Introduction

Infrared absorption spectroscopy is a powerful analysis tool to study the secondary structure of proteins. The most prominent absorption band of proteins in the mid-IR spectrum is the concentration-dependent temperature-induced (EC-QCL) for mid-IR transmission measurements at high optical paths of the protein amide I band in aqueous solution, despite the strong water absorption in this spectral region.

In this work, we apply the EC-QCL-based IR transmission setup for secondary structure analysis of proteins and polypeptides in deuterated solutions at concentrations as low as 0.25 mg mL$^{-1}$. Dynamic IR spectra acquired with the laser-based setup show excellent comparability with spectra obtained by conventional FTIR spectroscopy. By example of the concentration-dependent temperature-induced α-β transition of poly-L-lysine (PLL), we demonstrate the low accessible concentration range for the EC-QCL setup.

Experimental Setup

- Laser-IR transmission measurements were performed in pulsed mode using an external-cavity quantum cascade laser (Daylight Solutions, USA) with a tuning range between 1730–1565 cm$^{-1}$.
- Custom-made temperature-stabilized sample cell enabled IR transmission measurements in deuterated solutions at a path length of 478 μm.
- Advanced data processing routine was developed in Matlab employing a Correlation Optimized Warping (COW)-based routine. Inherent mode-hop structures of the laser emission curve are utilized for a rubberband type alignment of consecutive single beam scans. Scan-to-scan alignment allows for scan averaging and background-to-sample alignment reduces the noise level in the absorbance spectrum.
- Wavenumber calibration is performed by referencing the position of the water vapour absorption bands to positions taken from a spectra database. This type of wavenumber calibration accounts for aberrations originating in the light source and during data acquisition.

Protein Secondary Structure

- QCL-based IR transmission measurements were successfully employed to identify characteristic spectral features of proteins with different secondary structures at protein concentrations as low as 0.25 mg mL$^{-1}$.
- Protein spectra acquired by EC-QCL transmission measurements show excellent agreement with absorbance spectra recorded by FTIR spectroscopy.

Conclusions & Outlook

- EC-QCL IR transmission spectroscopy was successfully employed for monitoring temperature-induced conformational changes of PLL in deuterated solution.
- The concentration dependence of the transition temperature between 0.25 and 10 mg mL$^{-1}$ was investigated by QCL-IR, FTIR and CD spectroscopy.
- QCL-IR measurements at biomolecule concentrations as low as 0.25 mg mL$^{-1}$ allow the combination of CD and IR spectroscopy, which is beneficial for comprehensive understanding of complex biological samples.

References


Financial support was provided by the Austrian research funding association (FFG) under the scope of the COMET programme within the research project “Industrial Methods for Process Analytical Chemistry - From Measurement Technologies to Information Systems (imPACts)” (contract #8433546).

Andreas Schwaighofer$^1$, Mirta R. Alcaráz$^{1,2}$, Can Araman,$^3$ Héctor Goicoechea$^2$, Bernhard Lendl$^1$

$^1$Institute of Chemical Technologies and Analytics, Vienna University of Technology, Getreidemarkt 9, A-1060 Vienna, Austria
$^2$Laboratorio de Desarrollo Analítico y Quimiometría, FBCB, Universidad Nacional del Litoral-CONICET, Ciudad Universitaria, 3000 Santa Fe, Argentina
$^3$Department of Chemistry, Institute of Biological Chemistry, University of Vienna, Währinger Straße 38, 1090 Vienna, Austria