subunit C-terminal tails. The receptor sites that interact with these tails and their role in the activation process are at present unknown. Obtaining structural evidence with the whole protein will be the next step. While the crystal structure of a receptor-G protein complex will be valuable, the existing evidence for dynamic changes in the G protein and receptor during activation implies that other approaches will also be necessary. The role of the prenyl moiety also needs to be identified. Recent derivation of the prenyl group structure bound to proteins indicates the presence of specific sites for prenyl binding and conformational changes induced by prenyl group binding (e.g., [6]). It is clear that this lipid plays an important role in receptor-G protein interaction because the receptor is acutely responsive to the type of prenyl group attached to the G protein γ subunit [7, 8]. Whereas a role for the F64 residue in Gtγ (or its highly conserved homolog in other Gγs) maybe direct interaction with the receptor as proposed by Kisselev and Downs, this residue may also be the anchor that locates the prenyl moiety at the appropriate position for interaction with a receptor site. The precise location and orientation of this lipid in the G protein as well as its availability to the receptor when the γ subunit undergoes a conformational switch need to be defined.

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Structural and Energetic Aspects of Multispecific Immune Recognition by NKG2D

The multispecific immune receptor NKG2D binds different ligands using a different set of energetically dominant interface residues for each ligand.

Recognition in the immune system is critical for survival. Failure to recognize and destroy foreign molecules could allow a fatal infection to develop. Conversely, an inappropriate attack on nonforeign molecules could lead to a serious autoimmune disease. NKG2D is a homodimeric C-type lectin-like molecule that has recently been recognized as a key immunological receptor on natural killer cells and other immune effector cells [1]. It has multiple different ligands that resemble major histocompatibility class I molecules, but do not bind peptides or interact with β_2-microglobulin. NKG2D interacts with these ligands, which are upregulated on the surfaces of pathogen-infected or tumor cells. The interaction triggers killing of the cell expressing the ligand.

The mechanisms whereby this symmetrical homodimeric receptor can bind to multiple asymmetric ligands are of interest, especially as some of the interactions have tight affinities in the low nanomolar range [2]. One group has argued that NKG2D displays some plasticity and spatial reorganization upon binding and that this constitutes an "induced fit" [3]. Such conformational flexibility would allow a single receptor to adopt the different conformations necessary for interactions with structurally different ligands. Alternatively, multiple binding specificities could arise without a substantial conformational change, if different ligands interacted with the receptor at different sites, or at one site but in different fashions. Several NKG2D-ligand complex structures have already shown that NKG2D uses a similar surface and orientation to bind to different ligands.

In this issue of *Structure*, McFarland et al. [4] have now addressed the mechanism of the multispecificity of NKG2D by solving the structure of unliganded human NKG2D, comparing it to the ligand-bound complexes and performing a computational and mutational analysis of several NKG2D-ligand complexes. The structure of unliganded mouse NKG2D was already known [5]. The computational analysis considers shape complementarity and surface packing, polar interactions involving ion pairs and hydrogen bonds, and protein-solvent interactions including a penalty for buried polar groups that are unsolvated [6]. This approach was used to identify the energetically dominant interface residues on NKG2D in the different ligand-bound complexes. The results suggest that different receptor residues dominate each complex. Confirmation of this energetic modeling is provided by experimental analysis of changes in the free energy of binding of proteins with alanine substitutions

at these positions. Free energy changes were calculated from the equilibrium binding constants determined by surface plasmon resonance studies.

The authors' interpretation of the current structure and data already published is that NKG2D undergoes only minor structural changes on ligand binding and these are not consistent with an induced fit. Rather, they argue that despite using a similar part of the surface of NKG2D for the interface, the different ligands make interactions that are substantially different in terms of the residues that dominate the ligand-receptor interaction. Thermodynamic studies have previously shown that the NKG2D-ligand interactions can differ substantially, with different free energy changes and different relative entropic and enthalpic contributions to these changes [2].

To some extent, it is a semantic argument whether a given change with particular kinetics constitutes an induced-fit. What is demonstrated experimentally is that subtle conformational changes are associated with binding to some ligands, and that the key interface residues differ between the different interactions. Proteins are not static and an experimentally determined structural model typically represents the most energetically probable conformation present in the experiment. Other less energetically favorable conformations also exist with lower probabilities of occurrence. The interaction of a protein with a ligand may lower the free energy of one of these alternative conformations, making it the dominant conformation in the ligand-bound state. If the conformational change is large it may become termed an induced fit; if it is small, such as that of a few side chains, then it may become termed a "lock and key" fit.

A further aspect is the time course or kinetics of the conformational change. In this regard, chemists learn much about a reaction from studying the transition state characteristics, as well as the products and reactants. In protein-protein reactions, the practical challenges involved in studying transition states are significant, but progress is being made. Of relevance here are observations of the kinetics of antibody interactions using fluorescence studies [7]. These suggest that conformational diversity in the unbound antibody allows the rapid formation of multiple low-affinity promiscuous interactions. However, only certain ligands could then promote a slower induced-fit change in the antibody conformation. Nevertheless, in all these interactions the same forces are at play, with bond formation between the interacting molecules and entropic changes, including those arising from solvent interactions.

An interesting finding from this and other studies is that only a few residues dominate the energetics of the interactions. This is consistent with detailed studies such as those from the Genentech group [8]. They have experimentally established that in several interactions, key residues form hotspots that make major contributions to the binding-associated energy changes. For example, 8 residues accounted for 85% of the binding energy in one growth hormone-receptor interaction. In a biological world of protein-protein binding interactions, where the range of net free energy changes is from around 7 to 17 kcal/mol, [9] it is easy to see how a few residues can dominate the binding landscape. For example, the formation of just one hydrogen bond could contribute up to 5 kcal/mol to the free energy change of an interaction. When a net free energy change of 10 kcal/mol equates to an affinity in the nanomolar range, this is of great significance.

NKG2D is a highly conserved receptor, but even within individuals, there is a substantial diversity of ligands and now, it seems, of binding interactions. These results demonstrate how a family of ligand molecules, with surfaces that are broadly compatible with a receptor such as NKG2D, could evolve divergently. The selection and retention of key residues making strong interactions with the receptor would allow functionally important divergence elsewhere in the protein to expand the scope of immune surveillance by NKG2D.

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