Recent Advancements and Applications of EC-QCL based mid-IR Transmission Spectroscopy of Proteins

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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 amide I band (1600-1700 cm⁻¹) which is induced by vibrations of the peptide group and allows evaluating the secondary structure. 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.

For routinely used FTIR 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. These low optical paths considerable impair the robustness of the system and this has led to the introduction of a tunable external-cavity quantum cascade laser (EC-QCL) for mid-IR transmission measurements at high optical paths (38 µm) of the protein amide I band in aqueous solution [1].

This setup has been successfully employed to monitor dynamic changes of protein and polypeptide conformation induced by chemical denaturation and thermal perturbation [2,3]. Most recently, we applied EC-QCL mid-IR transmission spectroscopy for protein analysis in commercial bovine milk without sample pre-processing [4]. Casein, β-lactoglobulin and total protein content were determined by partial least squares (PLS) modelling after chemometric compensation of the matrix contribution employing science-based calibration (SBC).

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.
- A custom-made temperature-stabilized sample cell enabled IR transmission measurements at a path length of 38 µm.
- An advanced data processing routine was developed in Matlab employing a Correlation Optimized Warping (COW) 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 was performed by referencing the position of the water vapour absorption bands to band 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 2.5 mg mL⁻¹.
- Protein spectra acquired by EC-QCL transmission measurements show excellent agreement with absorbance spectra recorded by FTIR spectroscopy.

Monitoring Protein Conformational Change

- Dynamic protein conformational change was monitored by QCL-IR spectroscopy after exposure of α-chymotrypsin to 50% 2,2,2-trifluoroethanol (TFE).
- QCL-IR spectra reveal gradual α-helix aggregation, indicated by increasing bands at 1621 cm⁻¹ and 1697 cm⁻¹ accompanied by decreasing absorbance at 1655 cm⁻¹. Spectra were recorded between 2 and 240 min after dissolution in TFE.

Protein Quantification in Bovine Milk

- Partial least squares (PLS) regression models were employed for quantification of casein, β-lactoglobulin and total protein content in commercial bovine milk samples.
- QCL-IR spectra of homogenized milk were recorded after prior sample preparation.
- Science based calibration (SBC) was employed for compensating the background matrix signal.
- Concentration values obtained by QCL-IR spectroscopy and multivariate analysis show good agreement with reference methods (Kjeldahl, HPLC) requiring multiple sample preparation steps.
- Milk samples experiencing low (pasteurized, extended shelf life-filtered) and high heat load (extended shelf-life high temperature short time, ultra high temperature) were investigated.
- Discrimination between differently processed milk samples could be accomplished by evaluation of the β-lactoglobulin concentration.

Conclusions & Outlook

- EC QCL-based IR transmission measurements have been successfully employed to identify characteristic spectral features of proteins with different secondary structures and to monitor dynamic changes of protein secondary structure.
- Fast and simultaneous determination of casein, β-lactoglobulin and total protein content in commercial bovine milk samples has been accomplished by QCL-IR spectroscopy and multivariate analysis without prior sample preparation.
- The potential application of QCL-IR spectroscopy as a fast screening method for estimating the heat load applied to commercial bovine milk is demonstrated.

References