A Model of the Cyclophilin/Cyclosporin A (CSA) Complex from NMR and X-ray Data Suggests that CSA Binds as a Transition-State Analogue

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Received December 12, 1991

Cyclosporin A (CsA) (Figure 1A) is a clinically useful immunosuppressant that binds tightly to cyclophilin (CyP)[1] and inhibits its peptidyl-prolyl cis-trans isomerase (PPIase) activity.[2] The conformation of CsA when bound to CyP[3,4] and the secondary structure of free[5] and complexed[6,7] CyP have been determined by NMR. In addition, an X-ray structure of CyP[8] and a CyP/tetrapeptide complex[6] have appeared. However, no structure has yet been reported for the CyP/CsA complex, which would be a valuable aid in designing improved cyclosporin analogues, since this complex is responsible for the immunosuppressive properties of CsA[9] by binding to and inhibiting the calcium- and calmodulin-dependent phosphatase, calcineurin.[10]

In this communication, we present a model for the CyP/CsA complex that was generated using the NMR-determined bound conformation of cyclosporin A,[4] the X-ray structure of free cyclophilin,[8] and intermolecular NOEs which define how CsA binds to CyP.

From a ^{13}C-resolved 3D HMQC-NOESY spectrum[11] of [U-^{13}C]CsA bound to cyclophilin, the CsA protons in close proximity to the protein were unambiguously identified.[4] On the basis of the sequential assignments of the protein signals that were recently obtained[7] and from a ^{13}C-resolved 3D HMQC-NOESY spectrum of uniformly ^{13}C, ^{15}N labeled cyclophilin bound to unlabeled CsA, the CyP protons with NOEs to CsA were assigned. Figure 1B-E depicts representative ^1H-^1H NOESY planes of the 3D HMQC-NOESY spectrum at various CyP ^{13}C chemical shifts that illustrate key CyP/CsA NOEs used in defining intermolecular contacts in the complex. The complete list of CyP/CsA NOEs that were used in the structure calculations includes the following: F60(H^{ϵ})/MeVal^{11}($H^{\gamma 2}$), F60(H^{ζ})/MeVal^{11}($H^{\gamma 2}$), F60(H^{δ})/MeLeu^9($H^{\delta 2}$), F60(H^{ϵ})/MeLeu^9($H^{\delta 2}$), F60(H^{ϵ})/MeLeu^{10}(NCH_3), A101(H^{α})/Abu^2(H^{γ}), A101(H^{β})/Abu^2(H^{γ}), A101(H^{α})/MeVal^{11}($H^{\gamma 1}$), A101(H^{β})/MeVal^{11}($H^{\gamma 1}$), A103(H^{α})/MeBmt^1(H^{η}), A103(H^{α})/MeBmt^1(H^{ϵ}), F113(H^{δ})/MeVal^{11}($H^{\gamma 1}$), F113(H^{ϵ})/MeVal^{11}($H^{\gamma 1}$), W121($H^{\delta 1}$)/MeLeu^{10}($H^{\delta 2}$), W121($H^{\zeta 2}$)/MeLeu^9($H^{\delta 1}$),[12] W121(H^{η})/MeLeu^9($H^{\delta 1}$),[12] W121($H^{\zeta 3}$)/MeLeu^9-($H^{\delta 1}$),[12] W121($H^{\epsilon 1}$)/MeLeu^9(NCH_3), H126($H^{\epsilon 1}$)/MeBmt^1($H^{\delta Me}$), H126($H^{\epsilon 1}$)/MeVal^{11}(NCH_3).

In order to generate a model of the CyP/CsA complex, a starting structure of the complex was built by docking the bound NMR structure of CsA[4] into the X-ray structure of free CyP.[8] Structures were refined during 5 ps of restrained molecular dynamics (rMD)[15] at 300 K with the XPLOR program.[16] During the MD simulations, the α-carbons for the regular secondary structural elements of CyP were fixed to their crystallographic positions while the loop regions and all side-chain atoms were free to move. This strategy was adopted, since NMR studies[5-7] had indicated that the secondary structure of CyP in the CyP/CsA complex was the same as in the CyP/tetrapeptide complex and free CyP. Distance constraints obtained from the measured cross-peak volumes in the 3D HMQC-NOESY spectra were included in the rMD calculations to fix the previously determined bound conformation of CsA[4] and orient CsA relative to CyP in the complex. The intermolecular NOE-derived distance constraints used in the structure calculations are given as supplementary material. The final step in the refinement procedure

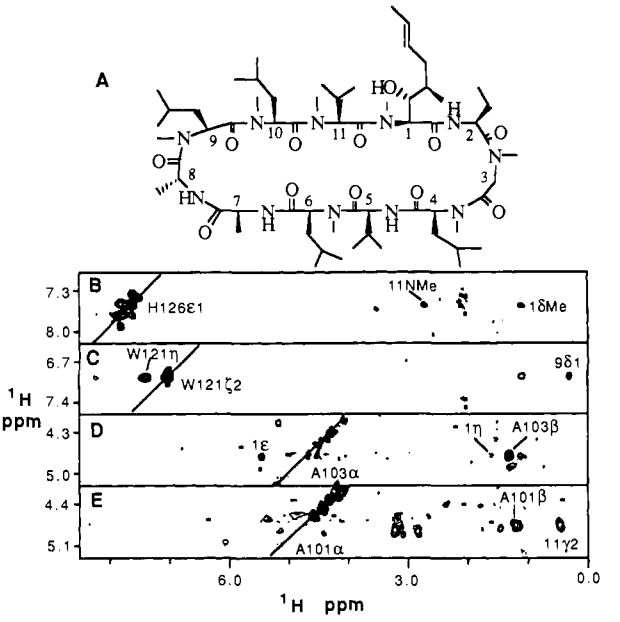

Figure 1. (A) Primary structure of cyclosporin A. (B-E) Cross sections (ω_2, vertical axis; ω_3, horizontal axis) from a 3D ^{13}C HMQC-NOESY data set of the [U-^{13}C,^{15}N]CyP/CsA complex at the following ^{13}C chemical shifts (ω_1): (B) 137.3, (C) 115.8, (D) 50.1, and (E) 51.4 ppm. The NOE data were acquired on a Bruker AMX 600 NMR spectrometer using a mixing time of 100 ms as a series of 45 complex (t_1) 2D experiments [96(t_2) × 2048(t_3)] using a spectral width of 6579 Hz in ω_1, 7463 Hz in ω_2, and 20000 Hz in ω_3. A skewed solid line in each plane indicates the diagonal peaks. The NOE cross peaks occur between protons at the diagonal peak frequencies and other protons along ω_3.

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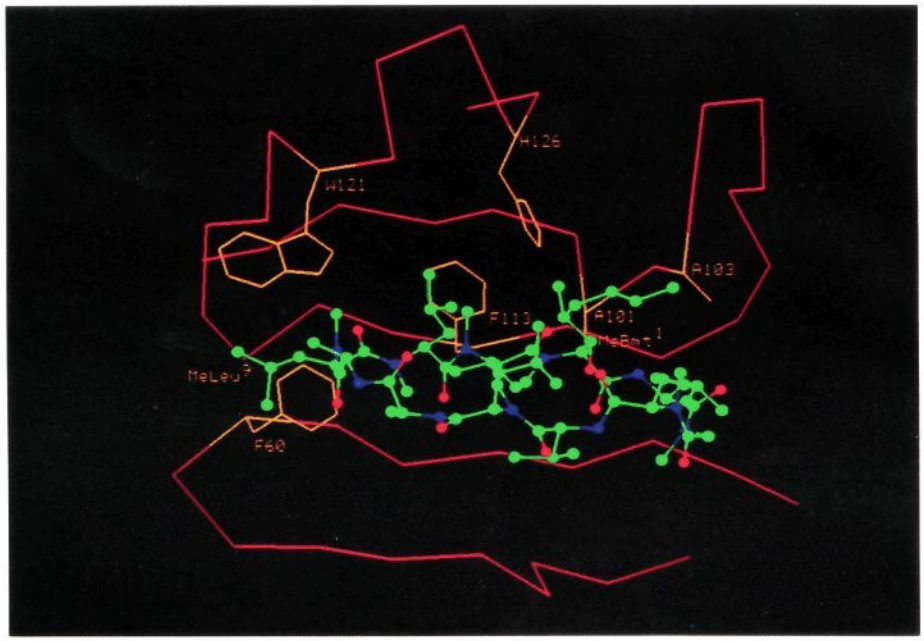

Figure 2. Portion of the CyP/CsA structure generated from the combination of NMR and X-ray data as described in the text. Only the α-carbon trace of the CyP regions involved in CsA binding is shown (red). The side chains of CyP showing NOEs to CsA are highlighted in yellow. The CsA heavy atoms are shown as a color-coded ball-and-stick model.

consisted of 500 cycles of constrained minimization followed by 100 steps of unconstrained minimization to relax the structure.

Figure 2 depicts the model of the CyP/CsA complex generated from the combination of NMR and X-ray data. As predicted from the changes in the chemical shifts of CyP observed upon CsA binding,[6,7] the portion of cyclophilin that binds to CsA is composed of four antiparallel β-strands (H54–I57, F60–G64, G96–N102, Q111–T116) and residues W121–H126. NOEs between cyclosporin A and F60, A101, A103, and F113 of cyclophilin define the binding orientation of CsA with respect to the CyP β-strands. The location of residues W121–H126 relative to CsA is characterized by several NOEs between the W121 aromatic ring and MeLeu[9] and MeLeu[10] CsA residues as well as NOEs between H126 (which has been implicated in the PPIase mechanism)[6] and MeVal[11] and MeBmt[1] of CsA. Consistent with the NMR data[3,4,17] and structure/activity relationships,[19] the MeLeu[9]-MeLeu[10]-MeVal[11]-MeBmt[1]-Abu[2] portion of cyclosporin A is binding to CyP. These CsA residues bind to a similar region of CyP as a tetrapeptide substrate (*N*-acetyl-Ala-Ala-Pro-Ala-amidomethylcoumarin)[6] but in an N- to C-terminal orientation opposite to that of the tetrapeptide. In the X-ray structure of the CyP/tetrapeptide complex, the N^{δ} of H126 was reported to be in close proximity ($\sim$6 Å) to the carbonyl carbon of the prolyl amide of the tetrapeptide. On the basis of our NOE data, the imidazole ring of H126 is in close proximity to MeBmt[1]($H^{\delta Me}$). Thus, from our NOE data and a comparison of the tetrapeptide/CyP X-ray structure (Figure 2, ref 6) and our model CyP/CsA structure, the MeBmt[1] carbinol group of CsA, which is required for immunosuppressive activity and CyP binding,[18] is located in approximately the same position and points in the same direction as the carbonyl group of the Ala-Pro peptide bond of the tetrapeptide substrate.[6]

PPIase mechanisms have been proposed that involve either a nucleophilic attack of a water molecule to form a tetrahedral intermediate that is stabilized by H126 and R55[6] or a twisted peptidyl-prolyl amide bond (induced fit mechanism).[19] In either case, the similar location of the CsA hydroxyl group and the carbonyl of the peptide bond that undergoes isomerization is consistent with cyclosporin A acting as a mimic of an intermediate or transition state in the PPIase reaction.[9] Unfortunately, a more detailed structural comparison of the bound tetrapeptide and CsA structures is unwarranted due to the expected inaccuracies of the model structures.

The approach used to generate the structure of the CyP/CsA complex from the bound conformation of CsA determined by NMR and the CyP X-ray structure using intermolecular NOEs represents a useful method of combining the results from these two complementary techniques. This approach may be generally applicable for generating structures of molecular complexes when protein/ligand NOEs and the structure of the uncomplexed protein are available.

Acknowledgment. We thank Robert Simmer and Rosalind Helfrich for the cloning and expression of cyclophilin, Tom Holzman and Jean Severin for the purification of cyclophilin, and Hengming Ke for the atomic coordinates of uncomplexed cyclophilin. P. Neri acknowledges support from a fellowship from the CNR, Italy (Bando No. 203.03.22, Com. Scienze Chimiche).

Supplementary Material Available: A complete list of intermolecular NOE-derived distance constraints used in the structure calculations (1 page). Ordering information is given on any current masthead page.

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