Collagen per protein analysis in fresh, frozen and formalin-fixed tissues

Introduction

Extraction efficiency of collagen from tissues is low and variable, probably due to the presence of crosslinks in mature collagen. Therefore a common way for quantitative analysis of collagen is acid hydrolysis of the tissue followed by measurement of hydroxyproline, either by HPLC or by colorimetric assays. This method is suitable for tissues and tissue extracts.
However, the major way in which tissues are stored is as paraffin-embedded formalin-fixed tissue. Until now histological staining followed by image analysis is the method to analyze paraffin-embedded tissue. Such methods do give qualitative information on localization of the collagen in the tissue, but are only semi-quantitative.

We have devised a method for quantitative and easy analysis of collagen in formalin-fixed paraffin-embedded tissues or tissue sections. Neither removal of paraffin with toxic solvents like xylene, nor special equipment is required. The sensitivity of the method is such that it is applicable to quantify collagen using only a few 10 µm tissue sections.

In this application note we show data and methods of easy collagen per protein analysis in fresh or frozen tissue, formalin-fixed tissue and paraffin-embedded tissue.

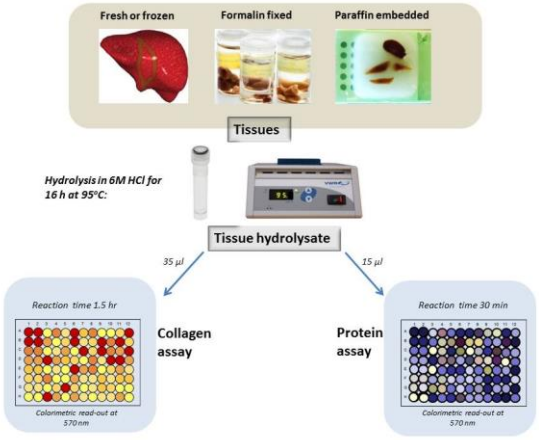

Fig. 1. Schematic overview of the analysis of total collagen and total protein in various types of tissues

Methods

See Fig.1 for a representation of the procedure. Five to ten 10 µm tissue sections (or a corresponding amount of fresh or formalin-fixed tissue) are transferred into a Sarstedt tube and upon addition of 150 µl 6M HCl hydrolyzed by o/n incubation at 95^{o}C in a heat block.

Upon hydrolysis, without any pretreatment, 35 µl is used for collagen quantification using the QuickZyme total collagen assay (assay time 90 min) and 15 µl is used in the QuickZyme protein assay (assay time 30 min), which has specifically been developed for protein analysis in acid hydrolyzates. The protein data are used as normalization for the unknown amount of tissue used for hydrolysis.

Results

Collagen and protein ratios are similar in frozen tissue, paraffinized and deparaffinized tissue sections

Treatment of samples, such as formaldehyde fixation, paraffin embedding and xylene treatment to deparaffinize the samples did not affect collagen and protein analyses (Fig 2).

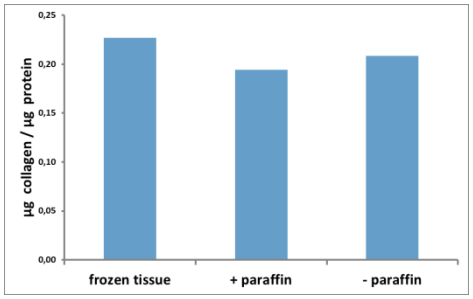

Fig. 2. Collagen/protein ratios in tissue processed in various ways
Collagen was analyzed in formaldehyde-fixed paraffin-embedded human arterial tissue. Either frozen tissue, ten 10 µm paraffin embedded tissue sections or ten 10 µm tissue sections pre-treated with xylene to remove paraffin were hydrolyzed and further analyzed using the QuickZyme total collagen assay and QuickZyme protein assay

Collagen per protein gives similar results as collagen per wet weight analysis

Collagen per mg tissue wet weight and collagen per mg protein give comparable results (see table 1A and 1B). Since not for all applications (e.g. when using formalin-fixed tissue) the wet or dry weight can be determined, the protein analysis in acid hydrolyzate gives new opportunities for collagen analysis in tissue.

Organ	lung	kidney	skin	liver	tendon
Collagen µg/mg wet weight tissue	10	5	150	1	300

Table 1A. Collagen per wet weight analysis of various mouse tissues

Organ	lung	kidney	skin	liver	tendon
Collagen µg/mg protein	60	34	333	5	656

Table 1B. Collagen per protein analysis of various mouse tissues

Collagen and protein analysis in sections from various types of (fibrotic) tissues

Starting from tissue sections, examples are given for mouse kidney fibrosis (UUO), lung fibrosis (bleomycin-induced), and liver fibrosis (NASH). Total collagen/total protein was measured from ten 10 µm paraffin embedded sections (Fig. 3) , and was found to be increased under fibrotic conditions.

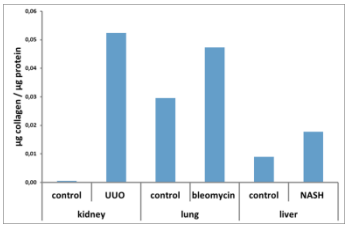

Fig. 3 *Collagen/protein ratios in tissue processed in various fibrotic and non-fibrotic tissue*
Total collagen/total protein was analyzed in formaldehyde-fixed paraffin-embedded tissues from mouse kidney fibrosis (UUO vs Sham) lung fibrosis (bleomycin vs PBS) and diet-induced NASH (western diet plus cholesterol vs control). Ten 10 µm tissue sections were hydrolyzed and analyzed using the QuickZyme total collagen assay and protein assay

Conclusions

Quantification of collagen and total protein in fresh, frozen or formalin-fixed paraffin-embedded tissues is now possible in a fast and easy assay format, without the need for HPLC or other special equipment.

QuickZyme Biosciences has developed a set of assays for the analysis of collagen from any species, each with a different application area

Collagen (related) assays available at QuickZyme Biosciences

- *QuickZyme Soluble collagen assay*
- *QuickZyme Total collagen assay*
- *QuickZyme Hydroxyproline assay*
- *QuickZyme Protein assay*

QuickZyme Soluble collagen assay

This assay recognizes soluble or (acid/pepsin) solubilized collagen.
The assay is colorimetric, has a 96-well plate format and is based on precipitation of collagen with Sirius-Red. This dye can bind the side-chain groups of basic amino acid residues. The dye is released from the precipitated complex at high pH followed by colorimetric detection. Sirius Red based assays recognize collagen, but also some other matrix related proteins may co-precipitate.
Application: The assay is used for the measurement of (soluble) collagen in e.g. cell culture media, and (acid or acid/pepsin) solubilized collagens e.g. from cellular extracts.

QuickZyme Total collagen assay

This assay recognizes all types of collagen irrespective of its form (mature, immature, procollagen, degraded collagen, cross-linked collagen, collagen from various sources).
The assay is colorimetric, has a 96-well plate format, and is based on the quantification of hydroxyproline, an amino acid exclusively occurring in collagen. Hydroxyproline is released from collagen upon acid hydrolysis of the collagen containing sample. Hydrolysis is carried out at 95 ^{0}C, and the product can directly be used for hydroxyproline analysis, without further washing or drying steps. The analysis is based on established Chloramine T/DMBA methodology.
Application: The assay is used for the measurement of total collagen. Since the first step is complete hydrolysis of the sample, difficulty in extraction of collagen plays no role. The assay is applicable for all types of samples, including tissue.

QuickZyme Hydroxyproline assay

This assay is similar to the total collagen assay, with the difference that no protocols and materials are included for collagen hydrolysis and instead of a collagen standard a hydroxyproline standard is provided.
Application: This assay has the same application area as the total collagen assay, but is intended for customers who have their own hydrolysis method, or have a collection of hydrolyzed samples to be analyzed.

QuickZyme Protein assay

This assay measures free amino acids resulting from hydrolysis of protein. The assay is colorimetric and can directly be performed on acid hydrolyzates.
Application: This assay is intended to measure total protein in acid hydrolyzates and provides normalization of the Total collagen and Hydroxyproline values when weight based normalization is not possible, inconvenient or too variable.

For more detailed information (including assay manuals) on
QuickZyme Soluble collagen assay: www.quickzyme.com/products/collagen-assay
QuickZyme Total collagen assay: www.quickzyme.com/products/total-collagen-assay
QuickZyme Hydroxyproline assay: www.quickzyme.com/products/hydroxyproline-assay

QuickZyme Biosciences
P.O.Box 2215
2301 CE Leiden
The Netherlands
T: +31-88-866 6114 / 6024
F: +31-88-866 0609
www.QuickZyme.com
E-mail: info@QuickZyme.com