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Inhibitors of the Cdc34 acidic loop: A computational investigation integrating molecular dynamics, virtual screening and docking approaches

Alberto Arrigoni *, Luca Bertini, Luca De Gioia, Elena Papaleo *

Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza 2, 20126 Milan, Italy

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Among the different classes of enzymes involved in the ubiquitin pathway, E2 ubiquitin-conjugating enzymes occupy a central role in the ubiquitination cascade. Cdc34-like E2 enzymes are characterized by a 12–14 residue insertion in the proximity of the catalytic site, known as the acidic loop. Cdc34 ubiquitin-charging activity is regulated by CK2-dependent phosphorylation and the regulatory mechanism involves the acidic loop. Indeed, the phosphorylation stabilizes the loop in an open conformation that is competent for ubiquitin charging.

Cdc34 is associated with a variety of diseases, such as hepatocellular carcinomas and prostatic adenocarcinomas. In light of its role, the discovery of potential inhibitory compounds would provide the mean to effectively modulate its activity.

Here, we carried out a computational study based on molecular dynamics, virtual screening and docking to identify potential inhibitory compounds of Cdc34, modulating the acidic loop conformation. The molecules identified in this study have been designed to act as molecular hinges that can bind the acidic loop in its closed conformation, thus inhibiting the Cdc34-mediated ubiquitination cascade at the ubiquitin-charging step. In particular, we proposed a pharmacophore model featuring two amino groups in the central part of the model and two lateral aromatic chains, which respectively establish electrostatic interactions with the acidic loop (Asp 108 and Glu 109) and a hydrogen bond with Ser 139, which is one of the key residues for Cdc34 activity.

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1. Introduction

Ubiquitination is a post-translational modification that was originally known as a signal for protein degradation by the 26S proteasome [1]. It is also involved in many other different signaling pathways, including cell cycle, endocytosis, transcription, DNA repair, signal transduction, apoptosis and the immune response [1–4].

In the ubiquitination pathway, E2 enzymes charged with ubiquitin (Ub) can be recruited by an E3 ligase, along with the target substrates. The C-terminal glycine of Ub can then be attached to a lysine residue on the target substrate. This can result in the transfer of only a single Ub molecule (mono-ubiquitination) or the addition of further Ub molecules to form a poly-Ub chain. Depending on the target lysine used to cross-link the Ub molecules in the chain, different poly-Ub chains can be formed, which adopt diverse three-dimensional (3D) structures and exert different biological effects [5–8].

Ubiquitination can thus be described as a molecular zip code, which is used to sort different ubiquitination products to different destinations. Errors in delivery of ubiquitinated proteins to the proteasome or other destinations are highly detrimental for the cell [3].

E2 enzymes (E2s) have a primary role in catalyzing, alone or with the cognate E3, the covalent attachment of Ub to the target proteins and they have a major role in defining the topology of the polyUb chain and thus the fate of the substrate [9]. E2s are often multi-domain proteins that all share a conserved Ub-binding domain (UBC characterized by a α/β fold) [9–11]. The highly

Abbreviations: MD, molecular dynamics; Sc, *Saccharomyces cerevisiae*; Ub, ubiquitin; UBC, ubiquitin-binding domain

* Corresponding authors. Current address: Structural Biology and NMR Laboratory, Department of Biology, University of Copenhagen, Ole Maaloes Vej 5, DK-2200 Copenhagen, Denmark (E. Papaleo).

E-mail addresses: arrigonialberto86@gmail.com (A. Arrigoni), elena.papaleo@unimib.it, elena.papaleo.78@gmail.com, elena.papaleo@bio.ku.dk (E. Papaleo).

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conserved active site Cys is located in a shallow groove of the UBC domain formed by a short loop connecting α-helix 2 and α-helix 3 and a long loop (β4-α2 loop) proximal to the active site [9–11].

E2 UBC domains have been recently classified by Michelle et al. [12] into 17 families of homologs by performing phylogenetic analyses on 207 E2 genes belonging to seven different species. Family 3 members, i.e. Cdc34-like enzymes are characterized by a conserved and disordered insertion in the β4-α2 loop in the proximity of the catalytic site. The insertion is known as the acidic loop of Cdc34-like enzymes.

In a previous work, we identified two co-evolving signature elements in Cdc34-like E2 enzymes: the acidic insertion in β4-α2 loop in the proximity of the catalytic cysteine and two conserved CK2 phospho-sites within the UBC domain [13]. We previously demonstrated by combining Ub-charging assays and MD simulations that the phosphorylation at one of this sites (S130) can modulate the opening and closing of the β4-α2 loop with respect to the catalytic cleft and, in turn, it modulates the accessibility of the catalytic Cys for Ub-charging [13]. More in details, this regulatory mechanism relies on electrostatic repulsive effects between the phosphorylated serine and the acidic residues in the β4-α2 loop. The loop can undergo a substantial shift and drift away from the catalytic cleft upon phosphorylation, promoting the accessibility of the catalytic Cys.

Cdc34 is known to be involved in a variety of diseases, such as hepatocellular carcinomas and prostatic adenocarcinomas [14–19]. In fact, Cdc34 stimulates cellular proliferation by enhancing the degradation of p53 and p27, which both act as inhibitors of cell cycle progression.

In light of the above scenario, recent studies have been focused in the design and identification of inhibitory molecules of E2 enzymes and of Cdc34 in particular. A small inhibitor (CC0651) was identified for Cdc34 [20,21]. CC0651 was able to inhibit proliferation of human cancer lines and caused accumulation of the p27 substrate. Another small inhibitor was identified for Rad6 E2s [22] with the capability to inhibit the thioester formation between the E2 catalytic cysteine and the C-terminal of Ub. These studies have opened the venue to study E2 enzymes as a suitable class of drug targets in the ubiquitination pathway.

We contribute to this scenario, proposing a group of molecules with the potential to directly counteract the Ub-charging activity of Cdc34 by acting on the acidic loop (β4-α2 loop) and keeping it in a closed conformation, shielding the catalytic cysteine needed for the attachment of the Ub molecule. The task was carried out by the integration of different computational approaches, as described by Sanders et al. [22]. In particular, we used a structural ensemble that was already available thank to our previous MD studies [13,23] to isolate the most representative conformations suitable for docking simulations. Subsequently, virtual screening and docking were performed to select putative compounds from 735,758 entries of the ZINC database [24]. We selected 20 molecules by both energy-based and structural-based screening of docking simulations of 500 compounds. We then provide a pharmacophore model with the aim of inhibiting Cdc34 Ub-charging activity acting as molecular zipper to stabilize the closed and inactive conformation of the acidic loop. The results here described can provide a valuable dataset for future experimental studies in the field.

2. Materials and methods

2.1. Rmsd-matrices and clustering

The Cdc34 MD ensemble previously published [13] along with an increased sampling achieved by performing new simulations [23] was used as a reference conformational ensemble for the present investigation. In particular, we post-processed the ensemble by rmsd-based structural clustering as described in the following.

C-alpha (Cα) root mean square deviation (rmsd) were calculated pairwise for each pair of frames of the available MD ensemble, collecting values ranging from 0.2 to 0.65 nm, indicating respectively nearly identical or highly diverging structures in the ensemble. The highest rmsd values are associated to a displacement of the acidic loop conformations, whereas secondary structures were conserved in all the MD structures and characterized by rmsd values lower than 0.25 nm [13]. The Cα rmsd matrix (Fig. 1S) was then processed to obtain structural clusters of similar conformations using the Gromos algorithm implemented in Gromacs (www.gromacs.org) with a clustering cutoff of 0.35 nm.

2.2. Virtual screening and docking simulations

The virtual screening and the docking calculations were performed with DOCK Blaster [25] and Autodock version 4.2 [26], respectively. DOCK Blaster is an online server that selects and scores thousands of compounds deposited in the ZINC database [24] for a target structure uploaded by the user. The center of mass of three residues (P110, I137 and N138) was used to set the grid for the calculation. In particular, we selected the ZINC subset 11 [24], containing 735,758 entries. Indeed, compounds belonging to this subset are described as lead-like and were selected to obey to the Lipinski rule [27], according to which an orally active drug has no more than one violation of the following criteria: not more than five hydrogen-bond (H-bonds) donors (nitrogen or oxygen atoms with one or more hydrogen atoms), no more than ten H-bond acceptors (nitrogen or oxygen atoms), a molecular weight under 500 Da and an octanol–water partition coefficient log *P* less than 5.

Once the virtual screening procedure was completed, we employed Autodock version 4.2 [26] for docking calculations of the first 500 compounds selected by DOCK Blaster energy-rank. For each molecule, DOCK Blaster provided one binding pose characterized by the lowest energy according to the DOCK Blaster energy function. The DOCK Blaster binding pose was used as a starting structure for docking simulations with Autodock for each molecule (500 Autodock simulations overall). The parameters used for Autodock simulations are reported in the Supplementary Table 1S.

Autodock provided different binding poses for each of the 500 molecules simulated. For each molecule, the binding poses generated by Autodock are at first clusterized by the Autodock internal routines. For each cluster referred to a specific molecule, the software generally returns the binding pose with the lowest energy (cluster binding pose). We then applied a further selection procedure by the Pymol Python APIs (application programming interfaces) on all the cluster binding poses provided by Autodock for each molecule. In particular, we used both energetic and structural criteria in this final selection step. The final goal is to obtain, for each of the 500 screened molecules, a unique binding pose that is not only characterized by the lowest energy but also that can structurally act as a molecular zipper contacting both the acidic loop and the surrounding region in the catalytic cleft, as also discussed in the Results. With this aim in mind, we ranked by the Python routine with the Pymol APIs the cluster binding poses referred to the same molecule according to their energy. Among the two cluster binding poses of a molecule that are characterized by the lowest energy, the API routine selects the one that has a center of mass at the minimal distance from the binding site (defined using as reference residues P110, I137 and N138).

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