Review Article

Challenge in protein-small molecule Interactions

Fumiaki Uchiyama

Department of Molecular Biosciences, Institute of Preventive and Medicinal Dietetics,

Nakamura-Gakuen University, 5-7-1 Befu, Jounan-Ku, Fukuoka, 814-0198, Japan

Corresponding author. E-mail: uchiya-f@nakamura-u.ac.jp
Phone: +81-(0)92-851-2598 fax: +81-(0)92-841-7762

Key words: conformational change, torsion strain, protein-protein interaction, protein binding surface areas, tumor necrosis factor, TNFR

(Received April 1, 2010)

Abstract

The potential for driving force in protein-ligand binding are discussed in this review. The conformational change in protein-ligand interaction is dependent on characteristics of proteins. Proteins which do not change conformation before ligand binding compose of constraint binding site residues. The conformation of ligand-binding sites of these proteins adopts disallowed Ramachandran angels. Conformational changes in protein-ligand binding result to adopt energetically unfavorable or disallowed Ramachandran angels. These findings show that an element of driving forces in protein-ligand binding may be the generation of torsion-angle strain.

Introduction

Foods in diet contain numerous chemical small compounds, which have some bioactivities in human. Enormous tests of food chemicals in vitro are not always linked to detect physiological activity in human body and also can not rationally explain the efficacy of traditional Chinese medical herbs except some compounds with strong activity. The efficacy of chemical compounds extracted from traditional Chinese medical herbs may be explained by either assembly in combination of compounds or little reaction of single compounds in the present biological evaluation. The interaction between proteins and small compounds has been studied in medicinal chemistry but poorly in food chemistry because of their different business values in success. Nevertheless, the use of herbs and supplements is prevalently popular in Japan. Yakuzen-Dietetics, which defines as diets contributed for prevention of diseases, delivery of nutritional elements and assisting medical treatments, needs to clarify the physiological efficacy of public herbs in physiological conditions by novel approaches such as omics-technologies and studies for protein-small moleule interaction. Protein-small molecule interaction is an important and confronted problem because all physiological actions are based on protein-ligand interaction.

Protein-ligand interaction has been advanced to determine 3D structures by X-ray crystallography, nuclear magnetic resonance spectroscopy, fluorescence, Raman spectroscopy and their combination. Although static structures are known for many proteins, the dynamic change of proteins in reactions is unknown. Proteins are dynamic molecules and often undergo conformational change upon ligand binding. Flexible loop regions in proteins are believed to have a critical functional role in protein-ligand binding. Rigid regions in proteins also are related to hot spots in protein-protein interaction. Therefore, there may be some patterns for protein dynamism in protein-protein interaction. In this review, I discuss studies related to conformation and

torsion strains in protein-ligand interaction and present the interaction between small compounds and TNF-TNFR system as a challenging example in protein-ligand binding.

Conformation and torsion strains in protein-ligand interaction

The process of ligand-receptor binding often contains conformational changes in structures. Conformational change includes both backbone and side chain movements[1]. The conformation in backbone structures changes domain motion such as hinge motion and loop structure around rigid structures. Side chain conformational changes are often in flexible loop structure on rigid structures. Conformational changes may perform in the range of own protein flexibility, which accompanies free energy changes. To consider driving force of conformational changes, proteins with metal binding sites is exampled. Metal binding in proteins contributes structural stability and acts as cofactors to function catalysis and protein binding. In the comparisons of 3D structures between unbound state and metal bound state in the Protein Data Bank (PDB), more than 40% of the binding sites revealed rearrangements of conformation including the backbones[2]. Metal ion interacts with ionic residues such as aspartate, glutamate, cystein and histidine to make conformation change. Since metal ion has concrete structure, the driving force of the conformational change is electrostatic energy of metal-ionic residue interaction. The electrostatic interaction provides energy to exceed transition state for the conformational change. In other driving force of conformational changes, hydrogen bonds, hydrophobic interactions and van der Waals packing relate to conformational change as a result of comparison of structures between bound state and unbound state. Conformational changes often accompany to change protein strain energies because ligands binding to proteins induce deformation. The intra-molecular electrostatic interaction in histidine-containig phosphocarrier protein (HPr) induces a conformational change to form the active center in the sequence of His-Ala-Arg[3]. The conformation of HPr active center adopts energetically unfavorable or disallowed Ramachandran angels. Conformational changes in protein-ligand interaction are dependent on characteristics of proteins. Proteins which do not change conformation before ligand binding compose of constraint binding site residues. Interestingly, the conformation of ligand-binding sites of these proteins also adopts disallowed Ramachandran angels[4]. The state having disallowed Ramachandran angels in ligand binding sites locally generates torsion-angle strain clusters. These findings show that one of the binding potential in proteins may prerequisite the generation of torsion-angle strain clusters rather than conformational changes. The release of torsion-angle strain may produce enough energy to form transition state of complex. In protein dynamics, energy landscape generally represented as the hierarchy of protein dynamics and energy barrire r[5,6]. For ligand-protein binding, the energy landscape is the free energy determined such as bond vibration, side chain rotation, loop motion, domain motion and solvation. Entropy changes may compensate by changing conformations of ligands and/or receptors. In fact, comparisons of 3D structures between bound and unbound structures in PDB show ligand-induced protein conformational changes in backbone structures as well as binding sites[7]. Nevertheless, the relationship between protein conformations and driving forces in ligand-protein binding is unclear. The torsion-angle strain is one of the candidates for critical driving force in protein-ligand binding. In designing torsion-angle strain clusters, the presence of glycine residues in functional loops may be important because glycine residues are the highest occurrence in loops to comply with the geometrical constraints. Since the glycine residue provides large allowed conformational space or is not restricted to the regions of Ramachandran plot appropriate to the other amino acids with side chains, the presence of glycine residue may be rational for adequate energy landscape. In fact, the relative frequency of glycine in loops is shown to correlate to the number of state having disallowed Ramachandran angels by analyzing a bank of loops from three to eight residues long from PDB[8]. These findings indicate that target regions in proteins for discovery of ligands may be in turns with glycine residues with disallowed Ramachandran angels.

Functional conformation and interfaces in protein-ligand binding

In the search of protein interfaces, the selection of loop motifs is important for designing peptides because binding site residues adopting disallowed conformations are classified as gamma-turn and beta-turn conformations. Loop structures are often present in the interface of protein-ligand binding and can change their conformations. Although loops were originally described as random coil, the increased loops in 3D structures could be structurally classified by loop geometry, three angles and one distance between connected secondary structures and Ramachandran pattern. The loop between connected secondary structures occupies 80% of short loops with less than 10 amino acids. The loops are functionally separated into two groups. The one is just to contribute globular formation of proteins to stabilize and prefers to the shortest loops broken alpha-helices or beta-sheets. The other is functional loops which generate interfaces for protein-ligand binding and binding pockets. Functional loops often make conformation change in protein-ligand binding. The areas of contact surface is broad from 100 Å to 3000 Å in protein-ligand binding and is dependent on the molecular size of ligands. The small ligands such as enzymes, G-protein-coupled receptors and allosteric pockets have been designed to be generated in medicinal chemistry. The large contact surfaces in protein-ligand binding have never been designed because of little example to know how to begin study on this case. Consequently, there is an interesting problem for understanding energy landscape in protein-protein interaction as well as an enormous therapeutic potential in challenging to design large contact surface in protein interaction. One of the approaches is used for antibodies which have been developed for humanization from mouse monoclonal antibodies, preparing from human peripheral lymphocytes, protein engineering for pharmaceutics and cell culture for antibody production, but the concept of antibody still remains in large contact surface interaction to oblige higher cost and time consume. Challenging approaches are now underway to understand the element of large contact surface interaction and to create small molecules bound or disrupted protein-protein binding to large contact surface. The practice is either high throughput screening (HTS) for chemical libraries or mimetics of interfaces of protein by fragmentation. These approaches seem to be technically independent each other, but have a common basic element. HTS must make libraries invest a lot of concept-based compounds with potentials to raise conformational change or binding energy. Equally, fragment mimics must not extract the only sequences of interfaces in complex but the potentials of conformational change or binding energy. In the behind, both approaches are introduced with tumor necrosis factor (TNF) and its receptor.

Energy landscape and binding active sites in proteins

The pathway of protein-ligand binding is two mechanisms[9,10], which are named as conventional induced-fit and population-shift[11] (conformational selection). Both mechanisms perform in the dependence on protein concentration[10] as well as types of proteins. The common element is conformational change before or after ligand binding. The motion of functional conformations accompanies to increase free energy of a protein in the unbound protein. Steric strain energy, which arises from rotation around disallowed torsion angles, is contributed to the increase of free energy in protein binding and is released to form a complex[12]. This finding agrees with the active sites of proteins with or without changing conformation often exist the state having disallowed Ramachandran angels. Therefore, steric strains may be necessary in designing ligands.

Interaction between TNF and TNF receptor families

Tumor necrosis factor (TNF) was identified as products of lymphocytes and macrophages and caused the lysis of certain types of cells, especially tumor cells. At the same time, TNF was cloned to be found identical to cachectin, mediator of cachexia. Then, several studies have demonstrated important roles in the field of inflammatory such as rheumatoid arthritis, Crohn's disease and septic shock. Moreover, TNF was a member of adipocytokines related to diabetes[13], atherosclerosis[14] and bone metabolism[15]. The DNA cloning of TNF receptors led to discovery of TNF receptor-related superfamily proteins (TNFR SFPs)[16],

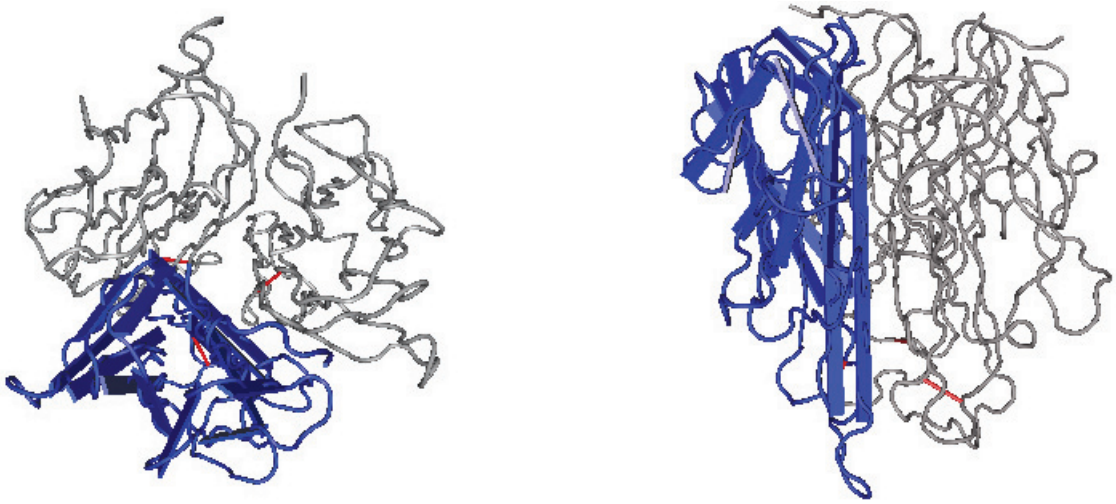

Fig. 1 The structure of mouse tumour-necrosis factor alpha (mTNF). The structures of mTNF and human TNF are very similar.

which related to host defense, inflammation, apoptosis, autoimmunity, and organogenesis. Since TNF is an important role in trigger of inflammatory pathway, the inhibition of TNF has been investigated as pharmacological target. The production or action of TNF was inhibited by TNF soluble receptor[17−20], glucocorticoids, phosphodiesterase inhibitors, anti-inflammatory cytokines (IL-10 and IL-4), cholinergic anti-inflammatory pathway, inhibitors of NF κ B, metalloprotease inhibitors, thalidomide and p38 mitogen-activated protein kinase inhibitors.

TNF is expressed as a membrane-embedded form, in which TNF is cleavaged to form soluble mature and is released as active trimers. The 17,350-dalton monomer forms an elongated, antiparallel beta-pleated sheet sandwich with a jelly-roll" topology. Three monomers associate intimately about a 3-fold axis of symmetry to form a compact bell-shaped trimer (Fig. 1)[21].

The TNF ligand family, TNF-beta (LT alpha) and TNF-alpha (LT beta) interact in a complex fashion of cross-binding to their receptors (TNFR p55, TNFR p75, and TNFR-RP). Two soluble TNF-binding proteins are derived from the extracellular domains of the p55 and p75 TNF receptors. The extracellular domains of the two receptors each contain four similar cysteine-rich repeats of about 40 amino acids, in common with several other cell surface proteins including the p75 nerve-growth-factor receptor and the CD40 and Fas antigens. The complex with TNF ligand and TNFR is considered to form TNF trimers covered with TNFR trimers. The structure of the complex[22] is shown as a monomeric view in Figure 2.

In the complex of TNF beta-TNFR p55, the flexible loop structure of TNF beta interacts with the longer face consisted of the sequence of HCLSCSKSRKEMGQ and the short loop with the sequence of FTASEN in TNFR. The static interface of the two sequences in TNFR provide the design of cyclic peptides, but TNF inhibition of their peptides were little activities[23]. On the other hand, beta-turn structure with the sequence of WSENL in TNFR, which is located near the interface of complex, was successful to peptidomimetics with

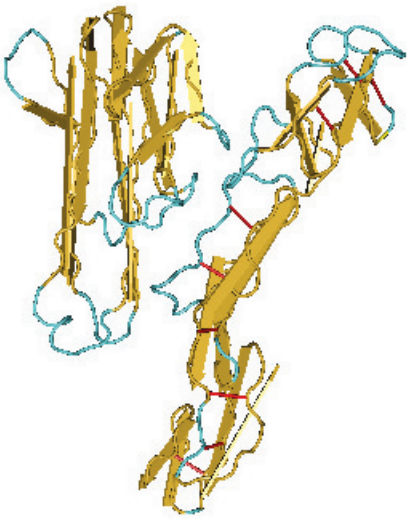

Fig. 2 The structure of the complex of the extracellular domain of the human 55 kd tumor necrosis factor (TNF) receptor with human TNF beta.

moderate TNF inhibitory activity (IC_{50} =5 μM in receptor binding assay)[23]. These findings indicate that the important dynamic region of TNFR to interact with TNF may be also present near the static interface regions. The region of WSENL sequence in TNFR may induce conformational changes in forming the complex of TNF-TNFR[24]. Therefore, the discovery of a key region for diriving force of conformational change may be important for designing ligands. On the other hand, HTS provided to discover non-peptidic small compound to inhibit TNF-alpha TNFR binding with K_D=13 μM[25]. The mechanism of this compound is to disrupt the trimer of TNF-alpha. This research indicates that there is a wedge-driven region to disrupt the trimer maintenance. Both compounds revealed the same order of inhibitory activity. Although two approaches are different technology, the potentials of the compounds was equivalent in the inhibition of TNF bound TNFR. The drug candidates for targets with small contact surface will make their biological activities improve by normal lead optimization, but the candidates with large contact surface provide no guarantee for lead optimization in conventional approach because of no precedents. Even lead optimization as well as discovery of leads for targets with large contact interface is a challenging task. In this way, the most advanced research just launches to elucidate protein-small-molecule binding. We will be encouraged to understand protein-small molecule binding by integrated information such as 3D structure and energy landscape in protein-protein interaction, identification of driving force for conformational changes and discovery of potential compounds.

Perspective in the future

Targets with large contact surface in protein-ligand binding such as growth factors and cytokines has been buried in fantasy. 3D structures in PDB provide the comparisons of protein-ligand complexes and their ligand-free proteins to understand the critical relation between loops and glycine residues with disallowed Ramachandran angels. Moreover, inhibitory compounds against targets with large contact surface have discovered. The protein-ligand interactions related to large contact surface will be an important therapeutics with advance on understanding energy landscape, especially steric strain energy in conformation, in protein-protein interaction.

References

1. Betts, M.J. & Sternberg, M.J. An analysis of conformational changes on protein-protein association: implications for predictive docking. *Protein Eng* **12**, 271-283 (1999).
2. Babor, M., Greenblatt, H.M., Edelman, M. & Sobolev, V. Flexibility of metal binding sites in proteins on a database scale. *Proteins* **59**, 221-230 (2005).
3. Jia, Z., Vandonselaar, M., Quail, J.W. & Delbaere, L.T. Active-centre torsion-angle strain revealed in 1.6 A-resolution structure of histidine-containing phosphocarrier protein. *Nature* **361**, 94-97 (1993).
4. Gunasekaran, K. & Nussinov, R. How different are structurally flexible and rigid binding sites? Sequence and structural features discriminating proteins that do and do not undergo conformational change upon ligand binding. *J Mol Biol* **365**, 257-273 (2007).
5. Henzler-Wildman, K. & Kern, D. Dynamic personalities of proteins. *Nature* **450**, 964-972 (2007).
6. Gutteridge, A. & Thornton, J. Conformational change in substrate binding, catalysis and product release: an open and shut case? *FEBS Lett* **567**, 67-73 (2004).
7. Najmanovich, R., Kuttner, J., Sobolev, V. & Edelman, M. Side-chain flexibility in proteins upon ligand binding. *Proteins* **39**, 261-268 (2000).
8. Kwasigroch, J.M., Chomilier, J. & Mornon, J.P. A global taxonomy of loops in globular proteins. *J Mol Biol* **259**, 855-872 (1996).
9. Okazaki, K. & Takada, S. Dynamic energy landscape view of coupled binding and protein conformational change: induced-fit versus population-shift mechanisms. *Proc Natl Acad Sci U S A* **105**, 11182-11187 (2008).
10. Hammes, G.G., Chang, Y.C. & Oas, T.G. Conformational selection or induced fit: a flux description of reaction mechanism. *Proc Natl Acad Sci U S A* **106**, 13737-13741 (2009).
11. Mourant, J.R. et al. Ligand binding to heme proteins: II. Transitions in the heme pocket of myoglobin. *Biophys J* **65,** 1496-1507 (1993).
12. Shim, J.Y. & Howlett, A.C. Steric trigger as a mechanism for CB1 cannabinoid receptor activation. *J Chem Inf Comput Sci* **44**, 1466-1476 (2004).
13. Flyvbjerg, A. Diabetic angiopathy, the complement system and the tumor necrosis factor superfamily. *Nat Rev Endocrinol* **6**, 94-101.
14. McKellar, G.E., McCarey, D.W., Sattar, N. & McInnes, I.B. Role for TNF in atherosclerosis? Lessons from autoimmune disease. *Nat Rev Cardiol* **6**, 410-417 (2009).
15. Kearns, A.E., Khosla, S. & Kostenuik, P.J. Receptor activator of nuclear factor kappaB ligand and osteoprotegerin regulation of bone remodeling in health and disease. *Endocr Rev* **29**, 155-192 (2008).

16. Locksley, R.M., Killeen, N. & Lenardo, M.J. The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell* **104**, 487-501 (2001).
17. Seckinger, P., Zhang, J.H., Hauptmann, B. & Dayer, J.M. Characterization of a tumor necrosis factor alpha (TNF-alpha) inhibitor: evidence of immunological cross-reactivity with the TNF receptor. *Proc Natl Acad Sci U S A* **87**, 5188-5192 (1990).
18. Lantz, M., Gullberg, U., Nilsson, E. & Olsson, I. Characterization in vitro of a human tumor necrosis factor-binding protein. A soluble form of a tumor necrosis factor receptor. *J Clin Invest* **86**, 1396-1402 (1990).
19. Gatanaga, T. et al. Purification and characterization of an inhibitor (soluble tumor necrosis factor receptor) for tumor necrosis factor and lymphotoxin obtained from the serum ultrafiltrates of human cancer patients. *Proc Natl Acad Sci U S A* **87**, 8781-8784 (1990).
20. Engelmann, H., Novick, D. & Wallach, D. Two tumor necrosis factor-binding proteins purified from human urine. Evidence for immunological cross-reactivity with cell surface tumor necrosis factor receptors. *J Biol Chem* **265**, 1531-1536 (1990).
21. Eck, M.J. & Sprang, S.R. The structure of tumor necrosis factor-alpha at 2.6 A resolution. Implications for receptor binding. *J Biol Chem* **264**, 17595-17605 (1989).
22. Banner, D.W. et al. Crystal structure of the soluble human 55 kd TNF receptor-human TNF beta complex: implications for TNF receptor activation. *Cell* **73**, 431-445 (1993).
23. Takasaki, W., Kajino, Y., Kajino, K., Murali, R. & Greene, M.I. Structure-based design and characterization of exocyclic peptidomimetics that inhibit TNF alpha binding to its receptor. *Nat Biotechnol* **15**, 1266-1270 (1997).
24. Murali, R. et al. Disabling TNF receptor signaling by induced conformational perturbation of tryptophan-107. *Proc Natl Acad Sci U S A* **102**, 10970-10975 (2005).
25. He, M.M. et al. Small-molecule inhibition of TNF-alpha. *Science* **310**, 1022-1025 (2005).