Structural Immunology

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THE MHC CLASS I HOMOLOG MICA AND THE NATURAL KILLER CELL RECEPTOR NKG2D

The MHC class I homolog MICA is a stress-inducible antigen that is recognized by T cells and natural killer cells through interactions with the stimulatory, C-type lectin-like natural killer cell receptor NKG2D. We have solved the structure of MICA, crystallized on its own, using native data collected at the ALS [1]. The crystal structure revealed a dramatically altered MHC class I fold, both in detail and overall domain organization, and explained the loss of association with β_2-microglobulin. Subsequently, we have solved the structure of a related protein, MICB, and the structure of the complex of NKG2D and MICA, both by selenomethionine-incorporation and MAD phasing at ALS. The crystal structure of the MICA/NKG2D complex reveals an NKG2D homodimer binding to a MICA monomer, an interaction that overlaps the footprint of T cell receptors on MHC class I proteins. Similar, dyad-related surfaces on each NKG2D monomer interact with strikingly different surfaces on either the $\alpha 1$ or $\alpha 2$ domains of MICA. The central section of the $\alpha 2$ domain helix, disordered in the structure of MICA alone, is ordered in the complex and forms part of the interface. A pocket, reminiscent of the peptide binding groove of classical MHC class I proteins, is created by ordering this loop. A manuscript describing the complex structure is in preparation. In addition, we have expressed, and in several cases crystallized, various related forms of MIC proteins and immunologically-related complexes that we plan to analyze crystallographically with data collected at ALS.

HUMAN NEUTROPHIL GELATINASE ASSOCIATED LIPOCALIN (NGAL)

Neutrophil gelatinase associated lipocalin (NGAL), a constituent of neutrophil granules, is a member of the lipocalin family of binding proteins. NGAL can also be highly induced in epithelial cells in both inflammatory and neoplastic colorectal disease. NGAL is proposed to mediate inflammatory responses by sequestering neutrophil chemoattractants, particularly N-formylated tripeptides and possibly leukotriene B_4 and platelet activating factor. Crystal structures of two forms of baculovirus-expressed NGAL display a typical lipocalin fold, albeit with an unusually large and atypically polar binding site, or calyx [2]. The fold of NGAL is most similar to the epididymal retinoic acid-binding protein, another lipocalin, though the overall architecture of the calyces are very different. The structures also reveal either sulfate ions or an adventitiously co-purified fatty acid bound in the binding site. Neither ligand is displaced by added N-formylated tripeptides. The size, shape and character of the NGAL calyx, as well as the low relative affinity for N-formylated tripeptides, suggest that neither the co-purified fatty acid nor any of the proposed ligands are likely to be the preferred ligand of this protein. The native data for these studies were collected at ALS.

Subsequent to these initial studies, we found that NGAL expressed in bacteria tightly binds to a red chromophore. We have tentatively identified this compound, using a variety of approaches including simple x-ray fluorescence experiments at ALS to narrow the elemental composition of the compound. The identity of the compound is such that we are able to propose a biological function for NGAL that explains many of the experimental results. We have since determined the structure of the NGAL/chromophore complex using selenomethionine-incorporation and MAD phasing at ALS. A manuscript describing these results is in preparation.

THE NON-CLASSICAL MHC CLASS I PROTEIN HLA-E

Human non-classical MHC class I proteins include HLA-E, F and G. HLA-E binds the leader peptides of classical class I proteins and interacts with natural killer cell killer inhibitory receptors as a check for normal expression of classical class I proteins. The 2.85Å crystal structure of HLA-E has been reported using protein re-folded *in vitro* [3]. We have expressed wild-type HLA-E and a point mutant with altered peptide specificity, both in complex with a series of four different peptides, in a similar expression system. We have crystallized these proteins, and have determined cryo-preservation conditions, unlike the original report. We have collected data at ALS and are in the process of refining the structures.

REFERENCES

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