Lipocalin-type Prostaglandin D Synthase (β-Trace) Is a Newly Recognized Type of Retinoid Transporter*

(Received for publication, December 17, 1996, and in revised form, March 21, 1997)

Toshiki Tanaka‡‡, Yoshihiro Urade‡, Hiromi Kimura‡, Naomi Eguchi**, Akemi Nishikawa‡, and Osamu Hayaishi¶

From ‡‡Protein Engineering Research Institute, 6-2-3 Furuedai, Suita, Osaka 565, Japan, the §Biomolecular Engineering Research Institute, 6-2-3 Furuedai, Suita, Osaka 565, Japan, Osaka Bioscience Institute, 6-2-4 Furuedai, Suita, Osaka 565, Japan, and **PRESTO, Japan Science and Technology Corporation, 6-2-4 Furuedai, Suita, Osaka 565, Japan

Lipocalin-type prostaglandin D synthase is responsible for the biosynthesis of prostaglandin D₂ in the central nervous system and the genital organs and is secreted into the cerebrospinal fluid and the seminal plasma as β-trace. Here, we analyzed retinoids binding of the enzyme by monitoring the fluorescence quenching of an intrinsic tryptophan residue, and appearance of circular dichroism around 330 nm, and a red shift of the UV absorption spectra of retinoids. We found that the enzyme binds all-trans- or 9-cis-retinoic acid and all-trans- or 13-cis-retinol, but not all-trans-retinol, with affinities (KD of 70–80 nM) sufficient for function as a retinoid transporter. All-trans-retinoic acid inhibited the enzyme activity in a noncompetitive manner, suggesting that it binds to the same hydrophobic pocket as prostaglandin H₂, the substrate for prostaglandin D synthase, but at a different site in this pocket. It is likely that this enzyme is a bifunctional protein that acts as both retinoid transporter and prostaglandin D₂-producing enzyme.

Retinoids play an important role in regulating a variety of biological processes, including differentiation, morphogenesis, and cell proliferation. The process is initiated by retinoid binding to the nuclear receptor for retinoic acid (RAR/RXR); RAR binds both all-trans- and 9-cis-retinoic acids, whereas RXR is specific for the 9-cis-isomer (1–3). The binding of retinoic acids to the dimerized receptor, RAR-RXR or RXR-RXR, activates or inhibits the transcription of retinoid-responsive genes. The proteins that transport retinoids are divided into two distinct families based on their sequence, structure, and function: the secretory transporters and the intracellular transporters (4). Secretory retinoid transporters, such as plasma retinol-binding protein (RBP) and β-lactoglobulin, circulate retinoids in a variety of body fluids and transport them to the intracellular retinoid transporters, cellular RBP (CRBP), and cellular retinoid acid-binding protein (CRABP), which finally transfer the retinoids to RAR or RXR.

Retinoids also regulate a number of genes expressed in the central nervous system and thus play a variety of important roles, particularly in development (5, 6). In the brain, mRNAs for RAR β1 and β3 (5, 7) and for RXR β and γ (5, 7) were found as well as CRBP and CRABP (8, 9). However, secretory retinoid transporters in the central nervous system have not been identified, although retinoids would need to change transporters at the blood-brain barrier as the barrier is impermeable to the secretory transporter.

Recently, a major protein in human cerebrospinal fluid, classically termed β-trace (10), was identified as prostaglandin (PG) D synthase (11–14). The enzyme is responsible for biosynthesis of PGD₂, which is a major PG in the brain of various mammals, including humans, and is proposed to be an endogenous sleep-promoting substance (15, 16), as well as a modulator of several central actions, such as the regulation of body temperature, luteinizing hormone release, and odor responses (17, 18). PGD synthase is produced in the choroid plexus, leptomeninges, and oligodendrocytes of the central nervous system (19, 20) and secreted into the cerebrospinal fluid. It is also localized in the pigmented epithelial cells of the rat retina (21) and in the epithelial cells of human male genital organs (22) and secreted into the interphotoreceptor matrix and seminal plasma, respectively, both of which are a closed compartment isolated by the respective blood-retina and blood-testicular barriers.

The results of cloning and sequence analyses of the rat and human cDNAs (23, 24) for the enzyme have already been reported. A homology search in data bases of protein primary structure revealed that the enzyme is a new member of the lipocalin superfamily (24–26), a group of proteins comprising a variety of secretory proteins that bind and transport small lipophilic molecules (27, 28). The gene structures for the rat and human enzymes are also comparable with those of other members of the lipocalin superfamily, in terms of the numbers and sizes of the exons and the phasing patterns of the introns (29, 30). Furthermore, the gene for the enzyme has been mapped within the lipocalin gene cluster in human chromosome 9 (30) and mouse chromosome 2 (31).

Secretory retinoid transporters, such as plasma RBP and β-lactoglobulin, are also members of the lipocalin family and show weak homology (20% identity) toward PGD synthase (24, 26). Thus, we considered that the enzyme may be involved in the transport of bioactive lipophilic substances in the central nervous system via the cerebrospinal fluid, analogous to the functions of other lipocalins, such as plasma RBP in the sys-
tomic circulation and β-lactoglobulin in milk. In this study, by measuring the fluorescence, UV, and circular dichroism (CD) spectra after incubation of the recombinant rat brain PGD synthase (32) with various isoforms of retinoid, we found that the enzyme binds all-trans- or 9-cis-retinoic acid and all-trans- or 13-cis-retinal with affinities comparable with those of other secretory retinoid transporters.

EXPERIMENTAL PROCEDURES

Materials—All-trans-retinoic acid, all-trans-retinal, all-trans-retinol, and N-acetyl-L-tryptophanamide were purchased from Sigma. 9-cis-Retinoic acid was purchased from Wako Junyaku (Osaka, Japan), and 13-cis-retinal was provided by Dr. K. Yoshihara, Suntory Institute for Molecular Biology. [3H]Retinoids (2.20 GBq/mmol) and [1-14C]arachidonic acid (2.20 GBq/mmol) were from DuPont NEN.

Expression and Purification of Recombinant Rat Brain PGD Synthase—The full-length cDNA for rat brain PGD synthase, which is composed of 189 amino acid residues (GenBank™ accession number M61900) (24), was ligated into the EcoRI-HindIII site of pUC119. The N-terminal 29 amino acid residues containing the signal peptide were composed of 189 amino acid residues (GenBank™ accession number M61900) (24), was ligated into the EcoRI-HindIII site of pUC119. The N-terminal 29 amino acid residues containing the signal peptide were expressed in Escherichia coli mutants were expressed in Escherichia coli JM109 and were purified to apparent homogeneity by Sephadex G-50 and S-Sepharose column chromatography (32). The Ala89,186 enzyme with a GS adduct at Cys 65 was prepared as reported previously (32).

Fluorescence Quenching—Retinoids were dissolved in ethanol to give a stock solution of 1 mM. The concentrations of retinoic acid, retinal, and retinol were determined spectrophotometrically in an ethanol solution based on their respective molar absorption coefficients of ε336 of 45,000 M⁻¹ cm⁻¹, ε383 of 42,800 M⁻¹ cm⁻¹, and ε325 of 46,000 M⁻¹ cm⁻¹, respectively (33). The retinoid solution (10 μl), which also contained a trace amount of [3H]retinoids (10,000 dpm), was added to 1 ml of 5 mM Tris-HCl, pH 8.0, containing 1.5 nmol of the purified enzyme. After incubation at 22 °C for 30 min, the fluorescence of the tryptophan residue was measured with a Shimazu Spectrofluorophotometer RF-5000 (Kyoto, Japan) with the excitation wavelength at 282 nm and emission wavelength at 338 nm. The loss of ligand, due to nonspecific adsorption to the incubation tubes, was corrected for by measuring the radioactivity of the [3H]retinoids in the incubation mixture after the measurements. The titration of N-acetyl-L-tryptophanamide was used to correct for the quenching of the tryptophan fluorescence due to nonspecific interactions with retinoids.

The apparent dissociation constant (Kₐ) of a single binding site was calculated by the method of Cogan et al. (34),

\[ P_{0m} = (R/n)(a/1-a) - K_a/n \]  

where \( a \) is the fraction of free binding sites, \( R \) is the total concentration of retinoid, \( P_0 \) is the total concentration of the enzyme, and \( n \) is the apparent molar ratio of the ligand to the enzyme at saturation. The value of \( a \) was calculated for every point on the titration curve by the following equation,

\[ a = (F - F_{\text{min}})/(F_0 - F_{\text{min}}) \]

where \( F \) is the relative fluorescence intensity at a certain \( R_0 \), \( F_{\text{min}} \) is the minimum fluorescence intensity after saturation of all ligand-binding sites of the enzyme (in the presence of a 5-fold molar excess of ligand), and \( F_0 \) is the initial fluorescence intensity in the absence of ligand.

Spectrophotometric Analysis—Absorption spectra were recorded at 25 °C after the addition of retinoids (20 μM, final concentration) or an equal volume of buffer (5 mM Tris-HCl, pH 8.0) to the enzyme (80 μM, final concentration) for a final volume of 1 ml in a cuvette (10-mm path length) in a DU 64 spectrophotometer (Beckman).

Circular Dichroism—CD spectra were measured after incubation of the protein (80 μM) with retinoids (20 μM) in 5 mM Tris-HCl, pH 8.0 (2 ml). They were recorded on a J-720 spectropolarimeter (Japan Spectroscopic, Tokyo, Japan) with the sample in a 10-mm path length cuvette at 10 °C. The spectra were recorded five times for each sample in the near-UV range from 300 to 400 nm, with a bandwidth of 1 nm and a resolution of 1 nm.

Enzyme Assay—The PGD synthase activity was measured by incubation at 25 °C for 1 min with [1-13C]PGH₂ (final 40 μM) in 50 μl of 0.1 M Tris-HCl, pH 8.0, in the presence of 1 mM GSH, unless otherwise stated (35). [1-13C]PGH₂ was prepared from [1-13C]arachidonic acid (35).

**FIG. 1.** Tryptophan fluorescence quenching by retinoids. A, the fluorescence spectra of Δ1–29 PGD synthase (1.5 nmol) were recorded after excitation at 282 nm in the absence (trace a) or presence of 1.5 μM (trace b) and 5 μM (trace c) of all-trans-retinoid acid. Representative results from five independent experiments are shown. The fluorescence intensity is shown relative to the intensity in the absence of retinoic acid. B, fluorescence titration curves indicate PGD synthase binds all-trans-, 9-cis-retinoic acid, and all-trans-retinal. Fluorescence intensities at 350 nm after excitation at 282 nm were recorded in the presence of varying amounts of all-trans-retinoic acid (open circles), all-trans-retinal (open triangles), all-trans-retinol (open squares), 9-cis-retinoic acid (closed circles), and 13-cis-retinal (closed triangles). C, the linear least squares plot of \( P_{0m} \) versus \( R_0(a/1-a) \) of PGD synthase with all-trans-retinoic acid is shown. This result is representative of the results of five independent experiments.
Retinoid Binding to Prostaglandin D Synthase

TABLE I
Retinoid affinities of recombinant PGD synthase and retinoid transporter proteins

| Protein (species)         | Retinoids                           | $K_d$ nM | Ref.     |
|---------------------------|-------------------------------------|----------|----------|
| Δ1–29 PGD synthase (rat)  | All-trans-retinoic acid             | 80 ± 15  | This study |
|                           | 9-cis-Retinoic acid                 | 80 ± 20  | This study |
|                           | All-trans-retinal                   | 70 ± 20  | This study |
|                           | 13-cis-Retinal                      | 70 ± 20  | This study |
|                           | All-trans-retinol                   | ND*      | This study |
| β-Lactoglobulin (bovine)  | All-trans-retinoic acid             | 39       | 40        |
|                           | All-trans-retinol                   | 47       | 40        |
|                           | All-trans-retinol                   | 85       | 47        |
| Plasma RBP (human)        | All-trans-retinoic acid             | 210      | 34        |
|                           | All-trans-retinol                   | 190      | 34        |
| Plasma RBP (bovine)       | All-trans-retinol                   | 90       | 37        |
| Plasma RBP (porcine)      | All-trans-retinoic acid             | 210      | 48        |
| CRBP (rat)                | All-trans-retinol                   | 50       | 49        |
|                           | All-trans-retinol                   | 50       | 49        |
| CRABP (rat)               | All-trans-retinoic acid             | 65       | 50        |
| CRABP (mouse)             | All-trans-retinoic acid             | 25       | 51        |
|                           | 9-cis-Retinoic acid                 | 69       | 51        |

* ND, not determined.

Fig. 2. UV maximum shift of retinoids by PGD synthase. UV spectra of PGD synthase (dotted line), retinoid (broken line), and a mixture of PGD synthase and retinoid (solid line) are shown. Note that PGD synthase does not absorb at wavelengths higher than 310 nm. A, all-trans-retinoic acid; B, all-trans-retinal; C, all-trans-retinol.

Modeling of Interaction between PGD Synthase and Retinoic Acid—
The model of PGD synthase was built with a homology modeling software HOMOLOGY (Molecular Simulations, Inc., San Diego, CA) as reported previously (36). Retinoic acid was built and manually docked into the cavity of PGD synthase by using Insight II molecular modeling software (Molecular Simulations, Inc.). During the process, we referred to the binding structure between rat epididymal retinoic acid-binding protein and retinoic acid (Protein Data Bank entry 1EPB), because this protein shows a sequence similarity of 19.7% identity to PGD synthase, and the amino acids lining the bottom of the hydrophobic cavity well correspond to those of PGD synthase. The model was further refined by manual modification of side chains to circumvent unfavorable atom-atom bumping. After the manual operation, molecular mechanics calculations were performed to relax side chains with the main chain fixed, by use of DISCOVER (Molecular Simulations, Inc.).

RESULTS

Fluorescence Quenching of PGD Synthase after Incubation with Retinoids—Several members of the lipocalin family, such as plasma RBP (37), β-lactoglobulin (38–40), and epididymal retinoic acid-binding protein (2), function as secretory retinoid transporters in a variety of body fluids. Since the binding of retinoids to those proteins is known to quench their intrinsic tryptophan fluorescence, we monitored the tryptophan fluorescence of recombinant PGD synthase after incubation with retinoids. For this purpose, we used recombinant Δ1–29 PGD synthase (32) from which the signal sequence had been deleted.

The Δ1–29 PGD synthase showed fluorescence quenching after addition of all-trans-retinoic acid in a dose-dependent manner (Fig. 1A). The fluorescence intensity decreased to 20% in the presence of excess amounts of all-trans-retinoic acid (Fig. 1B). The quenching was not observed after denaturation of the protein in the presence of 6 M guanidine hydrochloride. 9-cis-Retinoic acid resulted in fluorescence quenching, giving an almost identical titration curve to that of all-trans-retinoic acid (Fig. 1B). All-trans-retinal and 13-cis-retinal also quenched the fluorescence to about 40%; however, all-trans-retinol led to only 20% quenching under conditions of ligand excess.

When $P_{0\alpha}$ was plotted against $R_{0\alpha}(1 - \alpha)$ by the method of Cogan et al. (34), a positive regression line was obtained for the fluorescence quenching of the Δ1–29 enzyme with all-trans-retinoic acid (Fig. 1C). The number of apparent binding sites of the Δ1–29 enzyme was calculated from the slope to be 1.1 mol/mol, and the $K_d$ value was found to be 80 nM. The $K_d$ values for 9-cis-retinoic acid, all-trans-retinal, and 13-cis-retinal were 80, 70, and 70 nM, respectively, and the stoichiometries of the retinoid-protein complexes were about 1.1 for the three reti-
noids (Table I). The affinities of the enzyme for all-trans- and 9-cis-retinoic acid were comparable with or somewhat higher than those of other retinoid transporters. The $K_d$ value for all-trans-retinol was not determined.

In the presence of a 5-fold molar excess of all-trans-retinol, all-trans-retinoic acid and all-trans-retinal still exhibited fluorescence quenching and gave titration curves essentially identical to those in the absence of retinol (data not shown). These results indicate that retinol does not displace retinoic acid or retinal from the protein.

### Spectrophotometric and CD Analyses of Retinoid Binding to PGD Synthase

- Changes in the UV and CD spectra of retinoids have been reported to occur when retinoids bind to their transporter proteins, such as plasma RBP (41), $\beta$-lactoglobulin (42), CRBP (43), and CRABP (44). Therefore, we also examined the UV and CD spectra of retinoids after incubation with PGD synthase.

- Retinoic acid, retinal, and retinol yielded absorption spectra with peaks at 340, 380, and 317 nm, respectively. After forming the complex with PGD synthase, the spectra of all-trans-retinoic acid and all-trans-retinal were red-shifted approximately 30 nm, with peaks at 373 and 409 nm, respectively (Fig. 2, A and B). On the other hand, all-trans-retinol did not induce a significant change in the spectrum and instead produced a small shoulder around 373 nm (Fig. 2C).

- Incubation of the protein with all-trans-retinoic acid and all-trans-retinal induced changes in the CD spectra, with minima at 345 and 369 nm, respectively (Fig. 3, A and B). However, when the protein was incubated with all-trans-retinol, a weak positive change in the CD spectra was observed around 300 nm (Fig. 3C). These changes in the UV and CD spectra indicate that retinoic acid and retinal occupy fixed positions in the protein.

### Effect of Cys Residues on Retinoid Binding to PGD Synthase

We constructed three types of Cys→Ala-substituted mutants of PGD synthase, which contains three Cys residues at 65, 89, and 186: the Ala65 mutant, the Ala89,186 mutant lacking the intramolecular disulfide linkage between Cys89 and Cys186, and the Ala65,89,186 mutant lacking all of the Cys residues (32).

The titration curves of fluorescence quenching with all-trans-retinoic acid were almost identical between the Δ1–29 enzyme and the Ala65 mutant, indicating that the active thiol of Cys65 is not necessary for the retinoic acid binding (Fig. 4). The Cys65 residue is considered to be located in the hydrophobic pocket of the enzyme, and it is reactive with a sulphydryl modifier and reduced glutathione (GSH) (32). When the Cys65 residue of the parent and Ala89,186 enzymes was modified with GSH to form a glutathione adduct, the fluorescence quenching was inhibited by about 90% under conditions of ligand excess (Fig. 4). The Cys65 residue of the parent and Ala89,186 enzymes is also endogenously modified during expression in E. coli (32). The endogenously modified enzymes displayed only 10% of the fluorescence quenching after incubation with excess amounts of all-trans-retinoic acid (data not shown). Once the exogenous or endogenous modifiers were removed from Cys65 by treatment with dithiothreitol as reported previously (32), the retinoic acid binding activity was again recovered. These results suggest that retinoic acid binds in the hydrophobic pocket of PGD synthase, in which the active site of the enzyme is located.

When the disulfide linkage in the parent and Ala65 enzymes was cleaved by incubation with 1 mM dithiothreitol for 15 h, the fluorescence quenching was attenuated by about 40% in the presence of excess all-trans-retinoic acid (Fig. 4). Both the Ala89,186 and Ala65,89,186 mutants without the disulfide bond also exhibited weak fluorescence quenching with all-trans-retinoic acid, similar to the reduced forms of the parent and Ala65 enzymes. The $K_d$ values for the single binding sites of the

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**Fig. 3.** CD spectra change of retinoids by PGD synthase. CD spectra of PGD synthase (solid line), retinoid (broken line), and a mixture of PGD synthase and retinoid (solid line) are shown. Note that the protein and retinoids themselves are devoid of CD absorption in the range from 320 to 400 nm, where retinoids absorb. A, all-trans-retinoic acid; B, all-trans-retinal; C, all-trans-retinol.

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**Fig. 4.** Effect of Cys residues of PGD synthase on retinoid binding. Fluorescence quenching was recorded in the presence of varying amounts of all-trans-retinoic acid. Δ1–29 PGD synthase (open circles) and the Ala65 mutant (closed circles) with the disulfide bond between Cys89 and Cys186 show more intense quenching than the reduced form of Δ1–29 PGD synthase (open triangles) and the Ala65,186 mutant (closed triangles), both of which are devoid of the disulfide bond. Modification of Cys65 of Δ1–29 PGD synthase (open squares) and Ala65,186 mutant (closed squares) with GSH abolished retinoic acid binding. The conditions were described in the legend of Fig. 1.
for 1 min in the presence of 1 mM GSH in 0.1 M Tris-HCl, pH 8.0. The PGD synthase activity decreased with increasing concentrations of retinoic acid. On the other hand, the activity remained unchanged, when the enzyme was measured with 2.5 μM PGH₂ in the presence of various concentrations of all-trans-retinoic acid (closed circles). B, the enzyme was incubated with various concentrations of PGH₂ in the absence (squares) and presence of 5 μM (triangles) and 10 μM (circles) all-trans-retinoic acid. The Lineweaver-Burk plots show that the retinoic acid inhibits the enzyme activity in a noncompetitive manner.

**DISCUSSIONS**

The retinoid binding by PGD synthase was demonstrated by the measurement of fluorescence quenching of Trp residues of the enzyme (Figs. 1 and 4) and the red shift of the UV spectra (Fig. 2) and the appearance of CD spectral changes around 330 nm (Fig. 3) of retinoids. Among all-trans-retinoids, PGD synthase is specifically bound to retinoic acid and retinal, but not retinol (Figs. 1–3), suggesting that the functional groups, such as amino or hydroxy group exist in the ligand-binding pocket to interact with the carboxyl group of retinoic acid or retinal. We found that 9-cis-retinoic acid also bound PGD synthase with the same affinity as that of all-trans-retinoic acid (Fig. 1B). Both all-trans- and 9-cis-retinoic acids are ligands for RXR and RAR. All-trans-retinoic acid may be bound as a horseshoe conformation twisted at the C-8–C-9 bond, similar to that of the 9-cis-isofor. This is also found in the case of the epididymal retinoic acid-binding protein.

PGD synthase possesses three Cys residues: Cys^{65} is an essential thiol for the catalytic activity, and Cys^{89} and Cys^{186} form an intramolecular disulfide bridge. Our results indicate that Cys^{65} is crucial for the enzyme activity, but not for retinoid binding, because the substitution of Cys^{65} to Ala did not affect retinoid binding (Fig. 4). However, when Cys^{65} was modified with GSH, the retinoid binding was abolished (Fig. 4). Modification by GSH is a mild chemical modification, and therefore, it should not alter the conformation of the enzyme. We confirmed that CD spectrum of the Δ1–29 Ala^{89,186} enzyme was unchanged by the GSH modification of Cys^{65} (data not shown). It is, therefore, considered that all-trans-retinoic acid cannot enter the hydrophobic pocket of the modified enzyme, due to the steric hindrance by GSH. Furthermore, we speculated that retinoic acid binds in the same cavity as the substrate binds. In fact, all-trans-retinoic acid, but not all-trans-retinol, inhibited the enzyme activity in a noncompetitive manner (Fig. 5). Our results suggest that retinoic acid binds in the same cavity, but at a different site from where the substrate binds. Two types of β-lactoglobulin-retinol complex have been reported: one in which retinol binds to the external surface of β-lactoglobulin (39). Retinoid binding by PGD synthase corresponds to the former case.

We constructed a computer graphic model of PGD synthase and successfully placed retinoid acid within the hydrophobic cavity of the enzyme (Fig. 6). Based on the observed sequence homology, the enzyme is predicted to form an eight-stranded antiparallel β-barrel structure with a hydrophobic pocket, similar to other lipocalins (24, 36). Retinoids usually bind in the hydrophobic pocket of their transporters with the β-ionone ring of the retinoid toward the inside and the tail portion outside. In the model, retinoic acid binds at the bottom of the cavity with the distance from Trp^{143} short enough to cause the fluorescence quenching. The Trp residue is highly conserved among members of the lipocalin family. The Cys^{65} residue is located near the edge of the cavity, and PGH₂ binds relatively outside of the cavity. Binding of retinoic acid interferes with the interaction between PGH₂ and Cys^{65}, which is in good agreement with the fact that retinoic acid inhibited the enzyme activity.

PGD synthase was originally isolated as an enzyme converting PGH₂ to PGD₂. In the arachidonic acid cascade, arachidonic acid is converted to PGH₂ by PGH synthase, a membrane protein associated with both cyclooxygenase and peroxidase activities. Since PGH₂ is an unstable, highly reactive precur-
sor, PGD synthase should exist close to PGH synthase. As soon as PGH$_2$ is formed by PGH synthase from arachidonic acid, PGD synthase takes PGH$_2$ and converts it to the stable PGD$_2$. This reaction should be carried out near the membranes. In rat oligodendrocytes (19) and human arachnoid barrier cells (45), the enzyme was found by electron microscopy to be localized in the rough endoplasmic reticulum and outer nuclear membrane. The fact that retinoic acid inhibits production of PGD$_2$ (Fig. 5) suggests that retinoids may endogenously regulate the synthesis of PGD$_2$.

On the other hand, PGD synthase is also actively secreted into cerebrospinal fluid, interphotoreceptor matrix, and seminal plasma, in which a continuous supply of the substrate is unlikely. PGD synthase would thus have another function as a secretory protein. In fact, as shown in this study, the enzyme possesses high affinities for retinoids ($K_d$, 100 nM), sufficient for it to function as a secretory retinoid transporter (Table I). In the cerebrospinal fluid, the enzyme ($\beta$-trace) is found to be the second major protein following albumin. Moreover, different from albumin, which penetrates from the systemic circulation, PGD synthase is synthesized at the arachnoid membrane and choroid plexus in the brain, which form the blood-cerebrospinal fluid barrier. Transthyretin is also produced in the choroid plexus and mediates the transport of thyroxine from the blood stream to the thyroxine receptors in the brain (46). As judged by the affinities for retinoids, the content in the cerebrospinal fluid, and the sites of production, we propose that one of the functions of the secretory PGD synthase ($\beta$-trace) is to transport retinoids in the brain.

PGD synthase seems to be distributed where retinoids are required. For example, PGD synthase is detected in the neurons of infant rat brains, despite the absence of the corresponding mRNA. After the neurons have completely developed, PGD synthase is not detectable in them (19). There should thus be a mechanism that allows PGD synthase to be taken into the immature nerve cell but not into the completely developed cell. This suggests that PGD synthase plays a critical role in regulating the development of the neurons by, for example, the transfer of all-trans- or 9-cis-retinoic acid to RAR or RXR in the immature nerve cell. PGD synthase is produced in the retinal pigment epithelium and secreted into the interphotoreceptor matrix (21). The function there may be to supply retinal to the
photoreceptor. PGD synthase showed lower binding affinity for retinoid when the disulfide bond between Cys\textsuperscript{89} and Cys\textsuperscript{186} was reduced (Fig. 4). Such reduction might facilitate the release of retinoids in the cell.

Since PGD synthase binds all-trans- and 9-cis-retinoic acid, and all-trans- and 13-cis-retinol with the same affinity as reported for other retinoid transporters (Table I), the secreted metabolism as a secretory retinoid transporter. PGD synthase may play important roles in regulating retinoid metabolism in the central nervous system have not been identified yet, although many genes in the brain and various photoreceptor functions are regulated by retinoids. Thus, our results suggest that, as well as functioning as the PGD\textsubscript{2}-producing enzyme, PGD synthase may play important roles in regulating retinoid metabolism as a secretory retinoid transporter.

Acknowledgments—We are grateful to Dr. H. Kubodera, Mitsubishi Chemical Corporation, for the modeling of a binding structure between PGD synthase and retinoic acid. We also thank Y. Ono and S. Matsumoto, Osaka Bioscience Institute, for technical and secretarial assistance, respectively.

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