Journal of Molecular Graphics and Modelling 68 (2016) 147–157

Contents lists available at ScienceDirect

Journal of Molecular Graphics and Modelling

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

Explaining the autoinhibition of the SMYD enzyme family: A theoretical study

Nizar A. Al-Shar'i*, Soraya M. Alnabulsi

Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Jordan University of Science and Technology, P.O. Box 3030, Irbid 22110, Jordan

ARTICLE INFO

Article history:
Received 6 June 2016
Received in revised form 9 July 2016
Accepted 11 July 2016
Available online 13 July 2016

Keywords:
SMYD family
Autoinhibition
MD simulations
Energy correlation
Normal mode analysis

ABSTRACT

The SMYD enzymes (SMYD1-5) are lysine methyltransferases that have diverse biological functions including gene expression and regulation of skeletal and cardiac muscle development and function. Recently, they have gained more attention as potential drug targets because of their involvement in cardiovascular diseases and in the progression of different cancer types. Their activity has been suggested to be regulated by a posttranslational mechanism and by autoinhibition. The later relies on a hinge-like movement of the N- and C-lobes to adopt an open or closed conformation, consequently, determining the accessibility of the active site and substrate specificity.

In this study we aim to investigate and explain the possibility of the regulatory autoinhibition process of the SMYD enzymes by a thorough computational exploration of their dynamic, energetic, and structural changes by using extended molecular dynamics simulations; normal mode analysis (NMA); and energy correlations. Three SMYD models (SMYD1-3) were used in this study. Our results showed an obvious hinge-like motion between the N- and C-lobes. Also, we identified interaction energy pathways within the 3D structures of the proteins, and hot spots on their surfaces that could be of particular importance for the regulation of their activities via allosteric means. These results can help in a better understanding of the nature of these promising drug targets; and in designing selective drugs that can interfere with (inhibit) the function of a specific SMYD member by disrupting its dynamical and conformational behaviour without disrupting the function of the entire SMYD proteins.

1. Introduction

The human SET and MYND domain-containing proteins (SMYD) family is an important subfamily of the protein lysine methyltransferases (PKMTs) that catalyse the addition of one or more methyl groups, using *S*-adenosyl-L-methionine (SAM) as a cofactor, to the amino group of a lysine residue in the substrate resulting in a mono, di, or trimethylated lysine and the cofactor by-product *S*-adenosyl-L-homocysteine (SAH) [1]. The SMYD family is comprised of five members (SMYD1-5) of which three proteins (SMYD1-3) possess methyltransferase activity on histone protein H3 [2]. SMYD1-3 also show high sequence similarity [3], and they specifically catalyse the methylation of certain proteins in the cell. SMYD1 specifically methylates histone protein H3K4; SMYD2 specifically methylates histone proteins H3K36, H3K4 and non-histone protein p53-lys370; and SMYD3 catalyses the di- and tri-methylation of H3K4 and methylation of K831 of the vascular endothelial growth factor receptor-1 (VEGFR1) [4]. The catalytic activity of SMYD4 has not been determined, however, it has been identified as tumour suppressor in breast cancer [5]. SMYD5 has methyltransferase activity on histone H4, and it regulates pro-inflammation genes through trimethylation of H4K20 [6].

The SMYD enzymes have diverse biological functions including gene expression [7], regulation of skeletal and cardiac muscle development and function [8], cell-cycle regulation, DNA damage response, and chaperone machinery [3,9]. Recently, the SMYD family members have gained more attention as potential drug targets after the establishment of their involvement in cardiovascular diseases [10] and in the progression of different types of cancers such as breast, colorectal, hepatocellular, and esophageal squamous cell carcinomas [5,11–18].

The SMYD family is characterized by the presence of SET (Drosophila proteins **S**uppressor of variegation, **E**nhancer of Zeste, **T**rithorax) domain. The SET-domain is a conserved domain in many mammalian proteins, which contains a series of β strands folding

* Corresponding author.
E-mail addresses: nashari@just.edu.jo, pharma96@yahoo.com (N.A. Al-Shar'i), smalnabulsi@just.edu.jo (S.M. Alnabulsi).

http://dx.doi.org/10.1016/j.jmgm.2016.07.001
1093-3263/

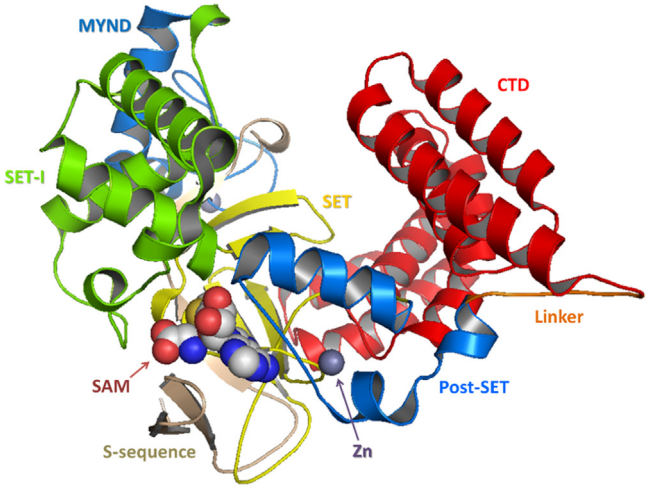

Fig. 1. Cartoon representation of the crystal structure of the SMYD2 enzyme (PDB code 3TG4) showing the different domains of the enzyme. The S-sequence, MYND, SET-I, core SET, post-SET, linker, and CTDs are depicted in wheat, dark blue, green, yellow, blue, magenta, and red respectively. SAM and zinc ions are shown in CPK. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

into three discrete sheets that surround a knot-like structure, and is responsible for lysine methylation [19,20]. In addition to the SET-domain, the family has the MYND (**My**eloid, **N**ervy and **D**EAF-1) zinc finger domain that split the SET-domain. MYND-domain is a cluster of cysteine and histidine residues, which form zinc-binding motif and is a protein–protein interaction module with preference for binding with a proline rich motif (PXLXP) [7,21]. A final feature found in SMYD1-4 is the C-terminal domain (CTD) which also functions as a protein–protein interaction module [3,10,22]. The overall structure of SMYD enzymes is bilobal. The N-terminal lobe is divided into four domains: SET located in the middle of this lobe and surrounded by MYND, SET-I, and post-SET domains (Fig. 1). The C-terminal lobe is composed of helices that adopt an open or closed conformation relative to the N-lobe. Up to date, only SMYD1-3 have solved crystal structures of which SMYD1 is the most open, SMYD3 is the most closed, and SMYD2 is intermediate between the two (Fig. 2).

The activity of the SMYD enzymes has been suggested to be regulated by a common posttranslational mechanism [23]; for example, association of SMYD2 with Hsp90 enhances its histone methylation activity [24]. Furthermore, Zhe Yang and co-workers have shown that the conserved C-terminal domain (CTD) of the SMYD enzymes negatively contributes to the regulation of their activity and could be partly responsible for their autoinhibition; and they proposed, based on different crystal structures of SMYD enzymes, that the CTD could undergo a hinge-like movement to adopt an open or closed conformation relative to the N-domain, consequently determining the accessibility of the active site [10,22,23,25]. They further investigated their hypothesis by running a short molecular dynamics simulation (2 ns) of the SMYD2 enzyme and they showed that this hinge-like movement is possible [26]. Therefore, these proposed conformational and dynamical changes of the CTD domain could regulate the activity and substrate specificity of the SMYD enzymes [25].

In this study we aim to investigate and explain the possibility of the regulatory autoinhibition process of the SMYD enzymes by a thorough computational exploration of the dynamic, energetic, and structural changes in this enzyme family. This in turn can help in a better understanding of the dynamical nature of these promising drug targets; and in designing selective drugs that can interfere with (inhibit) the function of a specific SMYD member by disrupting its dynamical and conformational behaviour and trapping it in a particular conformational state (inactive conformer) without disrupting the function of the entire SMYD proteins. In order to achieve this, extended (a total of 180 ns) molecular dynamics simulations; normal mode analysis (NMA); and energy correlations of three SMYD models (SMYD1-3) were conducted.

2. Computational materials and methods

2.1. Computational materials

Preparation of the simulated structures was performed using Discovery Studio (DS) 3.5 from BIOVIA® (formerly Accelrys®) Software Inc. [27]. MD simulations were performed using AMBER 12. Normal mode analysis was performed using Anisotropic Network Model (ANM) Web Server 2.0 (http://anm.csb.pitt.edu/cgi-bin/anm2/anm2.cgi) [28,29]. Energy correlation analysis was per-

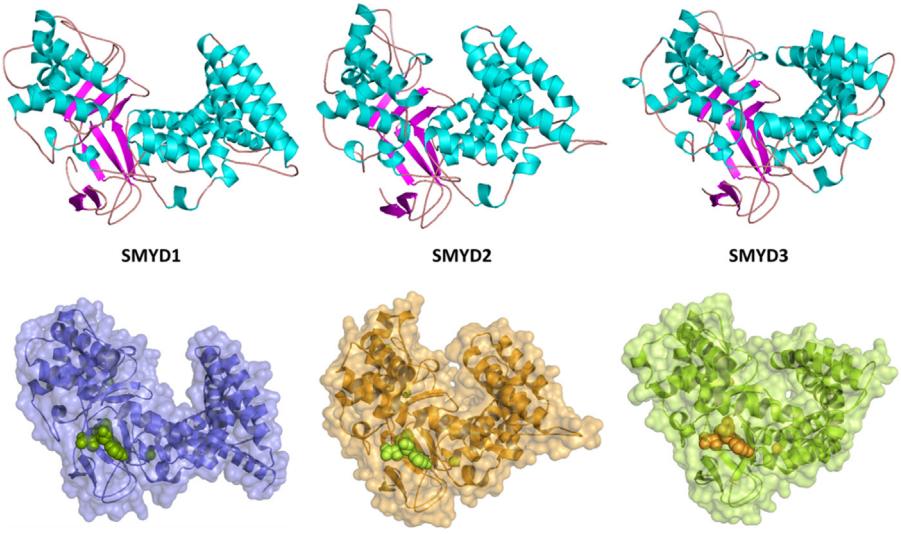

Fig. 2. The 3D crystal structures of the SMYD1-3 enzymes (PDB codes 3N71, 3TG4, and 3PDN respectively) showing the different conformations of the C-domain relative to the N-domain. Upper panel, is a cartoon representation of the backbone of the SMYD1-3 enzymes where α-helices are in cyan, β-sheets in magenta, and turns and loops are in brown (ligands are removed for clarity). Lower panel, transparent surface representation showing how closed or opened the conformation of the enzyme is. The co-crystallized ligands and Zn ions are shown in CPK.