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ARTICLE

NMR resonance assignments of mouse lipocalin-type prostaglandin D synthase/prostaglandin J_2 complex

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Abstract

Lipocalin-type prostaglandin (PG) D synthase (L-PGDS) catalyzes the isomerization of PGH_2 to produce PGD_2, an endogenous somenogen, in the brains of various mammalians. We recently reported that various other PGs also bind to L-PGDS, suggesting that it could serve as an extracellular carrier for PGs. Although the solution and crystal structure of L-PGDS has been determined, as has the structure of L-PGDS complexed PGH_2 analog, a structural analysis of L-PGDS complexed with other PGs is needed in order to understand the mechanism responsible for the PG trapping. Here, we report the nearly complete ^{1}H, ^{13}C, and ^{15}N backbone and side chain resonance assignments of the L-PGDS/PGJ_2 complex and the binding site for PGJ_2 on L-PGDS.

Keywords Prostaglandin D_2 · Prostaglandin H_2 · Prostaglandin J_2 · Lipocalin · Lypocalin-type prostaglandin D synthase

Abbreviations

CSF	Cerebrospinal fluid
ITC	Isothermal titration calorimetry
L-PGDS	Lipocalin-type prostaglandin D synthase
PG	Prostaglandin
PPARγ	Peroxisome proliferator-activated receptor γ

Biological context

Lipocalin-type prostaglandin (PG) D synthase (L-PGDS; EC 5.3.99.2) is isolated from the rat brain and identified as the PGD_2 synthesizing enzyme (Urade et al. 1985). L-PGDS is abundantly expressed in the central nervous system of various mammals, male genitals, human heart, and mouse adipocytes (Fujimori et al. 2007), and is the second most abundant protein after serum albumin in human CSF (Clausen 1961; Xu and Venge 2000). In addition, it has also been revealed that L-PGDS is the only enzyme among members of the lipocalin gene family that is composed of a group of lipid-transporter proteins (Toh et al. 1996; Urade and Hayaishi 2000). L-PGDS binds a large variety of ligands, such as retinoids (Tanaka et al. 1997), biliverdin, bilirubin, thyroid hormones (Beuckmann et al. 1999), gangliosides (Mohri et al. 2006), and amyloid β peptide (Kanekiyo et al. 2007). L-PGDS is thus thought to be a multifunctional protein possessing both the ability to synthesize PGD_2 and to serve as a carrier protein for lipophilic molecules.

Mouse L-PGDS is a 189 amino acid protein, and is post-translationally modified by the cleavage of an N-terminal signal peptide comprising 24 amino acid residues (Hoffmann et al. 1996; Urade and Hayaishi 2000). Mouse L-PGDS contains three cysteine residues, Cys65, Cys89, and Cys186. These three cysteine residues are conserved among all mammals. Two of these Cys residues, Cys89 and Cys186, form a disulfide bridge, which is highly conserved among most lipocalins. On the other hand, Cys65 residue is unique to L-PGDS and is essential for the catalytic reaction (Urade et al. 1995; Irikura et al. 2003). In previous mutational and structural studies, we reported that L-PGDS has a large hydrophobic cavity in its β-barrel fold and two substrate binding sites are present in the cavity (Shimamoto et al. 2021). In an isothermal titration calorimetry (ITC) analysis, we showed that L-PGDS binds not only its substrate, PGH_2, but also to various other PGs, such as PGD_2, PGE_2, and $PGF_{2\alpha}$ (Shimamoto et al. 2021). The affinity of some PGs ($K_d = 0.3 \sim 3$ μM) was higher than those of thyroid hormones ($K_d = 0.5 \sim 6$ μM), endogenous ligands of L-PGDS

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(Beuckmann et al. 1999), suggesting that L-PGDS has the ability to transport PGs (Shimamoto et al. 2021).

The PGD_2, produced by L-PGDS, is chemically unstable and readily undergoes non-enzymatically dehydration to produce the J series of PGs, such as PGJ_2, Δ^{12}-PGJ_2, and 15-deoxy-$\Delta^{12,14}$-PGJ_2 (Fitzpatrick and Wynalda 1983; Kikawa et al. 1984). These PGJs are actively taken into cells and accumulated in the nuclei, and especially 15-deoxy-$\Delta^{12,14}$-PGJ_2 is known as a natural activator for the peroxisome proliferator-activated receptor γ (PPARγ) (Kliewer et al. 1995).

The mechanism by which L-PGDS recognizes PG is not fully understood, because a structural analysis of L-PGDS in complex with PGs, except for the substrate analog, remains to be performed. In this study, we report that 15-deoxy-$\Delta^{12,14}$-PGJ_2, which is nonenzymatically derived from PGD_2, binds to L-PGDS by NMR and present the nearly complete assignment of the backbone and side chain resonances of the L-PGDS/15-deoxy-$\Delta^{12,14}$-PGJ_2 complex.

Methods and experiments

The gene corresponding to Δ1-24 mouse L-PGDS (165 residues) was synthesized and inserted into a bacterial expression vector pGEX-2T and transformed into *Escherichia coli* BL21(*DE3*) cells. The transformed colonies were grown in 3 mL of LB medium containing 100 mg/L of ampicillin at 37 °C for 4 h. One milliliter of the bacterial culture was diluted in 1 L of M9 minimal medium containing ^{15}N ammonium chloride (1 g/L) and/or ^{13}C glucose (2 g/L) as the sole nitrogen and carbon sources. The bacterial culture was grown at 37 °C to an optical density at 600 nm of 0.6. After induction with 1 mM isopropyl-β-D-thiogalactopyranoside, the protein was expressed as a glutathione S-transferase fusion protein in BL21(*DE3*) cells for 5 h at 37 °C. The cells were harvested by centrifugation for 30 min at 4000×g at 4 °C, resuspended in 30 mL of PBS and sonicated in 4 °C. The bacterial lysate was clarified by centrifugation at 20,000×g for 30 min at 4 °C. The protein was purified by affinity chromatography with Glutathione Sepharose 4B

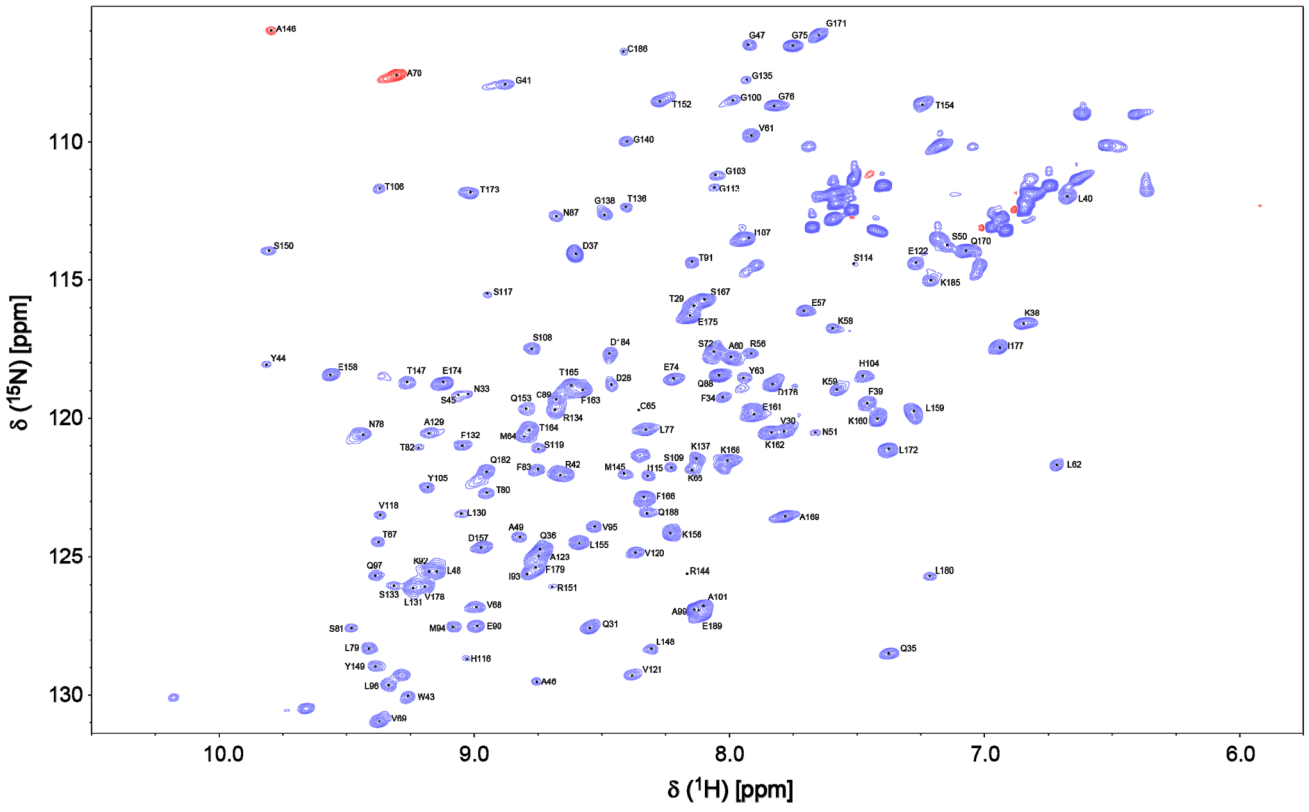

Fig. 1 ^{1}H-^{15}N HSQC spectrum of the L-PGDS/15-deoxy-$\Delta^{12,14}$-PGJ_2 complex. All assigned cross peaks are labeled with sequence number. The aliased peaks are shown in red

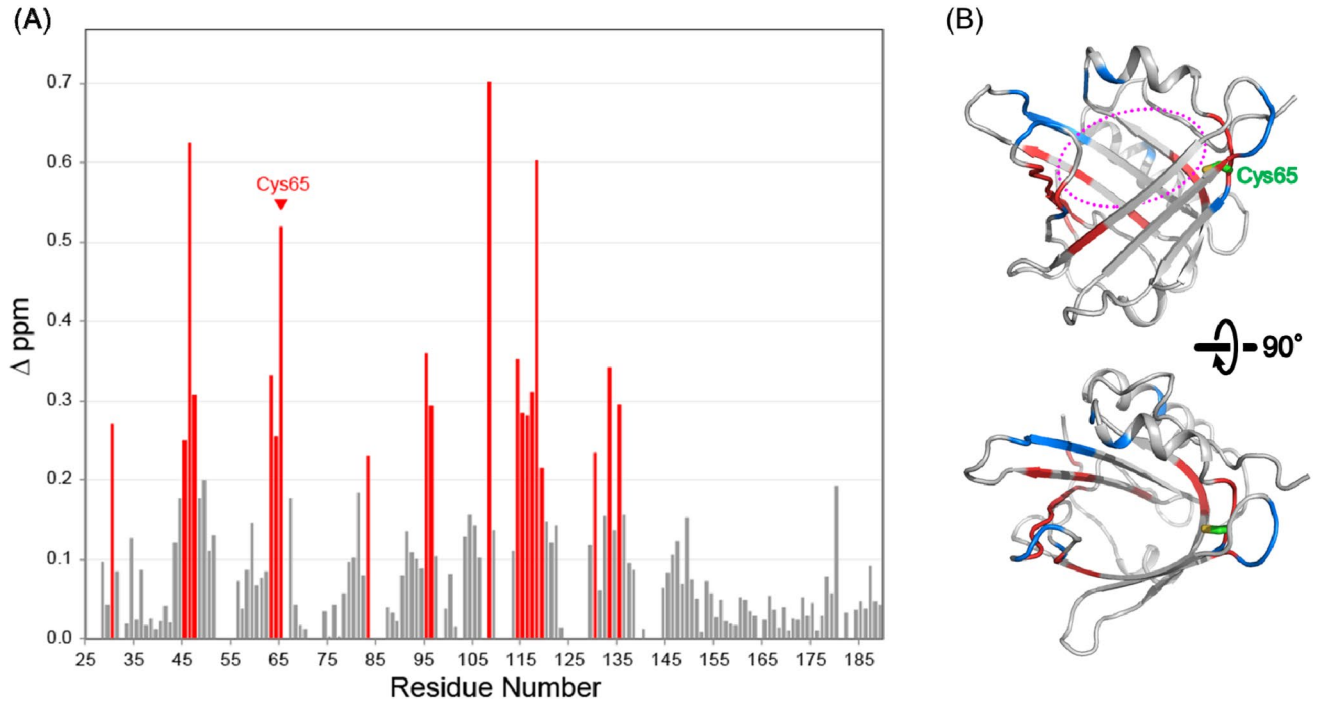

Fig. 2 Chemical shift perturbations of the ^{1}H-^{15}N HSQC spectrum of L-PGDS upon the binding of 15-deoxy-$\Delta^{12,14}$-PGJ_2. **A** ^{1}H and ^{15}N chemical shift differences versus the amino acid sequence. The chemical shift differences were calculated according to the empirical equation $\Delta ppm = \{(\Delta\delta_{HN} \times W_{HN})^2 + (\Delta\delta_N \times W_N)^2\}^{1/2}$ where, $\Delta\delta_{HN}$ and $\Delta\delta_N$ are the chemical shift changes of ^{1}H and ^{15}N, respectively. The weighting factors used were $W_{HN} = 1$, $W_N = 0.2$. The residues with relatively large changes in chemical shift ($\Delta ppm \geq 0.2$) are highlighted in red. **B** Mapping of NMR signal perturbation on L-PGDS backbone structure by binding of 15-deoxy-$\Delta^{12,14}$-PGJ_2. Backbone residues with relatively large changes in chemical shift ($\Delta ppm \geq 0.2$) are shown in red, whereas residues whose signals disappeared upon the binding of 15-deoxy-$\Delta^{12,14}$-PGJ_2 are shown in blue. Magenta dotted ellipses indicate the catalytic binding site of the PGH_2 substrate

(GE Healthcare, Tokyo, Japan) and size-exclusion chromatography with Superdex 75 (GE Healthcare, Tokyo, Japan), as reported previously (Inui et al. 2003). The L-PGDS/15-deoxy-$\Delta^{12,14}$-PGJ_2 complex sample was prepared by incubating a 1:1 ratio of L-PGDS and 15-deoxy-$\Delta^{12,14}$-PGJ_2 (Cayman, CA, USA). The complex sample were dialyzed and concentrated to approximately 0.5 mM in the NMR buffer (50 mM sodium phosphate, D_2O or an 85% H_2O/15% D_2O, pH 6.5).

To perform backbone and side chain resonance assignments of the L-PGDS/15-deoxy-$\Delta^{12,14}$-PGJ_2 complex, all 2D and 3D NMR experiments were carried out at 25 °C on an INOVA600 with a triple resonance $^{1}H/^{13}C/^{15}N$ cryoprobe. Backbone assignments were obtained from the standard double and triple resonance NMR experiments, ^{1}H-^{15}N HSQC, CBCA(CO)NH and HNCACB (Bax et al. 1994; Kay 1995). Side chain assignments were achieved by measurement of ^{1}H-^{13}C HSQC, HBHA(CO)NH, HCCH-TOCSY and CCH-TOCSY. All spectra were processed with NMRPipe (Delaglio et al. 1995). Resonance assignment was performed using the NMRviewJ (Merck Research Laboratories).

Extent of assignments and data deposition

Using the sequence-specific backbone assignments, 84% $^{1}H_N$, 84% ^{15}N, 91% C_α, and 90% C_β chemical shifts were assigned for the L-PGDS/15-deoxy-$\Delta^{12,14}$-PGJ_2 complex (Fig. 1). Signals for several residues in the N-terminus and loops (e.g., Gln25, Gly26, His27, Ser53, Trp54, Asn124, and Tyr125) were missing from the ^{1}H-^{15}N HSQC presumably due to ms-μs exchange line broadening and/or exchange with the solvent. These residues are also not defined in apo-L-PGDS (Shimamoto et al. 2007). For the aliphatic side chain moieties of the protein, 84% ^{1}H and 85% ^{13}C were assigned. The chemical shifts have been deposited in the BioMagnResBank (http://www.bmrb.wisc.edu) under the accession number 51128.

In a comparison of the ^{1}H-^{15}N HSQC spectrum of L-PGDS/15-deoxy-$\Delta^{12,14}$-PGJ_2 complex with apo-L-PGDS, numerous peaks in the HSQC showed significant perturbations upon 15-deoxy-$\Delta^{12,14}$-PGJ_2 addition, suggesting that 15-deoxy-$\Delta^{12,14}$-PGJ_2 binds to L-PGDS. Upon 15-deoxy-$\Delta^{12,14}$-PGJ_2 binding, large chemical shift changes were

observed at the catalytic site that is comprised of 7 residues, the Cys65 catalytic center, Ser45, Ala46, Gly47, Tyr63, Met64, and Phe83 (Shimamoto et al. 2021). In addition, the chemical shifts for 12 residues (Val95, Leu96, Ser108, Ser114, Ile115, His116, Ser117, Val118, Ser119, Leu130, Ser133, and Gly135) were changed substantially (Fig. 2A). These residues are located at the high affinity substrate binding site which is involved in trapping the substrate into the cavity during the catalytic reaction (Shimamoto et al. 2021). These results suggest that 15-deoxy-$\Delta^{12,14}$-PGJ_2 has the potential to inhibit L-PGDS activity.

In our previous study, we elucidated the binding of L-PGDS to several ligands, such as PGs (Shimamoto et al. 2007, 2021), NADs (Qin et al. 2015), retinoic acid (Shimamoto et al. 2007) and biliverdin (Miyamoto et al. 2010) by NMR titration experiments and demonstrated that high affinity ligands, such as retinoic acid and biliverdin ($K_d < 0.1$ μM), caused a significant broadening of the resonances of L-PGDS (Shimamoto et al. 2007; Miyamoto et al. 2010). In the ^{1}H-^{15}N HSQC spectrum of the L-PGDS/15-deoxy-$\Delta^{12,14}$-PGJ_2 complex, the HN signals of 13 residues disappeared due to the signal broadening and these residues were close to the residues that showed large chemical shift changes (Fig. 2B). This suggests that 15-deoxy-$\Delta^{12,14}$-PGJ_2 binding as well as biliverdin binding cause significant broadening in signals for residues in the binding site.

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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