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Protein Structures as Templates for the Design of New Drugs

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Einleitung

Die internationale Tagung vom 22. November 1996, organisiert von der Sektion Chemische Forschung der Neuen Schweizerischen Chemischen Gesellschaft, gab einen Überblick über das Gebiet der Proteine. Die Forscher können den räumlichen Aufbau der Proteine immer genauer ermitteln und in dreidimensionalen Darstellungen zeigen. Computerprogramme ermöglichen es Pharmakologen und medizinalen Chemikern, die Lage von Wirksubstanzen auf ihren Rezeptoren gezielter zu zeigen, sie masszuschneidern und ihre Eigenschaften zu verstehen. Die Technik wurde am Beispiel von Protein-Enzymen entwickelt und für die Schaffung von Enzym-Inhibitoren verfeinert.

Es gibt noch eine Vielzahl von Proteinen, die mindestens ebenso interessant und wichtig sind wie Enzyme. Gewisse Proteine machen Kontakte mit anderen Proteinen, um einer Zelle ein pharmakologisches Signal zu geben. Werden Struktur und Funktion von solchen Signal-Biomolekülen, wie Enzymen, mit Hilfe von kernmagnetischen Resonanzen in physiologischer Lösung und mit Hilfe von computergestützten Methoden untersucht, wird sich ein neuer Weg zu effizienten Medikamenten eröffnen.

Das Ziel dieser Tagung war, dieses Forschungsgebiet bekannt zu machen und ihm neue Impulse zu vermitteln. Zusammenfassungen der Vorträge dieser Protein-Tagung sollen den gleichen Zweck erreichen. Die Vorträge wurden entweder von den Autoren selbst oder von Spezialisten zusammengefasst.

R. Wenger, Chairman der Tagung

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Ligand Discovery Using Three-Dimensional Structures [1]

Malcolm D. Walkinshaw*

There are now over 4000 protein structures deposited in the Brookhaven Data Base which have been determined by protein X-ray crystallography or NMR spectroscopy. This still falls quite some way short of providing a structural model for each of the 100 000 or so gene products of the human genome. The rapidly expanding sequence and structure catalogue of these proteins is, however, already helping to answer many complex questions about signal transduction and cell-cell recognition. Of immediate pharmaceutical interest is how to use this structural information to discover new ligands. The various approaches can be classified in three general ways. *1*) Template Mimicry: a number of proteins are ligands in their own right and copies of small loops or fragments from the parent protein may provide biological activity. *2*) Lead optimization: in this approach, a known substrate or inhibitor is modified to enhance binding and inhibitory properties based on the 3D template structure of the protein. There are now over ten examples of this approach having been used to successfully design clinically tested drugs which include inhibitors for HIV protease, neuraminidase and thymidylate synthase. *3*) *Ab initio* design and database mining provide the third general approach which is being advanced by computer programs which fit molecules or molecular fragments into a defined protein template. This can involve for searching very large 3D databases of available or synthetically tractable molecules or the design of new molecules.

The immunoglobulin domain family of proteins including adhesion molecules provide examples of the possible application of template mimicry in ligand design. The vascular cell adhesion molecule (VCAM) consists of a linear array of seven immunoglobulin domains stretching out over 20 nm into the extracellular space to recognize and recruit passing leukocyte cells. Mutation studies show that six or seven residues (T37QIDSPL) at the tip of a loop region are the key to the recognition and binding. We have recently solved a new crystal form of the D1,D2 domains of VCAM which shows a conserved conformation of this exposed recognition loop when compared with the two other available X-ray structures of these domains [2][3]. An analogue cyclic hexapeptide of this loop (CQIDSPC) has been shown to inhibit VCAM binding to the integrin receptor [2]. The approach of making cyclic peptide mimics is particularly well suited to Ig folds as the recognition region normally consists of a peptide loop.

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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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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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