Molecular analysis and solution structure from small-angle x-ray scattering of the human natural killer inhibitory receptor IRp60 (CD300a).

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Introduction — Natural killer cells (NK) are part of the first line of innate immune defense against tumors or invading pathogens such as bacteria, virus and parasite. The inhibitory receptor protein of 60 kDa IRp60 (CD300a), was identified as a surface molecule, belonging to the immunoglobulin-super family and is expressed by all human NK cells. This molecule has one extracellular domain, a transmembrane region and a cytoplasmic tail that contains a signaling transduction motif. At the present there is no structural information for IRp60. Yet, the availability of several homology IRp60 three-dimensional immunoglobulin-like structures, warrant the possibility to employ structure based homology modeling to build a three-dimensional model for IRp60. A crucial part of homology comparative modeling is the experimental validation of the build model. Several experimental techniques could be used for the validation of a given homology model. The structural information of macromolecules in solution under physiological conditions obtained by small-angle x-ray scattering (SAXS) can be used to validate computer-modeled protein structures. In this study, we expressed IRp60 using a bacterial expression system and constructed a homology model based on its sequence similarity with NKp44 (PDB: 1HKF), another immunoglobulin-like NK cell receptor. Using SAXS, we also obtained an ab initio low-resolution model of IRp60 [1], which displays a good overall agreement with the structure of the homology model.

Results and Discussion.

Structure based homology modeling — To build the IRp60 homology model, we choose as template NKp44. Structure-based sequence alignment of IRp60 and NKp44 immunoglobulin-like domains reveals that they are very similar. In fact, they share an overall amino acid identity of 33% in 110 overlapping residues. SAXS measurements, data analysis and ab initio molecular shape determination — We investigated the solution structure of IRp60 using small-angle x-ray scattering. The processed experimental SAXS curve from IRp60 displayed in Figure 1A yields the effective molecular mass (MM) of the protein compatible with the value predicted from the IRp60 sequence (12,000 Da). The radius of gyration $R_g$ and the maximum particle size are 1.9 ± 0.1 nm and 7.0 ± 0.5 nm, respectively (Figure 1B). These values point to a rather extended structure of IRp60, which is further corroborated by the distance distribution function $p(r)$ having a typical appearance for elongated particles. To further assess the shape of IRp60, its low-resolution structure was reconstructed ab initio using the program GASBOR. Multiple ab initio runs were performed yielding reproducible models neatly fitting the experimental data with discrepancy $\chi$ around 1.1 (Figure 1C). The $R_g$ and $D_{max}$ values calculated from the homology model (1.7 and 6.5 nm, respectively) are similar although somewhat smaller than the experimental values (Figure 1B). The scattering pattern computed from this model yields an overall good fit to the SAXS profile (Figure 1A), but also displays some systematic deviations ({$\chi$}=1.7). These deviations along with the differences in the overall parameters indicate that the solution structure is somewhat more extended.
than the homology model. However, the comparison of the homology model with the ab initio low-resolution shape clearly shows that the former model is well positioned within the latter and displays a very similar appearance of an elongated domain with a protuberance (Figure 1D). It should be noted here that SAXS “sees” the hydrated molecule together with the solvation shell, so that the ab initio SAXS-derived low-resolution shape should display a somewhat bulkier appearance than the ribbon representation of the homology model.

**Figure 1:** (A) SAXS patterns from IRp60. The experimental data (dots with error bars), the scattering from the ab initio model (solid line) and the calculated scattering from the IRp60 homology model (dashed line), are shown. (B) Comparison of distance distribution functions. The pair distance distribution functions $p(r)$ of IRp60A (points), of IRp60 homology model (dashed line) and of the IRp60 ab initio model (dashed-points line) are shown. (C) Two independent IRp60 low-resolution SAXS models. (D) Orthogonal views of the IRp60 homology model (ribbon representation) superposed to the averaged ab initio model of IRp60 generated from SAXS data (semi-transparent envelope).

**References**