Study of PKA Binding Sites in cAMP-Signaling Pathway Using Structural Protein-Protein Interaction Networks

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Abstract

Background: Protein-protein interaction, plays a key role in signal transduction in signaling pathways. Different approaches are used for prediction of these interactions including experimental and computational approaches. In conventional node-edge protein-protein interaction networks, we can only see which proteins interact but 'structural networks' show us how these proteins interact which can give us so much information about the network. Structural networks help us understand the molecular basis of cellular functions and regulatory mechanisms in signaling pathways. In this study, we aimed to construct a structural network for a part of cAMP signaling pathway which has PKA (cAMP-dependent protein kinase catalytic subunit alpha) as the hub.

Materials and Methods: A part of cAMP signaling pathway was selected from kegg database and interactions of PKA as hub protein with some of its partners were achieved using Hex8.00 software. The interfaces of the resulted complexes were predicted by KFC2 server.

Results: Hex8.00, as a docking software, gave us the complexes from the interaction of PKA with 15 proteins of its partners. For each complex, the KFC2 server gave us the amino acid composition of the interfaces. Using this amino acid composition, we draw a structural network which shows the binding sites on PKA surface.

Conclusion: We have constructed a structural network for cAMP signaling pathway which shows how PKA interacts with its partners. This network can be used for understanding the mechanisms of signal transduction and also for drug design purposes.

Keywords: Structural network; cAMP Signaling pathway; Interface; PKA; Protein-protein interaction

Introduction

Protein-protein interactions play a key role in many biological processes such as signal transduction, gene expression control, enzyme inhibition, antibody-antigen recognition or even the assembly of multi-domain proteins (1). Different approaches are being used for the prediction and identification of protein-protein interactions. There are two main approaches: experimental methods such as yeast two hybrid and phage display, and computational prediction of interactions including protein-protein docking and template-based modeling. It is shown that only about 6% of the known human protein interactions have experimental complex structures (2). Because of the limitations of experimental approaches, computational methods have attracted much attentions in recent years. Computational methods for PPI prediction are based on protein sequence, structural and genomic features that are related to interactions and functional relationships (3). Protein-protein docking has now an increasing role in predicting protein-protein interactions, revealing the interacting mechanism between proteins and identifying interfaces and

hotspot residues for drug discovery (4). The interface refers to amino acids participating in the binding and physical adhesion of two proteins (5). Hot spots are a few residues that confer most of the binding energy in the interfaces (1). In conventional or classical protein interaction networks, which contain nodes and edges, nodes are proteins and edges represent interactions. But this classical node-and-edge representation cannot elucidate the details of mechanisms for understanding how the signals flow, and how the function and the regulation are executed in the cell (6). Structural networks can address this challenge and help us understand the interaction mechanisms. In fact, structural networks show 'how proteins interact' in addition to 'which proteins interact' (6). These structural networks are essential in understanding the molecular basis of cellular functions and for designing new therapies to regulate these interactions (2). Structural networks are also used for understanding regulatory mechanisms in signaling networks. In this paper, we aimed to construct a structural network for a part of kegg cAMP signaling pathway which contains PKA (cAMP-dependent protein kinase catalytic subunit alpha), as the hub in the network. cAMP signaling pathway has fundamental roles in cellular response to many hormones and neurotransmitters (7). cAMP regulates essential physiologic processes including metabolism, secretion, calcium homeostasis, muscle contraction, cell fate, and gene transcription. Three main targets of cAMP have been identified: protein kinase A (PKA), the GTP-exchange protein EPAC and the cyclic-nucleotide-gated ion channels. PKA is of great importance and modulates the cAMP response by phosphorylating other components of the cAMP signaling pathway (7).
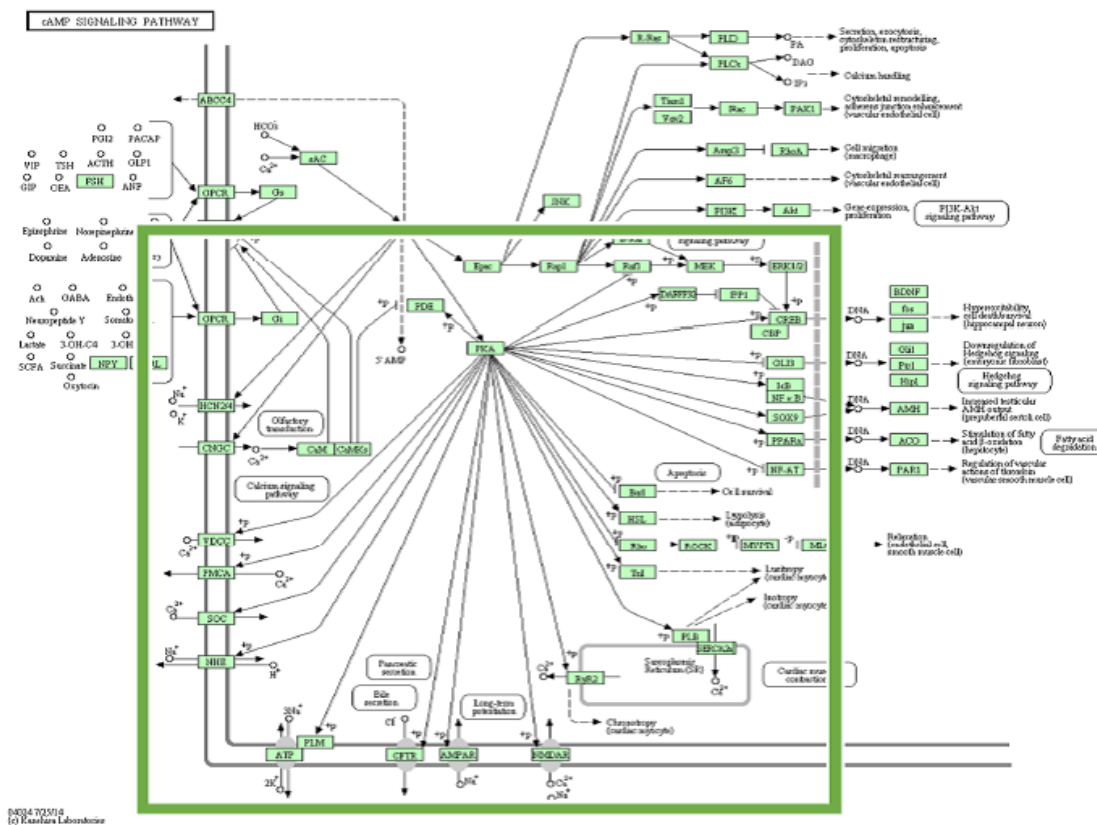

Figure 1. cAMP signaling pathway obtained from kegg pathway database. Interactions of PKA as a hub protein in this network is shown in green box.

Materials and Methods

A part of cAMP signaling pathway was selected to find its interactions (Figure 1). cAMP signaling pathway was obtained from kegg database (http://www .genome.jp/kegg/pathway.html) (8, 9). PKA was selected as the hub protein and the interaction of some of the partners were studied. Protein structures of the PKA and its partners were taken from RCSB database (www.rcsb.org) (10). For the proteins which did not have proper PDB structures or no PDB structures at all, we used model structures from Swiss-Model portal (11, 12). The

resulting protein 3D structures were then docked using Hex8.00 software (13).

The protein complexes resulted from Hex software were then used to find interaction interfaces by means of KFC-2 server (http://kfc.mitchell-lab.org/) (14, 15).
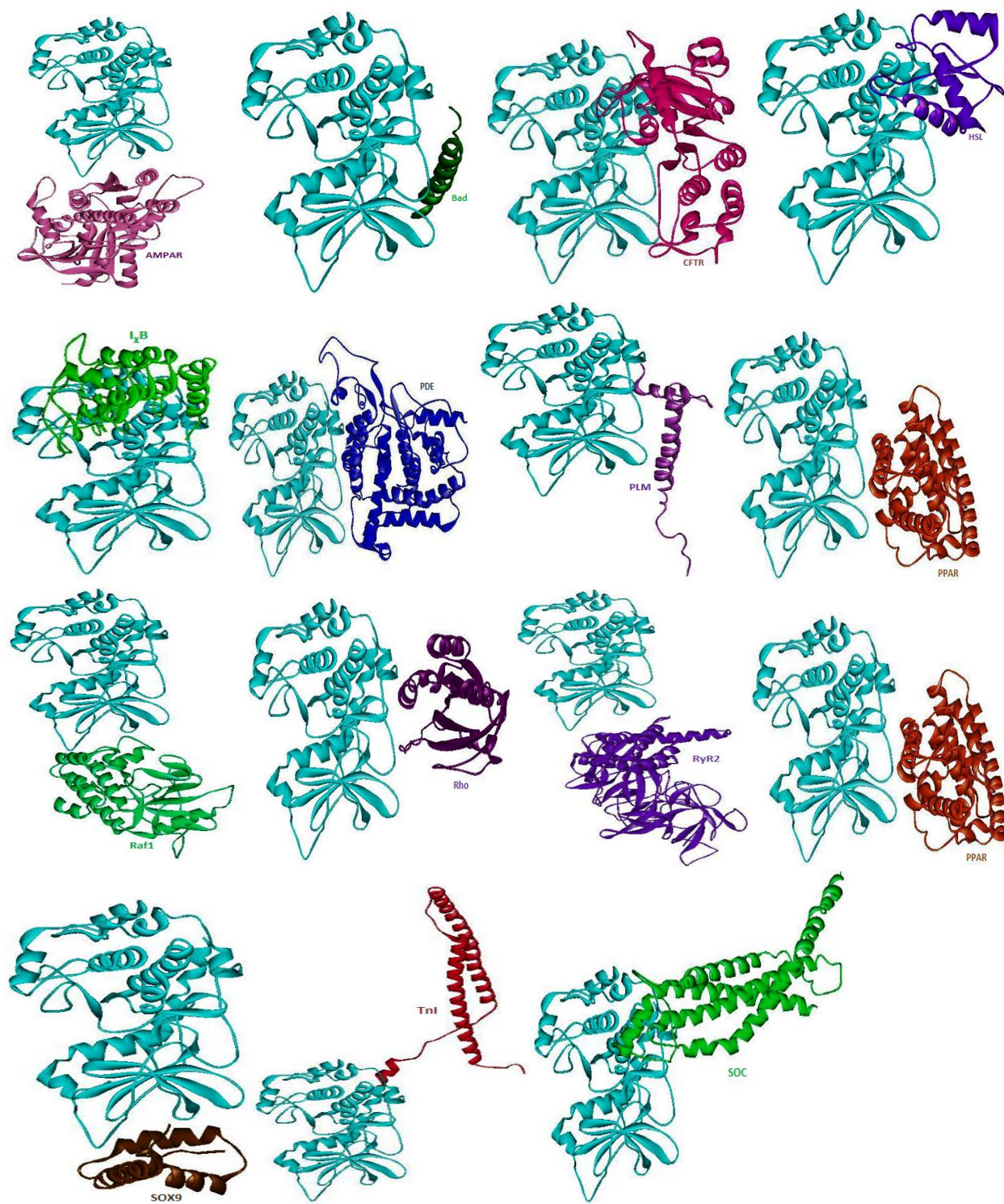
Figure 2. Structures of protein complexes of PKA obtained from Hex8.00 software. In all complexes, proteins colored cyan is PKA. Interface residues are used for constructing a structural network for PKA as hub protein.

which determines interfaces and hot spots in protein complexes. The interface residues for 16 complexes of PKA were obtained from KFC-2 server to find out how PKA interacts with its partners in cAMP signaling pathway. Results were used to construct structural network for these PKA interactions as an important hub in cAMP pathway.

Results and Discussion

In this study, we aimed to construct a structural network for a part of cAMP signaling pathway which had PKA as hub protein. Interactions of PKA with some of its partners were studied. The partners which we used their RCSB PDB structures included SOX9, PPAR, Rho, RyR2, CFTR, AMPAR, and PLM with PDB accession numbers of 4EUW, 2ZNN, 1KMQ, 4JKQ, 1XMJ, 2WJW, and 2JO1; respectively. For some of the other partners we used structures from Swiss-Model portal including PDE, Raf1, I$_\chi$B, BAD, HSL, TnI, NMDAR, and SOC. The Hex software was used for docking of PKA with these proteins. Hex is an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of protein and DNA molecules (16). The resulted complexes from Hex are displayed in Figure 2. We selected the structures with energies less than - 500 kj/mol. To construct a structural network, we had

to know the interface amino acids of the complexes. There are few servers available for calculating the interfaces based on different methods.
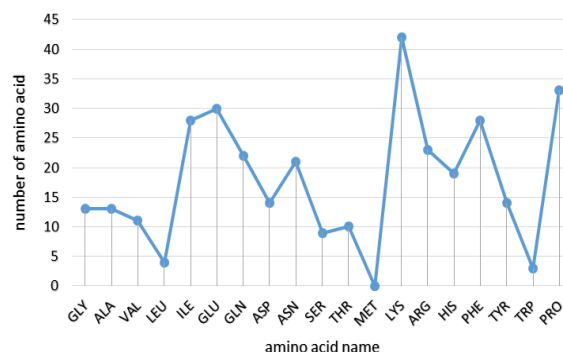
Figure 3. Frequency of amino acids of PKA binding sites in interaction with 15 partners according to KFC2 server.

We used KFC2 which gives us the amino acids of the interface and also hot spots. It is a web-based tool for predicting protein binding hot spots based on machine learning approaches. Figure 3 shows the statistics of amino acid frequencies of PKA interfaces in interaction with these 15 proteins. LYS, PRO, GLU, ILE, and PHE had the most and MET, TRP, and LEU had the least number of amino acids in PKA interfaces.
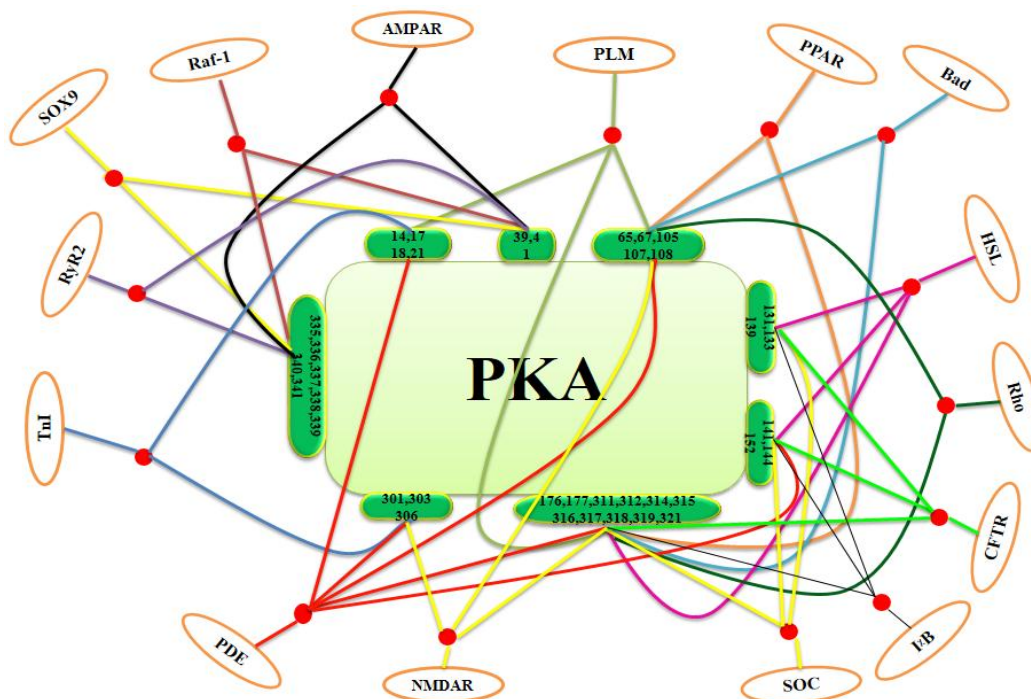
Figure 4. Structural network of PKA in cAMP signaling pathway. There are limited number of binding sites for PKA in interaction with its partners. Some proteins interact through the same or overlapping binding sites which means they are competitive. Common binding sites are colored green on PKA surface. Colored lines show interactions of partners with PKA.

Using this interface amino acid content, we draw a structural network (Figure 4) which shows the binding sites from which PKA interacts with its partners. Only the most common parts are shown on the surface of PKA. This network gives us information about how PKA interacts with other proteins in cAMP signaling pathway. As can be seen, PKA does not have many different binding sites but it has some limited parts in interaction with these 15 proteins. We can conclude that these binding sites are conserved sequences on the PKA surface. Some proteins have almost same binding sites with PKA and some have overlapping interfaces. This means that these proteins cannot interact simultaneously and they are competitive. We should also consider clashes for the proteins which have different binding sites. The 3D structures of some of these proteins may have steric hindrance so they can't be able to interact simultaneously. According to this network, we classified the partners in 4 groups based on their trait in interaction with PKA (Table 1). Proteins in each group interact with PKA through almost the same amino acids. Structural networks are also useful in drug design.

Table 1. Classification of PKA partners according to their trait in interaction with PKA. Common amino acids are shown.

	PKA interface residue number
AMPAR, Raf1, SOX9, RyR2	39, 41, 335, 336, 337, 338, 339, 340, 341
PPAR, Bad, Rho	65, 67, 105, 107, 108, 176, 177, 311, 312, 314, 315, 316, 317, 318
HSL, CFTR, SOC, I$_\chi$B	131, 133, 134, 135, 136, 137, 138, 139, 141, 144, 145, 176, 177, 311, 312, 314, 315, 316, 317, 318
TnI, PDE, PLM, NMDAR	14, 17, 18, 21,65, 67,105, 107, 108, 301, 303, 306, 176, 177, 311, 312, 314, 315, 316, 317, 318

When we know which proteins interact from which binding sites especially in signaling networks, we can design a drug with same interface properties to interact with our target and block an especial reaction in the pathway. One of the main limitations in drawing such structural networks is the lack of proper 3D structures for the study of protein interactions. For PKA partners in cAMP signaling pathway, no proper structures were found for the proteins, DARPP32, CREB, NHE, PMCA, and VDCC. For 2 proteins, GLI3, and NF-AT, the resulted Hex energies were more than -500 kj/mol, so we did not consider these proteins in the structural network. It is also desirable to expand this network to the PKA partners and show the other proteins interactions in this signaling network. Surely in the future, with the development of 3D structure databases, and also powerful computational methods, more protein 3D structures will be identified, therefore, it would be possible to construct a complete structural network.

Conflict of Interest
The authors declare that they have no conflict of interest in this work.

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