The study on molecular recognition of Serinhydroxymethyltransferase and bile acid conjugates

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1. Author contributions

M. Shirakawa: Organic synthesis, Structure-activity relationship study, and X-ray crystallography.

A. Senoo: Protein crystallization and X-ray crystallography.

2. Introduction

Serine hydroxymethyltransferase (SHMT) is located upstream in the one-carbon metabolism, catalyzing the reversible conversion of serine to glycine.[1)] The one-carbon units produced by this enzyme are involved in various biological processes, including maintaining amino acid homeostasis and de novo nucleic acid synthesis. Therefore, SHMT is an essential enzyme for cell survival. SHMT has garnered attention as a novel drug target because it is overexpressed in cancer cells, which have high energy consumption. [2)]

Focusing on humans, SHMT exists as two highly homologous and structurally similar isoenzymes, hSHMT1 and hSHMT2, which are localized in the cytosol and mitochondria, respectively. These isoenzymes play roles in maintaining one-carbon unit homeostasis and function as moonlighting proteins, thereby exhibiting distinct functions. Recently, they have been recognized as targets for elucidating new biological phenomena.[3, 4)] However, due to the high homology of these isoenzymes, distinguishing between hSHMT1 and hSHMT2 has been extremely challenging in SHMT research, posing a significant barrier in drug discovery and molecular probe development.

In this study, we focused on the compound Gly-DCA, identified from high-throughput screening using a fluorescent probe developed in our laboratory to detect SHMT activity. Gly-DCA exhibited stronger inhibitory activity against hSHMT2 compared to hSHMT1.[5)] We estimated the difference in molecular recognition of Gly-DCA by hSHMT1 and hSHMT2 from the co-crystal structures obtained by alumni of our laboratory and Mr. Senoo. Furthermore, considering that Gly-DCA is a naturally occurring bile acid conjugate, we conducted a structure-activity relationship study using other bile acid conjugates to deepen our understanding of their molecular recognition by hSHMT2.[6)] The outcomes of this research are expected to contribute to the development of selective chemical probes for hSHMT1 and hSHMT2, as well as aid in drug discovery efforts.

3. Result and Discussion

The complex structures of hSHMT1 and hSHMT2 with Gly-DCA are shown in Figure 1a. The non-covalent interactions between each enzyme and the ligand are indicated by red or black dotted lines. From these results, Gly-DCA interacts within the active sites of both hSHMT1 and hSHMT2. Notably, the cholesterol backbone of Gly-DCA appears to

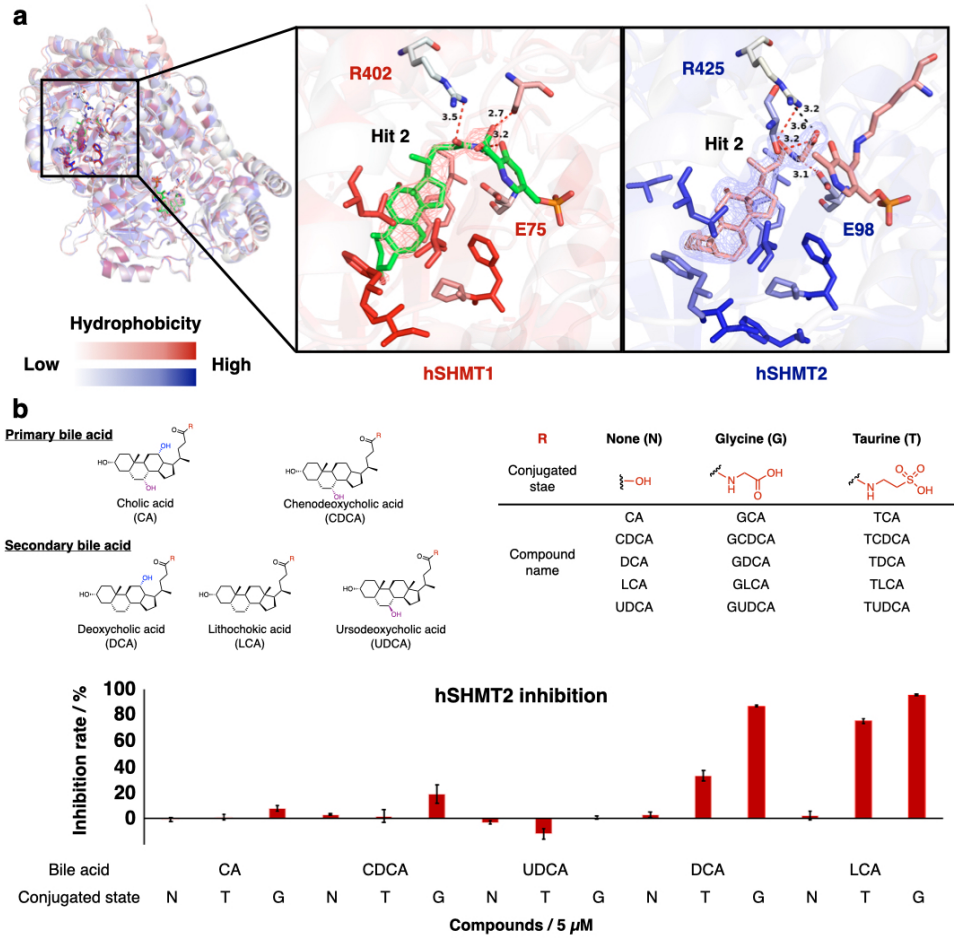

Figure 1. a. Crystal structures of hSHMT1-Gly-DCA complex and hSHMT2-Gly-DCA complex. b. Structure-activity relationship analysis of bile acid conjugates.

have affinity for the hydrophobic amino acid residues of each enzyme, while the glycine moiety is presumed to engage in electrostatic interactions with the enzymes. Additionally, it was suggested that Gly-DCA forms a stronger interaction with hSHMT2 through the formation of a salt bridge with the arginine residue (R425).

Next, a structure-activity relationship study was conducted using 15 different bile acid conjugates to evaluate their inhibitory activity against hSHMT2 (Figure 1b). The results indicated that secondary bile acids exhibited stronger inhibitory activity than primary bile acids. Furthermore, glycine conjugates demonstrated stronger inhibitory activity compared to taurine conjugates. This finding supports the notion from the previous co-crystal structure analysis that the glycine moiety significantly contributes to electrostatic interactions with the target enzyme.

In summary, the molecular recognition of Gly-DCA by hSHMT1 and hSHMT2 was analyzed from structural and enzymatic chemistry perspectives. Given that bile acid conjugates are endogenous compounds, future studies focusing on in cellulo and in vivo analyses are anticipated to elucidate the relationship between bile acid conjugates and SHMT in physiological environments. This could pave the way for the development of molecular probes and inhibitors mimicking bile acid

conjugates.

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5. References

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