Paper A23

Studies of Assembly and De-assembly of Collagen

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1. Introduction

Collagen type I is the main component of skin. There are still unanswered questions in the mechanism of tanning that make it necessary to continue research on this topic and try to understand tanning or rather the interaction between collagen and tanning agent better.

2. Materials and methods

We study the *in vitro* self-assembly of calfskin collagen from monomers to fibrils by UV/Vis spectroscopy and atomic force microscopy (AFM). Fibrillation in an acidic monomer solution is initiated by increasing the temperature to 30 °C and pH to neutral conditions [1].
The schematic build-up of fibrils out of monomers via microfibrils is shown in Figure 1. Iin Figure 2, the assembly process is displayed in the kinetic spectra taken by UV/Vis spectroscopy. For spectroscopy, the unspecific size effect which is measurable at wavelengths between 200 nm and 400 nm is used. Monomers do not scatter light at 340 nm whereas the fibrils do. The delayed slope (lag time) proves that there is a transition state between monomers and fibrils.

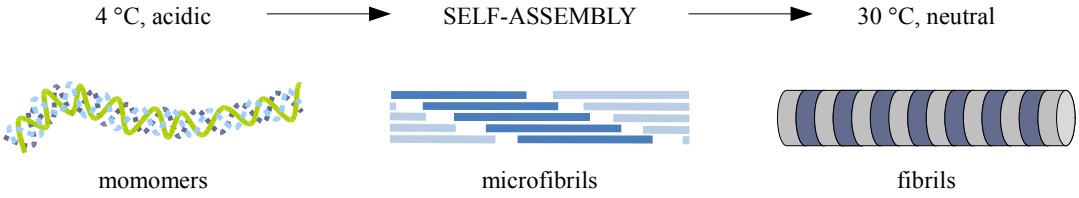

Figure 1. Schematic representation of the self-assembly of collagen.

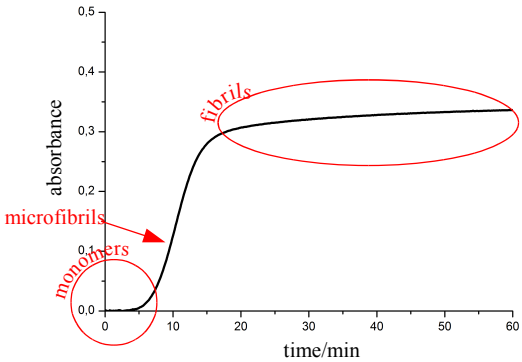

Figure 2. Time dependence of the assembly of collagen from monomers to fibrils.

Spectroscopic measurements of turbidity allow conclusions of the interaction between tanning agent and collagen at different hierarchical levels. To this aim, changes in the lag times and in the height of the turbidity plateaus are detected as function of the amount and type of additive [2]. The obtained results are analysed by means of a simple model, assuming so-called

microfibrils as an essential intermediate. Changes in the morphology of the assembled fibrils are - as complementary information - studied by AFM.

Further, we developed an additional method, to study the de-assembly of fibrils, where monomers get removed from the fibrils at low pH values. The obtained time-dependent turbidity data provide information about the stringency of the binding of additive to collagen [3].

3. Results and discussion

Tanning agents considerably influence the self-assembly and de-assembly of collagen. Polymers based on acrylates are used in tanning processes. In particular, we studied the influence of polyacrylic acid and polymethacrylic acid which differ only in one methyl side chain in their structure but in their tanning properties. Polymethacrylate has a large impact on the kinetics, whereas the use of polyacrylate leads to considerable changes in the morphology of the assembled fibrils [2].

The graphs presented in Figure 3 show assembly kinetics carried out with additives of up to 2 µmol polyacrylic acid (left) and polymethacrylic acid (right). In black, a standard curve as reference is shown. Longer lag times caused by polyacrylate as well as polymethacrylate can be observed. This effect is marked by the red arrows. When adding polymethacrylic acid this extension effect is much stronger.

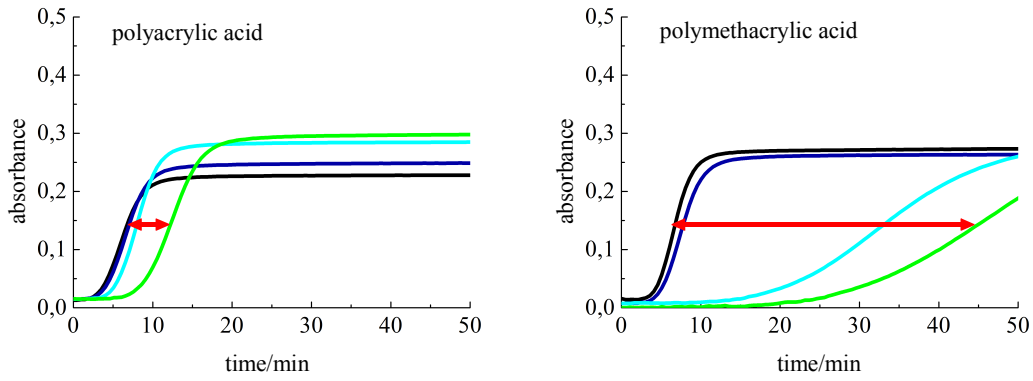

Figure 3. UV/Vis spectra of the assembly in presence of no (black), 0.1 µmol (dark blue), 1 µmol (light blue) and 2 µmol (light green) polyacrylic acid (left) and polymethacrylic acid (right).

As a second result, here we present AFM images that show the assembly products (Figure 4). In contrast to the fibril shown in the left image which was assembled with standard conditions, one can see sticking out ends at the fibril formed in presence of polyacrylate (right image) [3]. By the use of polyacrylic acid repulsion or defective assembly occurs which gives additional scattering centres, and thus, explains the enhanced plateau level in the UV/Vis spectra.

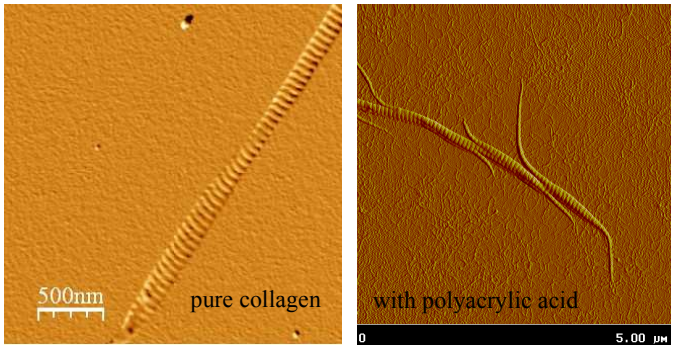

Figure 4. AFM images of collagen fibrils assembled without (left) and with 1 µmol polyacrylic acid (right).

Data obtained from de-assembly experiments are displayed in Figure 5. With increasing time, the concentration of collagen increases because more and more monomers become removed from the fibril matrix when 100 mM hydrochloric acid is added. This rise in the collagen concentration is measured in flow via the intensification of the peak at 200-220 nm caused by the peptide bond.

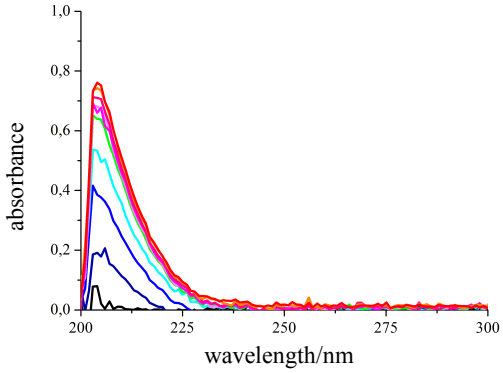

Figure 5. Increasing peptide peak during de-assembly of collagen.

4. Conclusions

The alternative approach of studying assembly and de-assembly of collagen with additional compounds provides insight into molecular level interaction of tanning agent and collagen matrix, and thus, helps to understand tanning better. For particular examples, we discuss results obtained in this combined theoretical-experimental approach.

5. References

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6. Acknowledgements

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