# Studying of Cyclosporin D by High Resolution NMR: Obtaining Information on the Spatial Structure

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## Introduction

Targeted synthesis of selective biologically active substances, i.e. substances that have no side effects, has been a point of interest for scientists for a long time. Many laboratories both in academics and in industry are aiming to establish a link between the structure of substances and their biological properties. Additional knowledge in the sphere of drug design [1] impacts chemistry, as well as biology and medicine. At first, scientists only paid attention to the chemical composition of substances, but they eventually realized that their molecular configuration is just as important. Although Barton [2] has clearly shown the role molecular conformation plays in chemistry and biology, there have only been weak attempts to study the relationship between conformation and biological activity [3].

There has been a great deal of interest in cyclic peptides as scaffolds in the development of drugs against difficult targets such as protein–protein interactions, based on the premise that large macrocycles are better suited to the inhibition of large binding surfaces. Solving conformations of cyclic peptides can provide insight into structure–activity and structure–property relationships, which can help in the design of compounds with improved bioactivity and/or ADME characteristics.

## Object

Considerable progress in transplantation of the last few decades is due to the development and introduction of immunosuppressive drugs to clinical practice, that increase the survival rates of both patients and transplants [4].

Cyclosporin D is a metabolite of cyclosporin A, an immunosuppressive drug that binds to cyclophilin, inhibiting the phosphatase activity of calcineurin in T cells. In the composition of CsD there are 11 amino acids, some of them have several instances:

1Bmt – 2Val – 3Sar – 4Mle – 5Val – 6Mle – 7Ala – 8D-Ala – 9Mle – 10Mle – 11Mva.

Different tendencies of cyclosporin molecules A, D etc. to formation of complexes with proteins (especially, cyclophilins) lead to observed differences in their biological activity. The reason for that lies in structural features of specific peptides, which in case of cyclosporin as a representative of cyclic peptides shows special properties [4]: relative stability within organism and the ability to adopt different conformations. Efficiency of CsA as an immunosuppressive drug is considerably higher than that of CsD. However, CsD interests us, because the question of significance of conformation as the main factor, which affects substance's properties, is raised. The only difference between CsD's and CsA's compositions is the second amino acid residue. The structure of CsA is presented below in figure 1; the picture also shows the part of the molecule, which is different in CsA and CsD.

## Method

Measurements were carried out on a Bruker Avance III HD 700 spectrometer. Signal assignment was made using a combination of 2D spectra: DQF-COSY, TOCSY, HSQC and HMBC, recorded at 25°C. Strong signal overlap regions hamper identification of some atoms, including Mle4 Hα and two protons at the double bond in Bmt1 (~5.35 ppm); atoms Bmt1 Hη, Hγ and $CH_2$δ all have close signals in the same region together with some of inequivalent $CH_2$

protons of other residues (1.6–1.65 ppm). Heteronuclear spectra were necessary to clarify these overlap regions, assign $NCH_3$ groups, and determine the place of each residue in the 11-member chain through the signals of carbonyl carbons. Homonuclear 2D spectra were recorded in the spectral window of 10x10 ppm and time domain size of 2048x512 points. HSQC spectra covered the spectral width of 10x140 ppm (centered at 65 ppm) with 2048x512 points; HMBC had the parameters 10x200 ppm (the center at 95 ppm) and 4096x512 data points. HMBC spectra were optimized for the long-range scalar coupling of 6 Hz.

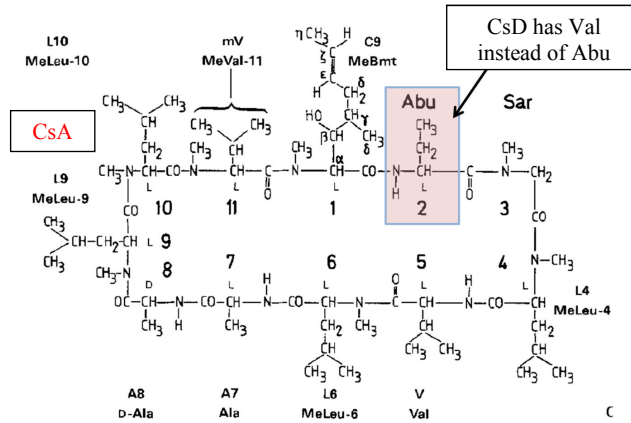


*Figure 1. The structure of CsA. The second residue of CsD is replaced with Val*

## Results

Cyclosporin D has been studied by NMR in $CDCl_3$. Chemical shift values were obtained from high resolution NMR spectra. Values for CsA were obtained earlier [5]. Results for CsD and CsA are shown in Table 1.

*Table 1. Chemical shifts for CsD and CsA*

| CsD | | | | | CsA | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Res. No. | Cα | Hα | C' | NH | Res. no. | Cα | Hα | C' | NH |
| 1 | 59.00 | 5.555 | 170.72 | | 1 | 58.97 | 5.461 | 170.6 | |
| 2 | 53.97 | 4.75 | 173.90 | 7.99 | 2 | 48.94 | 5.021 | 174 | 7.964 |
| 3 | 50.51 | 4.72 | 171.20 | | 3 | 50.5 | 4.725 | 171.4 | |
| | | 3.183 | | | | | 3.198 | | |
| 4 | 55.57 | 5.32 | 170.02 | | 4 | 55.63 | 5.334 | 170.2 | |
| 5 | 55.63 | 4.61 | 173.80 | 7.57 | 5 | 55.56 | 4.646 | 174 | 7.468 |
| 6 | 55.34 | 4.97 | 171.53 | | 6 | 55.48 | 4.973 | 171.8 | |
| 7 | 48.58 | 4.53 | 171.05 | 7.67 | 7 | 48.77 | 4.511 | 171.4 | 7.681 |
| 8 | 45.12 | 4.83 | 173.39 | 7.15 | 8 | 45.33 | 4.82 | 173.7 | 7.163 |
| 9 | 48.25 | 5.70 | 170.30 | | 9 | 48.35 | 5.687 | 170.5 | |
| 10 | 57.64 | 5.06 | 170.08 | | 10 | 57.69 | 5.069 | 170.3 | |
| 11 | 57.86 | 5.2 | 173.64 | | 11 | 58.06 | 5.119 | 173.7 | |

Then Table 2 was composed, consisting of the differences between the chemical shifts of the two studied peptides. Thus, the sites where the most noticeable changes of chemical shifts occur due to the replacement of the second residue can be revealed.

*Table 2. Differences between chemical shifts for CsD and CsA*

| ppm(CsD) – ppm(CsA) | | | | |
|---|---|---|---|---|
| Res. No. | Cα | Hα | C' | NH |
| 1 | 0.03 | **0.094** | **0.117** | |
| 2 | **5.033** | **-0.273** | **-0.098** | **0.031** |
| 3 | **0.016** | -0.003 | **-0.198** | |
| | | -0.015 | | |
| 4 | -0.057 | -0.012 | -0.176 | |
| 5 | **0.074** | **-0.032** | **-0.192** | 0.106 |
| 6 | -0.142 | -0.001 | -0.274 | |
| 7 | -0.187 | 0.021 | -0.35175 | -0.011 |
| 8 | **-0.208** | 0.015 | **-0.3085** | -0.01 |
| 9 | -0.102 | 0.011 | -0.19475 | |
| 10 | -0.047 | -0.009 | -0.223 | |
| 11 | -0.199 | **0.002** | **-0.055** | |

## Conclusions

Substitution of the second residue with different amino acids influences mainly the backbone chemical shifts in positions 2, its neighbors 1 and 3, and in more distant positions 5 (valine) and 8 (D-alanine). Residues 5 and 8 were the most sensitive to the amino acid substitution at the position 2. Further detailed information on the spatial structure is needed to clarify how the backbone and side chain orientations are altered, and how this may modify the biological behavior of cyclosporin.

## Acknowledgments

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