

## A Study on the Structural Stability of the Peptide 2.0<sub>5</sub>-Helix by Infrared Absorption Spectroscopy

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### Introduction

C<sup>α</sup>-Tetrasubstituted residues are known to induce the onset of helical structures in peptides with sequences even as short as 4-5 residues [1]. Due to their rigidity, such conformationally-constrained oligopeptides are often employed as spacers [2], or as simple models to study the stability and behavior of biologically-relevant helical structures [3]. C<sup>α,α</sup>-diethylglycine (Deg) is one of such residues. Its homo-peptides were found to adopt a rather unusual helical conformation, the 2.0<sub>5</sub>-helix, also known as fully-extended conformation [4]. The 2.0<sub>5</sub>-helix, characterized by  $\phi$  and  $\psi$  backbone torsion angles of 180°, is indeed the longest conformation a peptide of a given number of residues can adopt. Recently, we found that Deg homo-peptides are also able to switch their conformation between 2.0<sub>5</sub>- and 3<sub>10</sub>-helices in response to a change in solvent polarity [5]. Here, we present preliminary results obtained in our FT-IR study on the stability and behavior of the (Deg)<sub>n</sub> 2.0<sub>5</sub>-helix in the solid state. We found that the conformation adopted by our model peptide **Deg<sub>5</sub>** [Tfa-(Deg)<sub>5</sub>-OtBu (Tfa, trifluoroacetyl)] in CDCl<sub>3</sub> solution is not maintained when it is deposited as a film, and that we can govern the conformational equilibrium by applying an electric field (EF) during the deposition process [6].

### Results and Discussion

To study the influence of both the physical state and an applied electric field (EF=7.5 V/m) on the 3D-structure adopted by **Deg<sub>5</sub>**, we performed FTIR experiments under different experimental conditions. We chose to employ this spectroscopy because peptides have several IR active vibrational modes, some of which (amide I at 1700-1600 cm<sup>-1</sup> and amide II at 1600-1480 cm<sup>-1</sup>) are sensitive to the variation of secondary structure [7].

Experimentally, the FTIR spectrum of **Deg<sub>5</sub>** in CDCl<sub>3</sub> solution at 0.1mM peptide concentration has been obtained in the transmission mode using a NaCl cell, while that of **Deg<sub>5</sub>** as a film has been recorded using an ATR apparatus equipped with a ZnSe cell. In the latter case, CDCl<sub>3</sub> peptide solutions were deposited on the cell, and the solvent allowed to evaporate in the presence or absence of an external EF.

Analysis of the amide I and II regions of the **Deg<sub>5</sub>** FTIR spectrum as a film was performed and compared with the spectrum of the same peptide in CDCl<sub>3</sub> solution. In solution, the number, position, and relative intensities of the absorption bands in the two spectral regions are diagnostic of the presence of a fully-extended conformation [8,9]. One clear proof for the onset of a 2.0<sub>5</sub>-helix is indeed the presence in the amide I region of the FTIR spectrum of two, strong peaks near 1650 and 1670 cm<sup>-1</sup>, as shown in Figure 1-I (dashed line).

For the peptide film, however, the FTIR spectrum in the amide I region is clearly different (Figure 1-I). The peptide changes its most populated conformation, assuming a mainly  $\alpha/3_{10}$ -helical structure (a strong absorption band for  $\alpha/3_{10}$ -helices is usually seen in the 1655-1670 cm<sup>-1</sup> range). The minor component at about 1680 cm<sup>-1</sup> may be assigned to type-III  $\beta$ -turn and/or non H-bonded carbonyl groups [10,11]. An inspection of the intensities of the amide II bands (Figure 1-II), in comparison with those in the amide I region, confirms this conclusion, as the strong band near 1490 cm<sup>-1</sup>, expected for a fully-extended conformation, is much less evident in the peptide film.

When an external electric field is applied during the process of film formation, the FTIR spectrum of **Deg<sub>5</sub>** seems to indicate a somewhat increased helical content. This conclusion stems from a decrease in the intensity of the band centered at about 1680 cm<sup>-1</sup>, attributed to the 2.0<sub>5</sub>-helix, and a concomitant

shift of the band in the amide II region to higher wavenumbers with respect to  $1500\text{ cm}^{-1}$ , confirming the reduced presence of fully-extended structures.

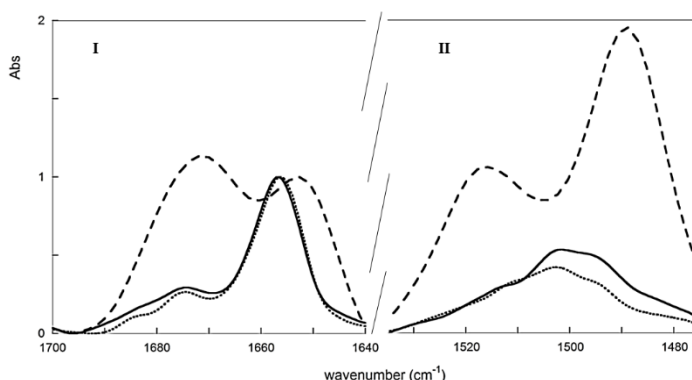


Fig. 1. Amide I (panel I) and Amide II (panel II) regions of the FTIR normalized spectra of *Deg5* ( $0.1\text{ mM}$ ) in  $\text{CDCl}_3$  solution (---) and in film obtained by a  $\text{CDCl}_3$  peptide solution in the absence (—) and presence (···) of an external electric field. Curves are normalized to the intensity at  $1656\text{ cm}^{-1}$  for film, and at  $1653\text{ cm}^{-1}$  for solution spectra.

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