CHIMIA 51 (1997) Nr. 1/2 (Januar/Februar)

Successful prediction of modifications to a ligand which result in stronger binding to the protein requires detailed high-resolution model structures. However, even small chemical changes to the ligand, like the addition of a Me group or changing a Me group to a OH group, can have a significant and unpredictable effect on binding strength. Our work with a series of cyclophilin-cyclosporin ligands provides a useful compilation of structural results which provides an insight into some of these problems. The cyclic undecapeptide cyclosporin A is an immunosuppressive drug which binds strongly to its cognate protein receptor cyclophilin. We have solved over 15 crystal structures of different derivatives of cyclosporin complexed with cyclophilin. The binding strengths of many of the cyclosporin derivatives can be correlated with subtle differences in nonbonding interactions or small changes in conformation. One complicating factor is the fact that the ligand undergoes a large conformational change on going from a lipophilic environment (possibly passing through the cell membrane) into a hydrophilic environment. The unliganded cyclophilin structure has also been used as a template for the design of small non-peptidic ligands which may provide interesting leads for the development of a new family of inhibitors.

Computer programs like DOCK, LUDI and MCSS are available to automate the procedures used to design and select small molecules as potential ligands. Over 150000 small molecule organic crystal structures are available in the Cambridge Crystallographic Database and reasonably reliable three-dimensional structures of most small organic molecules can also be generated using molecular mechanics programs. Large databases are therefor available for searching and ligand discovery becomes matchmaking process to find a ligand with the required shape and charge characteristics for the protein template. Our program LIDAEUS uses a site-point matching approach to search for ligands in a database of structures in which flexible subfragments are identified. Ultimate verification of a ligand comes from examination of a high-resolution NMR or X-ray structure of the protein-ligand complex. This has been achieved in our work with a number of thrombin and immunophilin complexes. The combination of conformational change and large entropic change on ligand binding makes the interaction energies difficult to compute. Ligand binding scores which should be related to experimental binding strength are still not very reliable unless extensive molecular dynamics calculations are carried out. Improvement in the estimation of binding strength and in modelling molecular flexibility are two of the major challenges in this field.

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Chimia 51 (1997) 16–17

ISSN 0009–4293

Structural Insight into Prion Diseases [1]

Kurt Wüthrich*

The appearance of bovine spongiform encephalopathy (BSE) in cattle and the links to a new variant of *Jakob-Creutzfeld* disease (CJD) in human has spurred intensive research into what is known as a central dogma in biology: pathogens need nucleic acids to perpetuate. However, there is belief today that, in contrast to this dogma, a proteinaceous particle (prion) is the infectious agent for some neurodegenerative diseases such as BSE, scrapie, and CJD [2][3]. A mechanism underlying the transition of the otherwise benign cellular prion protein PrP^C to the protease-resistant 'scrapie form' PrP^{Sc} has been proposed involving the partial unfolding/refolding of its polypeptide chain [4]. The three-dimensional structure of an autonomously folding domain of PrP^C comprising residues 121–231 was recently determined at the ETH-Zürich and now provides for the first time insight into the structural basis for prion diseases [5].

The NMR structure of mouse PrP^C-(121–231) in solution contains three α-helices and a two-stranded β-sheet. The molecule is V-shaped with the second and the third helix forming the structural scaffold onto which the β-sheet is anchored. In addition to hydrophobic interactions between side chains of residues in helix 2, helix 3, and the β-sheet, there is an additional disulfide bridge stabilizing the protein folding. The surface of the protein shows a markedly uneven distribution of positively and negatively charged residues and there is a hydrophobic surface patch near the β-sheet and the loop preceding the first helix.

Considerable differences between the predicted folding and the experimentally determined structure of the PrP^C domain are apparent. In particular, the observation of a β-sheet segment between residues 128–131 and 161–164 was unexpected from the secondary-structure prediction. This part of the protein is believed to become the nucleation site for a conformational transition from PrP^C to PrP^{Sc}, which is associated with an increase in β-sheet content in Pr^P. Interestingly, homozygosity for valine at the polymorphic codon 129 appears to increase the susceptibility for sporadic CJD [6]. Other residues associated with inherited prion diseases are located either in or adjacent to helix 2 or helix 3 and possibly interfere with the structural integrity of the protein or influence its ligand binding site.

The bipolar character of PrP^C(121–231) suggests that the protein would attach to the cell membrane with the positively charged surface and have a solvent-accessible negatively charged surface. Four out of eight residues associated with the species barrier of prion disease transmission between humans and mice are located within or adjacent to the first helix. Together with both glycosylation sites (Asn181, Asn197), these residues would then lie on the solvent-accessible site of PrP^C and might represent part of a single PrP^{Sc} binding site. This information on the

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structural details of crucial residues of PrP^C will be essential for the design of future experiments to elucidate the structure-function relationship of prion proteins and the mechanism of prion diseases. The next step structural biology will certainly aim at is the determination of variants of PrP^C with high susceptibility to prion disease such as the Met129Val mutation. Even more challenging will be to determine the structure of the entire PrP^C and finally the structure of PrP^{Sc}, the protein believed to be the infectious agent for prion diseases.

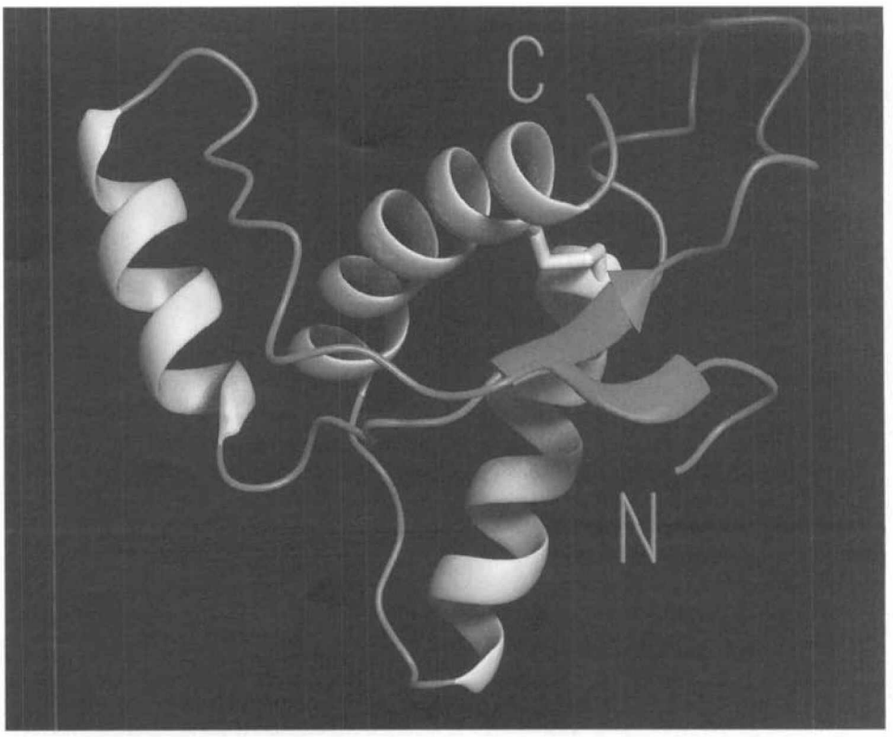

Figure. *Ribbon diagram of the structure of the mouse prion protein domain PrP(121–231).* Three helices are colored yellow, a short antiparallel two-stranded β-sheet cyan, the connectin loops green, except for a reddish loop in the upper right that is structurally disordered. There is a single disulfide-bond which is colored white.

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Chimia 51 (1997) 17–18

ISSN 0009–4293

Design of Superactive and Selective Ligands of the $\alpha_v\beta_3$ Integrin [1]

Horst Kessler*

Integrins play a major role in cell-cell and cell-matrix interactions. Most of the different integrins recognize the tripeptide sequence Arg-Gly-Asp (RGD) [2]. To explore the spatial requirements of the pharmacophore for receptor *selectivity* and *high activity*, a new procedure, the 'spatial screening', was used. The procedure is based on the experience that the conformation of small cyclic peptides are mainly determined by the chirality of the amino acids (and Gly or Pro). *E.g.* cyclic pentapeptides, with one D- and four L-amino acids prefer a βII'γ conformation. The sequence RGDFV was shifted around this spatial βII'γ template by synthesis of four peptides in which one of the L-amino acids was used in D-configuration [3] and the all-L-peptide (Gly represents the D-amino-acid analogue). It turned out that highest activity and selectivity was achieved with cyclo(RGDFV) for the $\alpha_v\beta_3$ integrin [4], which is strongly expressed on cancer cells. The same approach was also successful for the $\alpha_{IIb}\beta_3$ integrin using cyclic hexapeptides as templates [5]. Systematic variations with different turn mimetics [6], retro-inverso structures [7], reduced peptide bonds [8], and thiopeptides yielded in highly active, selective, and metabolically stable compounds.

It has been demonstrated that the inhibition of the $\alpha_v\beta_3$ integrins caused apoptosis in cancer tissue [9]. In addition, angiogenesis is strongly inhibited by blocking the $\alpha_v\beta_3$ and/or $\alpha_v\beta_5$ integrin [10][11]. As neovascularization is important for tumor growth and metastasis, our cyclic peptides are promising candidates for a new tumor therapy by 'starving'.

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