metal with sulfhydryl groups in the enzyme.

In the case of the kidney cortex, the NAD requirement for the metabolism of PGE and the absence of such an acute NAD requirement for PGA metabolism suggests that this portion of the kidney may contain two distinct PGDHs: one specific for PGAs similar to that present in the papilla, and a second capable of degrading PGEs. These observations contrast with reports that NAD is required for the metabolism of both PGEs and PGAs by the lung PGDH, and suggest that the enzyme that degrades PGEs in the kidney is incapable of degrading PGAs. Separation of these two enzymes is clearly required before these issues can be rigorously established.

Based upon the poor ability of the rabbit papilla to metabolize PGE in the presence of NAD, this portion of the rabbit kidney up to now has been considered to be largely devoid of a PGDH (17). Although this present report concurs that the rabbit papilla is incapable of converting PGEs to their 15-keto metabolites, it does establish the presence of a unique PGDH in this portion of the kidney specific for PGAs. Although the role of this class of prostaglandins in kidney function and hypertension has been controversial, the existence of this new enzyme poses the possibility that PGAs may play a regulatory role of some sort in the rabbit. We have also established the presence of a PGA-specific PGDH in papilla cytosol of the rat. As with the rabbit, cytosol derived PGDH from rat kidney cortex metabolizes PGEs as well as PGAs. The Km values determined in each case were virtually the same as those obtained for the rabbit systems. The question whether such an enzyme is also present in the human kidney where the papilla is less well defined remains to be established before supportive in vitro evidence for a role of PGA (medullin) in human kidney function and hypertension can be considered at hand.