Methods 46 (2008) 39–46

Contents lists available at ScienceDirect

Methods

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

Modulation of biomolecular interactions with complex-binding small molecules

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ARTICLE INFO

Article history:
Accepted 22 May 2008
Available online 20 June 2008

Keywords:
Structure-based drug design
Intermolecular pockets
Complex stabilization

ABSTRACT

Although numerous biomolecular interactions have been identified as potential targets for the development of therapeutic agents, modulation of these interactions with small molecules has historically been considered an extremely difficult task. This difficulty is largely due to the low effectiveness of the traditionally used competitive approaches in which inhibitors are designed and screened for their ability to block biomolecules from establishing contacts with their targets. We propose a novel approach to modulate biomolecular interactions by de novo structure-based design of noncompetitive small molecules that bind to the intermolecular complexes and make molecular contacts with both interacting partners. Similar complex-binding mechanism has been observed and well documented for many natural compounds that bind to protein–protein, protein–DNA or protein–small molecule complexes. To implement the paradigm for structure-based drug design, we have developed a complex-binding modulation (CBM) algorithm for the rational design of compounds (CBM compounds) that can affect biomolecular interactions by binding to the intermolecular pockets or cavities of biomolecular complexes. In this paper, we describe our methodology used to design the CBM compounds and to study their effects on biomolecular interactions including protein–protein and protein–small molecule interactions.

1. Introduction

1.1. Targeting intermolecular complexes for drug discovery

Biological processes are regulated by reversible interactions between macromolecules such as proteins and nucleic acids. Identification of critical protein interactions in human diseases by studies of cellular molecular pathways provide a rationale for pharmacological interference [1]. However, modulation of protein interactions remains a most challenging approach for drug discovery. Most current strategies to modulate biomolecular interactions are focused on the development of competitive inhibitors that are supposed to sterically block protein contact with the interacting molecule. The competitive approach has important drawbacks that limit its therapeutic effectiveness. Since biomolecular interactions often involve large hydrophobic interfaces, small and rather hydrophilic drug-like molecules usually cannot effectively compete for binding to such interfaces. It is also known that many proteins lack hotspots [2] for binding small molecules with high affinity and specificity [3]. Another typical problem with competitive inhibitors is lack of selectivity because their targets often belong to homologous families that are difficult to block selectively. Because of these drawbacks, the use of the traditional competitive compounds is typically limited to inhibition of enzymes containing deep druggable active sites. There are very few examples for successful design of inhibitors of a protein–protein interaction and there is clearly a need to develop of new paradigm for structure-based drug design.

Recently, we have developed two structure-based algorithms to allosterically modulate protein–protein interactions. The cavity-induced allosteric modification (CIAM) method [4] can be used to design compounds that bind to allosteric cavities located close to the protein–protein binding site resulting in propagation of inhibitory conformational changes to critical interaction-mediating residues. The complex-binding modulation (CBM) method described in this paper is based upon the concept that a compound that binds at the intermolecular space of a macromolecular complex can interfere with the normal function of the complex. Such complex-binding modifier compounds (CBM compounds) are designed to bind to complexes formed between two interacting molecules making molecular contacts with both molecules. Similar type of complex-binding modulation has been observed for many natural compounds that bind at protein–protein or protein–DNA intermolecular spaces [5]. Topotecan (Hycamtin) is a derivative of a natural alkaloid camptothecin. It is used clinically in patients with small-cell lung cancer. Topotecan binds to the topoisomerase I–DNA complex and prevents religation of the single strand breaks induced by the enzyme [6]. Brefeldin A is a fungal macrolide with a wide range of antibiotic activities. It has been characterized as an inhibitor of secretion [7] that targets activation of Arf, a small GTP-binding protein. Brefeldin A binds to the Arf–GDP–Sec7 complex and freezes

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1046-2023/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ymeth.2008.05.008

the complex in an inactive conformation [8]. We have developed a method to design compounds that can mimic complex-binding effects of some natural inhibitors (such as camptothecin and brefeldin A) by binding to the intermolecular space of complex-forming molecules. We have shown that depending on the mode of binding to an intermolecular complex (or to a complex formed between two separable sub-domains of the same protein), CBM compounds can either stabilize or destabilize the complex. They can also affect functional properties of the complex by introducing conformational changes or by other mechanisms.

2. Methodology

In this section, we first outline a novel structure-based algorithm to design small molecule modulators of intermolecular interactions, a CBM method that targets intermolecular complexes. We then describe some biophysical techniques used to characterize CBM compounds and to study their mechanism of action. Description of the methods is accompanied by illustrative examples based on the experimental data obtained in our laboratory.

2.1. CBM approach for targeting protein–protein interactions

We have developed a complex-binding modulation (CBM) method to design compounds (CBM compounds) that target intermolecular complexes formed between two interacting biomolecules. CBM compounds are designed to bind in the intermolecular pockets or cavities and thereby affect stability and/or functional properties of the complex (Fig. 1). This strategy is based, in part, on information gleaned from natural examples of CBM compounds, including brefeldin A (Fig. 2A) and fusicoccin (Fig. 2B) and numerous other compounds that have been shown to produce their effect by binding to protein–protein or protein–DNA complexes [5]. Binding of CBM compounds to a complex can either stabilize it by creating additional bonds between the interacting molecules (usually resulting in an agonistic effect) or, to the contrary, induce its dissociation by disrupting critical intermolecular interactions that hold the complex together (usually antagonistic or inhibitory effect). The effect of CBM compounds on a complex is not limited to stabilization/destabilization of the complex, but can also modify its functional properties.

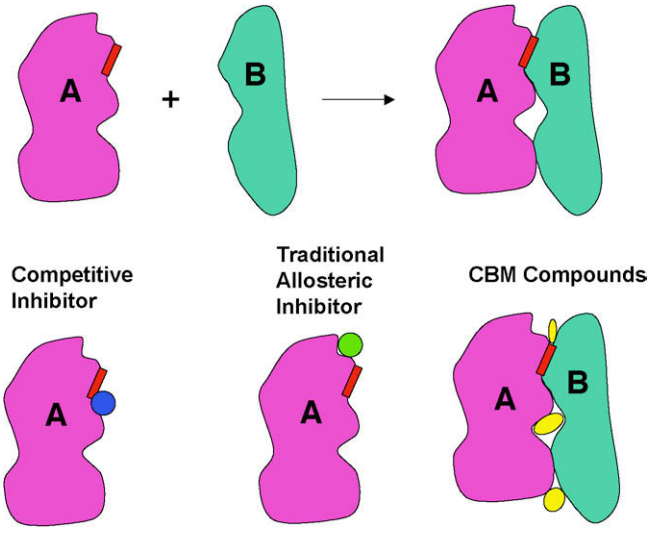

Fig. 1. Inhibition of protein–protein interactions. Two interacting proteins A and B are shown in magenta and cyan, respectively. Critical "hot spot" residues of protein A that are most important for binding with protein B are shown in red. Unlike competitive inhibitors (blue) or traditional allosteric inhibitors (green), CBM compounds (yellow) bind to the formed complex (AB) rather than to one of the interacting molecules (A or B).

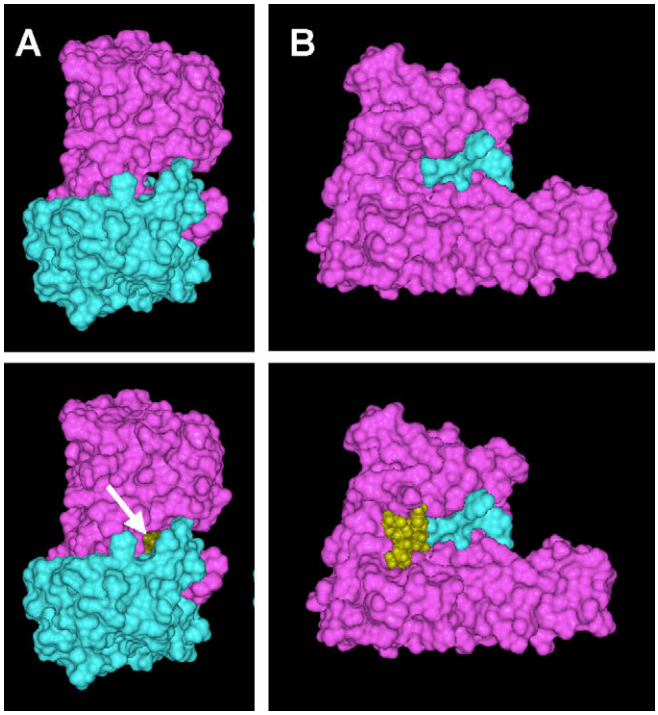

Fig. 2. Natural examples of CBM compounds. (A) Brefeldin A (shown in yellow on the lower panel and indicated by an arrow) stabilizes a complex between Arf (magenta) and Sec7 (cyan) in an inactive conformation [35]. (B) Fusicoccin (shown in yellow on the lower panel) over stabilizes a regulatory complex between H^+-ATPase (magenta) and 14-3-3 (cyan) [36]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

A traditional method for structure-based design of protein–protein interaction inhibitors involves targeting a cavity at the binding interface of one of the interacting partners (Fig. 1). Because of a relatively flat shape of many protein–protein interfaces, there are often few if any suitable pockets on the surface of any of the interaction partners that could bind small molecules with high affinity. Therefore, this approach is usually limited to targeting enzymes that have deep substrate-binding active site pockets. In contrast, surfaces of complexes formed between two proteins usually contain numerous intermolecular pockets or cavities suitable for structure-based design of the CBM molecules. Unlike traditionally used methods, the CBM-based approach targets a complex formed between the interaction partners and not an individual interaction partner. Instead of preventing complex formation like traditional inhibitors, CBM molecules bind to a complex that has already been formed and either stabilize or destabilize the complex. While compounds that target protein interface usually act as competitive inhibitors of protein–protein interactions and can be displaced by proteins at high protein concentrations, binding of CBM compounds is noncompetitive and is enhanced at high concentrations of complex-forming molecules.

Recently, complex-binding compounds have been described in a number of experimental and review papers where these compounds have been referred to as "interfacial inhibitors" [5]. We prefer using the term "complex-binding modulators" or "CBM compounds" to emphasize the mode of binding of the compounds rather than their effect on protein activity. Based on the results obtained in our laboratory and elsewhere, the effect of the CBM compounds is not limited to inhibition of protein functioning, but can result in complex activation. Thus CBM compounds can include but are not limited to the interfacial inhibitors.