Receptor-Induced Conformation Change of the Immunosuppressant Cyclosporin A

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CYCLOSPORIN A (CsA) IS A MEMBER OF A GROUP OF immunosuppressive agents that bind with high affinity to their respective immunophilins (immunosuppressant-binding proteins) and act as inhibitors of specific signal transduction pathways that lead to T lymphocyte activation (*1, 2*). At the present time CsA is the favored therapeutic agent for prevention of graft rejection in clinical organ transplantation and as such is one of the drugs with yearly multimillion dollar sales. In addition to the fundamental interest in the molecular basis of immunosuppressive action, the clinical importance of CsA has also spurred vigorous research activity on the structural basis of its function.

Important advances during the last decade include the determination of an x-ray structure of crystalline CsA (*3*), a nuclear magnetic resonance (NMR) structure of CsA in solution (*3, 4*), the identification of the cytosolic binding protein cyclophilin (CYP) as a cellular receptor of CsA (*5*), and the observation that CYP is identical with the enzyme peptidyl-prolyl *cis-trans* isomerase (*6*), of which CsA is a potent inhibitor (inhibition constant K_i = 2.6 nM) of enzymatic activity. The protein CYP has been found in diverse organisms from *Escherichia coli* to man (*7*) and is present in high abundance in all tissues studied. Furthermore, in the system of the macrolide FK506 and its receptor, FK-binding protein (FKBP)—a system unrelated to the CsA-CYP system in chemical structure—FK506 is a potent immunosuppressant with a biological profile similar to that of CsA (*2*). During the past few months research into the structures of both systems, CsA-CYP and FK506-FKBP, has made astonishing progress, with reports on the three-dimensional (3D) structures in crystals or in solution of FKBP (*8*), an FKBP-FK506 complex (*9*), receptor-bound CsA (*10, 11*) and CYP (*12, 13*), and the delineation of the intermolecular contacts in the 1:1 CYP-CsA complex by combined use of x-ray diffraction in single crystals and NMR spectroscopy in solution (*13*). The full impact of these structural data will be made when the chemists and molecular biologists working on the design of further improved immunosuppressive systems have had an opportunity to make use of this new information.

This perspective focuses primarily on the investigations of receptor-bound CsA. Both the results obtained and the methods used could be of general interest for studies of other receptor-effector systems.

Cyclosporin A is an undecapeptide with pronounced hydrophobic character (Fig. 1). In addition to the hydrocarbon moieties there is a single –OH group in the amino acid side chains, and along the polypeptide backbone 7 of the 11 residues are N-methylated. Prior to the studies of CsA bound to CYP discussed below, the structural basis for investigations into mechanistic aspects of the mode of action of CsA consisted of the 3D structure of free CsA in single crystals and in an apolar solvent [for example, (*14*)]. (Because free CsA is only sparingly soluble in aqueous media, the NMR structure was determined in chloroform solution). The NMR structure in chloroform at −20°C is virtually identical to the x-ray crystal structure. In both environments the backbone forms a twisted β sheet that involves residues 1, 2, 5, 6, 7, and 11 and a type II′ turn at Sar^3 and $MeLeu^4$. A loop formed by the remaining amino acids includes a *cis*-peptide bond between $MeLeu^9$ and $MeLeu^{10}$ (*3, 4*). The four amide protons (Fig. 1) are involved in the three transannular hydrogen bonds Abu^2 NH–Val^5 C′=O, Val^5 NH–Abu^2 C′=O, and Ala^7 NH–$MeVal^{11}$ C′=O, and one additional hydrogen bond from NH of D-Ala^8 to C′=O of $MeLeu^6$. The near identity of the CsA structures in the crystal and in chloroform solution extends also to the conformation of the amino acid side chains, which form two hydrophobic clusters. One of these includes Abu^2, Val^5, Ala^7, and $MeVal^{11}$ and is in front of the plane of the cyclic polypeptide backbone in the orientation of Fig. 1, and the other is made up of $MeBmt^1$, $MeLeu^4$, $MeLeu^6$, and $MeLeu^{10}$ and is behind the ring plane in Fig. 1.

In stark contrast to free CsA, CsA bound to CYP contains no regular secondary structure and no intramolecular hydrogen bonds. The two backbone segments from residues 4 to 7 and 9 to 2 (Fig. 1), which form the antiparallel β structure in the crystals, have their planar peptide bonds rotated out of the plane defined by the cyclic polypeptide backbone, in contrast with regular β-structures. Four of the seven NCH_3 groups line the inside of the cyclosporin ring, that is, those of residues 1, 4, 6, and 10, and all four amide protons are exposed to the outside. All of the peptide bonds are in the trans conformation; and the hydrophobic side chain cluster of $MeBmt^1$, $MeLeu^4$, $MeLeu^6$, and $MeLeu^{10}$ is located in front of the ring plane in the orientation of the molecule in Fig. 1. Overall, the conformation of CsA bound to CYP thus includes the following differences relative to that of free CsA. The peptide bond between residues 9 and 10 is trans rather than cis, all amide protons and carbonyl oxygens are exposed along the edges of the ring rather than hydrogen-bonded in the central region of the ring plane, and the dominant hydrophobic cluster formed by the side chains 1, 4, 6, and

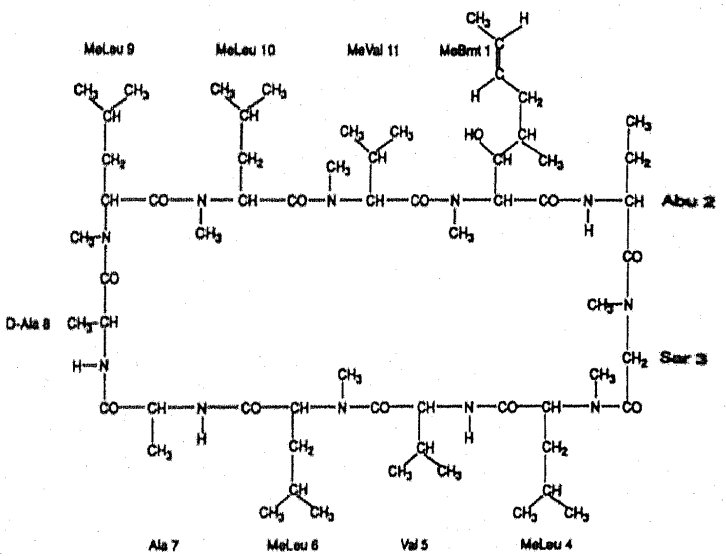

Fig. 1. Chemical structure of cyclosporin A (CsA) with the standard numeration of the amino acid residues. Abbreviations: MeBmt, (4*R*)-4-[(*E*)-2-butenyl]-4,*N*-dimethyl-L-threonine; Abu, L-α-aminobutyric acid; Sar, sarcosine; MeLeu, *N*-methylleucine; and MeVal, *N*-methylvaline.

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10 is located on the opposite side of the ring plane. For the polypeptide backbone the structural rearrangement upon binding to the receptor is reminiscent of the inversion of a glove, whereby the hydrophobic exterior edges formed by the N-methyl groups in free CsA are replaced by a polar surface of amide protons and carbonyl oxygens.

The conformation of the receptor-bound CsA indicates the possibility of hydrogen bonding with the receptor protein. For the amide proton of Abu[2] the presence of hydrogen bonding is directly supported by the observation of slowed exchange with the solvent (*10*). Hydrogen bonding is also compatible with ^{1}H-^{1}H nuclear Overhauser effects (NOEs) observed between CYP and the residues 1 to 3 and 9 to 11 of CsA (*10, 11*). The dramatic global rearrangement of the polypeptide backbone conformation in CsA, which enables recognition by hydrogen bonding, is particularly remarkable for a cyclic compound, which has a greatly reduced accessible conformation space when compared with a corresponding linear polypeptide. Considering the important role of molecular modeling in drug design, it is instructive to note that molecular dynamics calculations that started from the crystal structure or the solution structure of free CsA and that used different potential functions to represent the solvent (*15*) gave no indication of an imminent major conformation change away from the starting conformation. Thus CsA may well end up as a textbook case to illustrate the importance of experimental studies with both free and receptor-bound effector molecules for understanding structure-function correlations as a guide to improved molecular design.

The NMR investigations of receptor-bound CsA were performed with combined use of isotope-labeling and heteronuclear NMR experiments. In an unlabeled system the large number of protons from the receptor protein would interfere with the observation of the resonance lines of the ligand. However, binary complexes are ideally suited for studies with efficient labeling schemes, because the two components can be labeled separately with ^{13}C or ^{15}N before complex formation and subsequently combined with unlabeled partner molecules. Suitably chosen heteronuclear editing schemes (*16, 17*) can then be used to separate the ^{1}H NMR lines of the two molecules in the complex. In particular, use of the so-called heteronuclear half-filters (*17, 18*) in 2D ^{1}H NMR spectra represents a valid alternative to the use of three or higher dimensional experiments for improved resolution in such systems. An intrinsic advantage is that the 2D ^{1}H-^{1}H NMR spectra can be recorded with sensitivity and digital resolution comparable to those of corresponding conventional 2D ^{1}H NMR spectra. With ^{13}C-labeled CsA a double-half-filter technique was particularly helpful (*19*) because it produced different subspectra that contained either exclusively intramolecular NOE cross peaks between different protons of CsA or different protons of CYP or exclusively intermolecular NOE cross peaks relating protons of CsA with protons of CYP. These techniques are generally applicable with binary or multicomponent molecular assemblies, primarily in systems with very stable receptor-effector complexes, and represent an attractive avenue for the use of NMR in conjunction with projects on drug design.

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Rusting of the Lock and Key Model for Protein-Ligand Binding

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ATOMIC-LEVEL KNOWLEDGE OF THE GEOMETRIES OF protein-ligand complexes has only been accumulating since the mid-1970s. About 50 x-ray structures have now been determined for peptides or proteins bound to enzymes or antibodies. The traditional notion of rigid lock and key complementarity received support from the early and numerous studies of complexes of proteolytic enzymes with small protein inhibitors (*1*) and from the first example of an antibody-protein complex (*2*). However, the idea has become increasingly challenged.

In fact, conformational changes for enzymes upon ligand uptake are well known and range from modest loop motions to hinge bending (*3*). The prototypical case of strong binding, streptavidin-biotin, involves adjustments to streptavidin that include a loop flip (*4*), and the bear hug applied by human immunodeficiency virus type 1 (HIV-1) protease to a peptide inhibitor is a striking example of large-scale domain motions (*5*).

Recently, the effects of binding on ligand structure have received increased attention. Cases of profound conformational change have been provided by the determination of the structures of the immunosuppressive agents FK506 and cyclosporin A (CsA) complexed with their cytosolic binding proteins FKBP and cyclophilin. X-ray structures have been reported for the uncomplexed drugs and the FKBP-FK506 complex (*6*), and the structure of CsA bound to cyclophilin has been determined by two groups using multidimensional nuclear magnetic resonance (NMR) techniques (*7*).

Both binding proteins are peptidyl-prolyl-*cis-trans* isomerases and have been shown to interfere with T cell signaling upon forming ternary complexes with their respective immunosuppressive agents and the protein phosphatase calcineurin (*8*). FK506 is a macrocyclic organic molecule with a critical α-keto-homoprolyl subunit (upper right part of the structure) that acts as a transition-state surrogate (*9*), and CsA is a cyclic undecapeptide. Binding leads to substantial conformational change for FK506, including cis-trans isomerization

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