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Research paper

Study of the binding of copper (II) ions to the heavy chain of silk fibroin using the charmm22 force field

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Abstract

Silk fibroin is of great interest due to its unique mechanical properties and preparation of biomaterials. It is important to study the mechanisms of the interaction of fibroin with metal ions and the composition of the resulting complexes. We studied the possibilities of coordination binding of copper (II) ions to the heavy chain of the silk fibroin molecule of calculations using the CHARMM22 force field and determined the optimal parts for coordination bonds. To practically confirm the results obtained in theoretical calculations, samples of fibroin containing copper (II) ions were analyzed by ATR-FTIR spectroscopy, and it was proved that copper ions are coordinately bound to the groups in fibroin. The agreement of the results obtained in the theoretical calculations with the results of the actual analysis confirms that indeed, copper (II) ions correspond to the conditions studied by modelling.

Keywords: *Complex; Copper; Coordination Bond; Heavy Chain; Silk Fibroin.*

1. Introduction

Silk fiber is one of the most widely produced natural fibers in the world. Silk fibers consist of two types of proteins, and are composed of fibroin fibers coated with sericin. Fibroin protein contains 18 different amino acids, including glycine, alanine, and serine in varying amounts [1-4]. Many studies are being conducted on the possibility of obtaining and using various metal complexes of silk fibroin. X-rays of natural silk show that the fibroin protein is composed of crystal fragments - fibrils, hence the name "fibroin". Compared with other biomaterials, silk fibroin (SF) has good mechanical properties and exhibits high strength and toughness. Silk fiber shows very good elongation properties and can stretch up to 20÷25% under standard conditions. Mechanical properties are determined by the main hydrophobic protein domains [5]. These domains occupy a large part of SF and are responsible for insolubility, high strength of fibers, and thermal stability, and this is related to β-sheet secondary structure [6].

SF consists of three chains: light (25÷26 kDa), heavy (320÷390 kDa) and P25 glycoprotein (25÷30 kDa) fibroin. Heavy and light chains are connected by a disulfide bond. P25 glycoprotein binds by non-covalent bonds, with heavy and light chains linked by disulfide bonds.

S. Mizuno and others found that the P25 molecule plays an important role in maintaining the complex integrity of fibroin chains. The ratio of light, heavy and P25 glycoproteins in this complex is 6:6:1 according to studies [7-9].

The hydrophobic domains of the heavy chain contain repeating hexapeptide sequences Gly-Ala-Gly-Ala-Gly-Ser and repeating dipeptides Gly-Ala/Ser/Tyr, which can form stable antiparallel sheet crystal fragments. The amino acid sequence of the light chain is not repeated, so the light chain is more hydrophilic and relatively flexible. Compared to β-sheet parts of the heavy chain, among them, there are few random and α-conformational chains [10, 11].

Many studies have been conducted on the interaction of copper(II) ions with SF and the mechanisms of the formation of copper(II)-fibroin complexes.

It has been found that conventional activated carbon sorbents for ammonia removal are not as effective as expected. A new alternative method of removing ammonia by coordination and exchange reactions with metal-SF complex fibers are proposed and studied. Electron spin-resonance spectra (ESR) and atomic absorption spectrometry analyses show that the $Cu(O)_4$ complex with low copper(II) content is formed in a large amount of aqueous solution of silk fiber under acidic conditions. When the amount of copper(II) is high, a complex $Cu(N)_4$ structure is formed in the solution. According to the experimental results, it was found that the $Cu(O)_4$ complex is more effective than the $Cu(N)_4$ complex in terms of ammonia adsorption [12].

Due to the impressive mechanical properties of natural fibers, much attention has been paid to the natural mechanism of silkworm spinning. The analysis of EPR, ^{13}C NMR and Raman spectroscopy showed that the changes in the conformational components of the regenerated SF obtained from Bombyx mori fibers are significantly dependent on pH and Cu (II) ions. When the pH was lowered from 8 to 4, the coordination atoms of Cu (II) in SF were found to change from four nitrogen to two nitrogen and two oxygen, as well as one nitrogen and three oxygen. Adding a certain amount of Cu(II) ions to the SF solution can effectively induce a change from the soluble helix conformation to the insoluble β-conformation. SF has been proposed to coordinate with two detonated main chain nitrogen from two glycine residues and one nitrogen or oxygen from serine. Under weak acidic conditions, Cu(II)-amide bonds can be broken and Cu(II) can move from two histidine in adjacent peptide chains to bind two nitrogen, forming intermolecular His(N)−Cu(II). It has been studied that this type of coordination can cause β-sheet formation and aggregation, leading to crystalline structure [13].

Copper ions were used to induce water-insoluble crystallization of SF by freeze treatment. At less than 5% by weight of copper(II) chloride dihydrate, it was found that the Cu(II) ions forming the water-resistant materials formed silk I crystal networks. In addition, it has been studied that chelation of copper(II) ion further increases the stability of SF when a small amount of copper chloride is ingested. Increasing the amount of copper(II) chloride weakened the crystallization of silk I and Cu(II) ion chelation, which made the SFs unstable in water. Silk I with 1.5 wt % copper(II) chloride dihydrate showed the strongest water stability and the highest loading efficiency [14].

We examined the possibility of copper(II) ions complex formation with random chains of heavy chains of SF through theoretical calculations.

2. Theoretical part

Calculations are performed using certain types of CHARMM force fields for proteins. These include CHARMM19, CHARMM22 and its dual potential corrected variant CHARMM22/CMAP, as well as later versions CHARMM27, CHARMM36 and various variants such as CHARMM36m and CHARMM36IDPSFF [15].

The CHARMM22 force field takes into account quantum chemical calculations of protein atomic partial charges, and interactions between model compounds and water. In addition, CHARMM22 is fully parameterized for the TIP3P pure water model [16]. The CHARMM22 force field has the following potential energy function [17]:

$$V = \sum_{bonds} k_b (b-b_0)^2 + \sum_{angles} k_\theta (\theta - \theta_0)^2 + \sum_{dihedrals} k_\phi [1+\cos(n\phi - \delta)] + \sum_{impropers} k_\omega (\omega - \omega_0)^2 + \sum_{Urey-Bradley} k_u \ (u-u_0)^2 + \sum_{nonbonded} \left(\varepsilon_{ij} \left[\left(\frac{R_{\min_{ij}}}{r_{ij}} \right)^{12} - 2 \left(\frac{R_{\min_{ij}}}{r_{ij}} \right)^{6} \right] + \frac{q_i q_j}{\varepsilon_r r_{ij}} \right)$$

CHARMM software allows you to create and analyze a wide range of molecular simulations. The most basic types of simulation are the minimization of a given structure and the production processes of the molecular dynamics trajectory. Advanced features include free energy perturbation (FEP), quasi-harmonic entropy estimation, correlation analysis, and coupled quantum and quantum mechanics–molecular mechanics (QM/MM) methods.

Based on the above, the CHARMM calculation program was used to study the possibilities of complex formation between SF and copper(II) ions.

3. Results and discussion

The UniProt database contains precise scientific information on the structure and chemical composition of heavy, light and P25 macromolecular chains of Bombyx mori SF. The database shows that the heavy chain contains 5263 amino acid residues and has a molecular mass of 391593.33 Da. The amino acid residues with the largest share in the heavy chain are Ser (12.1 %), Ala (30.3 %) and Gly (45.9 %) [9].

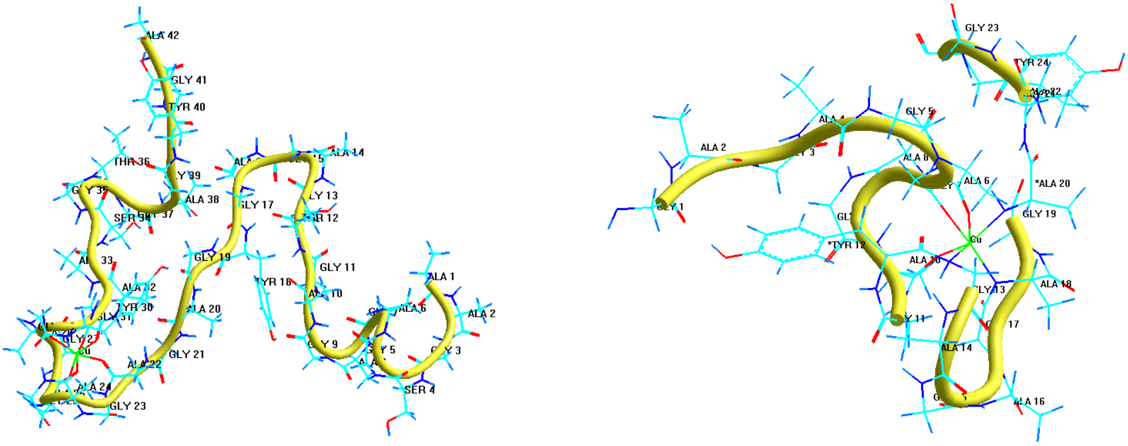
We studied the possibilities of binding copper(II) ions to the hydrophilic random chain structures in the heavy chain. The studied sites in the heavy chain are marked in blue (Scheme 1).

MRVKTFVILCCALQYVAYTNANINDFDEDYFGSDVTVQSSNTTDEIIRDASGAVIEEQITTKKMQRKNKNHGILGKNEKMIKTF
VITTDSDGNESIVEEDVLMKTLSDGTVAQSYVAADAGAYSQSGPYVSNSGYSTHQGYTSDFSTSAAVGAGAGAGAAAGSGAG
AGAGYGAASGAGAGAGAGAGAGYGTGAGAGAGAGYGAGAGAGAGAGYGAGAGAGAGAGYGAGAGAGAGAGYGAGAG
AGAGAGYGAGAGAGAGAGYGAASGAGAGAGYGQGVGSGAASGAGAGAGAGSAAGSGAGAGAGTGAGAGYGAGAGAGA
GAGYGAASGTGAGYGAGAGAGYGGASGAGAGAGAGAGAGAGYGTGAGYGAGAGAGAGAGAGYGAGAGAGYGAG
YGVGAGAGYGAGYGAGAGSGAASGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGTGA
GSGAGAGYGAGAGAGYGAGAGSGAASGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGYGAGAGAGYGAGAGAGY
GAGAGVGYGAGAGSGAASGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGVGY
GAGVGAGYGAGYGAGAGAGYGAGAGSGAASGAGAGAGAGTGSSGFGPYVANGGYSRSDGYEYAWSSDFGTGSGAGAG
SGAGAGSGAGAGSGAGAGSGAGAGSGAGAGYGAGVGVGYGAGYGAGAGAGYGAGAGSGAASGAGAGSGAGAGSGAGAG
SGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGVGSGAGAGSGAGAGVGYGAGAGVGYGAGAGSG
AASGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGVGYGAGVGAGYGAGYGAG
AGAGYGAGAGSGAASGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGA
GSGAGAGYGAGAGAGYGAGYGAGAGAGYGAGAGSGAASGAGSGAGAGSGAGAGAGSGAGAGSGAGAGSGAGAGSGAGA
GSGAGAGSGAGAGYGAGVGAGYGAGYGAGAGAGYGAGAGSGAASGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGA
GSGAGAGSGAGVGYGAGYGAGAGAGYGAGAGSGAASGAGAGAGAGAGTGSSGFGPYVAHGGYSGYEYAWSSESDFGTGS
GAGAGSGAGAGSGAGAGSGAGAGSGAGYGAGVGAGYGAGYGAGAGAGYGAGAGSGAGSGAGAGSGAGAGSGAGAGSGA
GAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGYGAGYGAGAGAGYGAGAGSGAGSGAGAGSGAGAGSGAGAGSGAGA
GSGAGAGSGAGAGSGAGAGSGAGAGYGAGVGAGYGAGYGAGAGAGYGAGAGSGAGSGAGAGSGAGAGSGAGAGSGAGV
GSGAGAGSGAGAGSGAGAGSGAGAGYGAGYGAGAGAGYGAGAGSGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGS
GAGAGSGAGAGSGAGVGYGAGVGAGYGAGYGAGAGAGYGAGAGSGAASGAGAGAGAGTGSSGFGPYVANGGYSGYE
YAWSSESDFGTGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGYGAGYGAGAGAGYGAGAGSGAGSGAGAGSGAGAGSGA
GAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGSGSGAGAGSGAGAGSGAGAGYGAGVGAGYGVGYGAGAGAGYGAGAGS
GAASGAGAGAGAGTGSSGFGPYVAHGGYSGYEYAWSSESDFGTGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAG
AGSGAGAGYGAGVGAGYGAAYGAGAGAGYGAGAGSGAASGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAG
AGSGAGAGSGAGAGSGAGAGSGAGAGYGAGAGAGYGAGAGSGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGS
GSGAGAGSGAGAGSGAGAGYGAGVGAGYGAGYGAGAGAGYGAGAGSGAGSGAGAGSGAGAGYGAGAGAGYGAGYGAG
AGAGYGAGAGTGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGSGSGAGAGSGAGAGSGAGAGSGAGA
GSGAGAGSGAGAGYGAGAGAGYGAGYGAGAGAGYGAGAGSGAGSGAGAGSGAGAGSGAGAGSGAGAGYGAGYGAGAGS
GAASGAGAGAGAGTGSSGFGPYVAHGGYSGYEYAWSSESDFGTGSGAGAGSGAGAGAGAGSGAGAGYGAGVGAGY
GAGYGAGAGAGYGAGAGSGTGSGAGAGSGAGAGYGAGVGAGYGAGAGSGAAFGAGAGAGSGAGAGSGAGAGSGAGA
GSGAGAGSGAGAGYGAGYGAGVGAGYGAGAGSGAASGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGYGAGVGA

GYGAGYGAGAGAGYGAGAGSGAASGAGAGSGAGAGAGSGAGAGSGAGAGSGAGAGSGAGSGAGAGSGAGAGSGAGAGY
GAGAGSGAASGAGAGAGAGAGTGSSGFGPYVANGGYSGYEYAWSSESDFGTGSGAGAGSGAGAGSGAGAGSGAGAGSGAG
AGYGAGVGAGYGAGYGAGAGAGYGAGAGSGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGYGAG
AGSGAASGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGYGAGVGAGYGVGYGAGAGAGYGAGAGSGAG
SGAGAGSGAGAGSGAGAGSGAGAGSGAGSGAGAGSGAGAGSGAGAGSGAGSGAGAGSGAGAGYGVGYGAGAGAGYGAG
AGSGAGSGAGAGSGAGAGSGAGAGSGAGSGAGAGSGAGAGSGAGAGSGAGAGYGAGVGAGYGVGYGAGAGAGYGAGAG
SGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGS
GAGAGSGAGAGSGAGAGSGAGAGYGAGVGAGYGVGYGAGVGAGYGAGAGSGAASGAGAGSGAGAGAGSGAGAGSGAGA
GSGAGAGSGAGAGSGAGAGSGAGAGYGAGYGAGVGAGYGAGAGVGYGAGAGAGYGAGAGSGAASGAGAGAGSGAGAGT
GAGAGSGAGAGYGAGAGSGAASGAGAGAGAGAGTGSSGFGPYVANGGYSGYEYAWSSESDFGTGSGAGAGSGAGAGSGAG
AGSGAGAGSGAGAGYGAGVGAGYGAGAGSGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGYGAGAGSGTGSGAGAGS
GAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGVGAGYGVGYGAGAGAGYGVGYGAGAGAGYGAGAGSGTGSGAGAGSGA
GAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGYGAGVGAGYGVGYGAGAGAGYGAGAGSGAGSGAGAGSGAGAGSGA
GAGSGAGAGSGAGSGAGAGSGAGAGSGAGAGSGAGSGAGAGSGAGAGYGVGYGAGAGAGYGAGAGSGAGSGAGAGSGA
GAGSGAGAGSGAGSGAGAGSGAGAGSGAGAGSGAGAGYGAGVGAGYGVGYGAGAGAGYGAGAGSGAGSGAGAGSGAGA
GSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGSGAGAGSGAGAGSGAGAGSGAGAGYGAGVGAGYGVGYGAGAGA
GYGAGAGSGAASGAGAGAGAGAGTGSSGFGPYVANGGYSGYEYAWSSESDFGTGSGAGAGSGAGAGSGAGAGYGAGYGA
GVGAGYGAGAGVGYGAGAGAGYGAGAGSGAASGAGAGAGAGAGSGAGAGSGAGAGAGSGAGAGYGAGYGIGVGAGYGA
GAGVGYGAGAGAGYGAGAGSGAASGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGYGAGYGAGVGAGY
GAGAGVGYGAGAGAGYGAGAGSGAASGAGAGAGAGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGA
GAGSGAGAGSGAGAGYGAGVGAGYGAGYGGAGAGYGAGAGSGAASGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAG
AGYGAGAGSGAASGAGAGAGAGAGTGSSGFGPYVNGGYSGYEYAWSSESDFGTGSGAGAGSGAGAGSGAGAGYGAGVGA
GYGAGYGAGAGAGYGAGAGSGAASGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGSGAGAGSGAGAGSGAGAGSGAGAGSGA
GAGSGAGAGYGAGVGAGYGAGYGAGAGAGYGAGAGSGAASGAGAGSGAGAGAGSGAGAGSGAGAGSGAGAGSGAGAGS
GAGAGSGAGSGAGAGSGAGAGYGAGYGAGVGAGYGAGAGVGYGAGAGAGYGAGAGSGAASGAGAGSGSGAGSGAGAGS
GAGAGSGAGAGAGSGAGAGSGAGAGSGAGAGYGAGYGAGAGSGAASGAGAGAGAGAGTGSSGFGPYVANGGYSGYEYA
WSSESDFGTGSGAGAGSGAGAGSGAGAGYGAGVGAGYGAGYGAGAGAGYGAGAGSGAGSGAGAGSGAGAGSGAGAGSGA
GAGSGAGAGSGAGAGSGAGAGSGAGAGYGAGYGAGAGAGYGAGAGVGYGAGAGAGYGAGAGSGAGSGAGAGSGSGAGA
GSGSGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGYGAGYGIGVGAGYGAGAGVGYGAGAGAGY
GAGAGSGAASGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGYGAGAGVGY
GAGAGSGAASGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGSGAGAGSGAGAGYGAGYGAGVGA
GYGAGAGYGAGYGVGAGAGYGAGAGSGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGSGAGAGYGAGAGAGY
GAGAGAGYGAGAGSGAASGAGAGAGAGSGAGAGSGAGAGSGAGSGAGAGSGAGAGYGAGAGSGAASGAGAGSGAGAGA
GAGAGAGSGAGAGSGAGAGYGAGAGSGAASGAGAGAGAGTGSSGFGPYVANGGYSRREGYEYAWSSKSDFETGSGAASGA
GAGAGSGAGAGSGAGAGSGAGAGSGAGAGGSVSYGAGRGYGQGAGSAASSVSSASSRSYDYSRRNVRKNCGIPRRQLVVKF
RALPCVNC

Scheme 1: *Sequence of amino acid residues in the heavy chain of silk fibroin*

Because the penetration of metal ions in an aqueous solution into these hydrophilic and random chains is very high. The probability of joining copper (II) ions to the specified parts was theoretically studied using the CHARMM22 calculation program. Different varieties of binding copper (II) ions were considered, and compounds with the lowest energy state were selected for each selected chain (Fig. 1-3).



AAGSGAGAGAGTGAGAGYGAGAGAGAGAGYG-AASGTGAGYGA
E=493.993648 kcal/mol

GAGAGAGAGAGYGAGAGAGAGAGY
E=496,808151 kcal/mol

Fig. 1: The Minimum Energy States of Copper (II) Ions Bound to AAGSGAGAGAGTGAGAGYGAGAGAGAGAGYGAASGTGAGYGA and GAGAGAGAGAGYGAGAGAGAGAGY Parts of the Heavy Chain.

GAASGAGAGAGAGAGTGSSGFGPYVANGGYSRSDGYEYAWS-SDFGT
E=391.131512 kcal/mol

AGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGA-GAGSGAGAGS
E=569.526807kcal/mol

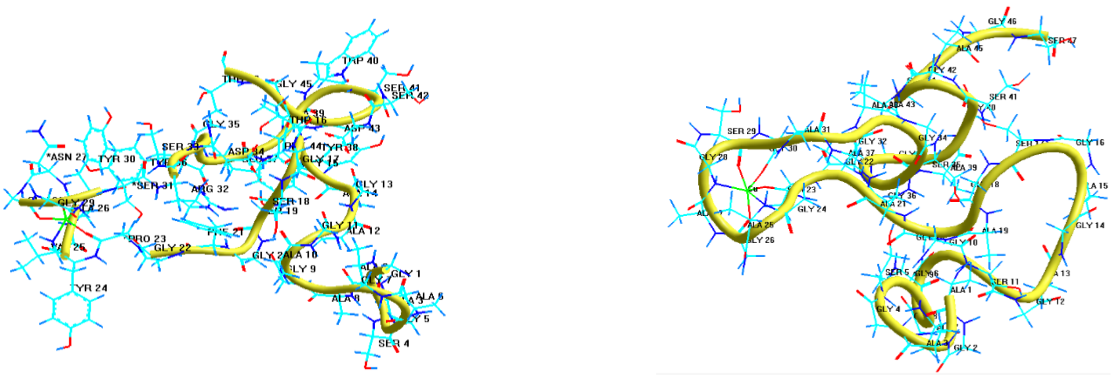
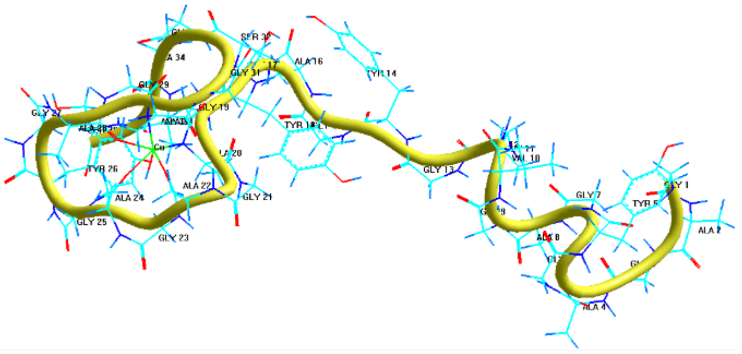


Fig. 2: The Minimum Energy States of Copper (II) Ions Bound to the GAASGAGAGAGAGAGTGSGSFGPGPYVANGGYSRSDGYEYAWSSDFGT and AGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGS Parts of the Heavy Chain.

GAGAGYGAGVGAGYGAGYGAGAGAGYGAGAGSGAAS
E=551.365128 kcal/mol



GAGAGGSVSYGAGRGYGQGAGSAASSVSSASSRSYDYSRRNVRKNCGIPRRQLVVKFRALPCVNC
E=-84.358574 kcal/mol

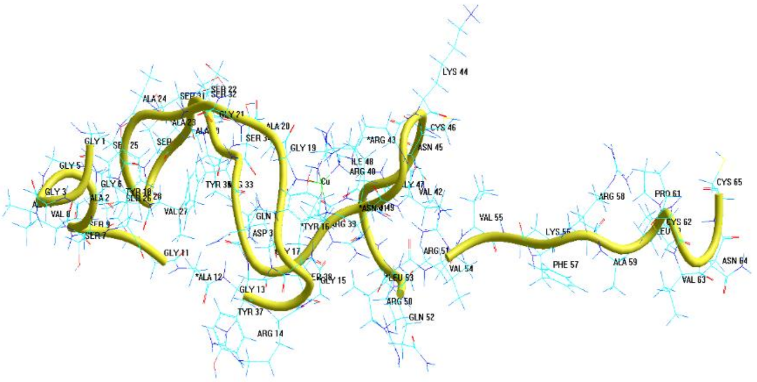


Fig. 3: Minimum Energy States of Copper (II) Ions Bound to the GAGAGYGAGVGAGYGAGYGAGAGAGYGAGAGSGAAS And GAGAGGSVSYGAGRGYGQGAGSAASSVSSASSRSYDYSRRNVRKNCGIPRRQLVVKFRALPCVNC Parts of the Heavy Chain.

According to the results obtained in the calculations, it was found that the connection of copper (II) ions with amino acid residues Ala22, Ala24, Ala26, Ala28, Tyr30, Gly31 in the first selected chain are energetically the most favourable. In this case, copper is modelled in the coordination-bonded state with 6 carbonyl groups of this amino acid residue ($[(C{=}O)_6Cu]$). The total energy has the lowest value when amino acid residues Ala6, Gly7, Ala10, Gly13, Ala20, Ala22 in the second chain bind with copper (II) ions. In this chain, 3 carbonyl groups and 3 nitrogen groups were coordinated with copper ($[(C{=}O)_3(NH)_3Cu]$). Amino acid residues Pro23, Val25, Ala26, Gly28, Gly29, Tyr30 in the third chain were found to bind with copper (II) ions ($[(C{=}O)_3(NH)_3Cu]$). It was found that amino acid residues Ser23, Ala25, Gly26, Gly28, Ser29, Gly30 in the fourth chain selected in the big chain are energetically favourable for binding with copper (II) ions. In this chain, 4 carbonyl groups and 2 nitrogen groups coordinated with copper ($[(C{=}O)_4(NH)_2Cu]$).

Amino acid residues Ala20, Ala22, Ala24, Tyr26, Ala28, Ala30 in the fifth chain were found to bind with copper (II) ions. In this chain, 3 Ala amino acid residues are coordinated to carbonyl groups, Tyr to –OH group, and 2 Ala amino acid residues to nitrogen groups to copper ($[(C{=}O)_3(OH)(NH)_2Cu]$).

As a result of calculations, it was found that the total energy of binding of amino acid residues Tyr16, Gly19, Arg39, Arg40, Asn41, Arg43 with copper (II) ions in the sixth chain has the smallest value. The nitrogen group of Gly, the carbonyl group of the Arg amino acid, the amino group of the Asn amino acid residue, and the -NHNH(NH_2) group of 2 Arg amino acids were modelled in a copper-coordinated state ($[(C{=}O)(OH)(NH)(NH_2)(NHNH(NH_2))_2Cu]$).

Based on the above calculation results, it can be considered that copper (II) ions are located in certain centres of the hydrophilic random parts of the large chain and bind to nitrogen and oxygen functional groups in different proportions.

Studies were conducted to study the connection of copper (II) ions with the fibroin molecule. Hydrolyzed fibroin (HF) samples were placed in aqueous solutions containing copper (II) ions. We have reported about the preparation of HF from silk fibroin fibers and its structure and properties in our previous publications [18, 19]. The main reason we chose HF for the experiment is that it has a large proportion of large chains. Copper (II) ions in solutions are sorbed to HF. At first, it was possible to desorb sorbed copper (II) ions. This phenomenon means that copper (II) ions are sorbed only on the porous surfaces of HF. It indicates that copper (II) ions are not coordinated with the groups in HF. However, it was observed that the HF samples (in the solution medium) that retained copper (II) ions for about 7 days turned yellow, and no desorption of copper (II) ions was observed in the samples. It was assumed that copper (II) ions in the yellow samples were coordinatively connected with functional groups in HF. ATR-FTIR spectroscopy was used to study changes in the composition of these samples (Fig. 4).

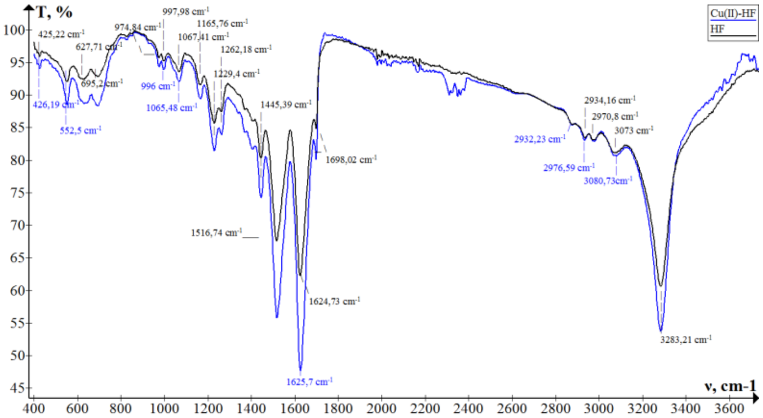


Fig. 4: ATR-FTIR Spectrum of Copper-Containing HF.

An increase in the intensity of the absorption of 426.191 cm^{-1} in the ATR-FTIR spectrum of copper-containing HF was observed. It is known that absorptions related to Cu-N, and Cu-O bonds are observed in the 470÷350 cm^{-1} range in the IR spectra of proteins bound by copper coordination bonds [20, 21]. The absorption area of 426.191 cm^{-1} in the ATR-FTIR spectrum indicates the formation of Cu-N and Cu-O bonds [22]. The increase in the intensity of absorption at 1625.7 cm^{-1} is of particular importance, which is explained by the formation of bonds of copper ions with the amide I group in the β-structure of fibroin. The increase in absorption in the region of 1165.76 cm^{-1} was considered to be related to changes in the Tyr amino acid residue.

Based on the results of theoretical calculation and ATR-FTIR spectroscopy analysis, it can be considered that copper(II) ions are coordinatively connected with amino acid residues in certain parts of the long chain.

4. Conclusion

It was assumed that copper (II) ions were connected to the hydrophilic parts of the heavy chain of silk fibroin with a random structure, and the most favourable energetic states of coordination binding and forming a complex compound were determined by theoretical calculations in the CHARMM22 force field. To verify the theoretical calculations in practice, it was observed that HF samples containing copper (II) ions turned yellow for 7 days, and it was found that copper (II) ions were not desorbed. The structural changes in the yellow samples were studied using ATR-FTIR spectroscopy and the presence of coordination bonds was proved.

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