

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 $\text{Cu}(\text{O})_4$ 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 $\text{Cu}(\text{N})_4$ structure is formed in the solution. According to the experimental results, it was found that the $\text{Cu}(\text{O})_4$ complex is more effective than the $\text{Cu}(\text{N})_4$ 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 deprotonated main chain nitrogen from two glycine residues and

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

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]:

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

[illegible]

[illegible]

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).

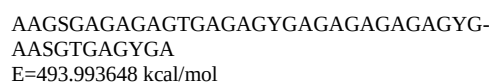
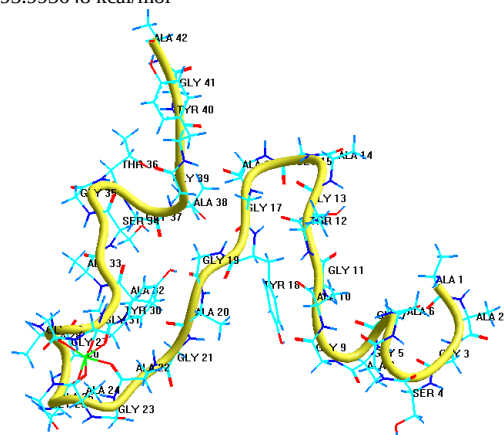
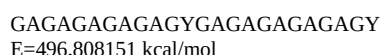
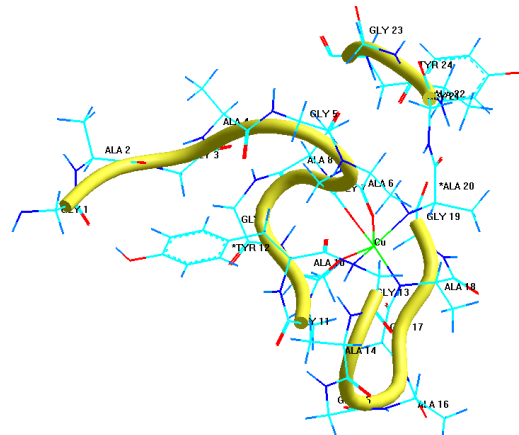
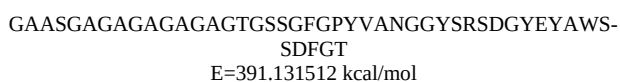
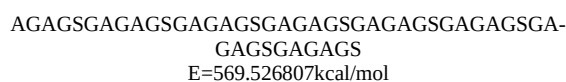


Fig. 1: The Minimum Energy States of Copper (II) Ions Bound to AAGSGAGAGAGTGAGAGYGAGAGAGAGAGYGAASGTGAGYGA and GAGAGAGAGYGAGAGAGAGAGY Parts of the Heavy Chain.





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).

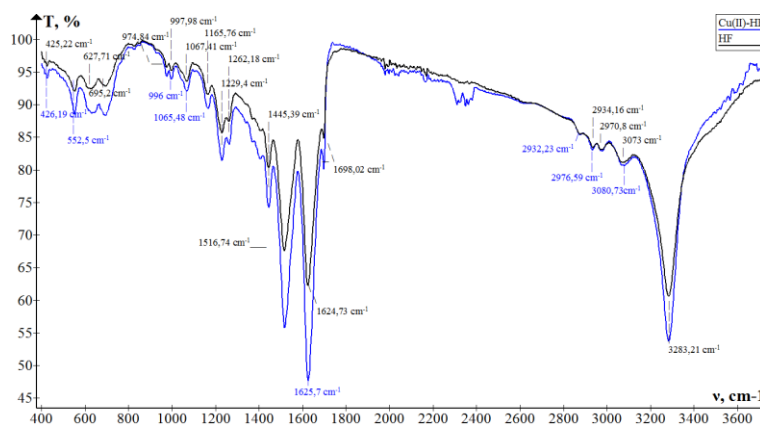


Fig. 4: ATR-FTIR Spectrum of Copper-Containing HF.

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 $470\div350\text{ cm}^{-1}$ 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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