Biochemical Pharmacology 115 (2016) 123–133

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

An engineered heterodimeric model to investigate SULT1B1 dependence on intersubunit communication

Zachary E. Tibbs, Charles N. Falany *

The Department of Pharmacology and Toxicology, The University of Alabama at Birmingham School of Medicine, Birmingham, AL 35294-0019, United States

ARTICLE INFO

Article history:
Received 25 May 2016
Accepted 17 June 2016
Available online 23 June 2016

Keywords:
Drug metabolism
Sulfation
Protein engineering
Half-site reactivity
SULT1B1

ABSTRACT

Cytosolic sulfotransferases (SULTs) biotransform small molecules to polar sulfate esters as a means to alter their activities within the body. Understanding the molecular mechanism by which the SULTs perform their function is important for optimizing future therapeutic applications. Recent evidence suggests each SULT isoform acts by a half-site reaction (HSR) mechanism, in which a single SULT dimer subunit is active at any given time. HSR requires communication through the highly conserved KxxxTVxxxE dimerization motif. In this investigation, we sought to test the intersubunit interactions of SULT1B1 as it relates to enzyme activity. We generated two populations of SULT1B1 isoforms that efficiently heterodimerize upon mixing by targeted point mutation of the KxxxTVxxxE motif to KxxxTVxxxK or ExxxTVxxxE. The heterodimer exhibited wildtype-like activity with regard to native size, thermal integrity, PAP affinity, and PAPS K_m, therefore serving as a valid model for investigating SULT1B1 dimer subunit interactions. The approach granted control over each independent subunit, permitting mutation of the critical 3′-phosphoadenosine 5′-phosphosulfate (PAPS) binding residue Arg258 and/or the catalytic base His109 in a single subunit of the dimer. Substitution of the dysfunctional subunits for fully active subunits yielded dimeric SULT1B1 with 50% the activity of the fully competent dimer, suggesting SULT1B1 intersubunit communication does not significantly contribute to the isoform's activity. These results are a testament to the unique properties of individual SULT isoforms. The dimerization system described in this manuscript can be used to study subunit interactions in other SULT isoforms as well as proteins in other families.

1. Introduction

Organisms regulate hormone and small molecule activities by a number of biotransformation pathways, one of which is sulfonate conjugation. Compared to a hydrophobic compound, its sulfate ester is generally more water-soluble, has an altered affinity for receptors, and can be actively exported out of the cell and eventually the body [1–3]. The cytosolic sulfotransferases (SULTs) are responsible for sulfo-conjugation of small molecules in the body, catalyzing the transfer of a sulfonate group from the ATP-like cofactor, 3′-phosphoadenosine 5′-phosphosulfate (PAPS), to the recipient compound [4,5]. Individual SULT isoforms have unique substrate specificity patterns but share a conserved reaction mechanism, reflected by their conserved architecture [6–8]. Aside from the catalytic residues in the SULT's pore, the most highly conserved region of the SULT includes the dimerization and PAPS binding domains, found directly adjacent to one another (Fig. 1) [6,9,10]. Each isoform must bind PAPS to perform its function therefore providing an understandable rationale for the conservation of PAPS-interacting amino acids. No discernable role has been identified for SULT dimerization; therefore its conservation remains unwarranted.

Since its identification in 2000, the abnormally small physiological dimerization motif (KxxxTVxxxE) of SULTs has been the subject of multiple investigations [9] (Fig. 1). Lu et al. reported no activity differences between a point-mutant monomeric isoform of hSULT1A1 and the wild-type dimer [11]. The group did, however, show the monomeric isoform was susceptible to thermal degradation and therefore determined the dimerization of hSULT1A1 played a role in enzyme integrity [11]. Another study reported loss of substrate inhibition upon monomerization of hSULT2A1, suggesting a link between SULT dimerization and

Abbreviations: PAP, 3′,5′-diphosphoadenosine; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; SULT, cytosolic sulfotransferase.

* Corresponding author at: Department of Pharmacology and Toxicology, University of Alabama at Birmingham, Birmingham, AL 35205, United States.
E-mail addresses: ztibbs@uab.edu (Z.E. Tibbs), cfalany@uab.edu (C.N. Falany).

http://dx.doi.org/10.1016/j.bcp.2016.06.011
0006-2952/

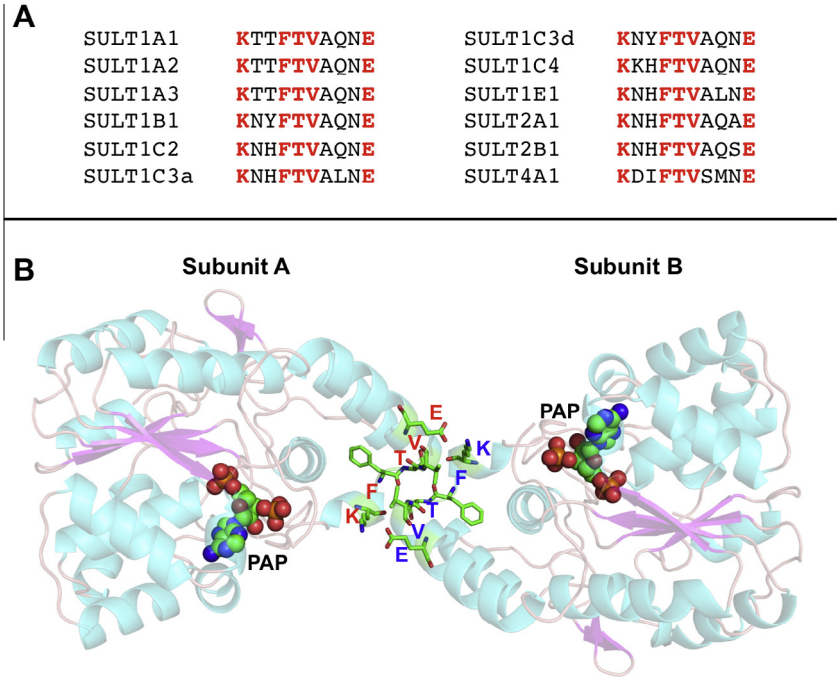

Fig. 1. The SULT dimerization domain. (A) Along with SULTs in other species, each human SULT has a conserved KxxFTVxxxE dimerization motif. (B) The SULT crystal structures show the interface between the conserved KxxFTVxxxE residues (sticks) of each subunit. PAP (cofactor) is represented by spheres.

substrate selectivity [12]. Though not directly targeted toward SULT dimerization, recent investigations insinuate a mechanistic role for SULT dimerization in at least three SULT isoforms; hSULT1A1, hSULT2A1, and hSULT1E1 [13–15]. These three isoforms have been reported as acting via a half-site reaction mechanism, a mechanism in which only half of the subunits catalyze the sulfonation reaction at any given time.

Half-site reaction mechanisms require intersubunit communication and often result in an entity with greater catalytic efficiency than the additive activities of the individual subunits [16]. Recent evidence suggests the binding of cofactor (PAPS) to the SULT induces structural asymmetry that drives communication between dimeric SULT subunits, contributing to SULT half-site reactivity. [17]. The aim of this investigation is to test the capacity of cofactor binding and catalysis to drive such intersubunit communication *in vitro* by engineering a SULT1B1 dimer with interchangeable functional and dysfunctional subunits.

2. Materials and methods

2.1. Approach

This study necessitated the complete control of SULT1B1 dimerization or the direct comparison of monomeric and dimeric isoforms. Based on previous experiments, the on-rate of SULT dimerization greatly outweighs the off-rate therefore complicating isolation of a wildtype monomeric species. Competition driven monomerization of hSULT1B1 by use of a peptide mimetic of the dimerization domain was unsuccessful (unpublished results) therefore we sought to monomerize hSULT1B1 by mutagenesis. The composition of the native dimerization domain of SULTs (KxxxTVxxxE) allowed for the design of a versatile system for control of SULT dimerization. Specifically, mutation of the complementary salt-bridging residues K266 and E275 to an E and a K, respectively, would theoretically result in two enzyme populations with either a predominantly negatively or positively charged dimerization domain. In theory, the independent populations would monomerize by repulsive forces (Fig. 2). Attractive forces could then be used to prompt heterodimerization upon mixing, forming a dimer with a scaffold resembling that of native hSULT1B1 (Fig. 2). Using this method, the effects of single subunit

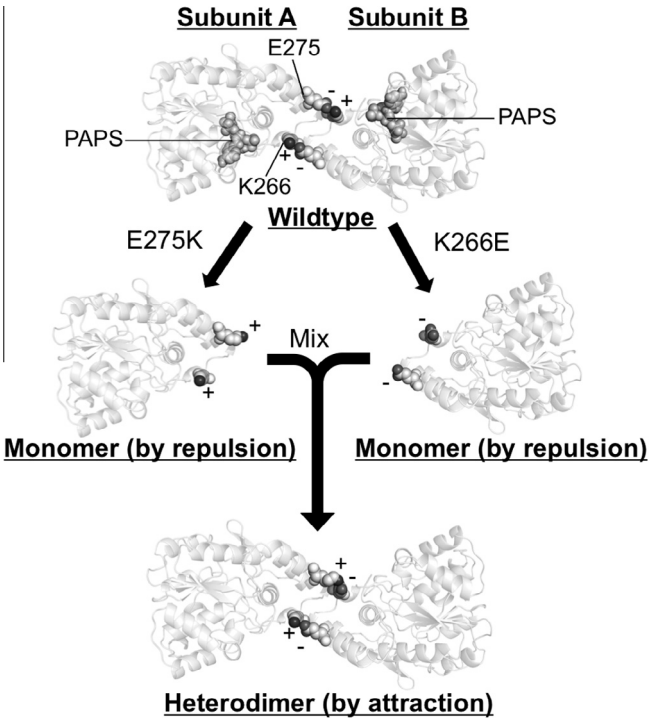

Fig. 2. Schematic for controlling SULT1B1 dimerization [28]. SULTs have salt bridging dimerization residues K266 (+) and E275 (−), nearby the PAPS (spheres) binding domain. Mutation of E275 to a K and K266 to an E results in either a dominantly positive or dominantly negative charged dimerization domain, disallowing dimerization via repulsive forces. Mixing of the two populations should yield a heterodimeric complex via attraction.