Beyond FT-IR: EC-QCL based mid-IR Transmission Spectroscopy of Proteins in Aqueous Solution

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Introduction

Infrared absorption spectroscopy is a powerful analysis tool to study the secondary structure of proteins. The most prominent absorption bands of proteins in the mid-IR spectrum are the amide I and amide II bands. Amide I band (1600-1700 cm⁻¹) is mainly induced by C=O stretching vibrations and allows evaluating the secondary structure of proteins. In this spectral region, the most important difficulty of IR investigations of proteins in aqueous solution is the strong absorbance of the HOH-bending band that overlaps with the amide I band. The amide II band (1500-1600 cm⁻¹) is derived from N-H bending and C-N stretching vibration. It provides information about protein secondary structure, but the correlation is less straightforward than for amide I [1].

For routinely used FT-IR spectrometers employing low intensity thermal emitters as light source, suitable path lengths for transmission measurements of proteins in aqueous solution are restricted to <10 µm. Recently, a setup based on a tunable external-cavity quantum cascade laser (EC-QCL) for mid-IR transmission measurements of the protein amide I band was introduced, utilizing a cell of 38 µm path length for analysis of protein solutions at concentrations as low as 2.5 mg ml⁻¹ [2].

In this work, a new setup is presented based on the latest generation broadly tunable EC-QCL for mid-IR transmission measurements of the protein amide I and amide II bands in aqueous solution. A new step to detect and remove highly shifted scans was included in the data processing routine, based on the product of the correlation coefficients between all single beam scans [3]. The spectra measured with the new setup showed good agreement with spectra obtained by conventional FT-IR.

Experimental Setup

- Laser-based IR transmission measurements were performed using an external-cavity quantum cascade laser (Hedgehog Daylight Solutions, USA) with a tuning range between 1730-1470 cm⁻¹.
- The EC-QCL was operated in pulsed mode, at a pulse rate of 100 kHz and pulse width of 5000 ns, resulting in a duty cycle of 50 %.
- A custom-made transmission cell enabled IR transmission measurements at a path length of 33 µm. The temperature of the transmission cell was controlled with thermo electrical cooling.
- A sapphire window of 2.5 mm thickness and a grid mesh were used to attenuate spectral regions causing detector overload and unravel the amide I and amide II bands simultaneously.

Data Processing

- A custom-made processing routine of the recorded data for alignment of consecutive single beam scans was applied in MatLab, based on Correlation Optimized Warping (COW).
- At a tuning speed of 700 cm⁻¹ s⁻¹, multiple scans presented a significant misalignment in the wavenumber axis.
- A method based on the product of the correlation coefficients between all single beam scans was used to detect and remove highly shifted scans with a low similarity index (< 0.99).
- For noise evaluation, 100 % lines of water were calculated with and without correlation coefficient analysis. The root-mean-square noise (RMS) was improved when highly shifted scans were removed prior to scan-scan alignment by COW.

Protein Spectra

- QCL-based IR transmission measurements have been successfully employed to identify characteristic spectral features of amide I and amide II bands of two proteins with different secondary structures in aqueous solution.
- Protein spectra acquired by EC-QCL transmission measurements show excellent agreement with absorbance spectra recorded by FT-IR spectroscopy.

Comparison with FT-IR

- 100 % lines of water obtained with the new EC-QCL setup (Hedgehog laser) were compared with the previous EC-QCL setup (1st generation laser) and two FT-IR instruments.
- RMS-noise of 100 % lines for the new setup was 4 times lower than for the old setup and 8 times lower than for the routine FT-IR.
- The signal-to-noise (SNR) ratio was significantly improved with the new EC-QCL setup, when considering larger absorbance values obtained with path length of 33 µm compared to 8 µm in the FT-IR.

Conclusions & Outlook

- The latest generation broadly tunable EC-QCL was successfully applied for transmission measurements of the amide I and amide II bands of two proteins in aqueous solution.
- The exclusion of significantly shifted scans, detected by the product of correlation coefficients between all scans, allowed to improve the processing routine and the RMS-noise of 100 % lines of water.
- The SNR obtained for the new setup outpaced high-end FT-IR instrument with comparable measurement time.

References