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Expression of NADP+-Dependent 15-Hydroxyprostaglandin Dehydrogenase mRNA in Monkey Ocular Tissues and Characterization of Its Recombinant Enzyme[1]

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Although prostaglandin (PG) $F_{2\alpha}$ and its analogue latanoprost decrease the intraocular pressure in a variety of animals, their intraocular metabolism has not yet been clarified. Here, we isolated the cDNAs for the monkey homologues of NAD+- and NADP+-dependent types of 15-hydroxy PG dehydrogenase (PGDH) from lung and eye, respectively, and investigated the distribution of their mRNAs in the monkey eye. The cDNAs for the NAD+- and NADP+-dependent types of PGDH contained an open reading frame of 798 and 831 bp encoding 266 and 277 amino acid residues with calculated molecular masses of 28.9 and 30.5 kDa, respectively. The amino acid sequences of the monkey NAD+- and NADP+-dependent enzymes showed less than 20% identity to each other, and the former enzyme shows 98.5 and 86.8% identity, and the latter 94.9 and 85.2% identity, to the human and mouse enzymes, respectively. Reverse transcription–PCR analysis revealed that the mRNA for NADP+-dependent PGDH, but not that for NAD+-dependent PGDH, was highly expressed in monkey ocular tissues. *In situ* hybridization analysis demonstrated that the mRNA for NADP+-dependent PGDH was localized in the epithelial cells of the cornea. The recombinant NADP+-dependent PGDH catalyzed the dehydrogenation of the 15-hydroxyl group of $PGF_{2\alpha}$ and the acid form of latanoprost in the presence of NADP+ as examined by HPLC. These results indicate that $PGF_{2\alpha}$ and the acid form of latanoprost are degraded to their 15-keto metabolites by NADP+-dependent PGDH localized in the monkey eye.

Key words: cDNA cloning, 15-hydroxyprostaglandin dehydrogenase, monkey, ocular tissues, prostaglandin metabolism.

Prostaglandins (PGs) contribute to numerous physiological events in various mammalian tissues, including the regulation of vascular tone, reproduction, sleep, body temperature, bronchoconstriction, pain, and sensitivity of photoreceptors (*1*). PGs are synthesized from PGH_2, a common precursor of all PGs, by their specific synthases, function in restricted areas, and are quickly metabolized (*1*). The general pathway of PG metabolism includes the oxidation of the 15-hydroxyl group, reduction of the 13,14-double bond, and ω-oxidation (*2*). Oxidation of the 15-hydroxyl group, the initial step in the metabolism of PGs, is catalyzed by 15-hydroxy–PG dehydrogenase (PGDH), which consists of two distinct isozymes (*2*). One is a homodimeric protein known as NAD+-dependent PGDH [EC 1.1.1.141], initially identified in the lung (*3*). The other is a cytosolic monomeric enzyme identified as NADP+-dependent PGDH [EC 1.1.1.189], and originally found in human liver (*4*). Both enzymes belong to the short-chain dehydrogenases/reductases (SDR) family that includes several mammalian hydroxysteroid dehydrogenases and oxidoreductases (*5*). The NAD+-dependent enzyme is termed type I PGDH, and its cDNA has been isolated from human (*6*), mouse (*7*), rat (*8*), and guinea pig (*9*) tissues. The NADP+-dependent PGDH is known as type II PGDH and also as a carbonyl reductase, and its cDNA has been isolated from human (*10*), mouse (*11*), rat (*12*), rabbit (*13*), and pig (*14*) tissues. The immunoreactivities and mRNAs of these two types of PGDH have been detected in various human (*15, 16*) and mouse (*7, 17*) tissues.

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Abbreviations: CBB, Coomassie Brilliant Blue; DIG, digoxygenin; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; IPTG, isopropyl-β-D-galactopyranoside; PG, prostaglandin; PGDH, 15-hydroxy PG dehydrogenase; RACE, rapid amplification of cDNA ends; RT, reverse transcription; SDR, short-chain dehydrogenases/reductases.

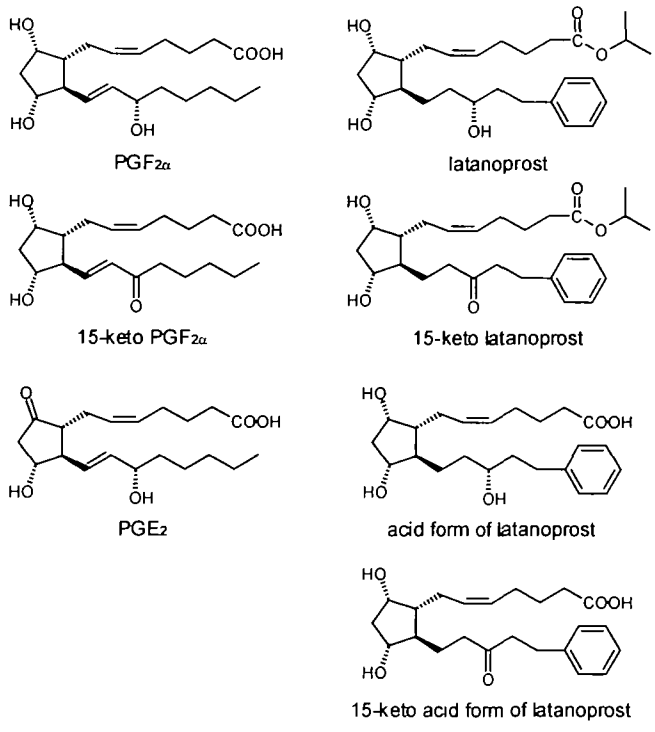

Fig. 1. **Structural formulae of PGs, latanoprost, and their metabolites.**

PGs also exhibit a variety of pharmacological effects in ocular tissues. $PGF_{2\alpha}$ decreases the intraocular pressure in a variety of animals as well as in humans (*18*). A synthetic $PGF_{2\alpha}$ analogue, latanoprost (Fig. 1), is used as an ocular hypotensive medication (*19*). However, the intraocular metabolism of $PGF_{2\alpha}$ and latanoprost has not yet been clarified and remains controversial. For example, PGDH activity has been detected in the ocular tissues of rabbits (*20*), rats (*21*), and pigs (*22*). Furthermore, the immunoreactivity of $NADP^+$-dependent PGDH has been found in the human retina (*16*). In contrast, Cheng-Bennet *et al.* (*23*) reported that human and rabbit ocular tissues lack PGDH activity.

In this study, we isolated the monkey cDNAs for the NAD^+- and $NADP^+$-dependent types of PGDH. We found by reverse transcription (RT)–PCR and *in situ* hybridization analyses that the mRNA for $NADP^+$-dependent PGDH, but not that for the NAD^+-dependent enzyme, was expressed in monkey ocular tissues and was localized in the epithelial cells of the cornea. We also expressed the recombinant monkey $NADP^+$-dependent PGDH in *Escherichia coli* and showed that the recombinant enzyme catalyzed the dehydrogenation of the 15-hydroxyl group of $PGF_{2\alpha}$ and the acid form of latanoprost. To our knowledge, this is the first molecular evidence for the expression and function of PGDH in monkey ocular tissues, and the substrate specificity of monkey $NADP^+$-dependent PGDH with $PGF_{2\alpha}$ and its analogue.

MATERIALS AND METHODS

Animals and Chemicals—Monkey (*Macaca fascicularis*) tissues were kindly provided by R-Tech Ueno (Sanda, Hyogo). PGE_2, $PGF_{2\alpha}$, 15-keto $PGF_{2\alpha}$ and 13,14-dihydro-15-keto $PGF_{2\alpha}$ were purchased from Cayman Chemical (Ann Arbor, MI, USA). Latanoprost, the acid form of latanoprost, and the 15-keto acid form of latanoprost (Fig. 1) were gifts from R-Tech Ueno. $NADP^+$, NAD^+, and NADPH were purchased from Wako Pure Chemicals (Osaka).

Rapid Amplification of cDNA Ends (RACE)—Total RNA was prepared from monkey tissues using ISOGEN (Nippon Gene, Toyama) according to the manufacturer's instructions. The cDNAs for the monkey NAD^+- and $NADP^+$-dependent PGDHs were isolated by the RACE technique (*24*). First-strand cDNAs were synthesized from total RNA (1 μg) prepared from monkey lung and cornea after annealing the RNA with Oligo dT-Adapter Primer (Takara Shuzo, Kyoto). RNA was denatured at 72°C for 3 min and incubated with avian myeloblastosis virus reverse transcriptase (Takara Shuzo) at 50°C for 30 min. After inactivation of the enzyme at 99°C for 5 min, the RT products from the lung and cornea were used as templates for RACE of the NAD^+- and $NADP^+$-dependent types of PGDH, respectively. The cDNAs for both types of PGDH were amplified by PCR with LA Taq DNA polymerase (Takara Shuzo), M13 M4 primer (Takara Shuzo), and PGDH1-F1 primer [5′-CGGAGCTCGCCCACCGCCCGC-3′; –31 to –19 of the cDNA for the human type I PGD (*25*)] or PGDH2-F2 primer [5′-TCCACGCAGGTGTTCCGCGC-3′; –33 to –14 of the cDNA for the human type II PGDH (*10*)]. The PCR conditions were as follows: initial denaturation at 95°C for 5 min and 30 cycles of 20 s at 94°C, 20 s at 55°C, and 1 min at 74°C. The resultant RACE products were separated by agarose gel electrophoresis, purified, and cloned into the pGEM-T Easy vector (Promega, Madison, WI, USA) to obtain pGEM-PGDH1 and pGEM-PGDH2, which contained the cDNAs for the type I and II PGDHs, respectively.

DNA Sequencing and Analysis—Nucleotide sequences were determined from both strands by the dideoxynucleotide chain termination method using a BigDye terminator cycle sequencing FS ready reaction kit (Applied Biosystems, Foster City, CA, USA) and an automated DNA sequencer Model 377A (Applied Biosystems). Sequence analyses were carried out using DNAIS-Mac ver. 3.4 software (Hitachi Software, Yokohama) and the BLAST algorithm of the National Center for Biotechnology Information (*26*).

RNA Analysis—Total RNA was prepared from monkey tissues as described above. Northern blot analysis was performed as described previously (*27*). First-strand cDNAs used for RT-PCR were synthesized from 1 μg of total RNA, and subsequent PCR amplification was conducted under the same protocol described above using PGDH1F-B (5′-CGGGATCCATGCACGTGAACGGCAAAGTGG-3′; *Bam*HI site and +1 to +22 of the monkey cDNA) and PGDH1R-H (5′-GCAAGCTTCACATAAGCTGTTCACTG-3′; *Hin*dIII site and +813 to +796) for type I PGDH, and PGDH2F-E (5′-CGGAATTCATGAAGTCCGGCATCCGTG-3′; *Eco*RI site and +1 to +19 of the monkey cDNA) and PGDH2R-S (5′-GCGTCGACAGCTCACCACTGTTCAACTCTC-3′; *Sal*I site and +837 to +816) for type II PGDH. A restriction site (underlined) was added to the 5′-end of each primer. The resultant PCR products were analyzed by agarose gel electrophoresis.

In Situ Hybridization—The *Pst*I fragment (672 bp) of pGEM-PGDH2, which contained the carboxyl terminal region, was subcloned into the *Pst*I site of pBluescript II vector (Stratagene, La Jolla, CA, USA). The resultant plas-

mid was used as a template for the synthesis of digoxygenin (DIG)-labeled antisense or sense RNA probe by *in vitro* transcription with DIG RNA labeling mix (Roche Diagnostics, Tokyo) according to the manufacturer's instructions. Monkey eyes fixed in 4% paraformaldehyde were sectioned at 10 μm thickness using a cryostat, mounted on glass slides, and post-fixed with 4% paraformaldehyde for 10 min. The sections were treated with 0.3% (v/v) Triton-X100 for 10 min, digested with 1 μg/ml proteinase K (Sigma Chemical, St. Louis, MO, USA) for 10 min at 37°C, and acetylated with 0.25% (v/v) acetate anhydride in 100 mM triethanolamine, 150 mM NaCl, and 32 mM HCl for 10 min. The sections were dehydrated through an ethanol series. Hybridization was performed at 50°C for 12–18 h using the DIG-labeled antisense or sense RNA probe, followed by washing in 50% (v/v) formamide/2× SSC. The sections were digested with 20 μg/ml of RNase A (Life Technologies, Rockville, MD, USA) for 30 min at 37°C, and

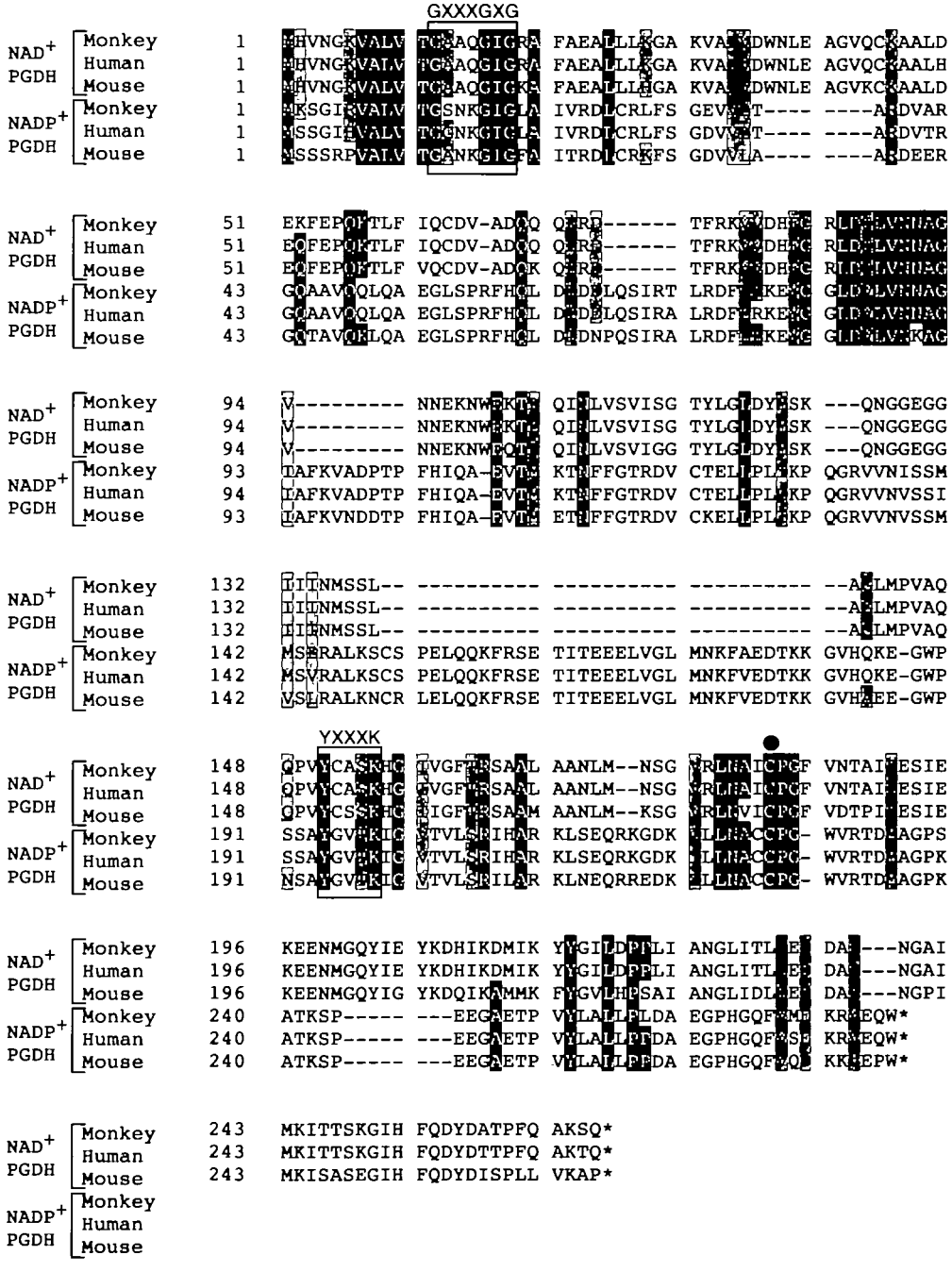

Fig. 2. **Multiple alignment of the predicted amino acid sequences of NAD⁺- and NADP⁺-dependent PGDHs.** Amino acid sequences of monkey NAD⁺- and NADP⁺-dependent PGDHs were compared with those of the human and mouse enzymes. The stop codon is shown as an asterisk. Black and gray backgrounds represent identical and similar amino acid residues in at least 4 of the 6 sequences, respectively. Amino acid motifs and a cysteine residue conserved in the SDR family are indicated by boxes and the closed circle, respectively, at the top.

then washed twice in 50% (v/v) formamide/0.1× SSC for 0.5–1 h at 50°C. Signals were detected and visualized using a DIG nucleic acid detection kit (Roche Diagnostics).

Expression and Purification of the Recombinant Enzyme—The coding region of monkey NADP$^+$-dependent PGDH cDNA was amplified by PCR using gene-specific primers, PGDH2F-E and PGDH2R-S, under the same conditions described above. After double digestion with *Eco*RI and *Sal*I, the resultant PCR products were ligated into the corresponding sites of the pET21a vector (Novagen, Madison, WI, USA) to obtain pET21-monkey PGDH2. *E. coli* BL21(DE3)pLysS strain (Stratagene) was transformed with pET21-monkey PGDH2 and cultured in LB medium supplemented with ampicillin (100 μg/ml) at 37°C until A_{600} = 0.5. Protein expression was induced by isopropyl-β-D-galactopyranoside (IPTG, final concentration of 0.5 mM) and cultivation was continued for 6 h. Cells were harvested and disrupted by sonication. The recombinant protein was produced as a fusion protein with a T7 tag sequence and purified with anti-T7 monoclonal antibody–conjugated resin (Novagen). The purified protein was applied to SDS-PAGE and stained with Coomassie Brilliant Blue (CBB) R-250 (Daiichi Pure Chemicals, Tokyo). Protein concentration was determined by the method of Bradford (*28*) with BSA as the standard.

Enzyme Assay—Enzyme assays were performed in 500 μl of 100 mM sodium phosphate (pH 7.0) containing 500 μM PGE_2, $PGF_{2\alpha}$, the acid form of latanoprost, or 100 μM latanoprost as the substrate and 50 μM NADP$^+$, NAD$^+$, or NADPH as the cofactor. After preincubation at 25°C for 5 min, the reaction was started by adding the recombinant protein or *E. coli* extracts, and the production of NADPH was monitored photometrically at 340 nm. Specific activity was calculated using 6,270 as the molar absorption coefficient (ε) for NADPH.

HPLC Analysis—15-Keto $PGF_{2\alpha}$ and the 15-keto acid form of latanoprost were detected by a reverse-phase HPLC apparatus (C_{18} column; YMC-Pack ODS-A, 0.46 × 10 cm; YMC, Milford, MA, USA) equipped with a 2487 dual λ absorbance detector (Waters, Milford, MA, USA). The mobile phases for the separation of 15-keto $PGF_{2\alpha}$ and the 15-keto acid form of latanoprost were 35 and 40% (v/v) acetonitrile, respectively, containing 0.2% (v/v) acetic acid, and were pumped at a flow rate of 1 ml/min. The chromatographs were recorded at 206 nm and analyzed by a Millennium^{32} Chromatography Manager (Waters).

RESULTS

Isolation and Characterization of Monkey cDNAs for NAD$^+$- and NADP$^+$-Dependent PGDHs—We isolated the full-length cDNAs for the monkey NAD$^+$- and NADP$^+$-dependent types of PGDH from the lung and cornea, respectively, by RACE with an Oligo dT-Adapter Primer and sequence-specific primers designed from the 5′ non-coding region of the human cDNAs for NAD$^+$-dependent PGDH (*25*) and NADP$^+$-dependent PGDH (*10*). Each PCR product was cloned into the plasmid, and several clones were obtained and sequenced to verify the completely identical nucleotide sequences for each type of PGDH. The amino acid sequences predicted from the cDNAs are presented in Fig. 2.

The full-length cDNA of the monkey NAD$^+$-dependent PGDH was approximately 2.6 kb long, and carried an open reading frame of 798 bp. Conceptual translation of the monkey NAD$^+$-dependent PGDH cDNA yielded a 28.9 kDa protein of 266 amino acids, which is very close to the respective 29.0 and 28.8 kDa for the human and mouse proteins. The monkey NADP$^+$-dependent PGDH cDNA was approximately 1.2 kb in length, and contained an open reading frame of 831 bp corresponding to 277 amino acid residues with a calculated molecular mass of 30.5 kDa, which is very close to the 30.4 and 30.7 kDa of the human and mouse proteins, respectively.

A comparison of the amino acid sequences of the NAD$^+$- and NADP$^+$-dependent types of PGDH among monkey, human, and mouse is also shown in Fig. 2. The amino acid sequence of monkey NAD$^+$-dependent PGDH showed 98.5 and 86.8% identity to the human and mouse enzymes, respectively, and the NADP$^+$-dependent PGDH showed 94.9 and 85.2% identity to the human and mouse enzymes, respectively. However, the NAD$^+$- and NADP$^+$-dependent enzymes shared less than 20% identity in each species.

In the SDR family, several conserved motifs or residues have been identified (*5*). The Gly-X-X-X-Gly-X-Gly motif for coenzyme binding in the amino-terminal part (*29*) and the Tyr-X-X-X-Lys motif for the proton-transfer reaction in the middle part (*30*) were also conserved as ^{12}Gly-Ala-Ala-Gln-Gly-Asp-^{18}Gly and ^{151}Tyr-Cys-Ala-Ser-^{155}Lys, respectively, in the monkey NADP$^+$-dependent PGDH, and as ^{12}Gly-Ser-Asn-Lys-Gly-Ile-^{18}Gly and ^{194}Tyr-Gly-Val-Thr-^{198}Lys, respectively, in the monkey NAD$^+$-dependent enzyme (Fig. 2). Furthermore, the ^{227}Cys residue essential for the activity of human NADP$^+$-dependent PGDH (*31*) was also conserved at position 227 in the monkey NADP$^+$-dependent PGDH and at position 182 in the monkey NAD$^+$-dependent PGDH (Fig. 2).

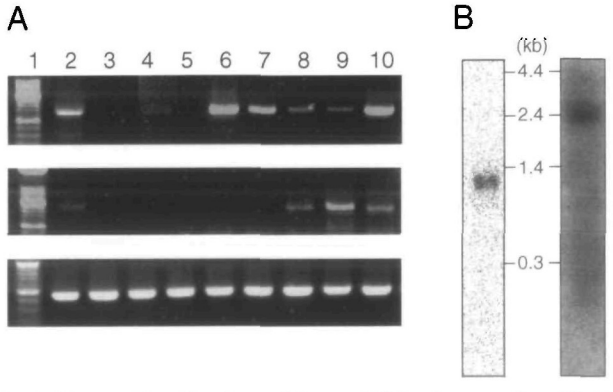

Fig. 3. **Tissue distribution of the mRNAs for monkey NAD$^+$- and NADP$^+$-dependent PGDHs.** (A) RT-PCR analysis of the mRNAs for NADP$^+$- (top panel) and NAD$^+$- (middle panel) dependent PGDHs in various monkey tissues. G3PDH mRNA (bottom panel) was amplified as an internal control using the primers 5′-ACCACAGTCCATGCCATCAC-3′ for the sense strand and 5′-TCCACCACCCTGTTGCTGTA-3′ for the antisense strand (*32*). Product sizes were 853 bp for NADP$^+$-dependent PGDH, 829 bp for NAD$^+$-dependent PGDH, and 452 bp for G3PDH. Lane 1, size marker; lane 2, cornea; lane 3, iris; lane 4, ciliary; lane 5, chorioretina; lane 6, optic nerve; lane 7, sclera; lane 8, conjunctiva; lane 9, lung; lane 10, liver. (B) Northern blot analysis for monkey NADP$^+$- and NAD$^+$-dependent PGDH mRNAs. Total RNA (10 μg) prepared from the optic nerve and lungs was hybridized with the monkey cDNA probe for NADP$^+$- (left panel) or NAD$^+$- (right panel) dependent PGDH.

Distribution of the Transcript for Monkey NADP⁺-Dependent PGDH in Ocular Tissues—We examined the tissue distribution profiles of the mRNAs for NAD⁺- and NADP⁺-dependent monkey PGDHs by RT-PCR (Fig. 3A). The mRNA for NADP⁺-dependent PGDH was expressed strongly in the liver and ocular tissues such as cornea, sclera, optic nerve, and weakly in the conjunctiva (Fig. 3A, top panel), whereas intense expression of the mRNA for NAD⁺-dependent PGDH was found in the lung but the levels in ocular tissues were very low (Fig. 3A, middle panel). The glyceraldehyde-3-phosphate dehydrogenase (G3PDH) transcript was ubiquitously detected in all tissues investigated (Fig. 3A, bottom panel).

Northern blot analysis with total RNA from lungs and optic nerve revealed that the full-length cDNAs for monkey NADP⁺- and NAD⁺-dependent PGDH hybridized with a single transcript of approximately 1.3 and 2.6 kb, respectively (left and right panels, respectively, in Fig. 3B), consistent with the size of each type of cDNA obtained.

The cellular distribution of the NADP⁺-dependent PGDH mRNA in the monkey cornea was investigated by *in situ* hybridization. Intense staining was restricted to the cornea epithelial cells when hybridization was carried out with the antisense probe (arrowheads in Fig. 4A), whereas the sense probe produced no positive signals (Fig. 4B). These results indicate that the NADP⁺-dependent PGDH mRNA is expressed widely in monkey ocular tissues and is localized in the epithelial cells of the cornea.

Enzymatic Properties of Monkey NADP⁺-Dependent PGDH—The recombinant NADP⁺-dependent PGDH was efficiently produced in *E. coli* transformed with the pET21-monkey PGDH2 plasmid following induction with IPTG (lane 3 in Fig. 5). The recombinant protein was purified from the soluble fraction of *E. coli* extracts by T7 Tag-affinity column chromatography to >90% homogeneity as judged by CBB staining after SDS-PAGE (Fig. 5, lane 5). The molecular mass of the purified protein was approximately 31 kDa, which is almost identical to the 30.5 kDa calculated from the predicted amino acid sequence of monkey NADP⁺-dependent PGDH cDNA.

We then examined the substrate and cofactor specificities of the purified recombinant monkey NADP⁺-dependent PGDH. As summarized in Table I, the enzyme showed its highest activity with $PGF_{2\alpha}$ and NADP⁺. When NAD⁺ was used instead of NADP⁺, the activity decreased to approximately 25%. The monkey NADP⁺-dependent PGDH also catalyzed the dehydrogenation of PGE_2, the acid form of latanoprost, and latanoprost with about 40, 8, and 5% of the activity for $PGF_{2\alpha}$, respectively, in the presence of NADP⁺.

One minor and one major product of $PGF_{2\alpha}$ were detected by HPLC after the reaction with the recombinant NADP⁺-dependent PGDH at retention times of 9.8 and 10.9 min, respectively, corresponding to the retention times of authentic PGE_2 and 15-keto $PGF_{2\alpha}$, respectively (Fig. 6A). The production of 15-keto $PGF_{2\alpha}$ was also confirmed by GC-MS (data not shown). In the case of the acid form of latanoprost, two products were found by HPLC at 6.2 and 8.3 min

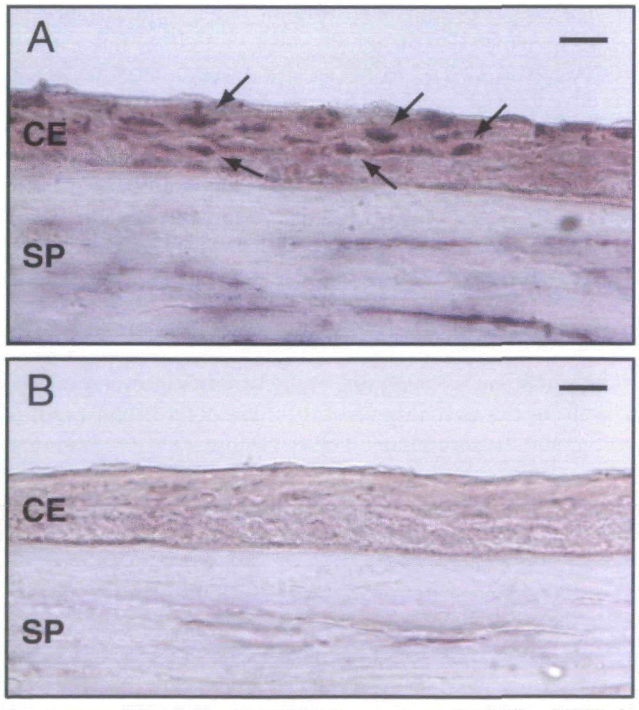

Fig. 4. **Localization of NADP⁺-dependent PGDH mRNA in monkey cornea.** NADP⁺-dependent PGDH mRNA in monkey cornea was detected by *in situ* hybridization analysis. (A) Antisense probe. Cells expressing the NADP⁺-dependent PGDH mRNA are indicated by arrows. (B) Sense probe. CE, cornea epithelium; SP, substantia propria. Scale bar: 10 μm.

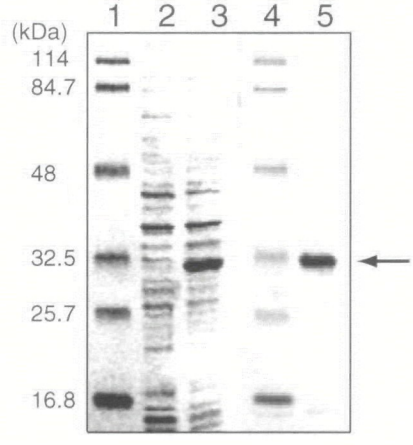

Fig. 5. **Expression of recombinant monkey NADP⁺-dependent PGDH in *E. coli*.** Recombinant monkey NADP⁺-dependent PGDH was expressed in *E. coli*. Extracts of the *E. coli* transformant were analyzed by SDS-PAGE followed by CBB staining before (lane 2) and after (lane 3) induction by IPTG. The recombinant protein was affinity-purified (lane 5). Molecular sizes of the markers (lanes 1 and 4) are shown on the left. The arrow indicates the recombinant monkey NADP⁺-dependent PGDH protein.

TABLE I. **Substrate specificities and cofactor requirement of the recombinant monkey NADP⁺-dependent PGDH.**

Substrate	Cofactor	Specific activity (nmol/min/mg protein)
$PGF_{2\alpha}$	NADP⁺	24.3
$PGF_{2\alpha}$	NAD⁺	6.08
$PGF_{2\alpha}$	NADPH	<0.1
PGE_2	NADP⁺	9.80
Acid form of latanoprost	NADP⁺	2.17
Latanoprost	NADP⁺	1.20

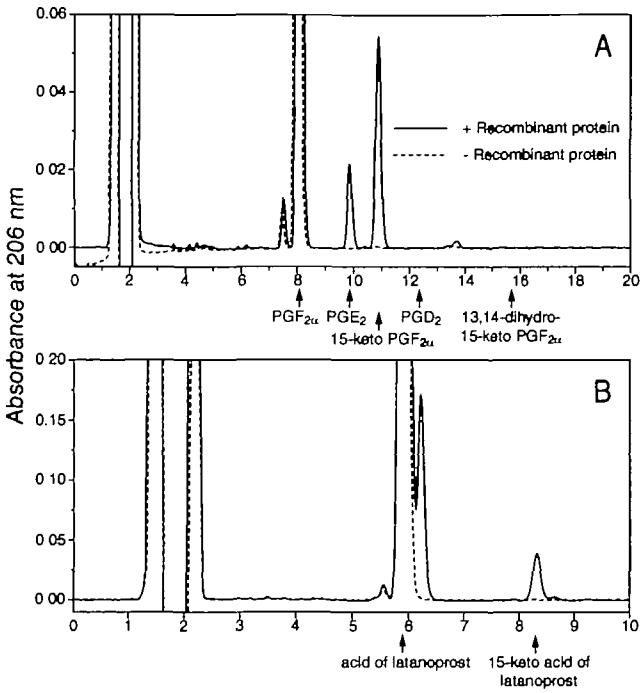

Fig. 6. **HPLC profiles of 15-keto metabolites produced from $PGF_{2\alpha}$ and from the acid form of latanoprost by the recombinant monkey NADP$^+$-dependent PGDH.** Conversion of $PGF_{2\alpha}$ (A) and the acid form of latanoprost (B) to their 15-keto metabolites in the presence (solid trace) or absence (dotted trace) of the recombinant monkey NADP$^+$-dependent PGDH was monitored by HPLC. The elution positions of the authentic PGs and their metabolites are indicated by arrows at the bottom.

(Fig. 6B). In GC-MS, the latter minor peak was identified as the 15-keto acid form of latanoprost, and the former major peak was identified as the 9- and/or 11-keto acid form of latanoprost (data not shown). In the absence of the recombinant NADP$^+$-dependent PGDH, no product was detected from $PGF_{2\alpha}$ or from the acid form of latanoprost. These results, taken together, indicate that the monkey NADP$^+$-dependent PGDH has the ability to dehydrogenate $PGF_{2\alpha}$ and the acid form of latanoprost to produce their 15-keto metabolites.

DISCUSSION

NAD$^+$- and NADP$^+$-dependent PGDH cDNAs have been isolated from several animal species as well as from humans (*6, 10*). In human and mouse, the mRNAs for these two distinct types of PGDH are widely expressed in many tissues (*2, 7, 15–17*), but the localization and characterization of the enzyme in ocular tissues have received little attention. Here, we report the molecular cloning of NAD$^+$- and NADP$^+$-dependent PGDH cDNAs from monkey, the tissue distribution of their transcripts, and the properties of the recombinant NADP$^+$-dependent enzyme. Monkey NAD$^+$- and NADP$^+$-dependent PGDHs show a high degree of homology to their counterparts in other species (more than 85%), but show lower identity (less than 20%) to each other (Fig. 2). Although the overall identity between them is low, their primary structures contain conserved amino acid motifs and residues responsible for coenzyme binding, proton-transfer reaction, and enzymatic activity, as found in other SDR family proteins (*5*). The three-dimensional structures of the SDR family proteins, including both NAD$^+$- and NADP$^+$-dependent PGDHs, are highly conserved even though the sequence homology among members of the SDR family is at the 20% level (*33*), indicating that SDR family proteins evolved from the same ancestral molecule, phylogenetically branched off at a very early phase of evolution, and evolved further independently while maintaining similar tertiary structures.

In this study, we clearly demonstrate that the NADP$^+$-dependent PGDH mRNA is actively expressed in the cornea, sclera, and optic nerve and weakly in the conjunctiva of monkey eye (Fig. 3A, top panel). Furthermore, we found that the NAD$^+$-dependent PGDH mRNA is expressed at very low levels only in the cornea and conjunctiva (Fig. 3A, middle panel). Previously, NAD$^+$-dependent PGDH activity has been detected in the ocular tissues of rabbit (*20*), rat (*21*), and pig (*22*). On the other hand, the PGDH expressed in the human retina was identified as the NADP$^+$-dependent enzyme (*16*). Our present results of the tissue distribution analysis, together with the previous findings, indicate that the NADP$^+$-dependent PGDH is dominant in ocular tissues rather than the NAD$^+$-dependent PGDH.

We expressed the recombinant monkey NADP$^+$-dependent PGDH in *E. coli* and characterized its enzymatic properties to investigate the PG metabolism in ocular tissues. We found that the enzyme utilized $PGF_{2\alpha}$ as the best substrate, and also PGE_2, latanoprost, and the acid form of latanoprost as weak substrates (Table I). The catalytic activity of this recombinant monkey enzyme for PGE_2 was on the same order with that of the native swine NADP$^+$-dependent enzyme (*34*). HPLC analysis revealed that $PGF_{2\alpha}$ was converted primarily to 15-keto $PGF_{2\alpha}$ and also weakly to PGE_2, which is the 9-keto form of $PGF_{2\alpha}$, however, no production of PGD_2, which is the 11-keto form of $PGF_{2\alpha}$, was observed (Fig. 6A). The acid form of latanoprost was dehydrogenated to the 15-keto acid form of latanoprost and to the 9- and/or 11-keto metabolites (Fig. 6B). However, identification of the 9- and 11-keto metabolites of the acid form of latanoprost was not accomplished in this study, because the authentic compound for each metabolite was not available. The recombinant NADP$^+$-dependent PGDH did not catalyze further conversion from 15-keto $PGF_{2\alpha}$ to 13,14-dihydro-15-keto $PGF_{2\alpha}$. These results indicate that NADP$^+$-dependent PGDH catalyzes the initial reaction of PG metabolism by oxidizing the 15-hydroxyl group of PGs, as well as the dehydrogenation of the 9-hydroxyl group of $PGF_{2\alpha}$ and its analogue. These results also suggest that $PGF_{2\alpha}$ and the acid form of latanoprost are metabolized by the NADP$^+$-dependent, but not by the NAD$^+$-dependent, PGDH in monkey ocular tissues.

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