P11 – KOKSCH

DETERMINATION OF THE ALPHA-HELIX PROPENSITY OF FLUORINATED AMINO ACIDS

LEARNED METHODS

- CD-spectroscopy
- Preparation of buffer solutions
- Concentration determination of a peptide solution with UV/VIS-spectroscopy
- Preparation of peptide solutions of different concentrations

EXPERIMENTAL PROCEDURE

The lyophilized peptide is dissolved in 1 M NaCl, 1 mM sodium phosphate, 1 mM sodium citrate, and 1 mM sodium borate buffer (pH 7.0) and the concentration of the obtained peptide solution is determined via the tyrosine absorbance in 6 M guanidinium chloride (ε_{276} = 1.455 $mol^{-1}cm^{-1}mL$) using PMMA cuvettes (10 mm path length, 1.5 mL). CD measurements are performed at peptide concentrations of 30, 50, and 80 µM at pH 7.0 and 0°C. CD spectra are recorded with a Jasco J-810 spectropolarimeter using Quartz cuvettes (1.0 mm path length). For each concentration at least 3 independent measurements are performed and the mean value is calculated. Data are collected from 250 to 200 nm at 0.2 nm intervals, 2 nm bandwidth, and 2 s response time. Spectra are background-corrected by subtraction of the corresponding buffer spectra. The measured CD data in mdeg are converted into molar ellipticity per residue [Θ]/ (10^3 deg cm^2 $dmol^{-1}$ $residue^{-1}$). Thus, the mean residue molar ellipticity is independent of the peptide concentration. The fractional helical content of the peptide (fhelix) is calculated from the mean residue molar ellipticity at 222 nm and the number of backbone amides (N = 19) using the equation fhelix = $[\Theta]_{222nm}/(40000(1-2.5/N))$. The α-helix propensity of the amino acid at the guest position **Xaa** is then calculated from the f_{helix} of the corresponding peptide based on a modified Lifson-Roig theory (Chakrabartty, A.; Kortemme, T.; Baldwin, R. L. *Protein Sci.* **1994**, *3*, 843-852; Doig, A. J.; Chakrabartty, A.; Klingler, T. M.; Baldwin, R. L. *Biochemistry* **1994**, *33*, 3396-3403; Andersen N. H.; Tong, H. *Protein Sci.* **1997**, *6*, 1920-1936.).