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COMPARISON OF CONFORMATIONS OF CYCLOSPORIN A AND MACROLIDE FK506 FRAGMENTS: LOCALIZATION OF PUTATIVE BINDING SITES WITH PHOSPHATASE CALCINEURIN

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The three-dimensional structures of two immunosuppressants, cyclosporin A and macrolide FK506, were compared. The sites N-methylglycine3-N-methylleucine4 and valine5-N-methylleucine6 of cyclosporin A were found to be similar to each other (the root-mean-square value was 0.29 Å for six reference points of the main chain) and also to the site C17-C22 of FK506 (the root-mean-square values were 0.33 Å and 0.13 Å, respectively). We suggest these fragments of cyclosporin A and FK506 make a major contribution to the interaction of the immunosuppressants with the phosphatase calcineurin.

The initial stage of the biological activities of both cyclosporin A (CsA) or macrolide FK506 results from the formation of complexes between these ligands and their specific peptidyl-prolyl isomerases, cyclophilin A (CyP) and FK506-binding protein (FKBP), respectively (1). At present, the X-ray structure of the FK506-FKBP complex has been solved (2, 3), and molecular models for the CsA-CyP complex have been constructed based on the X-ray crystallographic and NMR-spectroscopic data (4-11). The conformations of these two complexes have not been compared in detail so far. Nevertheless, it has been shown that, despite basic dissimilarities in the three-dimensional structures of CyP (12-14) and FKBP (15, 16), these two functionally related proteins have similar arrangements at nine of the amino acids at the substrate-binding pockets (17).

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Abbreviations: CsA, cyclosporin A; CyP, cyclophilin A; FKBP, FK506-binding protein; MLE, N-methylleucine; SAR, N-methylglycine (sarcosine); ABU, L-α-aminobutyric acid; RMS, root-mean-square.

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Moreover, their ligands, CsA (18-20) and FK506 (2, 3), have structural similarities at the region of interconvertible amide bonds (17).

It has been recently established that the complexes FK506-FKBP and CsA-CyP are specific inhibitors of the Ca^{2+}- and calmodulin-dependent phosphatase calcineurin (21-26). Phosphatase binding involves also the receptors of FKBP, CyP (27), and ligands FK506, CsA (28). In this work we compared the conformations of FK506 and CsA to search for the sites responsible for binding to the phosphatase calcineurin. The tertiary structures used for the analysis correspond to the ligand bound states. We found regions of FK506 and CsA that are conformationally similar and that are distinct from those containing the interconvertible amide bonds.

Materials and Methods

The atomic coordinates of FK506 were kindly provided by Prof. G.D. Van Duyne. Coordinates of CsA (18) were obtained from the Protein Data Bank (29; entry 2CYS).

Superposition of the fragments of CsA and FK506 was done using the molecular modelling software packages INSIGHT II (Biosym Technologies, Inc., San Diego, CA) on a Silicon Graphics Iris Crimson workstation and SYBYL (Tripos Associates, Inc., St. Louis, MO) on an Evans & Sutherland ESV 30 workstation.

Results

Superposition of the fragments C17-C22 of FK506 (2, 3) and N-methylglycine3 (SAR3)-N-methylleucine4 (MLE4) of CsA (18) is shown in Fig. 1. The root-mean-square (RMS) difference of the two compared structures indicates how similar the structures are. The RMS difference depends on the choice of the corresponding (bench-mark) atoms. We chose six pairs of atoms as bench-marks. Three pairs were atoms C17, C18, C19 of FK506 and N-, Cα-, C-atoms of the main chain of SAR3 of CsA, while the other three pairs were C20, C21, C22 of FK506 and the N-, Cα-, C-atoms of MLE4 of CsA. Their RMS value was 0.33 Å. Fig. 1 shows that upon superposition of the fragments C17-C22 of FK506 and SAR3-MLE4 of CsA, structural and chemical similarity is more pronounced at the termini of the compared regions. At the N-terminus there are methyl and hydrogen groups, while the C-terminus involves the side group of MLE4, the allyl group, and a pair of oxygen atoms. The greatest differences are observed at the region of the peptide bond of SAR3, MLE4 of CsA and atoms C19, C20 of FK506.

Superposition of the fragment VAL5-MLE6 of CsA and of the related fragment of FK506 is shown in Fig. 2. In this case we selected the following six pairs of atoms as bench-marks: on one hand, the same atoms C17-C22 of FK506, and on other hand - Cβ-, Cα-, C-atoms of VAL5 and N-, Cα-, C-atoms of MLE6 of CsA. Their RMS value was 0.13 Å. Comparisons of Figs.1 and 2 and the respective RMS values show that the second case of superpositions reveals much better similarity of the main chain fragments of FK506 and CsA. It should be noted, however, that the level of chemical similarity of the

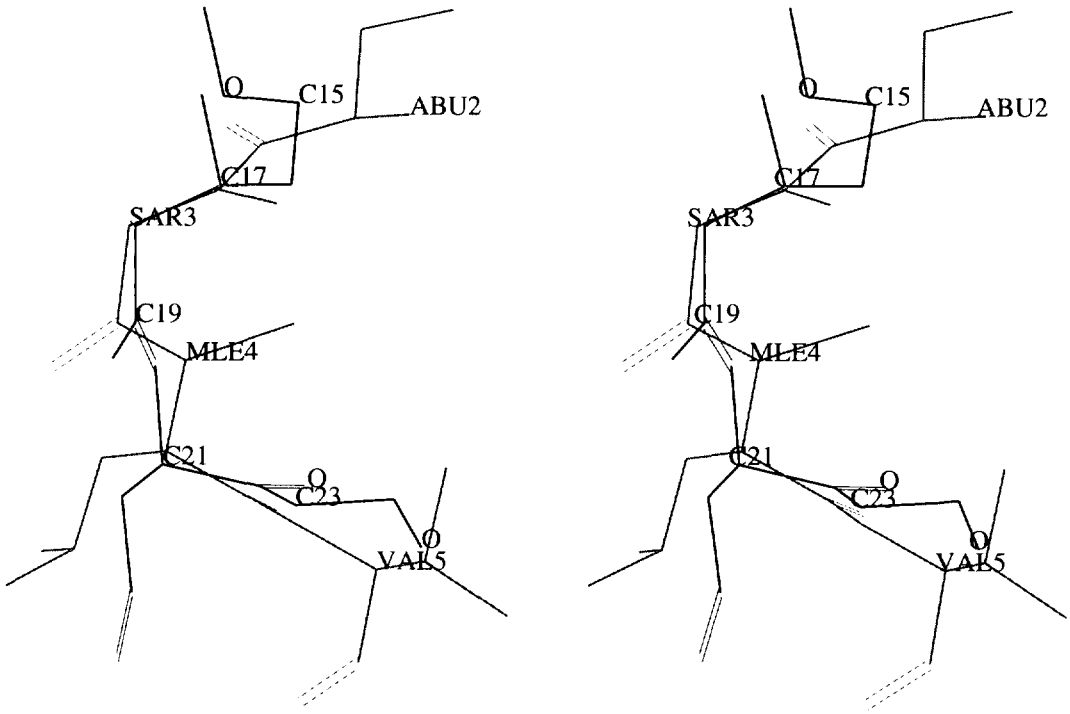

Fig. 1. Stereoview of the three-dimensional superposition of the C17-C22 (solid line) and SAR3-MLE4 (dashed line) fragments of the FK506 and CsA, respectively.

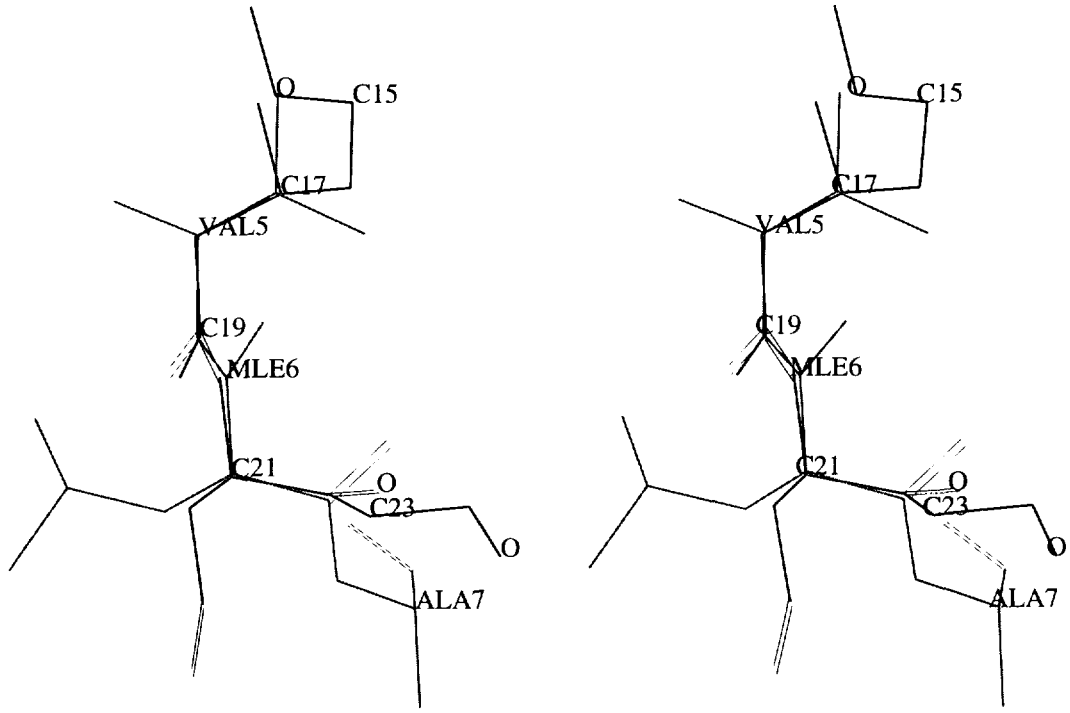

Fig. 2. Stereoview of the three-dimensional superposition of the C17-C22 (solid line) and VAL5-MLE6 (dashed line) fragments of the FK506 and CsA, respectively.

fragments compared was practically the same. Slight changes only occurred at the N-terminus. N-atom of VAL5 appeared to have no structural partner, and non-homologous pair C17-N-atom of SAR3 was replaced by a conserved pair C17-Cβ-atom of VAL5.

A comparison of spatial structures of two CsA fragments SAR3-MLE4 and VAL5-MLE6 is shown in Fig.3. As above, we used six atom pairs as bench-marks: the N-, Cα-, C-atoms of SAR3, MLE4 and the Cβ-, Cα-, C-atoms of VAL5, N-, Cα-, C-atoms of MLE6, respectively. The RMS value was 0.29 Å. Fig. 3 shows that structural similarity is observed not only for atoms of the main chain involved in the composition of bench-marks atoms, but also for the remaining atoms of fragments SAR3-MLE4 and VAL5-MLE6. Thus, CsA has two contiguously located and conformationally similar chain fragments. Distances between the atoms forming bench-mark pairs upon constructing of superpositions in Figs. 1-3 are summarized in Table 1.

Discussion

To construct the superpositions (Figs. 1-3) we used the fragments C17-C22 of FK506 and SAR3-MLE6 of CsA. Analysis of the conformations revealed that CsA has two contiguously located, structurally similar chain regions SAR3-MLE4 and VAL5-MLE6. In

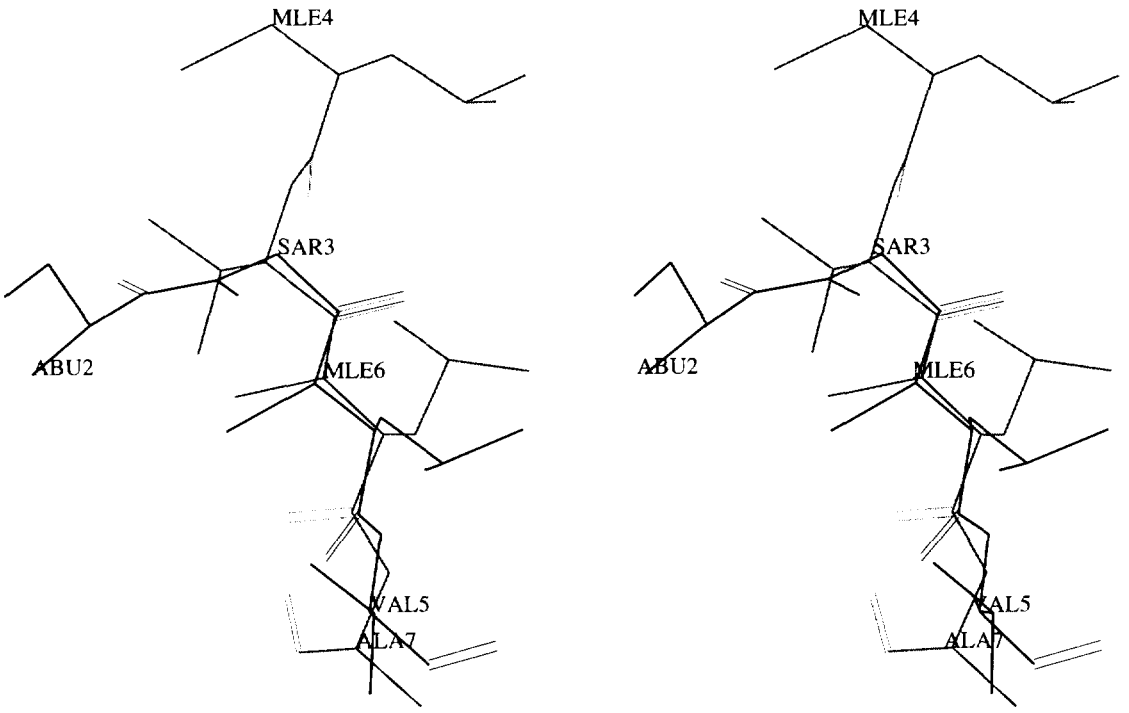

Fig. 3. Stereoview of the three-dimensional superposition of the SAR3-MLE4 (solid line) and VAL5-MLE6 (dashed line) fragments of the CsA.

Table 1. Dimensions (D) and RMS values between bench-mark atoms of CsA and FK506 fragments

CsA	FK506	D(Å)	CsA	FK506	D(Å)	CsA	CsA	D(Å)
SAR3			VAL5			SAR3	VAL5	
N	-C17	0.19	Cβ	-C17	0.11	N	-Cβ	0.27
Cα	-C18	0.17	Cα	-C18	0.11	Cα	-Cα	0.28
C	-C19	0.34	C	-C19	0.05	C	-C	0.29
MLE4			MLE6			MLE4	MLE6	
N	-C20	0.56	N	-C20	0.11	N	-N	0.46
Cα	-C21	0.24	Cα	-C21	0.17	Cα	-Cα	0.17
C	-C22	0.30	C	-C22	0.19	C	-C	0.14
RMS		0.33	RMS		0.13	RMS		0.29

addition, both sites are structurally similar to the FK506 fragment C17-C22. The RMS values calculated for the six atom pairs were approximately equal to each other.

The available data implies that the related fragments of FK506 and CsA participate in binding to the phosphatase calcineurin (28, 30-33). In particular, it was shown that substitution of the allyl group at position C21 of FK506 with an ethyl or methyl group does not lead to any changes in the nature of binding to FKBP but decreases the binding affinity of the FKBP-FK506 complex to calcineurin and correspondingly decreases the ability of the complex to inhibit its binding (28). Simultaneous substitution of the methyl group at C15 position of FK506 with a hydrogen and the allyl group at C21 with an ethyl group decreases immunosuppressive activity of phosphatase still further (28). Besides, two isomers, R- and S-[C18-OH]ascomycin (ascomycin is a potent immunosuppressant closely related to FK506 where the allyl group at C21 position is replaced with an ethyl group), bind tightly to FKBP but have little or no immunosuppresive activity (31). Similar results were obtained for CsA analogues. Replacements of D-N-methylphenylalanine, 3'-hydroxy-D-N-methylphenylalanine at position SAR3 or deletion of the isopropyl group (mutation of LEU to ALA) at position MLE6 leads to a decrease of immunosuppressive activity without any influence on the binding affinity to CyP (28, 32, 33).

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