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Polymolecular Complexes of Chitosan with the *Bombyx Mori* Protein

The interaction of chitosan (ChS) and the *Bombyx mori* protein on different pH ranges was studied, and the fundamental possibility of obtaining complexes of ChS with the *Bombyx mori* protein was revealed. The formation of a polymolecular complex of protein with ChS in aqueous solutions was confirmed by the results of physico-chemical methods. It is shown that the ChS structure is characterized by a certain rigidity and ionogenicity. The results indicate the complexation of the pupae protein with ChS in 2% acetic acid in the range of pH = 4.8–6.7. The detected changes and shifts of the absorption bands in the IR spectra confirm the occurrence of the complex formation reaction between the molecules of ChS and protein at pH = 4.8–6.7, which is characterized by absorption bands in the IR spectra at 1641 cm^{-1}, 1538 cm^{-1} and 1068 cm^{-1}. Quantum-chemical DFT study of ChS complexes with amino acids (AAs) was carried out. The stability of complexes of ChS with AAs (ChS-AA) was shown except for the complex formed with histidine in the gas phase. The calculation results indicate the presence of a strong thermodynamic driving force in the complexation of ChS with AAs.

Keywords: silk production waste, silkworm pupae, alkaline hydrolysis, protein, chitin, chitosan, polymolecular complexes, conformational characteristics.

Introduction

The main waste products of silk production are silkworm pupae, which have a high nutritional and biological value. The mass of a dry pupae consists of 60–65 % protein, 3–5 % chitin, 10–25 % lipids, and 2–6 % minerals [1]. Obtaining polymolecular complexes of protein and chitosan (ChS) from pupae of the silkworm *Bombyx mori* with valuable chemical and biological properties that will have antibacterial activity due to the natural ChS polysaccharide is an urgent task [2].

ChS is obtained from the natural biopolymer of chitin including the chitinous coatings of silkworm pupae in an aqueous solution of NaOH (30–50 %) in the temperature range of 90–150 °C [3]. This polysaccharide is considered as a promising biomaterial of the future; interest in it is associated with unique physiological and environmental properties such as biocompatibility, biodegradation, physiological activity in the absence of toxicity and the availability of raw materials as well as local sources for its production [4].

Since biopolymers including ChS are more capable of intermolecular interactions, one of the most effective ways to improve its characteristics is the formation of polymolecular complexes (PMCs) with other biopolymers and polar synthetic polymers [4]. Despite the fact that amino acids (AAs) are the building blocks of proteins elucidation of the nature of their interactions with ChS remains poorly understood. Understanding the strength of the interaction between ChS and AAs as well as studying the reactivity of the complexes formed as a result of these interactions is an urgent task. Despite the ubiquitous presence of such interactions in biological systems, there are few theoretical studies as well as experimental studies on the preparation of PMCs of *Bombyx mori* protein and ChS in solutions and in the solid state.

In this regard, physical and chemical phenomena and patterns of interaction of proteins with anionic and cationic polysaccharides are of great practical interest. A detailed study was made of the effect of both the ionogenic nature of the protein and the rigidity of ChS chains on its behavior in solutions with various concentrations subjected to various thermodynamic changes.

This paper presents the results of an experimental study of the production of PMCs preparations based on *Bombyx mori* protein and ChS, which have a different chemical structure of macromolecules due to the formation of complex compounds, and analyzes the role of ionogens in the structural and phase transformations of the biopolymer. Based on the density functional theory (DFT) method the formation of complexes between ChS and AAs (asparagine, threonine, serine, glutamine, alanine, tyrosine, histidine, and lysine), which are part of the protein isolated from the pupae of the silkworm *Bombyx mori* was analyzed.

Experimental

Protein hydrolysis was carried out in 1 % NaOH aqueous solution at 90 °C for 3 hours. The modulus of the reaction mass "silkworm pupae: alkaline solution" was 1:10. The protein was separated from chitin by filtration. It was revealed that the mass of chitin was 5.6 %. After filtration, 1 % alkaline hydrolysate contained about 8.83 % pupal protein, hydrolysate density was 1028 kg/m^3, pH was 10.96. After separation of the hydrolysate the resulting chitin was deacetylated with 50 % NaOH at 120 °C for 3 hours. ChS with a degree of deacetylation of 86 % was obtained. A sample of *Bombyx mori* chitosan is characterized by the elementary unit $[(C_6H_5O(OH)CH_2OH(NH_2)]_n$, the presence of an amino group (–NH_2), an aliphatic methylene (–CH)-group and hydroxyl (–OH)-groups at C-3 and C-6. The molecular weight of the elementary unit is $M_0 = 161$. The content of amine and carboxyl groups was determined by the method of conductometric titration (on a Seven Easy Conductivity Mettler-Totedo AG8603 instrument).

A simple and effective method of viscometry was used with the solvent outflow time $t_0 = 94$ s for acetic acid to determine the viscosity characteristics of ChS solutions depending on the concentration (C) of polymers. Sodium acetate (CH_3COONa) was added to the ChS solution to suppress the polyelectrolyte effect. At least 5 measurements were performed for each dilution of the solutions. The calculations were carried out using the Huggins equation:

$$\eta_{spec}/C \approx [\eta] + k[\eta]^2 C,$$

where $\eta_{spec} \approx \eta_{rel\,-\,1}$ — is specific viscosity, in which relative viscosity $\eta_{rel.} \approx t_{sol\text{-}n}/t_{sol\text{-}t}$ (where $t_{sol\text{-}n}$ is the solution outflow time and $t_{sol\text{-}t}$ is the solvent); k — is the Huggins coefficient; [η] — is the inherent viscosity of the solution, which is determined from the dependence of_{spec}/C on C extrapolating $C \rightarrow 0$ and used to calculate the relative molecular weight (M_η) of the polymer using the Mark-Kuhn-Houwink equation, i.e.:

$$M_\eta \approx ([\eta]/K)^{1/\alpha},$$

where $K = 1.4\times10^{-4}$ and $\alpha = 0.83$ [5].

Quantitative determination of the AA composition in protein samples was carried out on an amino acid analyzer (Amino acid analyzer T-339). Preliminarily freeze-dried portions (50 mg each) of the samples were hydrolyzed with 5.7 N HCl for 24 hours at a temperature of 110 °C in a vacuum. The obtained hydrolysates were evaporated on a rotary evaporator (DLAB RE 100-Pro). IR spectroscopic studies were carried out on an Inventio-S IR-Fourier spectrophotometer (Bruker, Germany) with a spectral resolution of 2 cm^{-1}. The IR spectrometer is equipped with an attenuated total internal reflection attachment in the range from 4000 to 500 cm^{-1} since absorption bands of almost all functional groups of organic molecules lie in this spectral range. The samples were prepared in the form of tablets with KBr under a pressure of 7×10^8 Pa. Turbidimetric studies were carried out on a Turbidimeter TB300IR instrument (Germany). The TB300 IR is a portable turbidity meter that complies with ISO 7027.

The instrument has an autoranging feature ranging from 0.01 to 1100 NTU/FNU. The light source is an infrared LED (light emitting diode) with a wavelength of 860 nm. The emitted light is reflected by haze in the sample. Stray light will be detected at a 90° angle by the photodiode. This principle is a part of ISO 7027. Formazin solution is the international standard for turbidity. The results associated with this standard are designated as FNU (formazine nephelometric units).

Computational methods

Although a huge number of experimental works are focused on the interaction of different amino acids/proteins and chitosan, only a few computational studies have been reported. The interactions between chemicals and chitosan toward two specifics emergent pollutants were studied by using the density functional theory (DFT) [6]. It is broadly used to understand and predict the interactions of a specific molecule over a polymeric structure, proving suitable correlations with the experimental results [7–13].

The calculations were performed using the GAUSSIAN 09 package and the Gaussview 5.0.9 molecular visualization program using DFT with the standard set basis 6-31++G (d,p) [6]. The first stage of the theoretical calculation was the determination of the optimized molecular structures of ChS and AA. The charges of atoms were calculated, diagrams of boundary molecular orbitals were constructed: the highest occupied (HOMO) and lowest unoccupied (LUMO) molecular orbitals and their energies were determined. Interactions of ChS with AAs were studied using reactivity descriptors. A monomeric link was taken as the structural unit of ChS; in calculations in the gas phase, AAs were considered as a nonionic form due to the greater intrinsic affinity for the proton of the carboxylate oxygen atom compared to the nitrogen atom of the amino group. The interaction energy (ΔE_{inter}) was calculated using the equation-based approach:

$$\Delta E_{\text{inter}} = E_{\text{ChS-AA}} - \left(E_{\text{ChS}} + E_{\text{AA}}\right) + E_{\text{BSSE}},$$

where $E_{\text{ChS-AA}}$, E_{ChS} and E_{AA} are the energy of the complex, ChS and AA, respectively. EBSSE is a base set superposition error (BSSE) correction calculated using the direct difference method for calculating molecular interactions based on a bivariate transcorrelation approach together with special methods for estimating other errors [14]. Of several reactivity descriptors, the energy of the HOMO, total rigidity, chemical potential and electrophilicity were taken into account to analyze the reactivity of ChS AAs complexes in the present study. The following reactivity descriptors [15] were calculated: chemical hardness (η)=1/2($E_{\text{HOMO}} - E_{\text{LUMO}}$), where E_{HOMO} is the energy of the HOMO, and E_{LUMO} is the energy of the LUMO, softness $\zeta = 1/\eta$, chemical potential $\mu = 1/2(E_{\text{HOMO}} + E_{\text{LUMO}})$, electrophilicity ($\omega$) is expressed as $\omega = \mu^2/2\eta$.

Results and Discussion

Since ChS molecules are more capable of intermolecular interactions, one of the most effective ways to improve its characteristics is the formation of PMCs with various compounds including proteins [4]. However, the preparation of PMCs of *Bombyx mori* protein and ChS, especially in electrolyte solutions, has not been sufficiently studied. In this regard, a detailed study of the influence of both the ionogenic nature of the protein and the rigidity of ChS chains on its behavior in solutions with different concentrations and pH was carried out.

Previously, we presented the results of an experimental study of protein structural changes in solutions and an analysis of the behavior of the R group of AA residues present in the *Bombyx mori* protein [16]. According to the amino acid composition, it is shown that the R-groups of AA residues present in the *Bombyx mori* protein consist of 16 AA residues, eight of which are non-polar, which is 1.98 %; five — uncharged, but polar, they make up 1.9 %, as well as three — charged, which make up 0.8 %. Conductometric titration showed that the content of $-NH_2$ groups in the *Bombyx mori* protein chain was 4.8 % and the obtained proteins in solutions exhibited polyampholytic properties characteristic of protein molecules. Based on the structural properties of the protein it is possible to obtain biologically active PMCs preparations with polysaccharides on its basis.

In polysaccharides, in particular, in ChS, amine and hydroxyl groups are functional, while in proteins, the chemical properties are determined by the nature of the amide bond and functional groups (carboxyl, hydroxyl, amine, disulfide) [17]. Through these functional groups and thermodynamic conditions the interactions of the polysaccharide and protein are carried out through hydrogen bonds, electrostatic forces, van der Waals and hydrophobic interactions.

The process of obtaining complexes based on *Bombyx mori* protein and ChS is inevitably accompanied by the breakdown of the supramolecular and molecular structure due to the interaction of individual functional groups and elements of its macromolecules. In this case, the rate of the complex formation process depends on the pH of the medium and temperature. However, the efficiency of complex formation is also largely determined by the initial behavior of the biopolymer macromolecule in bulk.

Table 1

Shows the physico-chemical characteristics of the *Bombyx mori* ChS sample used

Nitrogent content, %	Ash content, %	[η], dl/g	$M_{\eta\ (\text{kDa})}$	*DP*	*L*, nm	*N*	Solubility, %
8.20	2.6	2.7	14.6	910	465	23.3	94.0
Values: M_η is viscosity average molecular weight, *DP* is degree of polymerization, *L* is contour length and *N* is number of Kuhn segments for ChS samples.							

As can be seen from Table 1, ChS molecules are characterized by a certain rigidity and ionogenicity. These results are in good agreement with the literature data [18]. The influence of both ionogenicity and kinetic rigidity of ChS and protein on their behavior in solutions with different concentrations and pH was determined to select the optimal conditions necessary to obtain complexes based on the *Bombyx mori* protein and chitosan. The preparation of complexes depends on the initial concentration of protein substances. Therefore, protein alkaline hydrolysate and solution of *Bombyx mori* ChS in acetic acid were used to effectively carry out the process.

When obtaining complexes by direct titration of pH from alkaline to neutral media a monotonous increase in turbidity to pH = 7 occurs, the yield of the obtained complexes changes insignificantly. From the literature [19], it is known that at a pH value above 7 the amino group of ChS is deprotonated, exhibits nu-

cleophilic properties and participates in the nucleophilic substitution reaction. However, in an alkaline environment the protein molecule acquires a negative charge and exhibits ionogenic properties.

We have previously shown [16] that the *Bombyx mori* protein has five AAs that are uncharged but have polar side chains. They include asparagine, threonine, serine, glutamine and tyrosine, as well as three AAs, namely lysine, arginine and histidine with charged basic groups. These AAs are hydrophilic and can interact through electrostatic, hydrogen bonds, hydrophobic and steric interactions with water. They make up 1.9 % in one protein chain, at the N- and C-terminals of the polypeptide chains there are amino and carboxyl groups that contain functional groups capable of ionization. The degree of ionization of the functional groups of these radicals depends on the pH value.

In an alkaline environment at pH 10.96 with an excess of NaOH and the presence of a larger amount of Na^+ ions, the charge and degree of ionization in the protein molecule decreases and the conformation of the protein macromolecule looks like a coil [17]: $HONH_3$-R-COONa.

Due to the presence of a large ionization group the interaction of the *Bombyx mori* protein with ChS does not result in a nucleophilic substitution reaction. And also in protein molecules, due to the large number of hydroxyl ions the positive charge decreases and the protein behaves like an acid (according to the reaction shown in the diagram).

$$HONH_3\text{-R-}COO^- + H^+ + Na^+ + OH^- \rightarrow HONH_3\text{-R-}COO^- + Na^+ + H_2O$$

At pH = 7 all ionogenic groups of the protein are in an ionized state.

It is known [20] that at low pH values (pKa<6.5) the amino group is protonated, ChS is a cationic water-soluble polyelectrolyte and is capable of various types of interaction with the formation of 4 main types of bonds, namely ionic, hydrogen, hydrophobic, bonds by the type of complexation in which ChS acts as the core of the complex.

In this regard, the study is aimed at identifying the pH range from neutral to acidic during the formation of the complex of the protein and *Bombyx mori* ChS, as well as determining the special characteristics of the obtained samples. For titration of the protein solution 2 % solutions of ChS were prepared in a 2 % aqueous solution of CH_3COOH — pH=2.8. The resulting precipitate was filtered off, washed to pH=7 and freeze-dried for 2 hours.

The results of obtaining complexes by direct titration from neutral to acidic value are shown in Table 2. The influence of the pH of the titrant at the stage of obtaining the complex from the protein hydrolysate was evaluated by elemental analysis and the weight of the complex referred to the maximum possible.

Table 2

Influence of medium pH on the physico-chemical characteristics of the complex

No.	Protein hydrolysate, ml	2 % acetic acid, ml	2 % ChS pH 3.94	Solution 2 % ChS (pH 3.94), ml	pH	Yield, g	Nitrogen, %	Sulfur, %	Ash content, %
1	50	-	77	77	4.8	1.9	12.6	5.8	2.0
2	50	-	40	40	6.3	1.6	14.4	4.8	2.7
3	50	-	30	30	6.7	1.0	15.4	4.1	5.5
4	50 (control)	90	-	-	4.8	1.2	11.7	4.6	5.6

It can be seen from Table 2 that an increase in pH from 4.8 to 6.7 in the system (samples No. 1–3) leads to an increase in the degree of nitrogen content and ash content, as well as a decrease in sulfur and the complex yield. Undoubtedly, with an increase in pH from the isoionic point (IIP) to a neutral value the *Bombyx mori* protein macromolecule monotonously acquires a negative charge and entropy decreases while the coil of the macromolecule in this medium unfolds and the chain becomes flexible. At pH 6.3–6.7 (samples No. 2–3) the interaction of ChS with the *Bombyx mori* protein occurs efficiently, with a decrease in pH < 7 the protein and ChS have ionogenic properties: the protonated amino group of ChS is a cation and the protein is an anion. In this case, an increase in the nitrogen content occurs: an increase by 14–22 % (in relation to sample No. 1), respectively, due to the synergistic effect of the nitrogen content in the protein and ChS. With an increase in the acidity of the value pH = 6.7–4.8 the process of mineralization occurs and the ash content decreases in samples No. 1–3. This result is confirmed by the literature data [20]. As can be seen from Table 2 with IIP of the *Bombyx mori* protein which is at pH = 4.8 (sample 4), the yield of the control sample decreases by 60 % and the nitrogen content by 7.5 %, which is due to the absence of *Bombyx mori* ChS (in relation to sample No. 1).

IR spectroscopy is a reliable method showing the interaction between ChS molecules and the *Bombyx mori* protein at different pHs. Comparative studies by IR spectroscopy are shown in Figure 1.

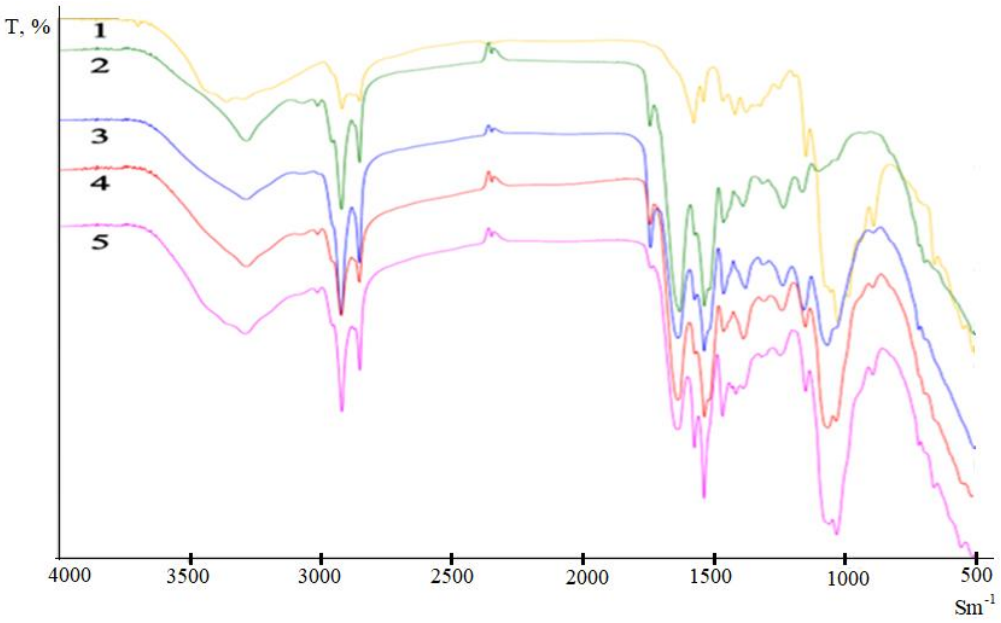


Figure1. IR spectra of the initial ChS (*1*), protein (*2*) and their complexes at pH = 4.8 (*3*), pH = 6.3 (*4*) and pH = 6.7 (*5*) of the value

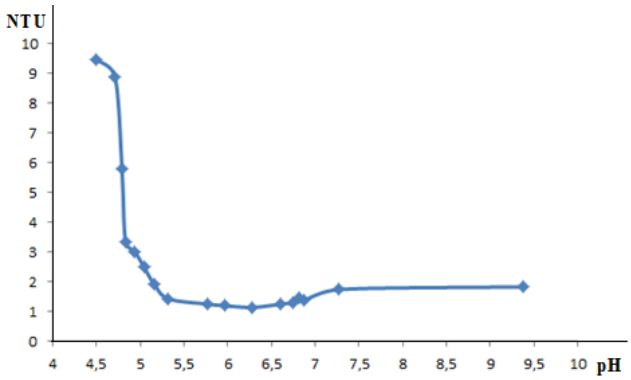
It can be seen that the IR spectrum of the initial ChS (1) and *Bombyx mori* protein (2) has characteristic absorption bands for these compounds. The IR spectrum of ChS (sample 1) has absorption bands at 3320 cm^{-1}, 3288 cm^{-1} and 2950 cm^{-1}, 2880 cm^{-1} corresponding to the stretching vibrations of –NH, –OH and –CH, CH_2-groups, respectively. Pronounced absorption bands at 1590-1620 cm^{-1} (amide I) and at 1510–1550 cm^{-1} (amide II), as well as 1440 cm^{-1}, correspond to the bending vibrations of NH-, CN-, CO- and CH-, CH_2-groups. Absorption bands characterizing CO–, C–O–C– ether bonds are observed at 1000–1150 cm^{-1}.

In the IR spectrum of the *Bombyx mori* protein (sample 2), several relatively strong absorption bands appear which, as a rule, refer to vibrations of the peptide group –CO–NH–, as a common structural component of protein molecules. There is a peak of –NH, –OH at wave numbers 3282 cm^{-1}, as well as peaks in the region of 2919 cm^{-1}, 2851 cm^{-1} and 1744 cm^{-1}, corresponding to the stretching vibrations of CH–, CH_2–, –COOH groups, respectively. The presence of two main absorption bands due to stretching vibrations of the –NH bond, a peak at 1631 cm^{-1}, and in-plane bending vibrations of the $–NH_2$ bond — a peak at 1537 cm^{-1}, are characteristic of the protein structure.

The complex should be realized at a pH above the isoelectric point of the protein (pH≈4.8), at which the ion-dipole interaction occurs between the negatively charged protein and ChS with polar $–NH_2$ groups. As a result of the formation of complexes in samples at pH = 4.8 (3), 6.3 (4) and 6.7 (5), some changes occur in the absorption bands of the above groups and bonds. Namely, pronounced bands appear at wavenumbers of 3000–3500 cm^{-1} due to the shift of stretching vibrations of the band of $–NH_2$ groups of ChS and –NH groups of the protein. Particularly there are pronounced some changes and shifts in the absorption bands in the IR spectra of sample (5) obtained at pH 6.7. The absorption bands of bending vibrations at 3287 cm^{-1} are shifted by 13 cm^{-1} (3300 cm^{-1}) and become more pronounced and the absorption intensity of asymmetric angular deformation at 2918 cm^{-1} is more pronounced. The absence of absorption bands of stretching vibrations at 1517 cm^{-1} characteristic of $–NH_2$ groups (amide I), as well as stretching vibrations in the region of 1467 cm^{-1} become pronounced in samples No. 3 and No. 4.

Natural polysaccharides and proteins have a pronounced optical anisotropy, which makes it possible to conduct studies at the molecular and supramolecular levels using optical methods [21]. Determination of optical density is an informative and widely used parameter for monitoring changes in the behavior of proteins and polysaccharides during the formation of complexes [22]. The most effective method is the turbidimetric method based on measuring the intensity of the light flux scattered by solid particles suspended in solution (usually at an angle of 90°). The intensity of scattered light depends on the number of suspended particles and

their size. A sample of complexes based on ChS and protein from *Bombyx mori* is practically insoluble and is a suspension at neutral pH. Figure 2 shows the results of a comparative study of changes in the regularity of optical density according to the precipitation of the complex at pH from an alkaline to an acidic medium determined by the turbidimetric method on a Turbidimeter TB300IR instrument (Germany). Graphically the dependence of optical density (NTU) determined by the turbidimetric method on pH is shown in Figure 2.



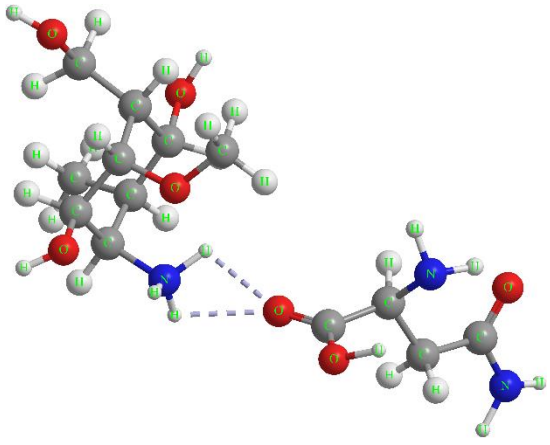
Fugure 2. The dependence of the optical density of ChS-protein complexes on pH

From Figure 2 it can be seen that the lowest optical density is observed in the range of pH = 5.7–6.8, the values of the optical index of the complex sample decrease. In this interval, the maximum precipitation of the complex occurs and the volume of the solution becomes more transparent.

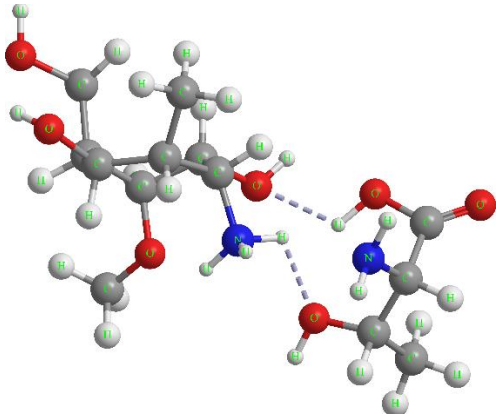
As studies have shown the interaction of ChS with protein is very strongly influenced by the pH of the medium and the ratio of components. ChS and protein with opposite charges at pH 5.5 and 6.0 can interact with each other through electrostatic attraction. In addition, at these two pH values the interaction was further influenced by the concentration of ChS. It can be concluded that under these parameters the greatest interaction between the components of the system occurs and ultimately the formation of the ChS-protein complex.

At low pH values the protonated amino group gives ChS the ability to bind to negatively charged molecules via electrostatic interaction [23]. In addition, a number of earlier studies noted the decisive role of hydrogen bonds in the formation of complexes of protonated ChS with electrically neutral nitrogenous bases. It is known that amino acids form hydrogen bonds with various carrier molecules [24, 25].

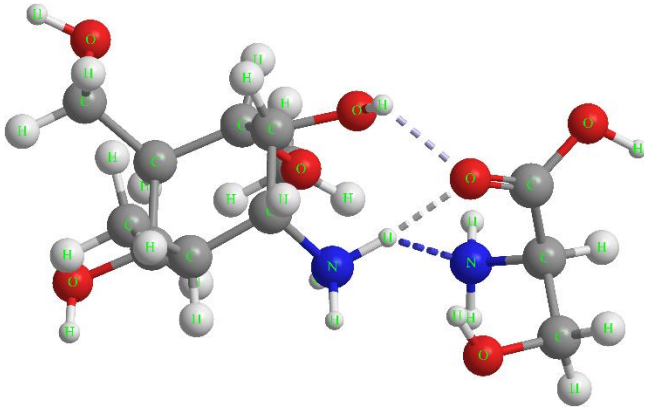
The calculated group charge distribution for the tertiary hydrogen atom of the protonated amino group of ChS is 1.54 a.u [26]. These values indicate that these hydrogen atoms have a tendency to form hydrogen bonds with electronegative centers. Similarly, a group charge of 1.54 a.e. for two atoms of nitrogen and oxygen in histidine allowed us to evaluate the atypical ChS-histidine interaction between electronegative atoms with –OH and $–NH_3^+$ ChS groups. The optimized structure of the complexes under consideration is shown in Figure 3.



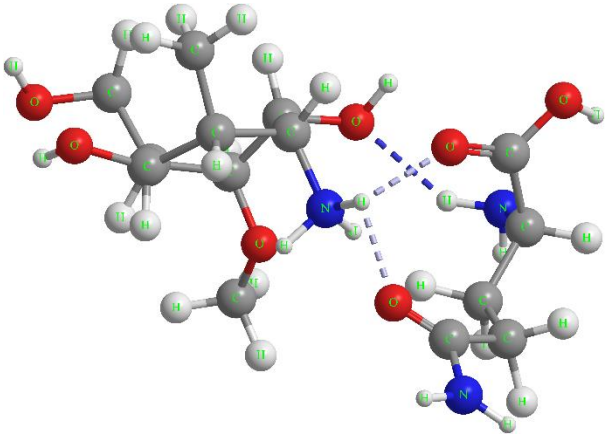
ChS-Asparagine



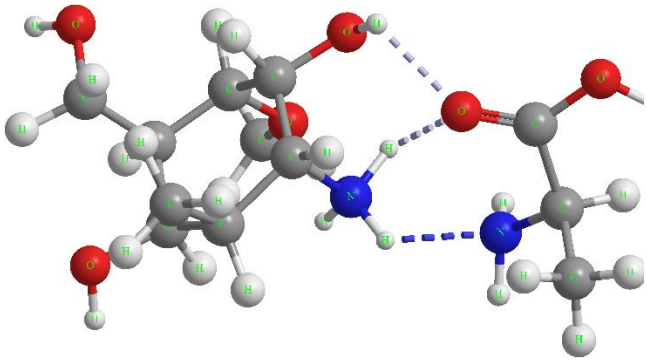
ChS-Threonine



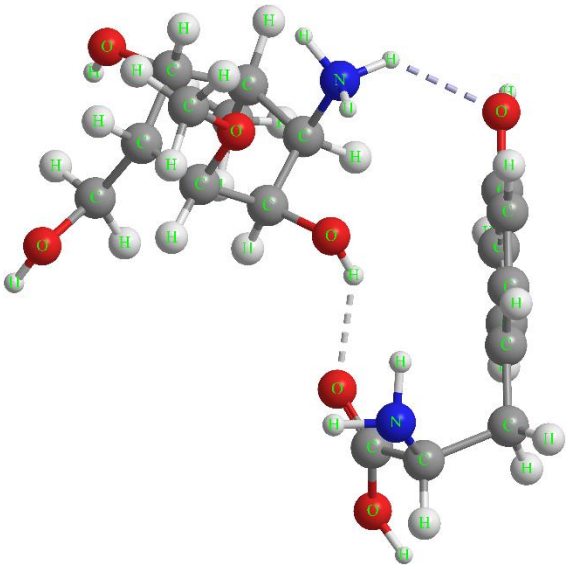
ChS-Serine



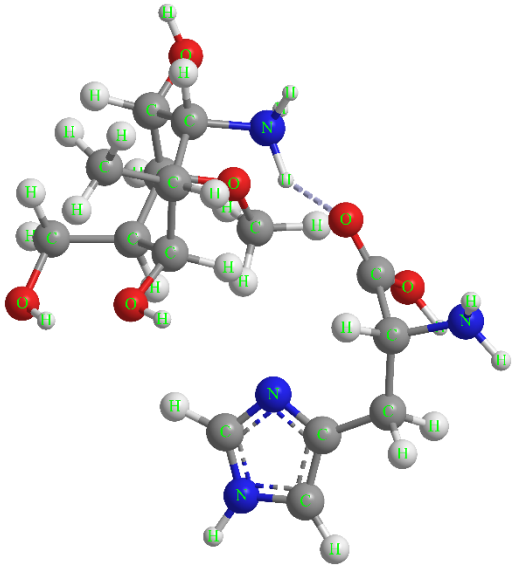
ChS-Glutamine



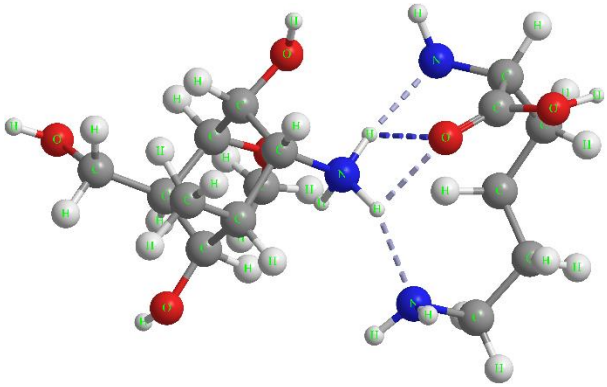
ChS-Alanine



ChS-Tyrosine



ChS-Histidine



ChS-Lysine

Figure 3. Optimized geometries of ChS complexes with AAs

The DFT calculations showed the presence of hydrogen bonds between the hydrogen atom of the amino group or –OH of the ChS group and the O atom of the $–COO^-$ or –OH groups of the studied amino acids (Table 3). According to Table 3, the distance between the H atom of the $–NH_3^+$ or –OH-group of ChS and the O atom of the $–COO^-$ or –OH groups of AAs is in the range of 0.96–1.34 Å, which is typical for hydrogen bonds [27]. This indicates that chitosan forms hydrogen bonds with amino acids during the formation of complexes. It is important to note the anomalously shorter H_{ChS}-$O_{Threonine}$ distance in the ChS-Threonine and $N_{Histidine}$-O_{ChS} system (0.96 Å) for the ChS-Histidine interaction. Various protonation states of the complexes were tested as starting points for geometry optimization. The results confirmed the proton transfer only in the ChS-Histidine system while denying the same in the case of other complexes. Cationic or anionic AAs are well known for their ability to form hydrogen bonds with oppositely charged species [28, 29]. However, the formation of such a salt bridge in the gas phase of the complexes under consideration was not observed.

Studies have shown that ChS and its derivatives form stable complexes with proteins and peptides [30, 31]. The magnitude of the interaction energy (ΔE_{inter}) of complexes is of decisive importance from the point of view of the resistance of the complexing protein to degradation during the transition of the protein into the cell, as well as the transfer of the protein near or inside the cell nucleus. A high value of ΔE_{inter} promotes strong binding between ChS and AAs in the complex while a decrease in energy values promotes the complex dissociation. Moreover, on the basis of the ΔE_{inter} values the suitability of the carrier with respect to a particular AA can be assessed. The calculated values of ΔE_{inter} in the gas phase are presented in Table 3. As can be seen from the Table 3 with the exception of the complex with Histidine (–11.45 kcal/mol) the calculated values of ΔE_{inter} are negative in all cases, which contributes to the formation of complexes in the range from –17.56 to –129.46 kcal/mol. Other similar studies report that the binding energy of the most stable conformation of the ChS-Insulin complex is –38.0 kcal/mol [32], the release energy of doxorubicin by polyethylene glycol-chitosan biopolymer is 122.41 kcal/mol [33].

The formation of a complex of protonated ChS with a positively charged methionine is obviously not a spontaneous reaction, which indicates a repulsive interaction between them. The value of ΔE_{inter} ranges from 11.45 kcal/mol (ChS-Lysine) to –129.41 kcal/mol (ChS-Serine) in the gas phase and from –11.03 kcal/mol (ChS-Lysine) to –22.09 kcal/mol (ChS-asparagine). As for the anomalously high value of ΔE_{inter}, this may be due to the strong Coulomb force of attraction, which leads to hydrogen migration. The strength of the interaction is in the following order: ChS-Asparagine > ChS-Alanine > ChS-Glutamine > ChS-Serine > ChS-Tyrosine > ChS-Threonine > ChS-Lysine > ChS-Histidine.

As can be seen from Table 3, the influence of the aqueous phase largely affects the interaction energy of these systems. There is a progressive destabilization of ChS complexes with AAs with the exception of histidine. In addition, it is worth noting that the complex with asparagine (in which the two functional groups are charged oppositely) have a significant decrease in the values of ΔE_{inter} compared to other complexes. For example, the ΔE_{inter} value for the ChS-Serine complex in water is –16.83 kcal/mol in the aqueous phase, indicating a difference of about 112.58 kcal/mol with the value in the gas phase. In the case of ChS-Tyrosine this difference is about 2.31 kcal/mol, respectively, the values of the interaction energy are –18.38 (gas phase) and –16.07 (water phase). The results show that monomers with opposite charges are indeed more separated and more stable than complexes in water, which leads to a decrease in ΔE_{inter}.

This can also be explained by the fact that in polar environments the interaction with the environment (solvation) is probably more important than the electrostatic interaction between the two interacting molecules, which leads to their preferential stabilization. In addition, it is interesting to note the invariably negative value of ΔE_{inter} in the aqueous phase for the complex with histidine, which contrasts sharply with what is observed in the gas phase. Due to the solvation of positively charged fragments, the repulsive interaction between them decreases, which can increase the strength of their interaction with the formation of hydrogen bonds. The results obtained ΔE_{inter} in the gas and water phases are very important from the point of view of protein delivery. The results indicate a strong interaction between ChS and AAs in a non-polar environment and a gradual weakening of the interaction in the aqueous phase. These results are of interest in modeling the process of penetration of complexes through a cell membrane, which is non-polar in nature. Thus, it is assumed that in the cytoplasm (which is polar in nature) the interaction will be the weakest, which can promote the dissociation of the complex into the corresponding fragments. It is important to note that the ability of chitosan to release amino acids into the cytoplasmic environment is comparable to that of ChS derivatives [34, 35]. Significantly high value of ΔE_{inter} in the gas phase at a very low energy value in the aqueous phase for the complexes indicates its suitability for use in biomedicine. An increase in the efficiency of ChS as a carrier of nitrogenous bases RNA and DNA was also studied in [36–39].

Table 3

Hydrogen bond distance and BSSE corrected interaction energy of ChS with AAs complexes

Complex	Aqueous phase, Å	$\Delta E_{inter.}$, kkal/mol (Gas phase)	$\Delta E_{inter.}$, kkal/mol (Aqueous phase)
ChS-Asparagine	1.07	–110.81	–22.09
ChS-Threonine	0.96	–127.60	–15.54
ChS-Serine	1.07	–129.41	–16.83
ChS-Glutamin	1.09	–80.56	–16.95
ChS-Alanin	1.026	–42.62	–19.65
ChS-Tyrosin	1.047	–18.38	–16.07
ChS-Histidin	0.96	–11.45	–11.03
ChS-Lysin	1.046	–17.56	–21.14

The use of pure therapeutic peptides and proteins in medicine is relevant but the main problem is stability in the gastrointestinal environment. They are vulnerable to electrophilic attack by various ions present in the gastrointestinal tract resulting in protein degradation. Therefore, understanding the chemical activity of the studied complexes in various media is important from the point of view of their medical applications. Reactivity descriptors determined [40] on the basis of a theoretical physico-chemical study by the DFT and electronic structure have become an auxiliary tool for interpreting the chemical and biological activity of compounds.

The calculated values of these parameters among the selected systems in the gas and water phases are presented in Tables 4 and 5.

Table 4

Calculated electronic parameters in the gas phase of amino acids and complexes

Amino acid	E_{HOMO}, eV	E_{LUMO}, eV	ΔE	Complex	E_{HOMO}, eV	E_{LUMO}, eV	ΔE
Asparagine	–9.98	0.80	10.78	ChS-Asparagine	–9.62	0.56	10.19
Threonine	–9.91	1.02	10.93	ChS-Threonine	–9.15	0.51	9.66
Serine	–10.12	0.83	10.96	ChS-Serine	–9.49	0.35	9.84
Glutamine	–10.23	0.66	10.9	ChS-Glutamine	–9.68	0.5	10.19
Alanine	–9.97	0.77	10.74	ChS-Alanine	–8.78	1.6	10.38
Tyrosine	–9.14	0.14	9.28	ChS-Tyrosine	–8.97	0.35	9.32
Histidine	–8.31	–0.02	8.34	ChS-Histidine	–7.09	–1.13	8.23
Lysine	–9.29	0.92	10.21	ChS-Lysine	–9.53	0.75	10.29

Table 5

Calculated descriptors of reactivity in the gas phase of complexes

Complex	Chemical hardness (η), eV	Electrophilicity Index (ω), eV	Chemical potential (μ), eV	Softness (ζ), eV^{-1}
ChS-Asparagine	4.52675	2.8691	–5.09665	0.220909
ChS-Threonine	4.32	2.7031	–4.8327	0.231481
ChS-Serine	4.56855	2.6514	–4.92205	0.218888
ChS-Glutamine	4.5875	2.8307	–5.0963	0.217984
ChS-Alanine	3.5879	3.7573	–5.1925	0.278715
ChS-Tyrosine	4.31255	2.5226	–4.66455	0.231881
ChS-Histidine	2.98245	5.9649	–5.9649	0.335295
ChS-Lysine	4.39235	3.0155	–5.14695	0.227669

The measurement of the E_{HOMO} of the complexes is an important factor since this characteristic indicates the electron donating capacity, i.e. reactivity of compounds. The narrow HOMO-LUMO band gap means that the molecule has low kinetic stability and high biological activity. According to the calculation results, there is a sharp drop in the HOMO energy in AAs during the formation of a complex with ChS, which speaks in favor of a more stable HOMO in complexes than in AAs. This result means that complexes of ChS with AAs are less prone to attack by any electrophile than the AAs themselves. Similarly, a high neg-

ative value of μ means a relatively large stability of the system. As can be seen from Tables 4 and 5, in accordance with E_{HOMO} and E_{LUMO}, data on the chemical potential also characterize the greater chemical stability of the complexes than AAs and also show differences in the nature of the interaction with respect to different AAs. The narrow energy gap characterizes the nucleophilic properties of the complexes. According to the calculation data given in Table 4, the smallest band gap ΔE (8.34 eV) is observed for the ChS-Histidine complex and 9.28 eV for ChS-Tyrosine. All complexes have a wide HOMO-LUMO band gap. This may be due to low chemical activity and high kinetic stability.

Chemical hardness quantitatively determines the chemical stability of a molecular system in various media [37]. The higher the electronegativity, the more electronegative the molecule is, and the higher the value of chemical hardness, the "harder" the molecule is [38–40]. According to Table 5, the value of chemical hardness (2.98) for ChS-Histidine indicates a greater stability of the complex than AAs but at the same time characterizes a large difference in the interaction energy compared to other AAs. The chemical mildness of ChS-Histidine increases and the activity increases. Moreover, in all cases, the maximum stiffness is not associated with a small value of electrophilicity. In addition, it is worth noting that the stability predicted by the change in the interaction energy of the gas phase coincides with the trend shown by the values of E_{HOMO} and η. The order of stability in the gas phase of the ChS-Histidine complex according to the values of E_{HOMO}, η, and ζ, which also correlates with the value of the interaction energy. However, this correlation of interaction energy values with reactivity descriptor values for the ChS-Histidine system is not consistent with those of other interactions of ChS with AAs. As in the case of complexes with lysine and tyrosine, the highest values of the interaction energy do not correlate with the values of η and ζ. This fact of discrepancy can be explained by the fact that the LUMO of AAs (acting as an H-donor/acceptor of electrons) in different complexes interact to a different extent. Although the nature of the change in η does not allow us to make any general conclusion about the chemical stability of the studied complexes, the values of HOMO and η do confirm the greater stability of the ChS-Histidine complex compared to histidine, as well as the orders of stability, the calculated values of η and ζ are in full agreement with calculated values ΔE_{inter}.

Conclusions

Thus, on the basis of the carried out studies, it was shown that the initial macromolecules of *Bombyx mori* protein and chitosan biopolymers have their own behavioral features. A protein alkaline hydrolysate was obtained from *Bombyx mori* pupae with a protein content that contains amino- and -carboxylic functional groups and amino acids radicals located in the protein chain, which are capable of ionization. It is shown that the structure of chitosan is characterized by a certain rigidity and ionogenicity. Based on the results of the studies, the fundamental possibility of obtaining complexes of chitosan with the *Bombyx mori* protein at various pH values was revealed.

The results of calculations based on the quantum-chemical theory of the density functional of the interaction of chitosan with amino acids that are part of the *Bombyx mori* protein confirm the presence of a hydrogen bond. Complexes are more chemically stable than pure amino acids. The calculated values of the reactivity descriptors and stability of the complexes are sensitive to the nature of the functional modification, as well as to the prevailing environment. It should be noted that the complex with histidine is unstable in the gas phase but acquires significant stability in the aqueous environment. Chitosan exhibits a stronger interaction in a nonpolar environment and a gradual weakening is observed with increasing polarity of the environment, although there is no linear correlation between the interaction energy and the permittivity of the environment.

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Хитозан *Bombyx mori* бар ақуыздың полимолекулалық кешендері

Хитозан (ХЗ) мен Bombyx mori протеинінің әртүрлі реакция жағдайларындағы әрекеттесуі зерттеліп, хитозанның *Bombyx mori* ақуызымен кешендерін алудың іргелі мүмкіндігі анықталды. Ерітінділердегі хитозанмен ақуыздың полимолекулалық кешенінің түзілуі физика-химиялық әдістердің нәтижелерімен дәлелденді. Хитозанның құрылымы белгілі бір қаттылықпен және ионогенділікпен сипатталатыны көрсетілген. pH = 4,8–6,7 диапазонында 2 % сірке қышқылында хитозанмен қуыршақ ақуызының комплексі көрсетілді. ИҚ-спектрлердегі жұтылу жолақтарының анықталған өзгерістері мен ығысулары pH = 4,88–6,7 кезінде хитозан мен ақуыз молекулалары арасында күрделі түзілу реакциясының болғанын растайды. ол 1641 $см^{-1}$, 1538 $см^{-1}$ және 1068 $см^{-1}$ ИҚ-спектрлердегі жұтылу жолақтарымен сипатталады. Жібек құртының *Bombyx mori* қуыршақтарынан бөлініп алынған ақуыздың құрамына кіретін аминқышқылдарымен (АК) хитозан кешендерін кванттық-химиялық зерттеу DFT әдісімен жүргізілді. Кешеннің түзілуі кезінде сутектік байланыс арқылы газ фазасында гистидинмен түзілген комплексті қоспағанда, ХЗ-АК кешендерінің тұрақтылығы көрсетілген. Термохимиялық талдау нәтижелері кейбір ерекшеліктерді қоспағанда, ХЗ-АК комплексінде күшті термодинамикалық қозғаушы күштің бар екенін көрсетті.

Кілт сөздер: жібек өндірісінің қалдықтары, жібек құртының қуыршақтары, сілтілі гидролиз, ақуыз, хитин, хитозан, полимолекулалық кешендер, конформациялық сипаттамалар.

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Полимолекулярные комплексы белка с хитозаном *Bombyx mori*

Исследовано взаимодействие хитозана (ХЗ) и белка *Bombyx mori* при различных условиях реакции, выявлена принципиальная возможность получения комплексов ХЗ с белком *Bombyx mori.* Образование полимолекулярного комплекса белка с ХЗ в растворах подтверждено результатами физико-химических методов. Показано, что структура ХЗ характеризуются определенной жесткостью и ионогенностью. Показано комплексообразование куколочного белка с ХЗ в 2% уксусной кислоте в диапазоне pH = 4,8–6,7. Обнаруженные изменения и смещения полос поглощений в ИК-спектрах подтверждают протекание реакции комплексообразования между молекулами ХЗ и белка при pH = 4,88–6,7,

которое характеризуется полосами поглощения на ИК-спектрах при 1641 $см^{-1}$, 1538 $см^{-1}$ и 1068 $см^{-1}$. Методом DFT проведено квантово-химическое исследование комплексов ХЗ с аминокислотами (АК), входящими в состав белка, выделенного из куколок тутового шелкопряда *Bombyx mori*. Показано, что стабильность комплексов ХЗ-АК, за исключением комплекса, образованного с гистидином в газовой фазе. Результаты расчетов свидетельствовали о наличие сильной термодинамической движущей силы при комплексообразовании ХЗ-АК.

Ключевые слова: отходы производства шелка, куколки тутового шелкопряда, щелочной гидролиз, белок, хитин, хитозан, полимолекулярные комплексы, конформационные характеристики.

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