Study of the interaction between collagen and collagenase molecules by optical methods in the presence of chromium ions

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Application in medicine:

1. Application in oncology, ophthalmology, surgery, dermatology.
2. Therapy of purulent and burn wounds.
3. Treatment of fibrosis of the liver and lungs.
4. Treatment of necrosis in diabetes mellitus.
5. Treatment of herniated discs of the spine.
6. Treatment of Dupuytren's contracture and others.

Purpose of study:
To evaluate the effect of chromium ions on the rate of biodegradation of type I collagen molecules under the influence of the collagenase enzyme in solutions using the method of dynamic light scattering.
Collagenolysis (biodegradation of collagen protein)

Activators: Ca$^{2+}$, Mg$^{2+}$, Zn$^{2+}$

Modifiers: EDTA, Cr$^{3+}$

Inhibitors: EDTA

Collagenase breaks down the collagen polypeptide chain in more than 200 sites [3]
Experimental results
pH dependence of the translational diffusion coefficient $D_t$ and hydrodynamic radius $R_h$ in collagen and collagenase buffer solutions with the addition of various salts ($\mu=10^{-3}$ mol/l, $c_{col}=0.03$ mg/ml)

$$D_t = D_0 \{1 + (2BM - [\eta])c\}$$
Temperature dependences of collagen and collagenase solutions

Logarithmic dependence of $R_h$ for collagen and collagenase solutions at different temperatures: $22^\circ C$ (1), $30^\circ C$ (2), $40^\circ C$ (3).

<table>
<thead>
<tr>
<th>Temperature, $^\circ C$</th>
<th>$k_1$, min$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>0,012±0,001</td>
</tr>
<tr>
<td>30</td>
<td>0,031±0,003</td>
</tr>
<tr>
<td>40</td>
<td>0,054±0,003</td>
</tr>
</tbody>
</table>

Collagen and collagenase solution at $T = 40^\circ C$. Collagen denaturation and precipitation is observed.

Time dependence of $D_t$ for collagen and collagenase solutions at different temperatures: $22^\circ C$ (1), $30^\circ C$ (2), $40^\circ C$ (3).
Chromated Collagen Research

Time dependencies $D_t$ and $\ln R_h$ in collagen solutions with the addition of various chromium salts $Cr^{3+}$:

![Graphs showing time dependencies $D_t$ and $\ln R_h$ in collagen solutions with various chromium salts.]

Cr$^{3+}$ ions of various salts have the same effect on the collagenolysis process.
Time dependencies $D_t$ and $\ln R_h$ in collagen and collagenase solutions:
1 – with the addition of $\text{CrCl}_3$, 2 – $\text{Col}_{Cr}$ with the addition of $\text{CaCl}_2$, 3 – clear, 4 - with the addition of $\text{CaCl}_2$

Collagenolysis slows down 3.8 times in the presence of $\text{CrCl}_3$. $\text{Col}_{Cr}$ is less susceptible to biodegradation under the influence of the enzyme, but when $\text{CaCl}_2$ is added to the chromium-plated protein, the $R_h$ dependence reaches the values that were observed in a pure solution after 90 minutes.

<table>
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<tr>
<td>collagen + collagenase</td>
<td>0.023±0.002</td>
</tr>
<tr>
<td>collagen + collagenase + $\text{CaCl}_2$</td>
<td>0.055±0.004</td>
</tr>
<tr>
<td>$\text{Col}_{Cr}$ + collagenase</td>
<td>0.006±0.001</td>
</tr>
<tr>
<td>$\text{Col}_{Cr}$ + collagenase + $\text{CaCl}_2$</td>
<td>0.009±0.001</td>
</tr>
</tbody>
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When the ZnCl₂ activator is added to the chromium-plated protein, the biodegradation of collagen molecules does not reach the final value observed in pure solution. Thus, Col_Cr is more resistant to biodegradation in solutions containing Zn²⁺ ions than in solutions with the addition of Ca²⁺ ions.

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<td>collagen + collagenase</td>
<td>0,023±0,002</td>
</tr>
<tr>
<td>collagen + collagenase + ZnCl₂</td>
<td>0,031±0,002</td>
</tr>
<tr>
<td>Col_Cr + collagenase</td>
<td>0,006±0,001</td>
</tr>
<tr>
<td>Col_Cr + collagenase + ZnCl₂</td>
<td>0,008±0,001</td>
</tr>
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</table>

When the ZnCl₂ activator is added to the chromium-plated protein, the biodegradation of collagen molecules does not reach the final value observed in pure solution. Thus, Col_Cr is more resistant to biodegradation in solutions containing Zn²⁺ ions than in solutions with the addition of Ca²⁺ ions.
Main results and conclusions

• It was found that Ca\(^{2+}\), Zn\(^{2+}\), Mg\(^{2+}\) and Cr\(^{3+}\) ions do not have a significant effect on collagen and collagenase molecules separately.

• It was found that heating collagen and collagenase solutions (from 22 to 40°C), as well as an increase in the concentration of the enzyme, accelerates the process of protein breakdown.

• It was revealed that chromium ions of various salts (CrCl\(_3\), Cr(NO\(_3\))\(_3\), Cr(CH\(_3\)CO\(_2\))\(_3\)) have approximately the same effect on the collagenolysis process.

• It was found that in the presence of chromium ions, the rate of collagen biodegradation decreases by 3.8 times compared to a pure solution, which indicates the strengthening of the protein structure.

• It was found that when Ca\(^{2+}\) and Zn\(^{2+}\) ions are added to a solution of chromium-plated collagen and collagenase, the reaction rate increases by 1.5 and 1.3 times, respectively, and decreases by 2.6 and 2.9 times, respectively, compared to a pure solution. non-chrome protein.
THANKS FOR ATTENTION!