EASY MEASUREMENT OF COLLAGEN IN TISSUES

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ABSTRACT

The quantification of collagen is of major importance in a wide range of research areas, including the study of diseases such as fibrosis as well as in tissue engineering. Especially collagen determination in tissues is difficult due to limitations in extraction procedures. A common way to analyze collagen in tissues is therefore measuring hydroxyproline after hydrolysis of the tissue. However, this method is laborious and requires special equipment for the removal of acid. This makes this analysis far from daily lab routine.
To circumvent the issue of acid removal we have developed a new hydroxyproline based collagen assay, which does not need special equipment and thus makes collagen analysis available for any research lab. The assay has a 96-well plate format, is easy and after acid hydrolysis data are obtained within 2 hrs. The assay is applicable to all types of samples: tissues, cell cultures and tissue engineered constructs.

INTRODUCTION

Collagens are the most abundant proteins in the vertebrate body, constituting about 30% of the total body protein. They play an important role in tissue structure and have many other functions such as in cell growth, differentiation, tissue repair and many pathological conditions.
Collagens are a family of extracellular matrix proteins; in vertebrates at least 27 collagen types with 42 distinct polypeptide chains are identified. Characteristic for collagen is the presence of hydroxyproline residues needed for stabilization of the collagen triple helix.
Collagen types I, II and III are most abundantly present in tissues.
Collagen is a molecule which is difficult to purify and to analyze. This is partly due to the extensive network that is formed by collagen molecules via different types of crosslinking which makes the collagen molecules insoluble and difficult to extract.

METHODS

A new assay was developed for the analysis of collagen in all kind of samples. The assay is based on the quantification of hydroxyproline, an amino acid exclusively occurring in collagen. Hydroxyproline is released upon acid hydrolysis of collagen. The hydroxyproline residues are modified using chloramine T/DMBA, resulting in a colored product which can be measured at 570 nm. Some characteristics of the assay: - 96-well plate format; - hydrolysis performed at 95°C; - no washing or drying steps upon hydrolysis. See Figure 1 for an assay overview.

Assay principle

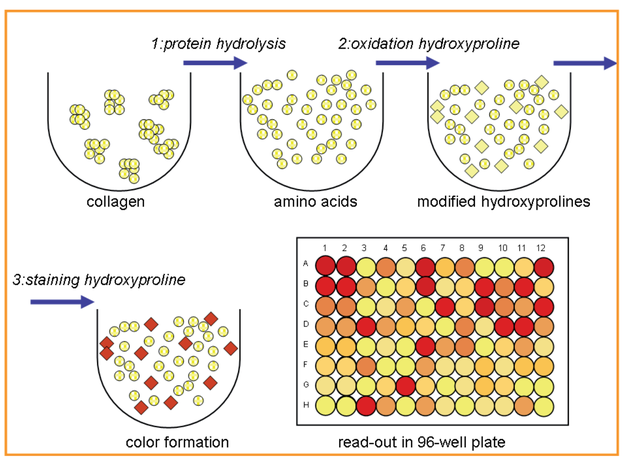

Figure 1: *Assay principle*

RESULTS

Collagen extraction

Extraction of collagen from tissue is difficult and inefficient. Extraction with acetic acid (+/- pepsin) as used for Sirius Red based assays resulted in solubilization of only a small fraction of the collagen (Fig.2). Similar results are obtained for solubilization by GuHCl (Fig. 2) or 1% SDS (data not shown).

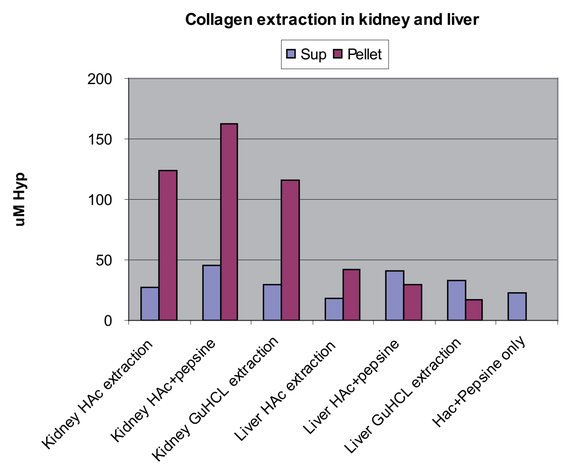

Figure 2: *Collagen extraction efficiency in kidney and liver tissue using various extraction methods*

New assay: assay development

A new assay was developed for the analysis of collagen in all kind of samples (see under methods).

New assay:hydrolysis temperature

We tested acid hydrolysis (using 6M HCl) efficiency of collagen at different temperatures. It was observed that at 95°C the same efficiency was observed as at regularly used 110°C (Fig. 3).

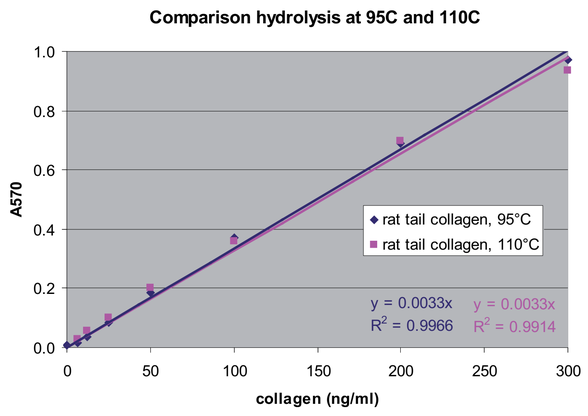

Figure 3: *Comparison collagen hydrolysis efficiency at 95°C and 110°C*

New assay: chromogenic read-out vs HPLC

In the validation of the new assay both purified collagen (data not shown) and cartilage (Fig. 4) were hydrolyzed and analyzed either by the new assay or by HPLC. It was observed that with both sample types similar results were obtained.

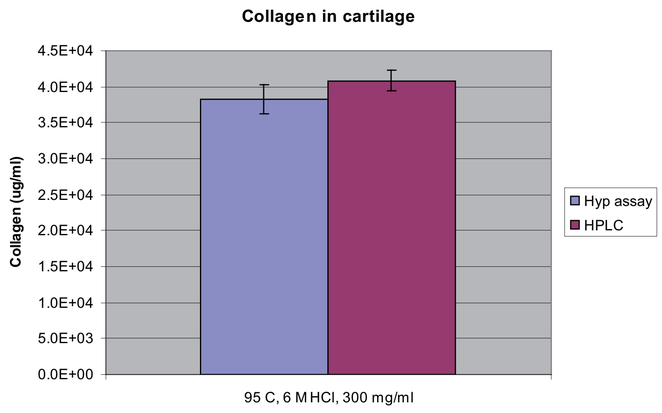

Figure 4: *Cartilage collagen content as measured by the new assay and by HPLC*

DISCUSSION

Extraction of mature tissue collagen for quantification is very difficult; only full hydrolysis of the tissue gives reliable results. This may be due to the extensive crosslinking that occurs during collagen maturation. Complete hydrolysis followed by hydroxyproline analysis may therefore be the only method available. However, this method is laborious and needs special equipment for drying and washing the acid hydrolysate.
We developed a chromogenic assay, which does not need this special equipment. The assay was validated and gives similar results as HPLC, both using purified collagen and tissue.

CONCLUSION

Quantification of collagen in tissues and other samples is now possible without the need for HPLC or special equipment.

The assay is made commercially available in kit format by QuickZyme Biosciences (www.quickzyme.com)