Available online at www.sciencedirect.com
SCIENCE DIRECT
Molecular & Biochemical Parasitology 143 (2005) 146–151
MOLECULAR & BIOCHEMICAL PARASITOLOGY

Mutational analyses of *Plasmodium falciparum* and human *S*-adenosylhomocysteine hydrolases

Masayuki Nakanishi [a], Saori Yabe [a], Nobutada Tanaka [b], Yasutomo Ito [a], Kazuo T. Nakamura [b], Yukio Kitade [a,c,*]

[a] *Department of Biomolecular Science, Faculty of Engineering, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan*
[b] *School of Pharmaceutical Sciences, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan*
[c] *Center for Emerging Infectious Diseases, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan*

Received 8 February 2005; received in revised form 5 April 2005; accepted 27 May 2005
Available online 21 June 2005

Abstract

S-adenosylhomocysteine hydrolase is a prospective target for developing new anti-malarial drugs. Inhibition of the hydrolase results in an anti-cellular effect due to the suppression of adenosylmethionine-dependent transmethylations. Based on the crystal structure of *Plasmodium falciparum S*-adenosylhomocysteine hydrolase which we have determined recently, we performed mutational analyses on *P. falciparum* and human enzymes. Cys59 and Ala84 of the parasite enzyme, and the equivalent residues on the human enzyme, Thr60 and Gln85, were examined. Mutations of Cys59 and Thr60 caused dramatic impact on inhibition by 2-fluoronoraristeromycin without significant effect both on its kinetic parameters and on inhibition constant against noraristeromycin. In addition, the impact was independent from the electronegativity of the side chain of the substituting residue. These results showed that steric hindrance between a functional group at the 2-position of an adenine nucleoside inhibitor and Thr60 of the human enzyme, not an electrostatic effect, contributed to inhibitor selectivity.
© 2005 Elsevier B.V. All rights reserved.

Keywords: *S*-adenosyl-L-homocysteine; *Plasmodium falciparum*; Site-directed mutagenesis; Nucleoside inhibitor; 2-Fluoronoraristeromycin

1. Introduction

S-adenosylhomocysteine hydrolase (SAHH) is the enzyme that catalyzes the breakdown of *S*-adenosylhomocysteine (SAH) to homocysteine and adenosine [1]. SAH is a strong inhibitor of numerous methyltransferases, enzymes that transfer methyl groups from *S*-adenosylmethionine to nucleic acids, proteins, lipids and other small molecules [2]. Since these methylations are required for the proliferation of viruses and tumor cells, the accumulation of SAH leads to anti-virus/anti-tumor effects [3,4]. A number of nucleoside inhibitors of SAHH have therefore been synthesized to develop anti-viral and -tumor agents [5,6]. Some of these inhibitors are expected to be drugs that act against malaria [7,8], a major parasitic disease that is a major cause of human morbidity and mortality, especially in tropical areas. The rapid emergence of drug-resistant parasites against currently available drugs generates an urgent need for novel therapeutic approaches.

To develop new anti-malaria drugs, it is essential to determine the structure of the enzymes or other molecules that must be inhibited. We recently reported the crystal structure of SAHH from *Plasmodium falciparum* [9], a causative agent of human malaria. Application of SAHH inhibitors to malaria therapy requires selective inhibition of the parasite enzyme (PfSAHH), since humans also possess an SAHH (HsSAHH). It is therefore necessary to obtain detailed knowledge about ligand binding to both enzymes. Less is known, however, about ligand binding and catalytic mechanisms of PfSAHH than about mammalian SAHHs [10–12]. In a previous study,

Abbreviations: SAH, *S*-adenosylhomocysteine; SAHH, *S*-adenoshylhomocysteine hydrolase; Pf, *Plasmodium falciparum*; Hs, *Homo sapiens*; NAM, noraristeromycin; 2FNAM, 2-Fluoro noraristeromycin

* Corresponding author. Tel.: +81 58 293 2640; fax: +81 58 293 2640.
E-mail address: kitade@biomol.gifu-u.ac.jp (Y. Kitade).

0166-6851/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.molbiopara.2005.05.012

we found that the introduction of fluorine to the 2-position of noraristeromycin (NAM), a potent nucleoside inhibitor of SAHH, selectively inhibits the parasite enzyme [13]. The enzyme structure of PfSAHH predicted that Cys59 is the key residue for selective inhibition by 2-fluoronoraristeromycin, in that the Cys59 residue of the parasite enzyme forms a depression around the 2-position of adenosine, enabling it to accommodate fluorine without steric hindrance [9]. In contrast, the side chain of the equivalent amino acid residue in the human enzyme, Thr60, plugs this depression. It is, however, not clear whether selective inhibition by 2FNAM is due only to steric hindrance or also to electrostatic effects, such as electronegativity and hydrogen bonding of the fluorine atom.

In this paper, we generate and analyse seven mutant enzymes biochemically in order to reveal the detailed mechanism of the selective inhibition of PfSAHH by 2FNAM.

2. Materials and methods

2.1. Bacterial strains and plasmids

Escherichia coli JM109 strain was used for all DNA cloning procedures and protein expression. pQEPfSAHH, a plasmid based on the vector pQE30 and which produces hexahistidine-tagged PfSAHH, and pKKHuS, a plasmid based on pKK223-3 and which produces non-fusion HsSAHH, have been described previously [9,14].

2.2. Construction of pQEHsSAHH

pKKHuS was digested with EcoRI, blunt ended with T4 DNA polymerase, and digested with *Hin*dIII. The 1.3 kb fragment containing the HsSAHH-coding sequence was ligated into pQE32 that had been digested with BamHI, blunt ended with T4 DNA polymerase and digested with *Hin*dIII. The plasmid thus generated was named pQEHsSAHH.

2.3. Site-directed mutagenesis

pQEPfSAHH and pQEHsSAHH were subjected to site-directed mutagenesis using the QuikChange method in conjunction with the primer sets listed in Table 1.

Table 1
Oligonucleotide primers used for point mutations

Mutation	Sequence[a]
C59T	ATGACTGTTGAAACTGCTTTATTAATTGAG
C59S	ATGACTGTTGAAAGTGCTTTATTAATTG
A84Q	ATATTTATTCAACACAGGATTATGCTGCAG
T60C	GACCGTGGAGTGTGCCGTCCTC
T60S	CATGACCGTGGAGTCGGCCGTCCTCATTG
T60A	GACCGTGGAGGCGGCCGTCCTC
Q85A	CTTCTCCACCGCGAACCATGCGG

[a] Complementary sequence primers were also used for mutations.

2.4. Expression and purification of mutant enzymes

Wild-type and mutant enzymes were expressed and purified as described [9]. Briefly, *E. coli* harboring the expression plasmid was grown at 25 °C in LB medium. The incubation was continued for 6 h after the addition of 0.5 mM isopropyl-1-thio-β-D-galactopyranoside. The cells were homogenized in a buffer containing 20 mM $KHPO_4$ (pH 7.6), 0.3 M NaCl and 5% glycerol, and the insoluble fraction was removed by centrifugation at 17,000 × *g* for 15 min at 4 °C. The supernatant (cell extract) was purified by TALON® metal affinity and Sephacryl S300 gel filtration chromatography, with each column pre-equilibrated with the same buffer.

2.5. Enzyme assay

Enzyme activity was measured in the synthetic direction, i.e. adenosine + DL-homocysteine → *S*-adenosylhomocystine. Each enzyme (0.05 μM) was incubated with adenosine (0.5–100 μM), inhibitor (0–500 μM) and 5 mM DL-homocysteine in 0.1 mL of 25 mM potassium phosphate buffer (pH 7.6) at 30 °C. Inhibitors were dissolved in 50% dimethylsulphoxide and added to the reaction so as not to exceed 10% by volume. The reaction was terminated by the addition of 10 μL of 0.67 M HCl. An aliquot of the supernatant of each reaction mixture was analyzed for *S*-adenosylhomocysteine by HPLC [14]. Analysis of the inhibition data was done by nonlinear least squares fitting to the equation $IC_{50} = K_i(1 + [S]/K_m)$ [15].

2.6. Structure analysis

Least squares comparisons of the molecular models and visualization of the models were carried out using the program CueMol (Ishitani, R., CueMol: Molecular Visualization Framework, http://cuemol.sourceforge.jp/). Molecular surface was calculated using the program MSMS [16].

3. Results

3.1. Construction of mutant SAHH genes

The structures of PfSAHH and HsSAHH used here are described in Fig. 1. The Cys59 residue of PfSAHH is thought to be responsible for inhibition by 2FNAM. The residue was substituted by Thr, the equivalent residue in HsSAHH, and Ser, a homologous residue with Cys. Conversely, Thr60 of HsSAHH was substituted by Cys, Ser and Ala. The Ala84 of PfSAHH is located at a distance of ca. 9 Å from 6-NH_2 group of adenosine. The equivalent of the Ala84 is Gln in HsSAHH and almost all known SAHHs. The residue was exchanged each other between PfSAHH and HsSAHH. Mutant genes containing a codon substitution were successfully prepared by the QuikChange method.

Download English Version:

https://daneshyari.com/en/article/9139947

Download Persian Version:

https://daneshyari.com/article/9139947

Daneshyari.com