Application of oligomer interface prediction method in Class A G-protein coupled receptors

Wataru Nemoto[1, 2]
wane@kuicr.kyoto-u.ac.jp

Hiroyuki Toh[1]
toh@kuicr.kyoto-u.ac.jp

[1] Bioinformatics Center, Institute for Chemical Research, Kyoto University, Gokashou, Uji, Kyoto 611-0011, Japan.
[2] Graduate School of Information Science, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192, Japan

Keywords: GPCRs, dimerization, oligomerization, interface, conserved residues, binomial distribution

1 Introduction

About 1000 genes encoding GPCRs (G-protein Coupled Receptors) exist in human genome. Over 30% of clinically marketed drugs are active at this family and GPCRs are one of the most important target classes of proteins for drug. Over the past 25 years, the formation of GPCR homo and hetero oligomer has been suggested by biochemical and pharmacological evidence. More recently, it was revealed that bovine rhodopsins form a homo oligomer in native membrane[2], by atomic force microscopy. Some subtypes form oligomers, while others do not. The oligomerization patterns differ with the subtypes of GPCRs. Signal transduction by GPCRs are considered to be associated with the oligomerization. To clarify the mechanism of signal transduction, it is important to elucidate the oligomeric patterns of GPCRs. Accordingly, prediction of interface for the oligomerization would be the first step to understand the mechanism of the oligomerization. When we examined the spatial distribution of conserved residues in the structure of bovine rhodopsin, significantly large number of conserved residues are observed at the oligomeric interface. Based on the observation, we tried to develop a method to predict the interface for the GPCR oligomers.

2 Method and Results

2.1 Projecting three-dimensional coordinates on two-dimensional plane

The principal component analysis was carried out with PDB coordinate data of bovine rhodopsin (1L9H). Because of its characteristic structure, the 1st principal component axis was almost perpendicular to the membrane. The geometric centers of the side chains for the exposed residues (ASA $\geqq$ 25%) in the transmembrane region were projected on a plane that was defined by the 2nd and the 3rd principal component axes. The two-dimensional coordinates of the projected residues were used in following study.

2.2 Assignment of conservation score

A multiple alignment of the opsin subfamily was constructed. Henikoff-Henikoff weighting[1] was used to reduce the taxonomic bias. Conservation score defined by Valdar et al.[3] was calculated at each aligned site. The score was assigned to the corresponding residue of bovine rhodopsin.

2.3 Searching candidates on GPCR oligomer interfaces

On the plane, projected residues were forming a ring. Consider a sector of θ degrees centering on the center of the ring. The number of conserved residues follows a binomial distribution. The probability that highly conserved residues found in the range is more than or equal to m is calculated as follows

$$p_{\theta}(i \geq m) = \sum_{i=m}^{n} \frac{n!}{i!(n-i)!} \left(\frac{X_{\theta}}{N}\right)^{i} \left(\frac{N-X_{\theta}}{N}\right)^{n-i}$$

Here n and N are the number of highly conserved residues and the total number of residues on GPCR surface. X_{θ} is the number of residues in the range of θ degrees.

3 Results

Shifting the range, the probability p_{θ} was calculated. If p_{θ} <0.01, the range was selected.

Table 1. Predicted interface residues about bovine rhodopsin oligomer

Region	Sequence Data	Cons(i)	p_{θ}	Selected Residues
I	Opsin 113 sequences	≧0.7	0.0077	152,155,162,175,201
II	Opsin 113 sequences	≧0.8	0.0077	152,155,162,175,201
III	Opsin 139 sequences	≧0.7	0.0077	152,155,162,175,201
IV	Opsin 139 sequences	≧0.8	0.0077	152,155,162,175,201
V	Opsin 163 sequences	≧0.7	0.0077	152,155,162,175,201
VI	Opsin 163 sequences	≧0.8	0.0077	152,155,162,175,201
VII	Opsin 163 sequences	≧0.5	0.0093	133,136,152,175,201,205,213,214,217,220,221 223,225,228,252,259,263,266,267,273,274,277
VIII	Opsin 163 sequences	≧0.5	0.0097	133,136,152,175,201,205,213,214,217,220,221 223,225,228,252,266,273,274,277

4 Discussions

Residues on helices IV and V play the most important interaction between monomers of the rhodopsin dimer[2]. Among them, it is currently believed that 175W is a critical role in oligomerization of bovine rhodopsin. Conserved residues in the regions I, II, III, IV, V and VI (152H, 155M, 162V, 175W and 201E) belong to helices IV and V and they are included in rhodopsin oligomeric interfaces (Figure 1). On the contrary, conserved residues in the regions VII and VIII are included in the interface between a pair of monomer as well as between monomers. Some atoms on the interdimeric surfaces of one molecule are located within 6Å from the opposite side. The observation suggests that the conserved residues are clustered at the interface between bovine rhodopsins and that our method can efficiently detect the clustering. Thus, the detection of the spatial cluster of the conserved residues would be useful to predict the interface. Since Class A type GPCRs have high sequence similarity with rhodopsin, precise model structures could be made. As described above, however, the oligomerization patterns are different among the subtypes, despite the high sequence similarity. By applying our method to the model structures, the interfaces of some Class A GPCRs could be predicted.

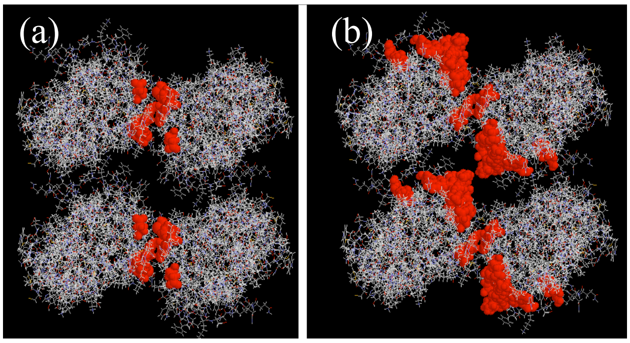

Figure 1. Mapping on oligomeric bovine rhodopsin structure model. Figures (a) and (b), respectively, corresponds to the mapping of the conserved residues on the region I and VII (Table 1). Both are the views from the extracellular side.

References

[1] Henikoff, S., and Henikoff, J. G., Position-based Sequence Weights, *J. Mol. Biol*, 243:574-578, 1994.
[2] Liang, Y., Fotiadis, D., Filipek, S., Saperstein, D. A., Palczewski, K., and Engel, A. Organization of the G Protein Coupled Receptors Rhodopsin and Opsin in Native Membranes, *J Biol Chem*, 278(24):21655-21662, 2003.
[3] Valdar, W. S., and Thornton, J. M., Protein-Protein Interfaces: Analysis of Amino Acid Conservation in Homodimers, *PROTEINS*, 42:108-124, 2001.