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Computational studies of the binding mechanism of calmodulin with chrysin

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Abstract

Calmodulin (CaM) plays a crucial role in metabolism and physiology of eukaryotes by regulating biological activities. Multiple lines of evidences indicate that the phosphorylated flavonoids possess relatively stronger affinities for proteins by forming non-covalent complexes with them, and that the cellular functions are often triggered by this kind of interactions. Chrysin is one of the phosphorylated flavonoids that exist ubiquitously in plants and have remarkably beneficial pharmacological effects. In this study, the molecular docking tools were utilized to investigate the interactions of CaM with chrysin. Two different favorable binding modes have been observed. To complement the results obtained by the molecular docking study, an in-depth investigation into the binding modes was conducted with the molecular dynamics (MD) simulation to explore the binding profile and energy landscape. Based on the results thus obtained, a clear definition of the binding pocket for each of the two binding modes has been revealed. These findings may shed light upon the binding interactions of CaM with chrysin, providing a solid molecular basis for subset analysis of its pharmacological benefits.

Keywords: Calmodulin; Phosphorylated flavonoid; Chrysin; Binding pocket; Molecular docking; Molecular dynamics; Escaping trajectory

Calmodulin (CaM) is a ubiquitous Ca^{2+} binding protein that plays a key role in numerous cellular Ca^{2+}-dependent signaling pathways and is actually involved in controlling many of the biochemical processes of cells. The convincing evidence from NMR study indicates that CaM bears the multifaceted and flexible structural feature [1,2], and this kind of feature is the key to its ability to bind a wide range of targets.

The crucial role of CaM in the metabolism and physiology of eukaryotes is derived from its capability of regulating biological activities of a massive amount of different protein targets and working as transmembrane ion transporters mainly in a Ca^{2+}-dependent manner. As is known, cells have developed a multitude of ways to control and make use of calcium ions. This kind of ions exist as a gradient across the plasma membrane with extracellular concentrations being about 10,000 times higher than the intracellular ones. The gradient changes resulted from the external signals, such as hormones, light, stress, or pathogenesis, can regulate many cellular processes. When the intracellular calcium level rises to 10^{-5} M, four Ca^{2+} ions will bind to CaM, and the Ca^{2+}–CaM complex thus formed will be linked to the target proteins, initiating various signaling cascades. CaM mediates biological processes, such as inflammation, metabolism, apoptosis, muscle contraction, intracellular movement, short-term and long-term memory, nerve growth, and the immune response by affecting many different cellular functions. CaM can bind up to four calcium ions, undergoing post-translational modifications, such as phosphorylation, acet-

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ylation, methylation, and proteolytic cleavage, each of which can potentially modulate its actions.

Recently, flavonoids, the polyphenolic compounds existing ubiquitously in plants, have attracted increasing interest due to their various beneficial pharmacological effects, such as antioxidant [3], antiviral [4], anti-diabetogenic [5], and anti-cancer effects [6]. Moreover, it is indicated from a series of researches that phosphorylated flavonoids possess relatively stronger affinities for proteins and are easier to form non-covalent complexes with proteins than the non-phosphorylated compounds. In addition, evidences about the phosphorylated flavonoids effecting on the Hela cell show an apparent anti-proliferation [7], and cell cycle arrest in G0/G1 or G2/M [8]. Also, induction of differentiation and apoptosis in special cell lines were reported [8,9].

Cellular functions were often triggered by weak interactions between protein and ligand [10]. To dig the pharmacological benefits of chrysin, a good candidate as a receptor for the ligand is CaM due to the following reasons: (1) CaM is widely distributed in the human body. (2) CaM plays an essential role in numerous biological processes as mentioned above. (3) CaM has the unique structural feature as a receptor: it consists of two structurally similar globular domains connected by a flexible linker, which allows the two domains to adopt different positions with respect to each other and to bind targets. The domains contain some determinants that can make distinct contributions in binding target molecules.

Computational methods

Many useful clues for drug design can be gained through molecular docking studies (see, e.g., [11–24]). In this study, the molecular docking with the Metropolis algorithm [25] was used to find the most favorable binding interaction. In our docking studies, the chrysin was flexible. The program generates a diversified set of conformations by making random changes of the chrysin coordinates [9,25]. When a new conformation of the chrysin was generated, the search for the favorable bindings was conducted within a specified 3D docking box, using either simulated annealing [26] or Tabu search [27,28]. Both methods seek to optimize the purely spatial contacts as well as electrostatic interactions. The interaction energy was calculated using the electrostatic and van der Waals potential fields. In all our computations, the CHARMM22 [29] force field parameters were used. The NMR solution structure of CaM (1CFF) was taken as the receptor. It has four calcium ions.

As mentioned, CaM bears the multifaceted and flexible structural feature [1,2]. To reflect this kind of dynamic feature, one of the feasible approaches is to use the molecular dynamics (MD) tool. The MD simulations [30] can solve the classical equations of motions for a system consisting of target protein (CaM) and small ligands (phosphorylated flavonoids) under specified ensembles. In our case, the energy favorable structures derived by the aforementioned docking operation were further studied with the molecular dynamics (MD) triggered by hydrogen bond braking and making events of the ligand and receptor interactions [31]. The results thus obtained would provide further information of the conformational searching in space. Constant temperature simulations were performed at 300 K and normal pressure. All the backbone atoms were fixed to maintain the correct protein structure with the side chains being allowed to move freely. Some fictitious degree of freedom was added to the system to represent the motion of heat in and out of the system. This would generate a series of conformations in the important phase space area, providing configuration and momentum information for each relevant atom, from which the thermodynamic properties of the system can be calculated. The trajectory represents an exploration of the energy landscape with chrysin sitting in a specific domain of the receptor. To simulate the interaction in the physiological condition, i.e., in the solution environment, 10 layer-water molecules around the active region between the chrysin and the receptor were added. Also, to study the temperature effect, the system with varying temperature was investigated.

The above approach by combining the molecular docking operation with the MD simulation would provide very useful information for the binding mechanism of CaM with chrysin.

Results and discussions

By means of the molecular docking operation, two stable structures for the CaM–chrysin complex were obtained. One is the interaction mode 1, where the chrysin is situated in the region between one of the EF fingers and the turn linking the two domains of CaM, allowing the chrysin to make the maximum contact with the side chains of the receptor and form proper bonds to stabilize the CaM–chrysin complex. Another stable structure is the interaction mode 2, where the chrysin is situated near Ca to allow the abundant oxygen atoms with negative charge of the chrysin molecule to form the electrostatic interactions with CaM.

To demonstrate that the two interaction modes are indeed the most favorable, we also investigated other four possible binding sites which fit the chrysin size very well. The results of the binding free energies are shown in Table 1, from which we can see that the energies of the other four interaction modes, i.e., modes A, B, C, and D, are much higher than those of modes 1 and 2. These findings have been further supported by the analysis with MD simulations later.

The two most favorable binding modes of CaM with the chrysin molecule obtained through the docking studies are shown in Fig. 1A (mode 1) and Fig. 1B (mode 2), respectively. As we can see from Fig. 1, the size of the chrysin molecule is well fitting into the active cavity of CaM. Also, the lipophilic and hydrophilic residues at the active cavity are one of the most important factors in favor to the electrostatic interaction between the chrysin and the receptor.

The MD simulation has made it possible to investigate the dynamic features of protein and DNA, such as low-frequency internal motion and its biological functions [32], among many others. These features are far beyond the reach of, or the imminent challenge to [33], X-ray

Table 1
List of different modes and their interaction energies (kcal/mol) obtained by docking the chrysin molecule to CaM

Mode	E (electrostatic)	E (van der Waals)	E (binding)
1	−10.61	−20.28	−30.89
2	−16.69	−13.73	−30.42
A	−5.58	−17.75	−23.33
B	−4.63	−17.72	−22.35
C	−6.01	−15.55	−21.56
D	−0.29	−15.75	−16.04