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Review article

Computational insights into function and inhibition of fatty acid amide hydrolase

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ABSTRACT

The Fatty Acid Amide Hydrolase (FAAH) enzyme is a membrane-bound serine hydrolase responsible for the deactivating hydrolysis of a family of naturally occurring fatty acid amides. FAAH is a critical enzyme of the endocannabinoid system, being mainly responsible for regulating the level of its main cannabinoid substrate anandamide. For this reason, pharmacological inhibition of FAAH, which increases the level of endogenous anandamide, is a promising strategy to cure a variety of diseases including pain, inflammation, and cancer.

Much structural, mutagenesis, and kinetic data on FAAH has been generated over the last couple of decades. This has prompted several informative computational investigations to elucidate, at the atomic-level, mechanistic details on catalysis and inhibition of this pharmaceutically relevant enzyme. Here, we review how these computational studies – based on classical molecular dynamics, full quantum mechanics, and hybrid QM/MM methods – have clarified the binding and reactivity of some relevant substrates and inhibitors of FAAH. We also discuss the experimental implications of these computational insights, which have provided a thoughtful elucidation of the complex physical and chemical steps of the enzymatic mechanism of FAAH. Finally, we discuss how computations have been helpful for building structure–activity relationships of potent FAAH inhibitors.

1. Introduction

Fatty acid amide hydrolase (FAAH – Fig. 1) [1–3] is a membrane-bound serine hydrolase that was initially identified as hydrolyzing the biologically active lipid oleamide (**1** – Scheme 1) [4]. However, it was later demonstrated that FAAH cleaves several other fatty acids, with remarkable preference for the endogenous cannabinoid anandamide [*N*-arachidonoyl-ethanolamine (**2**) – Scheme 1], a naturally occurring fatty acid agonist of cannabinoid receptors [2,3,5–9]. Therefore, inhibition of FAAH activity amplifies and extends the biopharmacological effects of these lipid-derived messengers, including the satiety factor oleoylethanolamide [10,11] and the anti-inflammatory/analgesic agent palmitoylethanolamide [12].

Pharmacological inhibition of FAAH allows modulation of the endogenous levels of cannabinoids. It is thus a promising drug target to treat pain, inflammation, and several other diseases, including cancer. Pharmacological inhibition of FAAH could also solve the main problems of therapeutic agents that act as direct agonists of the cannabinoid receptor CB1, which often cause severe side effects such as dysphoria and negative effects on motility, memory, and sleep [13]. In this regard, FAAH knockout ($FAAH^{-/-}$) mice show substantially increased levels of anandamide in the brain and reduced pain sensation without observable hypomotility, hypothermia, or catalepsy [14–19]. Thus, over the last decade, both academic and industrial groups have started drug discovery programs targeting FAAH. This has prompted computational studies on this enzyme with the common aim of deciphering, at the atomic level, both enzymatic function and inhibition.

Here, we review several informative computational studies on FAAH, including our own recent work. Overall, we aim to show how classical and first-principles-based computations have helped addressing relevant questions on the function and inhibition of FAAH in relation to the available experimental data on FAAH. We will examine the main structural features and energetics for ligand binding and hydrolysis during FAAH catalysis, as well as key

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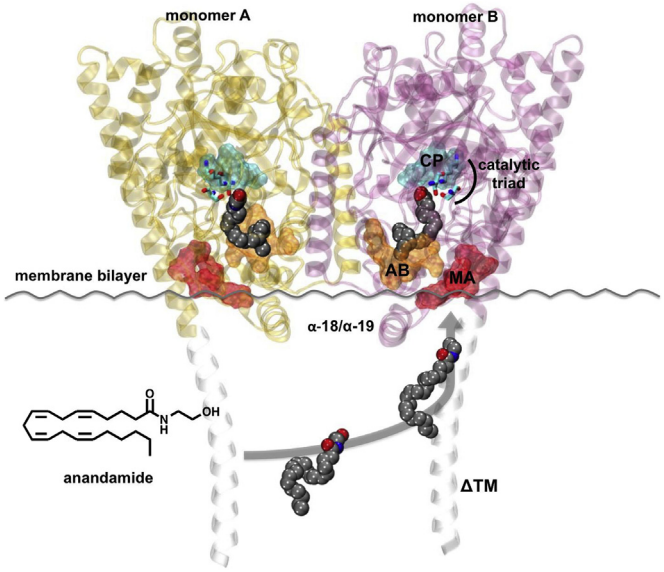
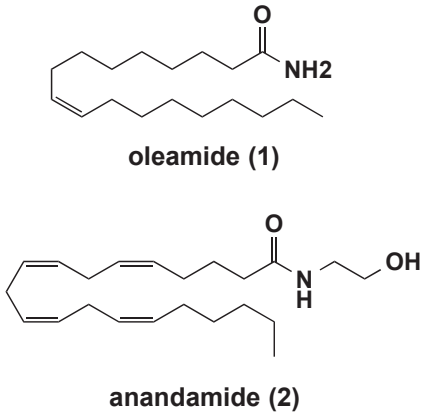


Fig. 1. Overview of the FAAH protein (pdb 1MT5) [1] in complex with anandamide (chemical structure at the bottom left) [119]. The enzyme is a membrane protein composed of two subunits, which are the monomers A (yellow ribbons) and B (magenta ribbons). The protein is inserted in the membrane thanks to the α-18 (residues 410–426) and α-19 (residues 429–438) transversal helices and the deleted transmembrane (ΔTM) domain (residues 9–29, shown as transparent ribbons). The membrane bilayer is indicated with a gray line. Anandamide (gray, in space-filling representation) is thought to reach the catalytic site via a "membrane access" channel (MA – shown in red molecular surface). An adjacent "acyl-chain binding" channel (AB – orange), which in the crystal structure is occupied by the arachidonoyl chain of the bound methyl arachidonyl fluorophosphonate (MAFP), likely contributes to the proper accommodation of the substrate during catalysis. At the top of the catalytic region, a third channel (cyan) constitutes the "cytosolic port" (CP) that allows the exit of the leaving group after hydrolysis. The Ser241-Ser217-Lys142 catalytic triad is indicated with cyan sticks. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

pharmacophoric elements for covalent inhibition of FAAH, as elucidated by several computational studies. Finally, we mention aspects related to FAAH catalysis and lipid selection that are still unclear and that could be the subject of future computational investigations.



Scheme 1. Chemical structure of oleamide (**1**) and anadamide [*N*-arachidonoyl-ethanolamine (**2**)] [4,6].

1.1. FAAH structure

FAAH is a 65 kD protein that was purified in 1996 by Cravatt et al. from rat liver membranes (*r*FAAH – Fig. 1) [2]. Based on sequence analysis, FAAH belongs to the amidase signature (AS) enzyme family. Unlike most AS enzymes, FAAH is an integral membrane protein with a transmembrane (TM) domain (amino acids 9–29). When expressed with deletion of the predicted TM domain (as TM-domain-deleted FAAH – ΔTM-FAAH), *r*FAAH maintains the wild-type protein's association with membranes as well as its enzymatic properties [20]. These results indicate that FAAH possesses multiple domains for membrane association, which were subsequently identified as the α-18/α-19 transversal helices [1].

The first X-ray crystal structure of the *r*FAAH was determined for ΔTM *r*FAAH in complex with an analogue of anandamide, the irreversible inhibitor methyl arachidonyl fluorophosphonate (MAFP) at 2.8 Å resolution (PDB code: 1MT5) [1]. This structure revealed a dimeric protein with a characteristic core structure of a twisted β-sheet surrounded by 24 α-helices. Interestingly, the crystal structure also revealed multiple pockets to the catalytic site of FAAH (Fig. 1). These cavities are the "membrane access" (MA) channel, located in proximity to the α-18/α-19 helices, which connects the membrane surface to the core of the FAAH active site. The substrate is thought to reach the catalytic site via this MA channel, reaching the hydrolytic Ser241–Ser217–Lys142 catalytic triad. The adjacent "acyl-chain binding" (AB) channel – found to be occupied by the arachidonyl chain of the bound MAPF [1] – likely provides an extra space for substrate binding. A cytosolic port (CP) is located at the top of the active site where it provides an exit route for the leaving group, after substrate hydrolysis, toward the cytosol.

Given the multiple membrane association possibilities of FAAH, protein expression and purification have been challenging for the human isoform (*h*FAAH) that shares ~82% sequence identity with *r*FAAH [21]. For this reason, a '*humanized*' rat FAAH (*h*/*r*FAAH) was resolved at 2.75 Å (PDB code: 2VYA) in complex with the piperidine-based inhibitor PF-750. In this structure, six amino acids of the active site were mutated into those of the human FAAH protein sequence (namely, Leu192Phe, Phe194Tyr, Ala377Trp, Ser435Asn, Ile491Val, and Val495Met) [21]. The *h*/*r*FAAH structure showed similar structural features as *r*FAAH. One remarkable difference, however, was detected at the interface between the AB and MA pockets, where a key residue (Phe432) was rotated by about 80° along the Cα–Cβ axis, due to the presence PF-750 in the *h*/*r*FAAH structure (Fig. 2). Thus, the MA channel of *h*/*r*FAAH in presence of PF-750 was remarkably larger than in *r*FAAH. This led to the suggestion that Phe432 might act as a "*dynamic paddle*" that directs the FAAH substrate into pre-active conformations for hydrolysis, located in either AB or MA during catalysis. Following the first *h*/*r*FAAH structure, several additional *h*/*r*FAAH structures bound with different covalent inhibitors were determined, confirming the marked structural flexibility of Phe432 [21–26].

1.2. Experimental findings on function and inhibition of FAAH

The mechanistic details of FAAH reactivity were extensively characterized by structural [1,21–26] and mutational [27] studies, further supported via several informative theoretical investigations, as discussed later in this review [28–32]. The hydrolytic function of FAAH is performed by an unusual Ser241–Ser217–Lys142 catalytic triad, which replaces the typical Ser–His–Asp motif of the serine hydrolases [1]. The enzymatic reaction comprises two main chemical steps (Scheme 2), which are the enzyme acylation (**A** → **D**) and subsequent deacylation (**D** → **E**), with release of the final product. During the enzyme