PROTEIN AND AMINO ACID CHEMICAL STUDIES

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Abstract

A cross-linking procedure not deteriorating the physico-chemical characteristics of wool has been elaborated. The thermal destruction processes in wool have been clarified. Solutions have been found for accelerating and inhibiting reactions taking place between wool and formaldehyde. The reactions between L-lysine and formaldehyde as well as L-arginine and formaldehyde have been identified. Procedures have been elaborated for the practical utilization of the reaction products.

Introduction

The work which began in 1970 was strongly connected to international research activities in the field of keratine (wool) chemistry. The three-dimensional tertiary structure of wool together with morphological features were already clear at that time.

The bonds fixing this tertiary structures, the amino acid sequences and the role of the accessibility of extrafunctional groups have already been known.

Our objective was to improve physico-mechanical properties, thermal and chemical stability and dyeability of wool keratine by chemical and physical modifications.

These modifications have been checked on other proteins and amino acids by model reactions.

3.1 Thermolysis of wool keratine

On chemically pretreated wools with various moisture contents the effect of thermal treatments on keratine has been studied in the temperature range of 110—230 °C for short periods (30—180 s). Thus processes taking place in industrial thermal fixation have been modelled [1].

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In the presence of air, a thermal treatment above 150 °C even for only 30 s leads to irreversible changes in wool keratine.

The structural water is liberated in the first 30 s irreversibly at 150—160 °C.

The decrease in the solubility in urea bisulfite is the largest in the first 30 s independently of the temperature of the heat treatment.

During thermal treatments, new cross-links (amide bonds, lanthyonine, lysino-alanine) where formed in wool keratine.

The increase in the acidic and alkaline dye-uptake proves that the number of acid and basic groups increased in the wool due to the hydrolysis of the peptide bonds.

3.2 Reactions of keratine with mono-, bi- and polyfunctional compounds

In choosing the compounds, we strived for possibilities of high reactivity, dimensions corresponding to the spatial structure of keratine and fibre protective reactions.

Without completeness, we found the following compounds or their combinations suitable for the treatment: F-DNB (1-fluor, 2,4 dinitro benzene), FF-DNB (1,5 difluoro, 2,4 dinitro benzene), FF-sulfon (4,4 difluoro, 3,3 dinitro diphenyl sulfon), F-DNB + formaldehyde, FF-DNB + formaldehyde, F-DNB, FF-DNB + β mercaptoethanol, DCHCD (dicyclohexyl carbodiimide), primary, secondary and tertiary amines, TMAH (tetramethyl ammonium hydroxide), isocyanates, maleic acid anhydride, mixed anhydride, phosgene, phosphasine, glutaric aldehyde, glyoxal, methyl glyoxal, malonic aldehyde, formaldehyde solution and [2] specific catalysts (tertiary amines, crown ethers, H_2O_2, HCOOH), treatment with paraformaldehyde in vacuum (gas phase treatment), acidic, reactive and dispersion dyes, sodium bor hydride [2, 3, 6],

By comparing the chemical and physical characteristics of wools modified with the above compounds we chose those modifying controllably the properties of wool.

The treatment with dilute (0.12 mol/l) formaldehyde solution in the presence of 0.02 mole H_2O_2 and 0.04 mole formic acid proved to be such a treatment.

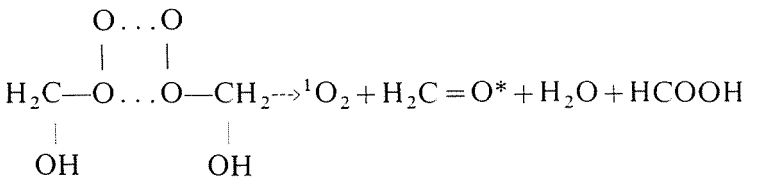
Formaldehyde activated by H_2O_2 reacts quickly with the basic amino acid segments of keratine and stabilizes wool by cross-linking.

Singlet oxygen ($^1O_2$) is also formed in the reaction, thus the wool, in addition to the significant increase of its tear resistance, also becomes whiter.

The singlet oxygen of high energy emits a red chemoluminescence at 633 nm in the emission band of 21 000 cm^{-1}.

$$2\,^1O_2 \dashrightarrow 2O_2 + h\nu\ (633\ nm)$$

The singlet oxygen and the activated formaldehyde are formed by the decomposition of a transition bis-hydroxymethyl diperoxide adduct:



This decomposition process is strongly catalyzed by the basic ε-amino groups of L-lysine present in wool.

The activated formaldehyde formed in the decomposition reaction is richer in energy by 3 eV (302 kJ/mol) than the unactivated formaldehyde, thus the former is much more reactive.

Activated formaldehyde emits a blue light measurable at 420 nm.

$$H_2C=O^* \dashrightarrow H_2C=O + h\nu \ (420 \text{ nm}).$$

The treatment with formaldehyde excited by H_2O_2 improves significantly certain physico-mechanical properties of wool. This advantage can be utilized in the treatment of Hungarian wools.

In the industrial check-up of the method, the treatment was carried out simultaneously with washing in the steep box. Thereby the rendement increased by 3—4%. This ensures 30—40 tons increase in rendement for 1000 tons of washed wool which corresponds to about 10 million forints.

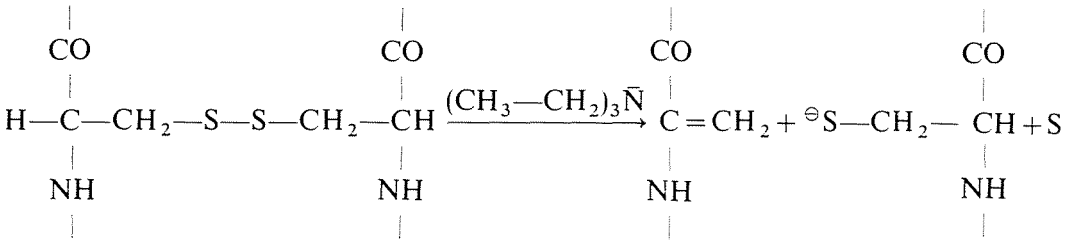
A para-formaldehyde treatment of wool in vacuum provided also favourable results [6]. At 65—85 °C, in vacuum, due to its high tension, the very reactive nascent formaldehyde is liberated from paraformaldehyde by its continuous decomposition.

$$HO(CH_2O)_nH \xrightarrow{60-100\ ^\circ C} n\ CH_2O + H_2O$$

Different catalysts (e.g. triethylamine) increase significantly the efficiency of the treatment by withdrawing structural water from wool keratine, and thus the extrafunctional groups become more accessible for formaldehyde molecules [4, 5].

$$(CH_3-CH_2)_3\bar{N} + HOH \rightleftharpoons (CH_3-CH_2)_3\overset{\oplus}{N}H + \overset{\ominus}{O}H$$

Triethylamine has also the function to cleave the cystine bridges fixing the keratine matrix in wool, increasing thereby the accessibility of wool. The extent of loosening can be controlled by the concentration of triethylamine and the temperature.



Owing to the treatment, the physico-mechanical properties of wool can be improved significantly. Tear resistance can increase by 20—30% so that in the meanwhile tearing length does not decrease, it can even increase by several per cents, thus the elasticity of wool improves.

Scanning electron micrographs prove that the scale layer of wool is not at all damaged by the treatment.

Sorption kinetic analysis during vacuum treatment showed that the formaldehyde binding of wool approached a saturation value, and the process could be described by a Langmuir isotherm. Sorption isotherms could be represented by a function based on the Elovich equation as interpreted by Landsberg which converged to a limiting value [6].

$$\frac{d\theta}{dt} = \alpha(1-\theta)^n$$

where

$\theta = \frac{q}{q\alpha}$ is the number of occupied active sites

q is the amount adsorbed at time t

n is the number of active sites occupied by a given molecule

α is the rate constant of chemisorption.

A significant result of the modification of wool keratine is the industrial realization of transfer printing by dispersion dyes of wool and wool-polyester mixtures, as well as panofix furs. The procedure elaborated for the simultaneous fixation and dyeing of panofix furs is of similar importance [4, 5].

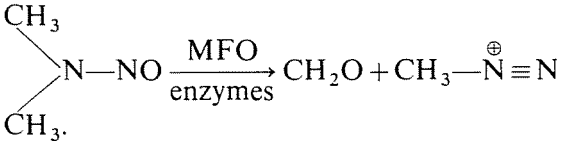
3.3 Specific reactions of proteins and some amino acids with formaldehyde and the biological applications of the reaction products

In studying the model reactions of proteins (e.g. caseine, fibroine) and amino acids with formaldehyde, new, so far not reported reactions have been discovered. Their essential feature is that on the ε-amino groups of L-lysine present in proteins, or on the L-lysine molecule itself, spontaneous methylation and formylation reactions take place effected by formaldehyde.

Reaction products have been isolated from the reaction mixture [7, 10].

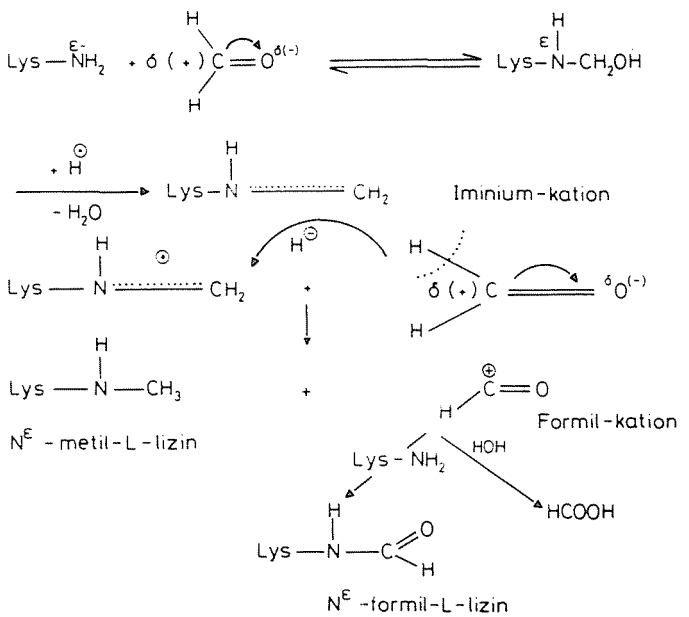
The isolated compounds have already been known in biology, but their formation has been assumed to occur only in an enzymatic way.

Indirect proof for this was provided by English authors in 1971, when they showed that by the biological oxidation of the carcinogenic dimethyl nitroseamine, a significant amount of formaldehyde was liberated, and this could methylate the ε-amino group of L-lysine.



However, they did not find any direct proof, and they had no idea about the reactions taking place [9, 10].

The studies on the reaction mechanism proved that methylation occurs via an intermediate iminium cation, and the hydride anion needed for reduction is provided by another molecule of formaldehyde [7, 9, 10, 12].



L-lysine methylated on the ε-amino group increases significantly the proliferation of cells. This property is utilized in agriculture: either soils rich in L-lysine (dung) are treated with formaldehyde, or N-ε-methyl L-lysine-containing preparations are used for spraying the plants in their appropriate fenophase. Thereby an increase of crop by 10—20% could be achieved. The procedures are patented.

Formaldehyde is not capable of the sponaneous methylation of any amino acid. L-arginine is not methylated either, instead the hydroxy methylated derivatives of the guanidine group are formed. The compounds are stable and can be isolated from the reaction mixture, to the contrary to the hydroxy methyl derivatives of L-lysine which are unstable [8, 13].

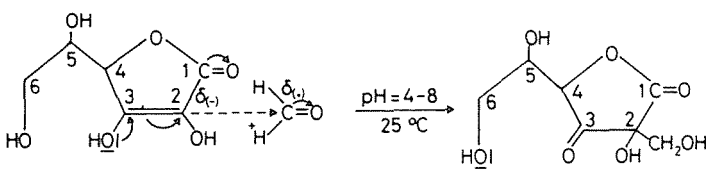
Based on experimental results, quantum chemical calculations also proved that the addition of formaldehyde occurs on the imino nitrogen of the guanidine group. The Náray-type electrostatic isopotential is the highest here: −749 kJ/mol.

$$\text{Arg—NH—C(=NH)—NH}_2 \xrightarrow{CH_2O} \text{Arg—NH—C(=N—CH}_2\text{OH)—NH}_2$$

These hydroxy-methyl L-arginine derivatives have been isolated also from human blood, and they proved to be of the contrary effect than the methylated L-lysine derivatives: they inhibit cell proliferation, also that of tumorous cells. The above results were reported on the XIV. International Congress on Cancer, in 1986 [3, 14, 15].

Finally, a new reaction taking place between L-ascorbic acid and formaldehyde has been recognized.

Formaldehyde is added to the C2 carbon atom of L-ascorbic acid as there is a partial negative charge on this carbon atom, and thereby the double bond of L-ascorbic acid is eliminated.



The new reaction may play an important role also in the biological world, because it protects the L-lysine groups of proteins from methylation by formaldehyde [9, 10, 11].

The reaction can be utilized also for binding the carcinogenic formaldehyde from tabacco smoke so that we prepare specific filters containing also L-ascorbic acid. The procedure is patented.

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