Research Article

Cation-Specific Effects on the Self-Assembly of Collagen Molecules Mediated by Acetate on Mica Surface Observed with Atomic Force Microscopy

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The well-organized collagen layers on mica surface have drawn extensive attention for its essential applications and studies on the process of self-assembly as a model system. In this work, collagen extracted from fish scales by acid-base method was used to explore the self-assembly characters, and atomic force microscopy was applied to observe the collagen assembled on mica surface mediated by acetate with four different cations, including K^+ , Na^+ , Mg^{2+} , and Ca^{2+} . It showed that cations might influence the interaction between collagen fibrils and mica surface at high ionic concentration. And a similar network structure was acquired with uniform pore size for four kinds of acetates; nearly ranged collagen fibrils in the same direction were collected in Mg^{2+} solutions, while flat films with some fibrils were achieved in K^+ solutions. The Hofmeister series and Collins' model were adapted to explain the effects of cations and acetate on the self-assembly of collagen. These results and analysis would be helpful for directing the pattern of collagen assembly on a solid surface with a potential application in food science and engineering.

1. Introduction

At present, collagen is widely added to the productions with high recognition and brilliant effective in cosmetic and functional food. And the collagen scaffolds were used for hemiacorneal reconstruction and regeneration. Collagen is a natural protein composed of glycine, alanine, proline, and 16 other kinds of amino acids; its molecular structure is rich in N-H, C=O, and -OH and other coordination groups which can be crosslinked with some metal ions [1]. Using transmission electron microscopy to determine three-dimensional organization and collagen fibril size has been reported. And collagen extracted forms are subjected to enhance their stability by chemical crosslinking. It has been found that collagen peptide fibers can radially self-assemble after being triggered by metals [2]. A new adsorbent can be prepared

by loading Zr (IV) and Fe (III) onto collagen fibers [3]. 20–40 nm clusters can be formed between collagen fibrils after being crosslinked with Cr^{3+} , but cycle structure of collagen did not change [4]. In this article, the effects of four acetates (KAc , $NaAc$, $MgAc_2$, and $CaAc_2$) were studied on the collagen assembly. Hofmeister series and Collins' model were used to explain the results.

In 1920s, the order of efficiency was followed by some cations as salting-out agents [5]; the order was expressed as $K^+ > Na^+ > \dots > Mg^{2+} > Ca^{2+}$ after more than eighty years [6, 7]. The numbers of papers involved in Hofmeister series related to various fields especially biomedicine are large and still keep growing every year [8–10]. Wang et al. demonstrated a sequence ($SO_4^{2-} > Ac^- > Cl^-$) which has the stabilizing and salting-out effects on proteins and macromolecules [11]. Acetate was selected to investigate the effect of cations on

collagen self-assembly in this study. However the results in this experiment was not fully carried out with the law especially Ca^{2+} ($\text{K}^+ > \text{Na}^+ > \dots > \text{Mg}^{2+} > \text{Ca}^{2+}$), and the ion-specific interactions in the water [12–15] need to be explained.

In this study, collagen was extracted from fish scales by acid-base method and purified by dialysis. At the same time, collagen is a natural extract that can form film because of its molecules self-assembly [16–20]. Furthermore, to make the collagen film more controllable and stable, the metal ions were used to regulate the self-assembly of collagen molecules [21]. It can provide reference for products of cosmetic and food additions.

2. Materials and Methods

2.1. Materials and Chemical Reagents. Live grass carp (*Ctenopharyngodon idellus*) was purchased from a supermarket in Yangling City, Shaanxi, China. Grass carp scales were manually removed from the fish and washed with fresh water. The harvested scales were stored in polyethylene bags at 4°C until experimentation. Potassium acetate, magnesium acetate tetrahydrate, and acetic acid (36%) were purchased from Guangdong Chemical Reagent Engineering-Technological Research and Development Center (Guangdong, China). Calcium acetate monohydrate and sodium acetate trihydrate were purchased from Chengdu Kelong Chemical Reagent Factory (Chengdu, China). All reagents in analytical grade were used as received without further purification. All solutions were prepared with deionized water supplied by the Northwest A&F University. The mica substrate [$\text{KAl}_2(\text{AlSi}_3\text{O}_{10}(\text{OH})_2$] was purchased from Tosai (Hong Kong).

2.2. Preparation of ASC from the Scales of Grass Carp. Acid-soluble collagen (ASC) from the scales was isolated following the reported methods in reference [22] with some modifications. All steps to equip ASC were prosecuted at room temperature (22–24°C). In detail, the scales of grass carp were drought in an automatic blast drying oven and then immersed in 20 volumes of 0.5 M Na_2CO_3 with a magnetic stirring for 3 h, and the operation was repeated three times. After filtrating by cheesecloth, the insoluble components were rinsed with deionized water until a neutral pH was reached. The insoluble components were reimmersed in 0.3 M EDTA for 12 h. After rinsing to neutral pH, the insoluble components were reimmersed in 40 volumes of 0.5 M acetic acid using a magnetic stirring for 24 h and repeated again. Both of the supernatants were collected and merged as the original ASC solution. The NaCl was added to the ASC original solution to a salt concentration of 0.9 M. The collagen precipitation would be salted out for 24 hours and then redissolved completely with 0.5 M acetic acid. Then, the collagen solution was dialyzed with 0.1 M acetic acid and deionized water for 12 h, respectively. The ASC supernatant was lyophilized into solid and then stored at 4°C for further experiment.

2.3. Preparation of Collagen Solution for AFM Imaging. 1% acetic acid at pH 2.8 was used as the solvent to dissolve collagen solutions and diverse acetate solutions. Collagen

was dissolved in 1% acetic acid solution to obtain 5.0 mg/mL collagen solution which was further diluted to prepare other collagen solutions in different concentrations. All of solutions were stored at 4°C for next experiment. Original acetate solutions in 5.0 M were used to dilute metal salts to various concentrations for further use. 10 μL collagen solution with different cations was deposited onto the freshly cleaved mica sheets in $1.0 \times 0.5 \text{ cm}^2$, respectively, and was dried in sealed desiccators at room temperature (15–20°C). Then, the mica sheets were fixed onto the sample mounting disk in diameter of 12 mm for AFM imaging.

2.4. AFM Imaging of Collagen Assembly. A Multimode-8 AFM (Bruker Co., Santa Barbara, USA) was employed in ScanAsyst mode with ScanAsyst-AIR probes for topology of collagen assembly at 0.977 Hz. Typical height images, roughness, and surface area difference were statistically analyzed using the offline software NanoscopeAnalysis V1.10 (Bruker Co., Santa Barbara, USA). Root mean square roughness (Rq) and average roughness (Ra) were collected to describe the topological change, and the surface area difference was gathered to indicate the degree of surface coverage which was named the difference between the analyzed region's three-dimensional surface area and its two-dimensional, projected area.

3. Results and Discussion

3.1. Self-Assembly of Collagen in Different Collagen Concentration. Typical AFM height images of collagen assembly on mica in different collagen concentrations were shown in Figure 1. It was shown that the morphology of self-assembled collagen in different concentrations has a significant difference. Section profiles of height images, roughness (Ra, Rq), and surface area difference were shown in Table 1. At 5 $\mu\text{g/mL}$, the collagen molecules were self-assembled into single fibers on the mica sheet with the width of $16 \pm 4 \text{ nm}$ and the height of $1.027 \pm 0.055 \text{ nm}$, and Ra was 0.142 nm, Rq was 0.188 nm, and surface area difference was 0.311% (Figure 1(a)). Then, the length of collagen fibers was gradually increased and interwoven into porous network structure with the width of $27 \pm 9 \text{ nm}$, the height of $1.165 \pm 0.077 \text{ nm}$, and Ra of 0.233 nm, Rq of 0.317 nm, and surface area difference of 0.313% (Figure 1(b)). At low concentrations, single fibers were the mainly self-assembled morphology of collagen molecules. Collagen fibers got thicker and crosslinked on the mica sheet with each other at the width of $41 \pm 15 \text{ nm}$, the height of $1.462 \pm 0.030 \text{ nm}$, Ra of 0.296 nm, Rq of 0.364 nm, and surface area difference of 0.340% at 20 $\mu\text{g/mL}$ (Figure 1(c)). After the concentration of collagen was increased to 50 $\mu\text{g/mL}$, collagen molecules arranged evenly just like rough film on the mica sheet with the width of $42 \pm 9 \text{ nm}$, the height of $1.923 \pm 0.168 \text{ nm}$, Ra of 0.385 nm, Rq of 0.493 nm, and surface area difference of 0.662% which was much higher than other concentrations (Figure 1(d)). The porous structure was observed in the range of $2 \times 2 \mu\text{m}$, as shown in Figure 1(d). There was large difference in height and white particles caused by large local concentration. It was not suitable for observing the details of the self-assembled collagen morphology. So the 20 $\mu\text{g/mL}$

FIGURE 1: Typical AFM height images of collagen fibrils assembled on mica surface at different concentrations of collagen: (a) 5, (b) 10, (c) 20, and (d) 50 µg/mL. Scale bar = 0.4 µm.

TABLE 1: Width, height, roughness (Ra, Rq), and surface area difference of the collagen molecular self-assembly in different collagen, KAc, NaAc, MgAc₂, and CaAc₂ concentrations.

Cation	Concentration	Width/nm	Height/nm	Ra/nm	Rq/nm	Surface area difference
—	5 μ g/mL	16 $\pm$ 4	1.027 $\pm$ 0.055	0.142	0.188	0.311%
	10 μ g/mL	27 $\pm$ 9	1.165 $\pm$ 0.077	0.233	0.317	0.313%
	20 μ g/mL	41 $\pm$ 15	1.462 $\pm$ 0.030	0.296	0.364	0.340%
	50 μ g/mL	42 $\pm$ 9	1.923 $\pm$ 0.168	0.385	0.493	0.662%
K ⁺	25 mM	49 $\pm$ 7	1.906 $\pm$ 0.104	0.489	0.572	0.412%
	50 mM	60 $\pm$ 24	1.413 $\pm$ 0.098	0.369	0.442	0.163%
	100 mM	95 $\pm$ 19	2.279 $\pm$ 0.029	0.651	0.781	0.275%
	300 mM	73 $\pm$ 30	3.349 $\pm$ 0.352	0.999	1.210	0.388%
Na ⁺	50 mM	24 $\pm$ 4	1.302 $\pm$ 0.252	0.264	0.349	0.633%
	100 mM	27 $\pm$ 4	1.397 $\pm$ 0.449	0.289	0.360	0.388%
	200 mM	46 $\pm$ 14	2.817 $\pm$ 0.170	0.591	0.743	0.625%
	400 mM	45 $\pm$ 8	1.401 $\pm$ 0.084	0.378	0.471	0.179%
Mg ²⁺	50 mM	43 $\pm$ 10	2.378 $\pm$ 0.078	0.544	0.188	0.501%
	100 mM	76 $\pm$ 14	2.665 $\pm$ 0.058	0.643	0.317	0.594%
	300 mM	81 $\pm$ 5	2.435 $\pm$ 0.143	0.604	0.364	0.208%
	400 mM	59 $\pm$ 17	2.056 $\pm$ 0.048	0.441	0.493	0.634%
Ca ²⁺	100 mM	38 $\pm$ 4	0.892 $\pm$ 0.021	0.168	0.233	0.147%
	200 mM	47 $\pm$ 8	1.319 $\pm$ 0.058	0.453	0.536	0.238%
	300 mM	56 $\pm$ 13	1.378 $\pm$ 0.068	0.346	0.516	0.375%
	400 mM	58 $\pm$ 10	1.083 $\pm$ 0.111	0.222	0.311	0.205%

collagen solution was selected to study the impact of metal ions.

3.2. Effect of KAc on Self-Assembly of Collagen Molecules.

To investigate the effect of cation concentration, 20 μ L of 20 μ g/mL collagen solutions in five different KAc concentrations (25, 50, 100, 300, and 500 mM) was deposited onto the fresh cleaved mica sheet and used for AFM imaging after drying in the air. Typical AFM height images of collagen fibrils assembled on mica surface at 20 μ g/mL collagen in the presence of different concentrations of KAc were shown in Figure 2. Section profiles of height images, roughness (Ra, Rq), and surface area difference were shown in Table 1. In the presence of 25 mM KAc, fibril-like collagen mesh was formed on mica surface with the width of 49 $\pm$ 7 nm and the height of 1.906 $\pm$ 0.104 nm. Ra was 0.489 nm, Rq was 0.572 nm, and surface area difference was 0.412% (Figure 2(a)). And the mesh pore had less difference in size which on average was 1.906 nm, as shown in Figure 2(a). Instead of the fibril-like collagen mesh at 25 mM KAc, the root-like collagen fibrils were shown at higher KAc concentrations and changed from straight alignment at 50 mM to regular assembly at 100 mM KAc in detail. The fibers overlap to root-like structure with numerous branches at a diameter of 26 nm and the width was 60 $\pm$ 24 nm and the height was 1.413 $\pm$ 0.098 nm. Ra was 0.369 nm, Rq was 0.442 nm, and surface area difference was 0.163% at 50 mM KAc (Figure 2(b)). Then the pluralities of fibers are aggregated in fibrous bundles with a diameter of 38–138 nm and fibers were 1.46–5.31 thicker than at 25 mM KAc (Figure 2(c)). The width was 95 $\pm$ 19 nm, the height was 2.279 $\pm$ 0.029 nm, Ra was 0.651 nm, Rq was 0.781 nm,

and surface area difference was 0.275% at 100 mM KAc. The numbers of dispersed collagen fibers were significantly increased and the porous network was reconstructed instead of the fiber bundles (Figure 2(d)). The width was 73 $\pm$ 30 nm, the height was 3.349 $\pm$ 0.352 nm, Ra was 0.999 nm, Rq was 1.210 nm, and surface area difference was 0.388% at 300 mM KAc. It was shown that the collagen molecules assembled into the fibrous network on the mica sheet at low concentrations, the fibers gathered into bundles with the increase of the concentration, and the salinity had a great influence at high concentrations.

3.3. Effect of NaAc on Self-Assembly of Collagen Molecules.

AFM images of collagen assemblies on mica surface in the presence of various concentration of NaAc (50, 100, 200, and 400 mM) were shown in Figure 3. Section profiles of height images, roughness (Ra, Rq), and surface area difference were shown in Table 1. In the presence of 50 mM NaAc, it was clearly observed that the collagen fibers were evenly distributed on the mica surface with the width of 24 $\pm$ 4 nm and the height of 1.302 $\pm$ 0.252 nm, and Ra was 0.264 nm, Rq was 0.349 nm, and surface area difference was 0.633% (Figure 3(a)). Then the collagen fibers were woven into beautiful pattern of flowers with uniform pores at the width of 27 $\pm$ 4 nm, the height of 1.397 $\pm$ 0.449 nm, Ra of 0.289 nm, Rq of 0.360 nm, and surface area difference of 0.388% at 100 mM NaAc (Figure 3(b)). When it came to 200 mM NaAc, the multilayers of fibrils assembled on the basis of dense porous mesh organized on the mica sheet with the width of 46 $\pm$ 14 nm and the height of 2.817 $\pm$ 0.170 nm, and Ra was 0.591 nm, Rq was 0.743 nm, and surface

FIGURE 2: Typical AFM height images of collagen fibrils assembled on mica surface at 20 $\mu\text{g/mL}$ collagen in the presence of different concentrations of KAc: (a) 25, (b) 50, (c) 100, and (d) 300 mM. Scale bar = 0.4 μm .

FIGURE 3: Typical AFM height images of collagen fibrils assembled on mica surface at 20 $\mu\text{g/mL}$ collagen in the presence of different concentration of NaAc: (a) 50, (b) 100, (c) 200, and (d) 400 mM. Scale bar = 0.4 μm .

area difference was 0.625% (Figure 3(c)). At 400 mM NaAc, smooth, flat, and flaky membrane appeared with fibers (the width was 45 ± 8 nm) attaching at the film thickness of 1.401 ± 0.084 nm and Ra was 0.378 nm, Rq was 0.471 nm, and surface area difference was 0.179% which was much lower than other concentrations (Figure 3(d)). Obviously, the collagen molecules assembled into fibers or multilayer fibers on the mica sheet at the low concentrations, and flat membrane at higher concentrations. It demonstrated that the solubility of collagen increased with the NaAc concentration; that is, collagen stability was becoming weaker and completely denatured when the collagen films emerged.

3.4. Effect of MgAc_2 on Self-Assembly of Collagen Molecules.

The effect of four MgAc_2 concentrations (50, 100, 300, and 400 mM) on 20 $\mu\text{g/mL}$ collagen self-assembly is shown in Figure 4. Section profiles of height images, roughness (Ra, Rq), and surface area difference were shown in Table 1. In the presence of 50 mM MgAc_2 , the porous network can be clearly observed on the mica sheet and it had strong directionality making the pores elliptical. The width was 43 ± 10 nm, the height was 2.378 ± 0.078 nm, Ra was 0.544 nm, Rq was 0.188 nm, and surface area difference was 0.501% at 50 mM MgAc_2 (Figure 4(a)). The collagen molecules were assembled into dense fiber mesh with small uniform pores at the width of 76 ± 14 nm and the height of 2.665 ± 0.058 nm, and Ra was 0.643 nm, Rq was 0.317 nm, and surface area difference was 0.594% at 100 mM MgAc_2 (Figure 4(b)). When the MgAc_2 concentration increased to 300 mM, single fibers got thicker with consistent directionality at the width of 81 ± 5 nm and the height of 2.435 ± 0.143 nm, and Ra was 0.604 nm, Rq was 0.364 nm, and surface area difference was 0.208% (Figure 4(c)). The multilayered fibers were distributed on the mica sheet at the width of 59 ± 17 nm and the height of 2.056 ± 0.048 nm, and Ra was 0.441 nm, Rq was 0.493 nm, and surface area difference was 0.634% at 400 mM MgAc_2 (Figure 4(d)). It indicated that collagen molecules assembled into dense mesh after the MgAc_2 was added, which was similar to the effect of NaAc.

3.5. Effect of CaAc_2 on Self-Assembly of Collagen Molecules.

The effect of six CaAc_2 concentrations (100, 200, 300, and 400 mM) on 20 $\mu\text{g/mL}$ collagen self-assembly was proved in Figure 5. Section profiles of height images, roughness (Ra, Rq), and surface area difference were shown in Table 1. In the presence of 100 mM CaAc_2 , collagen microfibers were observed on the mica sheet with the width of 38 ± 4 nm and the height of 0.892 ± 0.021 nm, and Ra was 0.168 nm, Rq was 0.233 nm, and surface area difference was 0.147% at 100 mM CaAc_2 (Figure 5(a)). The collagen fibrils changed from disordered arrangement to regular network with uniform pores with the width of 47 ± 8 nm, the height of 1.319 ± 0.058 nm, Ra of 0.453 nm, Rq of 0.536 nm, and surface area difference of 0.238% at 200 mM CaAc_2 (Figure 5(b)). When it came to 300 mM, single fibers were organized on the mica sheet with longer length at the width of 56 ± 13 nm and the height of 1.378 ± 0.068 nm, and Ra was 0.346 nm, Rq was 0.516 nm, and surface area difference was 0.375% (Figure 5(c)). Then the long fibers were disconnected into short ones with similar

length and they gathered into bundles at the width of 58 ± 10 nm and the height of 1.083 ± 0.111 nm, and Ra was 0.222 nm, Rq was 0.311 nm, and surface area difference was 0.205% at 400 mM CaAc_2 (Figure 5(d)). It was confirmed that the collagen molecules assembled into the fibrils on the mica sheet at low concentrations (Figure 5(a)); then the fibers got longer and intertwined into network at medium concentrations (Figures 5(b)-5(c)); finally they gathered into bundles and tend to be randomly dispersed at high concentrations (Figure 5(d)).

The effects of cations on the self-assembly of collagen molecules were demonstrated in four acetates. With the increase of Na^+ and Mg^{2+} concentration, the morphology of self-assembled collagen molecules changed from single-layer structure to multilayer network or flat membrane on the mica sheet, which indicated that Na^+ and Mg^{2+} could promote the dissolution of collagen in acidic solution. Collagen molecules self-assembled into coarse fibers at low and medium concentrations and better aggregation especially at high concentrations. It was shown that K^+ and Ca^{2+} can effectively reduce the solubility of collagen and make the collagen macromolecules more stable in acidic solution.

The order of $\text{K}^+ > \text{Na}^+ > \text{Mg}^{2+} > \text{Ca}^{2+}$ was mentioned in Hofmeister series, which was followed on the efficiency of cations as salting-out agents. In this article, however, Collins' model was required to analyze it which did not fully comply with Ca^{2+} . At the atomic level, Ca^{2+} has a larger radius than Na^+ and Mg^{2+} ; there would be a bigger amount of water molecules combined with Ca^{2+} to hydrated molecules. A large and loose hydrated shell was formed at Ca^{2+} outer layer, so that the two ions cannot form a close ion pair, leading to a large number of Ca^{2+} freely dispersed in the solution. Due to electrostatic interaction, numbers of Ca^{2+} were adsorbed to the protein, making hydrogen bonds greatly weakened on the polypeptide chains, and finally, the solubility of collagen was decreased.

4. Conclusions

The studies on the morphology and stability of collagen self-assembly in presence of four metal cations (K^+ , Na^+ , Mg^{2+} , and Ca^{2+}) by AFM can lead to the following conclusions. The strong effect of K^+ on collagen self-assembly belongs to kosmotropes in the Hofmeister series, which can increase the stability of collagen. The binding of K^+ and Ca^{2+} with collagen molecules will lead to the decrease of collagen hydrogen-bonding and the increase of the protein hydrophobicity; and the combination of both ions and collagen leads to a decrease in hydrophobicity on the collagen molecules. In contrast, the effects of Na^+ and Mg^{2+} on collagen self-assembly were typically chaotropic and will cause collagen assembly homogeneous dense network or flat membrane and the increase of collagen solubility. In this study, Hofmeister series [11] and Collins' model [12–15] were used to explain the interaction mechanism of collagen and different cations at the molecular level. It provides theoretical guidance and data supports for the biological, medical, food, and cosmetic manufacturing industries.

FIGURE 4: Typical AFM height images of collagen fibrils assembled on mica surface at 20 $\mu\text{g/mL}$ collagen in the presence of different concentration of MgAc_2 : (a) 50, (b) 100, (c) 300, and (d) 400 mM. Scale bar = 0.4 μm .

FIGURE 5: Typical AFM height images of collagen fibrils assembled on mica surface at 20 µg/mL collagen in the presence of different concentration of CaAc_2 : (a) 100, (b) 200, (c) 300, and (d) 400 mM. Scale bar = 0.4 µm.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

- [1] H. Liu, Y. Xi, S. Ge, and S. Wang, "Synthesis, characterization, and antibacterial activity of collagen-metal complexes," *Journal of Zhengzhou University (Medical Sciences)*, vol. 67, pp. 1675–1686.
- [2] D. E. Przybyla and J. Chmielewski, "Metal-triggered radial self-assembly of collagen peptide fibers," *Journal of the American Chemical Society*, vol. 130, no. 38, pp. 12610–12611, 2008.
- [3] B. R. Locke, M. Sato, P. Sunka, M. R. Hoffmann, and J. Chang, "Electrohydraulic discharge and nonthermal plasma for water treatment," *Industrial & Engineering Chemistry Research*, vol. 45, pp. 3896–3901, 2006.
- [4] B. Wu, C. Mu, G. Z. And, and W. Lin, "Effects of Cr³⁺ on the structure of collagen fiber," *Langmuir*, vol. 25, no. 19, pp. 11905–11910, 2009.
- [5] M. Randall and C. F. Failey, "The Activity coefficient of the undissociated part of weak electrolytes," *Chemical Reviews*, vol. 4, no. 3, pp. 291–318, 1927.
- [6] T. Oncsik, G. Trefalt, M. Borkovec, and I. Szilagyi, "Specific ion effects on particle aggregation induced by monovalent salts within the Hofmeister series," *Langmuir*, vol. 31, no. 13, pp. 3799–3807, 2015.
- [7] L. He, S. Cai, B. Wu, C. Mu, G. Zhang, and W. Lin, "Trivalent chromium and aluminum affect the thermostability and conformation of collagen very differently," *Journal of Inorganic Biochemistry*, vol. 117, pp. 124–130, 2012.
- [8] P. Lo Nostro and B. W. Ninham, "Hofmeister phenomena: An update on ion specificity in biology," *Chemical Reviews*, vol. 112, no. 4, pp. 2286–2322, 2012.
- [9] X. Tadeo, B. López-Méndez, D. Castaño, T. Trigueros, and O. Millet, "Protein stabilization and the hofmeister effect: The role of hydrophobic solvation," *Biophysical Journal*, vol. 97, no. 9, pp. 2595–2603, 2009.
- [10] W. Kunz, J. Henle, and B. W. Ninham, "'Zur Lehre von der Wirkung der Salze' (about the science of the effect of salts): Franz Hofmeister's historical papers," *Current Opinion in Colloid & Interface Science*, vol. 9, pp. 19–37, 2004.
- [11] L. Wang, Y. Guo, P. Li, and Y. Song, "Anion-specific effects on the assembly of collagen layers mediated by magnesium ion on mica surface," *The Journal of Physical Chemistry B*, vol. 118, no. 2, pp. 511–518, 2014.
- [12] K. D. Collins, "Ions from the Hofmeister series and osmolytes: effects on proteins in solution and in the crystallization process," *Methods*, vol. 34, no. 3, pp. 300–311, 2004.
- [13] K. D. Collins, "Ion hydration: Implications for cellular function, polyelectrolytes, and protein crystallization," *Biophysical Chemistry*, vol. 119, no. 3, pp. 271–281, 2006.
- [14] K. D. Collins, G. W. Neilson, and J. E. Enderby, "Ions in water: Characterizing the forces that control chemical processes and biological structure," *Biophysical Chemistry*, vol. 128, no. 2–3, pp. 95–104, 2007.
- [15] J. Heyda, T. Hrobárik, and P. Jungwirth, "Ion-specific interactions between halides and basic amino acids in water," *The Journal of Physical Chemistry A*, vol. 113, no. 10, pp. 1969–1975, 2009.
- [16] P. D. Yurchenco, H. Furthmayr, and A. Coritz, "Self-assembly of basement membrane collagen," *Biochemistry*, vol. 23, no. 8, pp. 1839–1850, 1984.
- [17] D. A. Cisneros, C. Hung, C. M. Franz, and D. J. Muller, "Observing growth steps of collagen self-assembly by time-lapse high-resolution atomic force microscopy," *Journal of Structural Biology*, vol. 154, no. 3, pp. 232–245, 2006.
- [18] G. D. Pins, D. L. Christiansen, R. Patel, and F. H. Silver, "Self-assembly of collagen fibers. Influence of fibrillar alignment and decorin on mechanical properties," *Biophysical Journal*, vol. 73, no. 4, pp. 2164–2172, 1997.
- [19] F. H. Silver, J. W. Freeman, and G. P. Seehra, "Collagen self-assembly and the development of tendon mechanical properties," *Journal of Biomechanics*, vol. 36, no. 10, pp. 1529–1553, 2003.
- [20] C. M. Franz and D. J. Muller, Humana Press, 736, 97–107, 2011.
- [21] W. Cai, S. W. Kwok, J. P. Taulane, and M. Goodman, "Metal-assisted assembly and stabilization of collagen-like triple helices," *Journal of the American Chemical Society*, vol. 126, no. 46, pp. 15030–15031, 2004.
- [22] H. Wang, Y. Liang, H. Wang, J. Zhou, and H. Zhang, "Isolation and partial biological properties of scale collagens from grass carp (*Ctenopharyngodonidellus*)," *Journal of Fisheries of China*, vol. 36, pp. 553–561, 2012.