Comparative modelling for predicting the different conformations assumed by a protein during its different activities

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Introduction

The knowledge of structural organization of proteins is crucial in understanding their role in the cell and the related molecular mechanisms. Comparative modelling has already become one of the most effective computational approaches in facilitating structural/functional characterization of many protein-coding sequences across genomes and it is based on the assumption that homologous proteins adopt the same fold to have the same function. On this basis, it is possible to model the 3D structure of a protein if it is known at least one experimental model of an homologous protein. However, it is evident that a large number of proteins, probably all, may assume different conformations depending on the different environmental conditions or the interaction with other molecules. Conformational modifications occur when a protein changes its monomeric / oligomeric state, enzymes adapt their conformation to the substrate when it is recognized, but also, very different secondary structures are observed in the normal and pathological forms of the prion protein.
We are interested to apply the comparative modelling to predict the different conformations assumed by a protein to exert its biological activities or in different environmental conditions. In this work we applied the comparative modelling methods to create models of the interleukin 1beta (IL-1beta) and to investigate the conformational changes occuring when this protein interacts with its receptor (IL-1R).

Materials and Methods

The search for sequence similarity within databases and the alignment of the selected sequences were performed with BLAST program. The programs MODELLER and Quanta were used to build 10 full-atom models of each sequence according to the comparative protein modelling method. To select the best model, their stereo-chemical quality was verified with the program PROCHECK. Secondary structure in three-dimensional models has been assigned with the program DSSP and solvent accessibility of amino acids was evaluated by the program NACCESS Moreover, the IL-1beta/IL-1R complexes were created by using as reference the experimental model of *human* IL-1beta complexed with its receptor (PDB code:1ITB), using InsightII to superimpose the chains of the new complex to the corrisponding chains of the experimental model of complex. The energies of interaction for IL-1beta/IL1R complexes were evaluated after energy minimization of the complex by using 500 steps of energy minimization under conjugate gradient algoritm of DISCOVER (InsightII).

Results

Two different experimental models of human IL-1beta are available in PDB, related to the protein complexed with its receptor [1] (PDB code 1ITB, chain A) and without receptor, or free [2] (PDB code 1IOB). We used these two templates to create a theoretical model of human IL-1beta (h-ThM). The differences between the three conformations were evaluated by structural superposition obtaining the RMSD values, and by comparing their secondary structure elements. The comparison between the two experimental structures has evidenced that the most of the differences regards Il-1beta amino acids involved for the binding with the receptor. The RMSD values suggest that h-ThM is more similar to the conformation of the free protein, although (even if) the differences are very subtle, probably within the range of errors accepted for the comparison of experimental structures. This finding is in agreement with expectable features, because the modelling procedure is performed without consideration of complexed molecules and the model is created as a free protein. Starting from the experimental structure of human IL-1beta complex with its receptor (PDB code:1ITB), a theoretical complex protein-receptor was created by substituting the human IL-1beta in the complex with the experimental structure of human IL-1 beta in the non-complexed state, obtaining a complex named h-ThCOMPL-X, as well as with the theoretical model h-ThM, obtaining a complex named h-ThCOMPL-M. The experimental and theoretical

complexes were compared and the energies of intermolecular interaction were evaluated after a similar procedure of energy minimization. The energy of interaction in the experimental complex results the most favourable, compared to the theoretical complexes. In particular, the electrostatic interactions are strongly reduced in h-ThCOMPL-X, probably as a consequence of the different conformation of IL-1beta . The h-ThCOMPL-M results to have energy of interaction more favourable than the h-ThCOMPL-X. This may be explained because the h-ThM was obtained by using as templates both the experimental conformations, and therefore it may represent a mean structure, more similar to the free protein but anyway with some structural feature of the complexed form of IL-1 beta.
Comparative modelling was also applied to create the model of mouse IL-1R [3] and two models of the mouse IL-1beta protein, using the two human models. We named these two models as m-ThF (mouse Theoretical Free model) and m-ThC (mouse Theoretical Complexed model), corresponding to the features of the human template used. The differences between the experimental mouse IL-1beta model and the two theoretical models were evaluated by structural superposition and RMSD values indicate that m-ThF is more similar to the experimental model of mouse IL-1beta, obtained in absence of the receptor, as expected, because m-ThF was modelled on the template of the human non-complexed protein. Indeed, on the basis of the experimental structure of the human IL-1beta/IL-1R complex, we simulated the interaction of the two obtained models of mouse IL-1beta with the model of their receptor, and named m-ThCOMPL-F and m-ThCOMPL-C, respectively, the complex obtained using m-ThF and m-ThC as IL-1beta conformation. Moreover, we have simulated the complex between the X-Ray solved structure of mouse IL-1beta (PDB code: 2MIB) and its receptor, and named this complex m-ThCOMPL-X. For each simulated complex, the energies of interaction have been evaluated and results have shown that the energy of interaction is more favourable in the complex m-ThCOMPL-C, as expectable, because it is the only complex in which IL-1beta has been modelled by using as template the complexed form of IL-1beta.
We are applying this approach to IL-1beta and IL-1R from other organisms [3]. Our work suggests that very subtle differences in the conformations assumed by a protein can be translated by the comparative modelling. Therefore, comparative modelling of complexes can be applied to evaluate conformational changes occurring in the association of molecules.

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