0273-2289/1559-0291 (Online)/07/136/0001/$30.00

Computational Prediction of the Coupling Specificity of G Protein-Coupled Receptors

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Received January 23, 2006; Revised April 17, 2006;
Accepted May 16, 2006.

Abstract

G protein-coupled receptors (GPCRs) represent one of the most important categories of membrane proteins that play important roles in signaling pathways. GPCRs transduce the extracellular stimuli into intracellular second messengers via their coupling to specific class of heterotrimeric GTP-binding proteins (G proteins) and the subsequent regulation of a diverse variety of effectors. Understanding the coupling specificity of GPCRs is critical for further comprehending their function, and is of tremendous clinical significance because GPCRs are the most successful drug targets. This minireview addresses the computational approaches that have been created for the prediction of coupling specificity of GPCRs and highlights the perspective of bioinformatics strategies that may be used to tackle this important task. In addition, some of the important resources of this field are also provided.

Index Entries: G protein-coupled receptor; coupling specificity; orphan receptor; bioinformatics.

Introduction

G protein-coupled receptors (GPCRs) constitute one of the largest superfamilies of membrane proteins. The high level of attention to GPCR discovery and characterization derives largely from their amenability to drug intervention *(1,2)*. It is estimated that over 50% of the current drugs on the market are targeting GPCR proteins. Recent work revealed that the human genome may encode between 800 and 1000 GPCRs *(3)*. As the data

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continues to grow with the completion of more genome sequencing projects, so does the need for fast and reliable elucidation of the functions of GPCRs, which will not only provide novel insights into cell biology but also offer opportunities to therapeutic intervention *(4)*. The specificity of the interactions between GPCRs and heterotrimeric guanine nucleotide-binding proteins (G proteins) determines the scope and characteristics of the ultimate cellular response *(5)*. The coupling information between GPCRs and G proteins has a tremendous impact on choosing the right heterologous cell system for receptor expression and understanding the physiological responses triggered by signal transduction *(6)*. During recent years, many computational approaches have been developed to predict the coupling specificity of GPCRs, which are playing increasingly important roles in designing effective experimental systems for GPCR coupling research *(7)*.

In this minireview, we discuss the application of computational techniques for the prediction of GPCR coupling specificity and highlight the perspective of bioinformatics strategy that may be used to tackle this important task.

Computational Approaches for Predicting the Coupling Specificity of GPCRs

The Coupling Specificity of GPCRs

GPCRs are characterized by an extracellular N-terminus, intracellular C-terminus, and seven-transmembrane domains. This class of membrane proteins can respond to a wide range of agonists, including photon, amines, hormones, neurotransmitters, and proteins. When activated by an agonist, the receptor molecule undergoes a conformational change, which facilitates its interaction with and the activation of specific intracellular signaling proteins, such as heterotrimeric G proteins *(8–10)*. Previous experimental evidence has revealed that the intracellular loops, the C-terminal, and the cytoplasmic ends of the transmembrane helices of GPCRs contribute to their recognition of specific G proteins *(5)*.

G proteins represent a well characterized class of signal transduction proteins in mammalian cells *(11)*. There exist two types of G proteins: heterotrimeric G proteins and monomeric G proteins (or small G proteins). The term of G protein generally refers to the heterotrimeric G proteins that GPCRs couple to. G proteins can be further divided into four classes including $G_{i/o}$ proteins whose members are typically PTX-sensitive and inhibit adenylyl cyclase, $G_{q/11}$ proteins that stimulate phospholipase C, G_s proteins that stimulate adenylyl cyclase and phosphodiesterases, and $G_{12/13}$ proteins whose functions are incompletely characterized to date *(12,13)*. Different agonists may stabilize the interaction of GPCRs with G proteins belonging to different subfamilies, resulting in the activation of different signaling pathways *(14–16)*. There is increasing evidence that several domains of Gα subunits are involved in the control of the coupling specificity of GPCRs,

including the extreme N- and C-terminus, the α4/β6-loops, and the loop linking the N-terminus α-helix to the β1-strand of the ras-like domain *(17)*. Experimental evidence has revealed that the intrinsic structural features in the extreme C-terminus of the Gα subunit and the extreme N-terminus of Gα appear to be critical for its interaction with the receptor, especially for the coupling specificity of GPCRs *(18–23)*. Particularly, the C-terminus domain has been thought to play an important role in constraining the basal activity or coupling selectivity by regulating the accessibility of G proteins to the GPCR surface *(5,15)*. Moreover, there are indications that the Gβγ subunits may also take part in binding to the receptors, although the structural elements involved in this interaction are less well characterized until now *(24)*.

The coupling specificity of GPCRs is crucial for understanding the physiological functions of receptors and developing cell-based bioassays for drug screening. The coupling specificity between GPCRs and G proteins is primarily determined by biochemical experiments until now *(25,26)*, which is still a major challenge as it is difficult to construct such experiment systems.

Resources for the Research of the Coupling Specificity of GPCRs

During the past 10 yr, many databases and analysis tools related to GPCRs and G proteins have been developed *(27–32)*. Some of the public databases are listed in Table 1. The gpDB database is one of the most comprehensive ones, which provides a starting point for predicting the coupling specificity of GPCRs to G proteins. The gpDB contains species homologs, G proteins, the detailed information for G protein monomers, and GPCR sequences with known coupling to G proteins *(32)*. Besides the category of both G proteins and GPCRs, one of the major innovations of this database is the relational model describing the known coupling specificity of GPCRs to the respective α-subunit of G proteins.

Predicting the Coupling Specificity of GPCRs

A considerable amount of computational approaches have been developed to tackle the problem of predicting the coupling specificity of GPCRs during the last decade. According to the feature information utilized, these approaches can be divided into three broad categories: (1) the approaches based on n-grams of amino acids, (2) the approaches based on sequence alignment information, and (3) the approaches based on physiochemical properties of amino acids *(33)*.

- The approaches based on n-grams of amino acids. The approaches based on n-grams have been widely used for sequence analysis. N-grams are subsequences of length n from given sequences, which have the ability to capture the motif information *(33,34)*. Möller et al. *(35)* proposed a novel method to explore the n-gram information based on the hypothesis that the patterns of amino acid residues in

Table 1
Representative Online Tools and Databases Related to G Protein-Coupled Receptors (GPCRs) and G Proteins

Server and database	WWW URL	Descriptions
GPCR-GRAPA-LIB	http://www.affymetrix.com/community/publications/affymetrix/index.affx	The library of HMMs for GPCRs
GPCR patterns tool	http://www.biochem.ucl.ac.uk/bsm/dbbrowser/GPCR	GPCR pattern recognition server
GPCR classification	http://www.soe.ucsc.edu/research/compbio/gpcr–subclass	GPCR classification server
GPCRpred	http://www.imtech.res.in/raghava/raghava/gpcrpred	GPCR prediction server
GPCRsclass	http://www.imtech.res.in/raghava/gpcrsclass	GPCR amine type classification server
GRIFFIN	http://griffin.cbrc.jp	GPCR/G protein interaction feature finding server
GPCRDB	http://www.gpcr.org/7tm	Information system for GPCRs
gpDB	http://bioinformatics2.biol.uoa.gr/gpDB	Database of GPCR/G coupling information
JHP	http://bbsrc–bioinf.leeds.ac.k/BIOINF/jhp	GPCR signatures and refinement generating sever
PRED-COUPLE2	http://bioinformatics.biol.uoa.gr/PRED–COUPLE2	Prediction server of the coupling specificity
PRED-GPCR	http://bioinformatics.biol.uoa.gr/PRED–GPCR	GPCR recognition and classification server
SWISS-PROT	http://www.expasy.ch/cgi–bin/lists?7tmrlist.txt	Information list of GPCR proteins

receptor sequences may contain enough information of the coupling specificity for a class of G proteins. Using a pattern recognition algorithm and combining with a membrane topology prediction approach, they identified class-specific sequence patterns in the intracellular loops of GPCRs that perhaps determine the coupling specificity of GPCRs, and this strategy achieved a high accuracy on a small test set *(35)*. In addition, Cheng *(33)* proposed a method that took advantage of the location information of n-grams selected in GPCR sequences for coupling specificity prediction. In order to identify the top discriminative n-grams for each class, they first ranked the n-grams for each class by computing the chi-square statistic. Then, they selected the top discriminative n-grams and located them in the sequences for each class. The results show that n-grams have the ability to capture the motif information related to coupling specificity *(33)*.

- The approaches based on sequence alignment information. During recent years, some researchers attempted to tack the problem of predicting coupling specificity on the basis of the sequence alignment information. Cao et al. proposed a naïve Bayesian method to predict the coupling specificity of GPCRs based on the sequence alignment information derived from their intracellular domains, which achieved an encouraging accuracy for the $G_{i/o}$ class and $G_{q/11}$ class *(6)*. In their study, each intracellular domain of GPCRs was first treated as a discrete random variable, conditionally independent with each other. Then, the phylogenetic trees constructed by the multiple sequence alignment tool ClustalW *(36)* were used to cluster the intracellular domain sequences with the aim to determine the conditional probability distributions of these variables. HMM is another effective statistical approach that is very well suited to many tasks of biological sequence analysis *(37)*. Sgourakis et al. (2005a) achieved a significant success of predicting the coupling selectivity of GPCRs to the G_s, $G_{i/o}$, and $G_{q/11}$ subfamilies based on the idea of generating a refined library of HMM statistical models *(38)*. Furthermore, they further improved the prediction performance by combining the feed-forward artificial neural network (ANN) method *(39)*.
- The approaches based on physiochemical properties of amino acids. It has become increasingly clear that the physiochemical features of amino acids can be used to predict the coupling specificity of GPCRs efficiently *(7,40–42)*. Henriksson *(41)* constructed classifiers to predict the coupling specificity of GPCRs by utilizing the physiochemical properties reflecting the size, lipophilicity, and rigidity of the amino acids of intracellular domains. Yabuki et al. *(7)* revealed that the ligand, extracellular loops, intracellular loops, and the transmembrane domains of GPCRs were essential for describing the coupling specificity of GPCRs based on the assumption that the ligand,

GPCR, and G protein form a complex. They constructed the SVM- and HMM-based classifiers to predict the coupling specificity by integrating the ligand information (e.g., molecular weight of the ligand) and the GPCR sequence information (e.g., averaged hydrophobicity of transmembrane helices). Their results show that this strategy could obtain better prediction performance *(7)*. In addition, good results have also been achieved by analyzing and extracting the features of amino acid compositions and physiochemical properties (e.g., hydrophobicity, polarity of amino acids) from the full-length GPCR sequences *(42)*.

Discussion

This minireview summarized some of the bioinformatics techniques and web resources related to the prediction of the coupling specificity of GPCRs. Despite the promising results of these techniques, there still exist several potential problems in their application to the research of the coupling specificity of GPCRs.

One problem of current methods for predicting the coupling specificity of GPCRs is that most of the currently available methods, except the method proposed by Sgourakis et al., are limited to three classes of G proteins, not including $G_{12/13}$ proteins that are very important mediators of GPCRs actions since they are known to couple with many diverse receptors *(33,39)*. Additionally, these methods have good effects only for predicting the coupling of GPCRs to single class of G proteins, however increasing evidence suggests that some GPCRs are able to couple to G proteins belonging to different classes, which motivates the need for a uniform framework that can predict the multiple coupling of GPCRs to G proteins *(6)*. Another problem is the technical challenges of analyzing and modeling signal transduction pathways in systems, which is crucial for the coupling specificity research. G proteins are involved in a wide range of physiological signaling processes, however, the mechanism of G protein-mediated cellular signaling pathways is poorly understood. Furthermore, the power and utility of protein microarray technology, which may provide experimental evidence for understanding the interaction between GPCRs and G proteins has not been extended to GPCRs.

In addition, how do GPCRs activate G proteins and cause specific responses in cells? What are the changes in GPCRs triggered by agonist binding? All of these unanswered questions heavily depend on detailed structural information *(43)*. However, despite their importance as drug targets, very little about the actual three-dimensional structures of GPCRs are currently known *(44)*, due to their overall lipophilic nature and membrane-bound localization that make their crystallization notoriously difficult. This difficulty motivates the importance of developing computational methods that can predict their structures with high reliability *(45)*.

The Prospect of Future Work

The continued discovery of genes encoding GPCRs has provided an extensive reserve of potential therapeutic targets *(46)*. Unfortunately, a number of receptors still remain poorly characterized and orphaned with unknown ligand specificity *(47,48)*. It is important to develop new approaches to understand the coupling specificity of GPCRs, which can gain an insight into the physiological functions of oGPCRs *(49)*.

Current approaches can only be realized to their fullest potential with the wealth of experimental data and powerful bioinformatics tools. Therefore, there are several potential avenues that could be explored to improve the performance of predicting the coupling specificity of GPCRs. First, a considerable amount of literatures concerning the topic of the coupling specificity of GPCRs have been accumulated, and many biological databases offer access to various formalized facts including different information related to GPCRs, which are expected to aid in elucidating the mechanisms that these receptors exert their numerous physiological roles. Mining and integrating these diverse data resources to effectively predict the coupling specificity of GPCRs are an important direction for future research. Second, several lines of evidence suggests that GPCRs function as dimers or higher order oligomers *(10,50)*. It has been reported that GPCR dimerization plays important roles in the processes of ligand binding, receptor activation, desensitization and trafficking, as well as receptor signaling *(51)*. Furthermore, the heterodimerization of a variety of GPCRs may alter the G protein specificity. Therefore, we believe that the increasing knowledge of GPCR dimerization can lead to novel strategies to accelerate the coupling specificity prediction. Third, most of previous studies have focused on the intracellular domains of the receptor sequences; however, the extracellular domains, transmembrane segments, ligands, and G proteins might also hold the coupling specificity information. Thus, integrating the information effectively may improve the prediction accuracy and provide useful clues to analyze the signal transduction pathways.

It should be noted that although computational approaches can be used to predict the coupling specificity of GPCRs, the validation of the predictions is still a process that requires understanding the role of GPCRs and G proteins in the cellular signaling pathways. However, we expect that novel powerful computational techniques will allow better understanding of the biological function of GPCRs that can facilitate elucidating the coupling specificity of GPCRs.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant Nos. 30370354 and 90203011), the Ministry of Education of China (Grant No. 505010), and the Specialized Research Fund for the Doctoral Program of Higher Education (Grant No. 20050487037).

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