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Study of the interaction between collagen and collagenase molecules by Dynamic Light Scattering in the presence of various modifiers

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The aim of this work is to investigate the interaction between collagen and collagenase molecules in the presence of various activators, inhibitors and tanning agents by dynamic light scattering in buffer solutions. It is necessary to identify and analyze the nature of the interaction as a function of time.

Collagen-Collagenase Interaction in Medicine

Today in medicine enzyme drugs based on the collagenase enzyme are widely used for treatment many diseases in various fields:

- Application in oncology, ophthalmology, surgery, cosmetology
- Therapy of purulent and burn wounds, frostbite
- Treatment of necrosis in diabetes mellitus
- Treatment of spinal disc herniation
- Treatment for Dupuytren's syndrome

Results

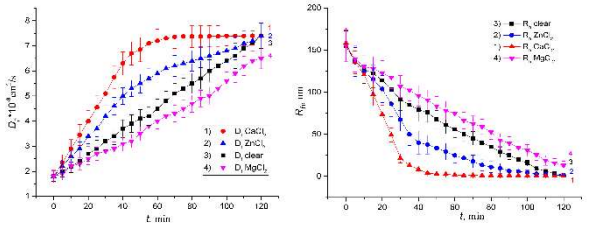


Fig. 1. Time dependences of D_t and R_h in collagen and collagenase buffer solutions with the addition of activators ($CaCl_2$, $ZnCl_2$) and inhibitor ($MgCl_2$)

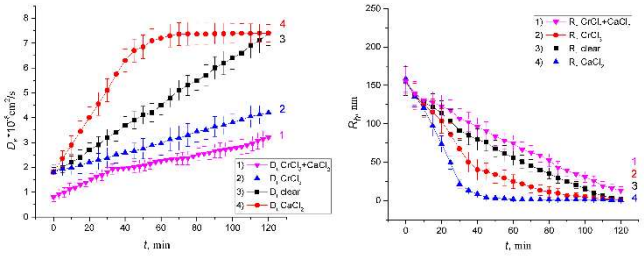
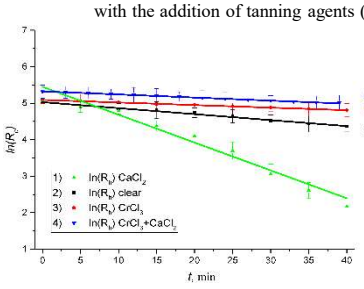


Fig. 2. Time dependences of D_t and R_h in collagen and collagenase buffer solutions with the addition of tanning agents ($CrCl_3$) in different variations

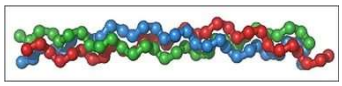


Solution	k_1
Col+coz	0.02
Col+coz+$CaCl_2$	0.07
Col+coz+$CrCl_3$	0.006
Chromed Col+coz+$CaCl_2$	0.009

Fig.3. lnR on time dependencies, where R_h is hydrodynamic radius of scattering particles, in pure "collagen+collagenase" solution, with added $CaCl_2$ and $CrCl_3$, "chromed collagen+collagenase" with $CaCl_2$.

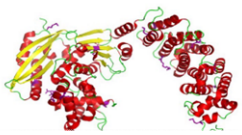
Investigated Objects

collagen



the most abundant protein
is 30% by weight of all proteins
molecular weight 300 kDa
length 300 nm

collagenase



cleaves the collagen protein
molecular weight 79,8 kDa
activator is Ca^{2+}

Dynamic Light Scattering

Allows to define:

- Diffusion coefficient D_t
- Hydrodynamic radius R_h

Experimental equipment 'Photocor Complex'

- Photocurrent power spectrum - Lorentzian curve with half-width $\Gamma=q^2D$
- Correlation function - exponential with relaxation time $t_{rel}=1/\Gamma$

$$D_t = \frac{k_B T}{6\pi\eta R_h}$$

Conclusions

It was found that the addition of $CaCl_2$ and $ZnCl_2$ activators increases the rate of collagenolysis by 2 and 1.5 times, respectively. In turn, the addition of the inhibitor $MgCl_2$ slows down the rate of collagenolysis by 1.3 times. In solutions containing $CrCl_3$, the decrease in R_h is less noticeable, which indicates the formation of collagen crosslinks. For a more accurate estimation process were calculated rate constants k_1 for reactions.

Cr^{3+} really strengthen the collagen structure, and the rate of collagenolysis is reduced by 70%. The reaction rate can be increased by 50% by adding Ca^{2+} or Mg^{2+} to the solutions. However, the rate of collagenolysis that is observed in a pure solution is still not reached. The maximum rate of breakdown of protein molecules is achieved by the activation of collagenase by Ca^{2+} ions.