Compact Packing of Lipocalin-Type Prostaglandin D Synthase Induced by Binding of Lipophilic Ligands

Lipocalin-type prostaglandin D synthase (L-PGDS; prostaglandin-H_2 D-isomerase, EC 5.3.99.2) is responsible for the biosynthesis of prostaglandin D_2 (PGD_2) from prostaglandin H_2, which is a common precursor of all prostanoids [1]. L-PGDS is also known as a member of the lipocalin superfamily, which comprises lipid-transporter proteins such as β-lactoglobulin (βLG), retinol-binding protein (RBP), major urinary protein, aphorodisin, and tear lipocalin. It is well known that the lipocalins are small proteins of approximately 200 amino acid residues and 20 kDa in molecular weight. Their tertiary structures are very similar despite their wide range of functions and high levels of sequence divergence. That is, the lipocalin proteins have a highly symmetrical all-β structure dominated by a single 8-stranded antiparallel β-sheet closed back on itself to form a continuous hydrogen bonded β-barrel. This β-barrel commonly encloses a ligand binding site composed of both an internal cavity and an external loop scaffold [2]. So far, we have clarified that L-PGDS can act not only as an enzyme but also as a lipid transporter. The results of such studies also revealed that L-PGDS can bind a large variety of lipophilic ligands such as retinoids, biliverdin, bilirubin, thyroid hormones, gangliosides, and amyloid beta peptides *in vitro* with high binding affinity. We defined such a feature as 'broad ligand selectivity' [3]. However, in contrast to the extensive data obtained on L-PGDS from many biochemical studies, detailed information about the conformational changes that it undergoes when it forms a complex with a ligand still remains to be forthcoming. Thus, in the present study, we measured the structure of L-PGDS and its complexes with three kinds of lipophilic ligand in solution by the small-angle X-ray scattering (SAXS) method.

Before the SAXS measurements, we first measured the ability of lipophilic ligands to bind L-PGDS by conducting an intrinsic tryptophan fluorescence quenching assay. The fluorescence intensity decreased to less than 13, 10, and 14% of that of L-PGDS itself in the presence of 10 μM RA, BV, and BR, respectively, and the K_d values of L-PGDS were calculated from the quenching curves to be 138 ± 8 nM for RA, 103 ± 5 nM for BR, and 70 ± 7 nM for BV. These results demonstrate that L-PGDS exhibits high binding affinities for RA, BV, and BR. We then determined the SAXS of L-PGDS in the presence or absence of those lipophilic ligands to investigate the structural changes caused by the ligand binding. All SAXS data were collected at beamline **BL40B2** using an R-AXIS IV^{++} system (RIGAKU, Tokyo Japan) as a detector. The X-ray wavelength was tuned to 1.000 Å, and the camera distance was set at 1050 mm. The temperature of the samples was kept at 25°C. For each polypeptide, SAXS profiles were collected at the concentrations of 2.5, 5.0, 8.0, and 12.5 mg ml^{-1}. The molecular structures of the polypeptides were predicted by applying the *ab initio* structure determination program GASBOR [4]. The 10 predicted molecular structures having almost the same overall structures were averaged, and then aligned. The scattering intensity curves of L-PGDS in both the presence and absence of ligands showed a large central peak at a reciprocal vector (S) < 0.032 Å^{-1}, a second peak at S = 0.044 Å^{-1}, and a small maximum at S = 0.075 Å^{-1} (Fig. 1). These curves revealed that L-PGDS has a globular shape in both the presence and absence of ligands. The binding of ligands to L-PGDS caused clear changes in the scattering curve only in the small-angle region (S < 0.02 Å^{-1}, Fig. 1 *inset*). From the Guinier analysis of each scattering curve, the radius of gyration $R_g(0)$ was calculated to be 19.4 ± 0.03 Å for L-PGDS, 18.8 ± 0.10 Å for L-PGDS/RA, 17.3 ± 0.12 Å for L-PGDS/BR, and 17.8 ± 0.04 Å for L-PGDS/BV, indicating that L-PGDS became compact after binding these ligands.

The molecular weights of L-PGDS and its complexes with the ligands were calculated to be 2.0×10^4 – 2.1×10^4 Da from the linear relation

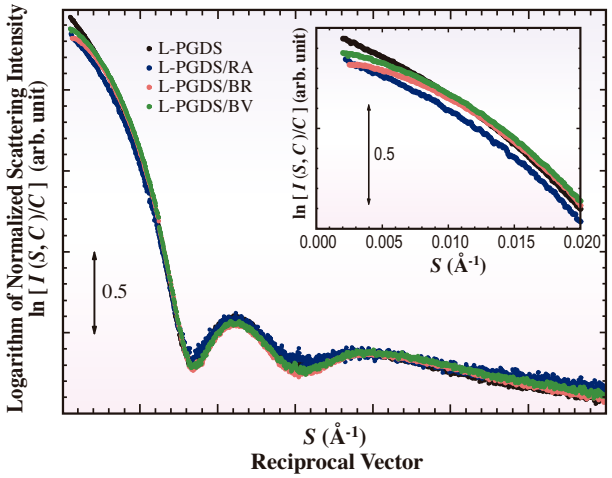

Fig. 1. SAXS profiles of L-PGDS and each L-PGDS complex. SAXS profiles of L-PGDS (black), L-PGDS/RA (blue), L-PGDS/BR (orange) and L-PGDS/BV (green) are shown. The logarithm of scattering intensity is shown as a function of reciprocal vector (S). The inset shows the logarithm of scattering intensity in the small S region.

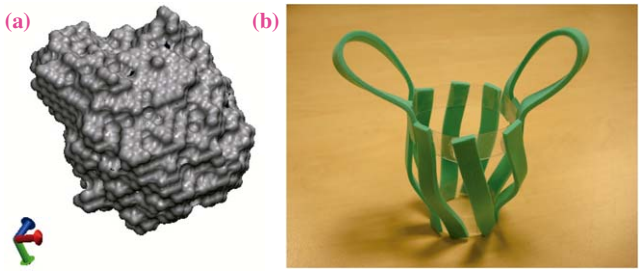

Fig. 2. **(a)** *Ab initio* model of L-PGDS, and **(b)** conceptual 3D molecular model. The *ab initio* model was calculated from $P(r)$ functions using GASBOR. The χ^2 of each model was less than 2.0.

between the forward scattering intensity and the molecular weight of the protein. These values are in good agreement with the molecular weight of L-PGDS calculated from its amino acid sequence (1.9×10^4 Da). To visualize the structure of L-PGDS and its complexes, we calculated their molecular models by the *ab initio* method. The *ab initio* model of L-PGDS showed that the overall structure of the molecule was globular in shape (Fig. 2(a)). Furthermore, to compare the shapes of L-PGDS and its complexes, we superimposed the model of each complex onto that of L-PGDS. The other three models of complexes also had globular shapes, and they became more compact than L-PGDS upon ligand binding (Figs. 3(b), 3(c) and 3(d)). Our data also revealed that the magnitude of compactness may depend on the size of the ligand that binds to L-PGDS. We consider that these results directly indicate the structural flexibility of the L-PGDS molecule. Such structural flexibility may be due to the particular molecular structure of L-PGDS itself and the origin of the compact packing observed only in L-PGDS.

We propose the following model for the binding of the ligands to L-PGDS: a lipophilic ligand enters the large cavity of the β-barrel, which triggers a conformational change such that the molecule of L-PGDS becomes compact, and thus, the ligand is held tightly inside the L-PGDS molecule. This study was published in the Journal of Biochemistry [5].

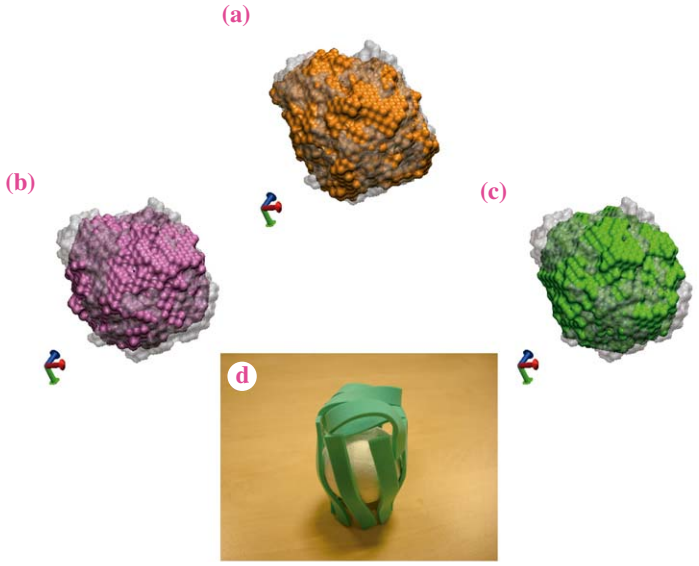

Fig. 3. **(a)** The *ab initio* model of L-PGDS/RA (yellow-colored model) is superimposed on that of L-PGDS (gray-colored transparent model). **(b)** The *ab initio* model of L-PGDS/BR (pink-colored model) is superimposed on that of L-PGDS (gray-colored transparent model). **(c)** The *ab initio* model of L-PGDS/BV (green-colored model) is superimposed on that of L-PGDS (gray-colored transparent model). **(d)** Conceptual 3D molecular model of the L-PGDS complex. Each *ab initio* model was calculated from $P(r)$ functions using GASBOR. The χ^2 of each model was less than 2.0.

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