

Study of receptor-ligand pair NKR-P1F and Clrg

Mouse NKR-P1F:Clr-g receptor:ligand pair is important component of the receptor “zipper” that occurs at the contact between natural killer cell and its target cell, and represents a recently discovered example of lectin-lectin interactions important for recognition among immune cell subsets.

In order to study structure of these proteins and interactions between them, we have prepared pET-30a(+) bacterial expression vectors coding parts of extracellular domains of the two receptors. After induction of protein production with IPTG, the proteins precipitated into inclusion bodies, from which they could be refolded *in vitro*. Refolded proteins were purified using combination of ion exchange and size exclusion chromatography.

NKR-P1F construct yielded only small amounts of soluble protein using standard refolding protocols. Furthermore we have experienced difficulties with reproducibility of the refolding results. In the case of Clrg the standard protocols for protein refolding were not sufficient. In order for the Clrg to fold properly, the odd cysteine which does not fit into the pattern usual for this family of receptors was substituted for serine and resulting C148S construct was shown to be more useful.

Further, using (benzyltrimethylammonio)propanesulfonate in refolding buffer has brought better yields of soluble protein Clrg. Identity, purity and quality of refolding of both proteins was confirmed using ion cyclotron resonance mass spectrometry. Using modified electrophoresis and digest protocols in combination with liquid chromatography and mass spectrometry we found that disulfide bonding of both proteins fitted into the pattern expected for C-type lectin like molecules.

Protein Clrg was analyzed by analytical ultracentrifugation. Using sedimentation velocity method we obtained sedimentation coefficient and found that the protein was mostly monomeric in solution. The crystalization of Clrg was successful and diffraction data were collected at the synchrotron radiation source Bessy II of the Helmholtz Zentrum Berlin. Phase problem was solved by molecular replacement using structure of human CD 69. The structure of Clrg protein is the first resolved structure in the whole Clr family. (In Czech)

Supported by grants from Ministry of Education of Czech Republic (MSM_21620808 and 1M0505), and from The Grant Agency of Czech Rep. (GACR 305/09/H008 and 303/09/0477).