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Crystal strutcture of modified Human Serum Albumin complexed with Δ^{12}-Prostaglandin J_2 and myristate

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Introduction

The prostaglandins (PGs) are a family of structurally related molecules that are produced by cells in response to a variety of extrinsic stimuli and regulate cellular growth, differentiation, and homeostasis. Among them, PGD_2 is a major cyclooxygenase product in a variety of tissues and cells and has marked effects on a number of biological processes, including platelet aggregation, relaxation of vascular and nerve cell functions. The two major pathways for the catabolism of PGD_2 are an 11-ketoreductase and NADP-linked 15-hydroxy-PG-dehydrogenase. The latter pathway leads to the formation of 13, 14-dihydro-15-keto-PGD_2, which does not appear to be biologically active. PGD_2 is known to be unstable and readily undergoes dehydration in vivo and in vitro to yield biologically active PGs of the J2 series, such as PGJ_2, Δ^{12}-PGJ_2, and 15d-PGJ_2. These cyclopentenone-type PGs (cyPGs) are actively transported into cells and accumulated in the nuclei, where they act as potent inducers of cell growth inhibition and cell differentiation, and exhibit their own unique spectrum of biological effects. HSA is known to be an endogenous catalyst of the PGD_2 metabolism. HSA catalyzes the conversion of PGH_2 to PGD_2. In addition, in aqueous solutions containing HSA, PGJ_2 isomerizes to yield Δ^{12}-PGJ_2. Δ^{12}-PGJ_2 is suggested to be a natural PGD_2 metabolite that is formed in vivo and excreted in the urine. Δ^{12}-PGJ_2 is stable in serum suggest that Δ^{12}-PGJ_2 may be produced and actively carried by HSA in the bloodstream and transported into cells to exhibit its biological effects. Here, we describe the structure of HSA complexed with Δ^{12}-PGJ_2 and show that two molecules of Δ^{12}-PGJ_2 uniquely bind at a fatty acid binding site.

Experimental

The HSA fraction V fatty acid free was further purified by anionexchange chromatography on UNO Q-6. The purified protein was concentrated to 100 mg/mL. Myristate dissolved in buffer A (20 mM phosphate buffer, pH 7.0) was heated to 50 °C to facilitate the dispersal of myristate, and then mixed with solution containing HSA to achieve the myristate molar ratio of 4. To gain the tertiary complex of HSA, myristate, and Δ^{12}-PGJ_2 , the Δ^{12}-PGJ_2 solution was concentrated under a gentle stream of nitrogen, and then a 5-fold molar excess of Δ^{12}-PGJ_2 was directly dissolved in the HSA-myristate solution. Crystals were obtained by the vapor diffusion hanging drop technique against a reservoir solution containing 50 mM phosphate buffer, pH 6.5-7.0, 28-32% (w/v) PEG 3350. Colorless crystals with the dimensions of 0.5 × 0.5 × 1.0 mm grew within 2 weeks. The crystals were harvested by the gradual addition of 2 μL of cryoprotectant solution containing Δ^{12}-PGJ_2 and slightly higher PEG 3350 concentrations than were used for crystallization to the hanging drop. The diffraction data were collected at 98 K using the beamline BL5A of the Photon Factory. The crystals are in the space group *P*1, with unit cell dimensions a = 38.1 Å, b = 92.1 Å, c = 94.7 Å, α= 74.8°, β= 89.5°, γ= 80.2°, and contain two molecules in the asymmetric unit. Model building in the electron density map and crystallographic refinement were performed using the programs in the CCP4 program suite and Coot. the resulting electron density maps were used to guide positioning of the ligands and bound water molecules and further refined in Refmac using 3 TLS groups. The final model, refined to 2.20Å, contains two proteins, three Δ12-PGJ2, 12 myristic acids, and the solvent molecules.

Results and Discussion

The facts that (i) arachidonic acid metabolites bind to human serum albumin (HSA) and the metabolism of these molecules is altered as a result of binding, (ii) HSA catalyzes the transformation of PGJ_2 into Δ^{12}-PGJ_2, and (iii) Δ^{12}-PGJ_2, is stable in serum suggest that HSA may bind and stabilize Δ^{12}-PGJ_2, in a specific manner. A molecular interaction analysis using surface plasmon resonance (Biacore) indeed suggested the presence of a specific Δ^{12}-PGJ_2-binding site in HSA. To investigate the molecular details of the binding of this PGD_2 metabolite to albumin, we analyzed the cocrystal structure of the HSA-Δ^{12}-PGJ_2-myristate complex by X-ray crystallography and found that two Δ^{12}-PGJ_2 molecules bind to a primary site in subdomain IB of the protein. The electron density results suggested that one of the two Δ^{12}-PGJ_2 molecules that specifically bind to the site covalently interacted with a histidine residue (His146). Using nano-LC-MS/MS analysis of the HSA-Δ^{12}-PGJ_2 complex, the formation of an unusual Δ^{12}-PGJ_2-histidine adduct at His146 was confirmed. Thus, our crystallographic and mass spectrometric analyses of the HSA-Δ12-PGJ2 complex provided intriguing new insights into the molecular details of how this electrophilic ligand interacts with its primary producer and transporter.

References

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