# PHYSICAL MECHANISM OF KERATIN SWELLING

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The mechanism of swelling of keratin in an aqueous solution of thioglylicolic acid has been studied. X-ray diffractograms, IR-spectra, and the dependence of the acoustic modulus on the static tension in nonswollen and swollen keratin fibers are obtained. Based on experimental data, we found that the molecules of thioglycolic acid are arranged in unordered intrafibrillar regions at the swelling of keratin, which stimulates the orientation of spiral segments. Disulfide bonds between oriented segments appear to fix the newly formed structure.

## 1. Introduction

Keratin is a substance referred to the family of fibrous proteins. Modern conceptions concerning the keratin structure (see Fig. 1) are expounded in works [1–4]. The main component of the keratin structure is a fibril 7.5 nm in diameter. Fibrils are separated from one another by unordered interfibrillar layers called an amorphous matrix, with their axes being arranged in parallel. Ordered and unordered intrafibrillar regions alternate along every fibril.

An ordered region is a crystalline lattice formed by keratin chains with their axes oriented in parallel to the fibril one. These chains look like $\alpha$-helices, with the distance between neighbor coils amounting to approximately 0.51 nm (Fig. 2,*a*). The chains are connected with one another by chemical (disulfide) and hydrogen bonds. In unordered regions, such bonds are broken or substantially weakened in comparison with those in the crystal. Chains in such regions are composed of sequences of helical and nonhelical segments (Fig. 2,*b*).

While experimentally studying the properties of keratin, a human hair is traditionally selected as an object of investigation. The swelling of keratin is put into the basis of a technology aimed at making the hair curly [5]. An aqueous solution of thioglycolic acid is one of liquid

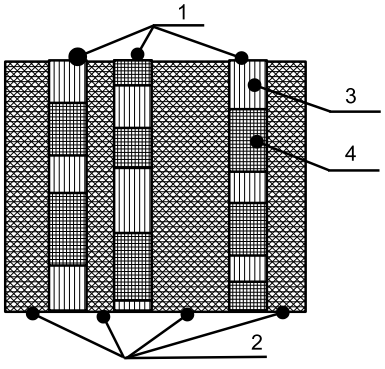


Fig. 1. Keratin structure: fibrils (*1*), unordered interfibrillar regions (*2*), crystallite (*3*), unordered intrafibrillar region (*4*)

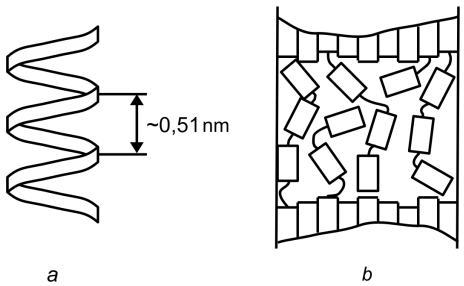


Fig. 2. Shape of keratin chains in ordered (*a*) and unordered (*b*) intrafibrillar regions. Helical segments are denoted by rectangles, nonhelical by twisting lines

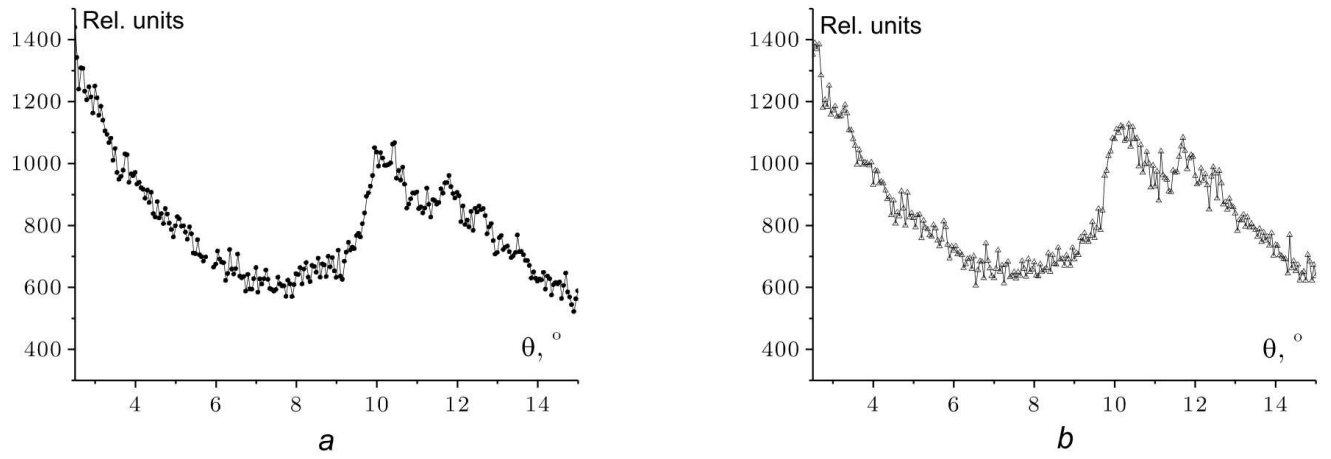


Fig. 3. Angular dependences of the intensity of X-ray radiation diffracted at untreated ($a$) and treated ($b$) specimens. The specimen axes are oriented along the meridian

systems used in this technology. This work aimed at determining the physical mechanism of swelling of keratin in the aqueous solution of thioglycolic acid. In other words, we tried to find structural modifications occurring in keratin under its swelling. To the authors' knowledge, this issue has not been considered earlier in the literature.

## 2. Preparation of Specimens

Traditionally, a human hair is an object of investigation. Its average diameter is $d = 69$ $\mu$m.

Specimens of two types were studied. They were conditionally named untreated and treated ones. Untreated specimens were no more than as-cut hairs. Treated specimens were obtained by keeping them in a 10-vol.% aqueous solution of thioglycolic acid at a temperature of 35 °C for 10 min. After removal from the solution, specimens were washed out with distilled water and dried up.

## 3. Wide-Angle X-ray Diffraction Study of Keratin

As specimens for X-ray diffraction study, we took bundles of hair 6 mm in diameter. They were attached to a quartz cuvette, which was afterward mounted in a DRON-3M X-ray diffractometer.

Figure 3 demonstrates X-ray diffraction patterns registered for untreated and treated specimens in the case of meridian diffraction. The figure shows that the meridian reflex was observed at the angle $\theta_1 = 9.9°$ for untreated specimens and $\theta_1' = 10.3°$ for treated ones. Those values correspond to the distances $d_1 = 0.518$ nm and $d_1' = 0.501$ nm, respectively. The accuracy of angle determination was $\Delta\theta = 0.1°$. Therefore, we can reliably assert that the position of the meridian reflex changes after the treatment.

The meridian reflex is known [1] to correspond to the distance between neighbor coils of an $\alpha$-helix in a crystallite. Based on the data presented in Fig. 3, we can assert that this distance decreases under the swelling of keratin.

In our opinion, when finding the structural changes induced by the swelling, the priority task consists in answering the question concerning the localization places of thioglycolic acid molecules. We tried to answer this specific question basing on the results of our X-ray diffraction research.

The situation becomes somewhat simplified owing to the presence of a significant amount of the unordered substance in keratin. It is clear that it is more beneficial for thioglycolic acid molecules to be deposited in unordered regions, because it is associated with a lower growth of the energy of the system. The results of the specific experiment confirm this almost evident conclusion. Really, let a molecule of thioglycolic acid be in the lattice. After the removal of this molecule, the lattice contains a void, which actually plays a role of the point defect in the lattice. It is known that such defects do not affect the reflex position, their emergence can change the reflex intensity only, and a considerable number of defects may bring about a growth of the reflex smearing degree. However, in our experiment, we did not observe such a growth after the treatment, which confirms once more that the crystallites remain almost inaccessible for

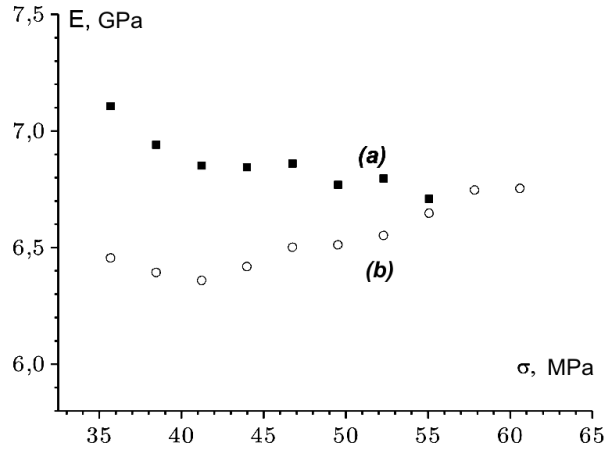


Fig. 4. Dependences of Young modulus $E$ on the static tension $\sigma$ for untreated ($a$) and treated ($b$) specimens

thioglycolic acid molecules. The same conclusion was made by the authors of work [6].

Now, the answer to the formulated question is reduced to a solution of the dilemma: Where are the molecules of thioglycolic acid located—in unordered intrafibrillar regions or in unordered interlayers between fibrils? It is known that a change of crystal lattice parameters can be induced by either a phase transition or the emergence of macroscopic stresses. There also exists another crystal modification of keratin; it is the so-called $\beta$-form [5]. However, the reflexes obtained by us for the treated fiber differ from those, which are characteristic of the $\beta$-keratin. Therefore, our experimental results lead to the conclusion that a squeezing tension takes place in treated specimens along the axis of fibrils, which gives rise to a reduction of the distance between the neighbor coils of $\alpha$-helix in the crystallite. Hence, we obtained the answer to the formulated question, because such tension can arise only in the case where thioglycolic acid molecules are located in unordered intrafibrillar regions.

## 4. Acoustic Parameters of Keratin

The acoustic characteristics of keratin were measured with the use of the acoustic interferometry method [7]. The applied frequency was $f = 12930$ Hz. The sound velocity $c$ was measured at various static tensions $\sigma$. The measurements were carried out at the temperature $T = 153$ K to exclude a possible influence of relaxation processes on measurement results. The elastic modulus $E$ was determined by the formula

$$E = \rho c^2, \tag{1}$$

where $\rho$ is the density. According to work [8], the dependence $E(\sigma)$ was taken in the form

$$E = E_0 + \frac{E_0'}{E_0}\sigma, \tag{2}$$

where $E_0$ and $E_0'$ are the elastic moduli of the second and third orders, respectively.

In Fig. 4, the dependences of the Young modulus $E$ on the static tension $\sigma$ are depicted for the untreated and treated specimens. The figure demonstrates that, after the treatment of keratin, (i) the elastic modulus decreases, and (ii) the sign of the third-order modulus changes from negative for untreated specimens to positive for treated ones. Let us consider how the indicated facts agree with the keratin structure model presented above.

Actually, the unordered interfibrillar regions are transition layers. Therefore, their thickness is considerably less than the dimensions of other structural regions. As a result, those layers weakly affect the elastic characteristics of a fiber, so that, while analyzing the results of the acoustic experiment, they can be neglected, and the examined system can be regarded as composed of regions of two types, unordered intrafibrillar and ordered fibrillar ones.

Let us designate the elastic moduli of those mentioned regions as $E_1$ and $E_2$, and the relative volume occupied by unordered regions as $\kappa$. Neglecting the interaction between fibrils, let us assume that every fibril makes an independent contribution to the total deformation. The ordered and unordered regions alternate along the fibrils. This means that the fibril compliance is a sum of compliances for separate regions, being expressed by the formula

$$\frac{1}{E} = \frac{\kappa}{E_1} + \frac{1-\kappa}{E_2}. \tag{3}$$

In the ordered regions, the chain axes are oriented along the fiber axis; in the unordered ones, they are disoriented. As a result, it should be admitted that

$$E_1 \ll E_2. \tag{4}$$

The dimensions of unordered intrafibrillar and ordered regions are of the same order of magnitude. Approximately assuming those quantities equal, from formula (3) and inequality (4), we obtain the expression

$$E \approx 2E_1, \tag{5}$$

i.e. the measured acoustic elastic modulus is a doubled elastic modulus of unordered regions.

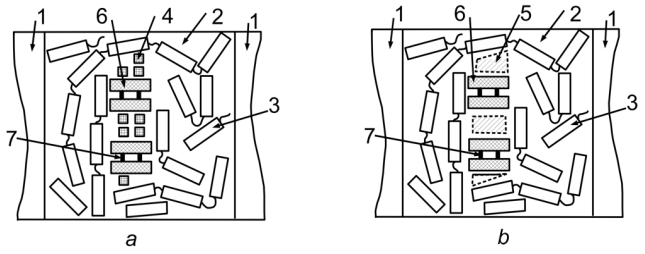


Fig. 5. Structure of the unordered intrafibrillar region under the swelling (*a*) and after washing out and drying (*b*): ordered region (*1*), unordered intrafibrillar region (*2*), non-oriented helical segment (*3*), thioglycolic acid molecule (*4*), void (*5*), oriented helical segment (*6*), new disulfide bond connecting oriented segments (*7*)

Above, we emphasized two facts revealed in the acoustic experiment. The first one – a reduction of the elastic modulus after the treatment – can be regarded now as a consequence of the reduction of the elastic modulus for unordered regions. In combination with the data of the X-ray diffraction experiment, this interpretation seems reasonable. Really, the experiment showed that thioglycolic acid molecules are located in the unordered regions (Fig. 5,*a*). After the removal of those molecules, the unordered regions contain voids (Fig. 5,*b*). It is the presence of those voids that induces a reduction of the elastic modulus for unordered regions.

The second fact revealed by the acoustic experiment – the change of the third-order modulus sign – also finds the explanation in the model of structural modifications depicted in Fig. 5,*b*. Really, as was shown in work [8], the negative sign of the third-order modulus is typical of non-oriented structures. On the contrary, the positive sign of this modulus is inherent to oriented ones. Hence, we may assert that, owing to the treatment, some of the helical segments in unordered intrafibrillar regions become oriented along the fibril axis, as is illustrated in Fig. 5,*b*.

## 5. Infra-Red Spectra of Keratin

The infra-red spectra were obtained with the help of a Nicolet 4700/6700 FT-IR device. In Fig. 6,*a*, the IR spectra of treated and untreated specimens are exhibited. Figure 6,*b* demonstrates the low-frequency interval of this spectrum. One can see that the treatment leads to the appearance of an absorption band at a frequency of 520 $\mathrm{cm}^{-1}$. In the literature [9], this band is associated with vibrations arising, when a disulfide bond together with neighbor bonds forms chains of the *gauche–trans–gauche* configuration.

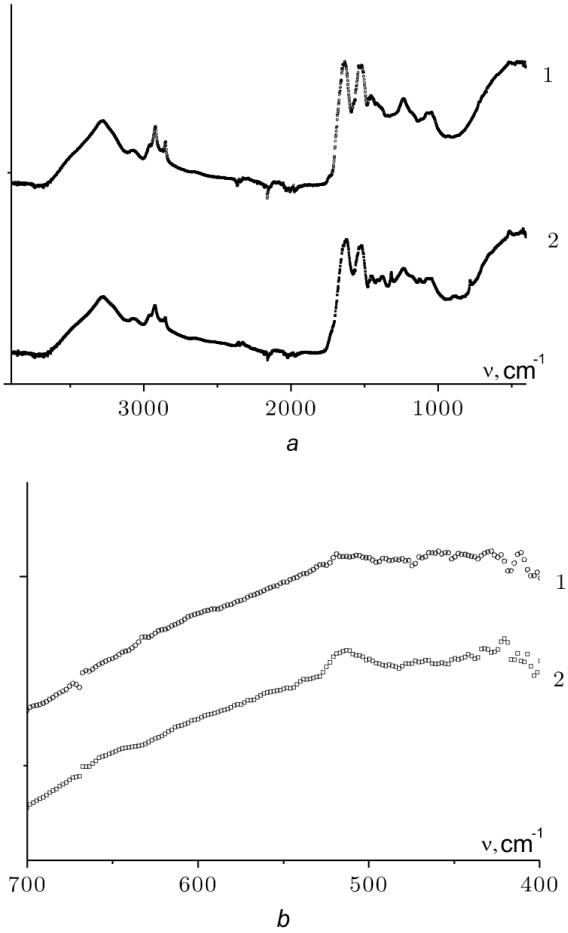


Fig. 6. Infra-red spectra of untreated (*1*) and treated (*2*) specimens

The appearance of the 520-$\mathrm{cm}^{-1}$ band is an additional argument in favor of the proposed mechanism of structural modifications. Really, as is shown in Fig. 5, the axes of helical segments in the regions that separate voids are in parallel to one another. Therefore, disulfide bonds can emerge between them (in Fig. 5, they are conditionally represented by bold lines). However, in spite of the parallel arrangement of helical segment axes, the atoms belonging to those segments do not form a lattice. Therefore, the configuration formed by the disulfide bond and the neighbor bonds in the chains differs from the configuration characteristic of the lattice. For this reason, the mentioned band is absent from the spectrum of untreated specimens.

Owing to newly formed disulfide bonds, the new structure, which is characterized by oriented helical segments in unordered intrafibrillar regions, remains preserved after the removal of thioglycolic acid molecules.

## 6. Conclusions

The following features are typical of the physical mechanism of swelling of keratin in the aqueous solution of thioglycolic acid:
1) thioglycolic acid molecules are arranged in unordered intrafibrillar regions;
2) the emergence of those molecules in the mentioned regions results in an orientation of some helical segments of keratin chains in a vicinity of the indicated molecules;
3) new disulfide bonds emerge between oriented helical segments, owing to which the newly formed structure of keratin remains preserved after the removal of thioglycolic acid molecules.

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ФІЗИЧНИЙ МЕХАНІЗМ НАБУХАННЯ КЕРАТИНУ

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Р е з ю м е

Досліджено механізм набухання кератину у водному розчині тіогліколевої кислоти. Отримано рентгенівські рефлектограми та ІЧ-спектри, залежність акустичного модуля від статичного напруження для ненабухлого та набухлого кератину. На основі цих експериментальних даних встановлено, що при набуханні кератину молекули тіогліколевої кислоти розташовуються у внутрішньофібрилярних невпорядкованих областях, приводячи до орієнтації спіральних сегментів. При цьому між орієнтованими сегментами виникають нові дисульфідні зв'язки, які фіксують новоутворену структуру.