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Research Article

Molecular Modeling Study of Enzyme-Substrate Interaction in the Synthesis of Anandamide Synergists

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Keywords

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Abstract

A computational analysis about lipase-catalyzed synthesis of anandamide analogs with pharmacological activity was carried out. A thorough molecular modeling study of the interaction between seven representative different substrates and the lipase used in the synthesis allowed to explain the observed relation between structure and reactivity. A trend that unravels the molecular determinants behind our experimental results was obtained, giving us not only information to improve further syntheses, but also showing the great potential and utility of molecular docking as a tool to explain and predict experimental results.

ABBREVIATIONS

AEA: *N*-Arachidonoyl-Ethanolamine; CB_1: Cannabinoid Receptor Type 1; CB_2: Cannabinoid Receptor Type 2; TRPV1: Transient Receptor Potential Cation Channel Subfamily V Member 1; CAL B: *Candida antarctica* Lipase B; RCSB: Research Collaboratory for Structural Bioinformatics; PDB: Protein Databank; AM1: Austin Model 1.

INTRODUCTION

Amongst the broad family of compounds that constitute the endocannabinoid family, anandamide (*N*-(2-hydroxyethyl) arachidonoylamide or *N*-arachidonoyl-ethanolamine, AEA) stands out as a promising substrate for the treatment of an increasing number of pathologies. AEA is a neuromodulatory lipid that, like Δ^9-tetrahidrocannabinol, activates the central and peripheral cannabinoid receptors CB_1 and CB_2 and is also a ligand for transient receptor potential vanilloid receptor 1 (TRPV1).

Considerable research has shed light on the impact of endocannabinoids on human health and its applicability as pharmacological drugs. Anandamide and its derivatives are involved in basic biological processes in the brain, gastrointestinal tract, skeletal muscle, liver, bone and skin as well as in the immune response and reproduction. Moreover, they have been recognized as key mediators of several aspects of human pathophysiology and thus are considered one of the most widespread and versatile signaling molecules ever discovered.

Recently, we reported the application of lipases to the synthesis of anandamide and two series of fatty acid derivatives with various alkanolamines and the biological studies carried out to evaluate their antitumoral activity (Figure 1). The antitumor effect of AEA was extensively demonstrated and we determined that two AEA analogues are capable to enhance the AEA cytotoxicity on rat C6 glioma cells, indicating a possible role as therapeutic agents in cancer treatment.

Biocatalysis proved to be a good alternative for the synthesis of organic compounds through a Green Chemistry approach. Over the last years, biocatalysis using lipases in non-aqueous media has been widely used in synthesis of pharmaceuticals catalyzing several synthetic reactions such as esterification, transesterification, aminolysis, polymerization, etc. Enzymes are also well-known by their high enantioselective behavior and this property formed the basis for their widespread use for the synthesis of enantiomerically pure compounds. In addition, this methodology presents important advantages such as mild reaction conditions, economy and low environmental impact.

We strongly believe that a better understanding of reaction determinants in a growing discipline such as biocatalysis could lead to better results in further synthesis of novel compounds. Hence, in order to explain the experimental results in the lipase catalyzed synthesis of anandamide analogues, herein we report a docking study of enzyme-substrate interaction for different alkanolamines.

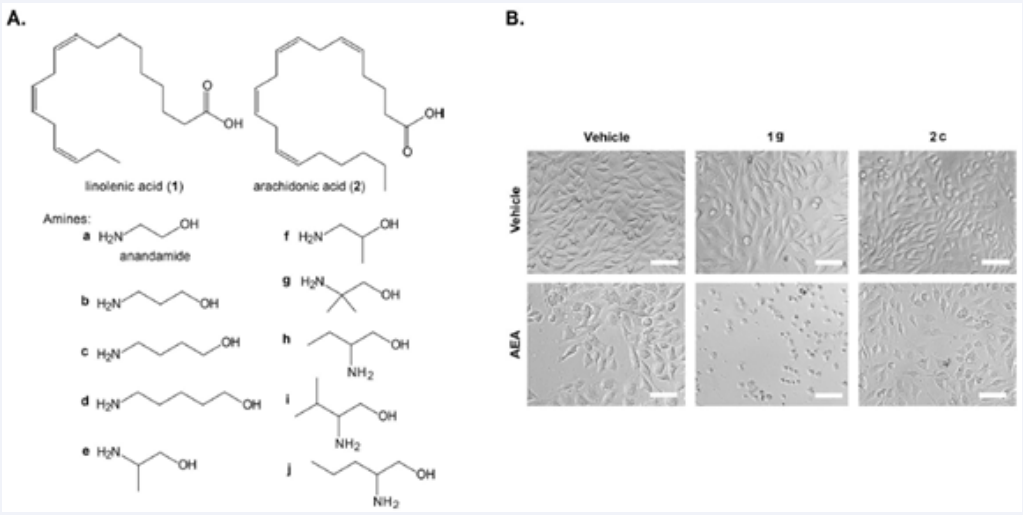

Figure 1 A. Structures of fatty acids and alkanolamines used to synthesize AEA analogs. B. Representative phase contrast images of the combined effects of AEA (15 M) and its analogs 1g and 2c (15 μM). Scale bar: 50 μm.

MATERIALS AND METHODS

CAL B structure was downloaded from RCSB Protein Databank (http://www.rcsb.org/pdb/). PDB code for CAL B is 1TCA. Structure of all substrates was optimized using semi empirical AM1 method with the algorithm Polak-Ribiere using Hyperchem software. Docking calculations were carried out using Autodock 4.2 program considering all significant bonds rotatable both for linolenic acid and every alkanolamine, and the protein was defined as if it remains non flexible during reaction. A grid of 64x52x50 points with a spacing of 0,447 Å centered in the enzymatic site of CAL B was calculated and used to obtain 200 runs of the genetic algorithm method. In a first step interaction between linolenic acid and lipase was modeled and the best conformer was selected to carry out a consecutive simulation using each selected alkanolamine as ligand. Finally, considering binding energy, cluster population, and distance to the catalytic site, the observed experimental trend was explained.

RESULTS AND DISCUSSION

As it was mentioned, the use of enzymes constitutes an excellent alternative to the synthesis of pharmaceuticals derivatives and allows carrying out different chemical transformations without the need for tedious protection and deprotection steps, especially in compounds with several functional groups. Considering this, we used lipases for the synthesis of twenty AEA derivatives. Amongst various lipases, *Candida antarctica* B showed very good yield and a chemoselective behaviour in the conditions reported lately by our group, using three different pathways for reaction of linolenic and arachidonic acid with alkanolamines. It was observed that product yield depended on the fatty acid chain length and number of unsaturations. Also, the alkanolamine structure played a major role on it (Table 1).

The best yield was obtained using linolenic acid and ethanolamine as nucleophile. We noted a decrease in yield proportional to the increase in the number of insaturations of the fatty acid: in every case, linolenic acid derivatives were obtained in higher yield than those corresponding to arachidonic acid. Besides, comparing linear alkanolamines the decrease in yield was proportional to the chain length (Table 1, entries 1-4). Regarding the branched alkanolamines derivatives, some differences in product yield were observed among the different examples studied, ranging from 60 to 87% (Table 1, entries 5-10). These nucleophiles have another polar group (hydroxyl) in β-position of the amino group which favors the *N*-acylation reaction. The alkanolamines with two methyl groups (g) and isopropyl group (i) as substituent afforded the products in the lowest yield (Table 1, entries 7-9). These results were similar for both fatty acids and could be attributed to some steric hindrance in the alkanolamines.

Puzzled by this, we made a series of computer simulations to find an explanation to this experimental result. Using molecular modeling techniques we were able to understand the relation between substrate structure and reaction rate. Considering that both acids showed the same trend, molecular docking studies were performed using acid **1** as model to analyze the interaction between this acid and the catalytic site of CAL B, and therefore obtain a model for our acyl-enzyme complex. The next step was

Table 1: Enzymatic synthesis of *N*-linolenoyl (**1**) and *N*-arachidonoy-lalkylamines (**2**).

Entry	Alkanolamine	Product Yield (%)	
		1	2
1	$NH_2(CH_2)_2OH$, **a**	92	81
2	$NH_2(CH_2)_3OH$, **b**	83	73
3	$NH_2(CH_2)_4OH$, **c**	75	69
4	$NH_2(CH_2)_5OH$, **d**	68	66
5	$NH_2CH(CH_3)CH_2OH$, **e**	86	78
6	$NH_2CH_2CH(CH_3)OH$, **f**	87	80
7	$NH_2C(CH_3)_2CH_2OH$, **g**	63	60
8	$NH_2CH(CH_2CH_3)CH_2OH$, **h**	78	68
9	$NH_2CH(CH(CH_3)_2)CH_2OH$, **i**	62	63
10	$NH_2CH(CH_2CH_2CH_3)CH_2OH$, **j**	77	75

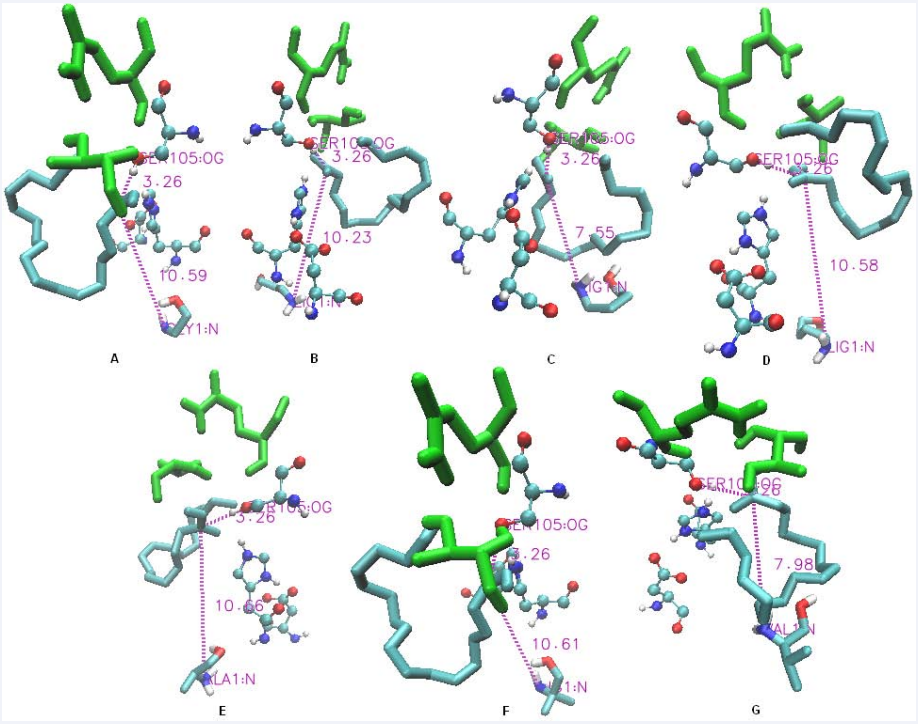

Figure 2 Docking results for seven representative N-linolenic acid alkanolamines: A) etanolamine; B) propanolamine; C) butanolamine; D) pentanolamine; E) 2-amino-1-propanol; F) 2-amino-2-methyl-1-propanol; G) 2-amino-3-methyl-1-butanol.

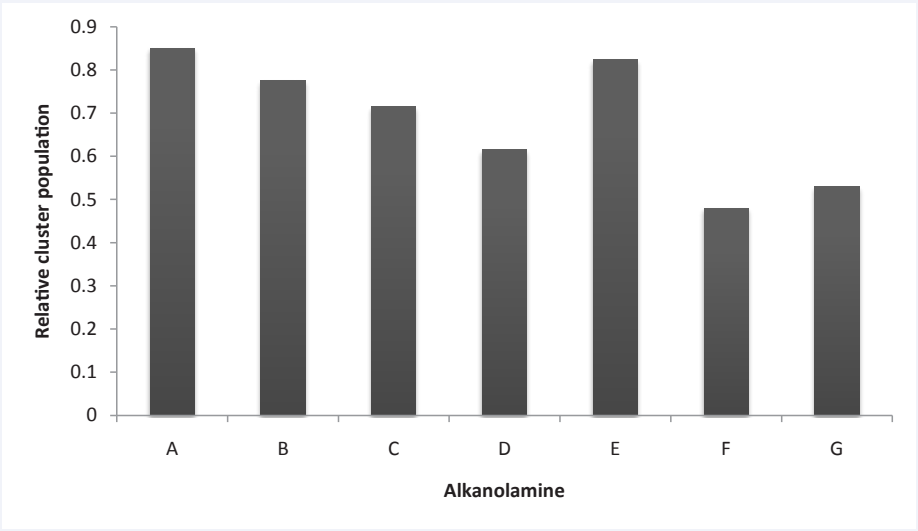

Figure 3 Docking results: relative cluster population vs alkanolamine structure: A) etanolamine; B) propanolamine; C) butanolamine; D) pentanolamine; E) 2-amino-1-propanol; F) 2-amino-2-methyl-1-propanol; G) 2-amino-3-methyl-1-butanol.

to study how each nucleophile attack the complex to form the desired product.

The analysis of the results led to the selection of a possible conformation for the fatty acid, considering the interaction with the aminoacids of the catalytic site (Asp201-His258-Ser146), binding energy and cluster population as selection criteria (Figure 2). A correlation between active cluster population and yield was observed as shown in Figure 3. This correlation reflects the probability of an effective conformation between substrates, showing a decrease as alkanolamine chains get larger. Also, using this approach, we were able to explain the difference in reactivity amongst substrates e and g. The presence of another methyl group adds steric hindrance to the nucleophile, thus reducing the probability of it attacking properly the acyl-enzyme complex. Lastly, comparing e and i, the decrease in cluster population, and therefore reactivity, between them can be explained for the presence of the much bigger isopropyl group in the latter.

CONCLUSION

This work describes the computational analysis made in order to explain the experimental results obtained in a previous work for the synthesis of anandamide analogs with pharmacological activity.

A trend that unravels the molecular determinants behind our experimental results was obtained, giving us not only information to improve further syntheses, but also showing the great potential and utility of molecular docking as a tool to explain and predict experimental results.

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