

In situ study of protein aggregates by FTIR spectroscopy coupled to multivariate analysis

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Keywords: Amyloid, biomarkers, infrared (micro-)spectroscopy, multivariate analysis, protein misfolding, systemic amyloidosis, spectroscopic biomarkers.

Deposition of misfolded proteins as extracellular amyloid aggregates is the pathological hallmark of systemic amyloidoses. In particular, light chain (AL) amyloidosis is the most common systemic form in industrialized countries. The mechanisms of amyloid formation in vivo and the bases of organ targeting and dysfunction are still open questions. In this perspective, important information could be provided by investigating amyloid deposits in their natural environment. To pursue this goal, in a previous work [1], we applied Fourier transform infrared (FTIR) (micro-)spectroscopy - a label-free vibrational tool that provides a biochemical fingerprint of the sample under investigation - to the in situ analysis of unfixed tissues recovered from the heart and subcutaneous abdominal fat of patients affected by AL amyloidosis. We demonstrated that the IR marker band of intermolecular β -sheets, typical of protein aggregates, can be detected in situ in LC amyloid-affected tissues. We also disclosed a possible role of lipids, collagen and glycosaminoglycans in amyloid deposition in vivo [1].

Recently, by means of Attenuated Total Reflection (ATR) FTIR spectroscopy - supported by multivariate analysis - we explored the possibility of discriminating between amyloid-positive and -negative samples, analyzing subcutaneous fat acquired by fine needle aspiration, the preferred screening tissue in suspected patients [2]. We found that the ATR-FTIR approach makes it possible to differentiate fat aspirates containing amyloid deposits, Congo Red (CR) positive, from control specimens, CR negative, with high sensitivity and specificity.

Interestingly, in agreement with the results obtained by FTIR microspectroscopy on heart and subcutaneous abdominal fat tissues [1], the wavenumbers most important for the discrimination indicated that changes both in the protein conformation and in resident lipids are intrinsic features of affected subcutaneous fat in comparison with the CR negative controls.

Overall, these results suggest that FTIR spectroscopy could be a useful tool for diagnostic purposes, as well as for the acquisition of novel knowledge on the molecular bases of the disease.

[1] D. Ami, et al. "In situ characterization of protein aggregates in human tissues affected by light chain amyloidosis: a FTIR microspectroscopy study" *Sci Rep.* 2016; 6: 29096.

[2] D. Ami, et al. "ATR-FTIR spectroscopy supported by multivariate analysis for the characterization of adipose tissue aspirates from patients affected by systemic amyloidosis." *Anal Chem.* 2019; 91: 2894-2900.

