Studying cyclosporin D – micelle complex by high-resolution NMR: Obtaining information on the spatial structure

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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 [1].

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 [2].

For an in-depth understanding of the mechanism of cyclosporin's activity, it should be considered in combination with micelles. Since micelles can be used to model biological membranes (on a basic level), such an experiment will allow us to draw certain conclusions regarding the conformation of cyclosporin when interacting with a cell [3].

Cyclosporin D is a congener of cyclosporin A, an immunosuppressive drug that binds to cyclophilin, inhibiting the phosphatase activity of calcineurin in T cells. The molecule of CsD consists of 11 amino acids.

Different tendencies of cyclosporin variants A, D etc. to formation of complexes with proteins (especially, cyclophilins) lead to observed differences in their biological activity. CsD interests us because the question of significance of conformation as the main factor, affecting substance's properties, is raised. The only difference between CsD's and CsA's compositions is the second amino acid residue.

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. Also ROESY technique was used for obtaining structural parameters (distances between nuclei).

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.

In experiments with DPC micelles a severe overlap of signals in the high-field region (side chains) is observed; nevertheless, we can still assign the signals of the main chain. The assignment of the backbone signals should be performed anew, since the new spectrum is different from what we observed in chloroform. Since the spectra were recorded in heavy water, the signals of all α-CH groups appear quite well. Changes in their resonance frequencies allow concluding that the backbone conformation has become different after binding of the peptide molecules to the micelles, like it was reported in [4] for CsA.

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