Viscosity Properties of Collagen Solutions: Influence of pH, Salt Type and Salt Concentration

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Abstract

In this work, the effects of pH, salt type, and salt concentration on the viscosity behaviour of collagen solutions were investigated by a photoelectric viscometer. The results show that the viscosity behaviour as polyelectrolytes or polyampholytes depended on the charge distribution on the polypeptide chains, which can be tuned by the value of pH of the collagen solution. The intrinsic viscosity of collagen in Na_2SO_4 solution at pH2.0 first decreases and then increases with increasing salt concentration, while a monotonic decrease of the intrinsic viscosity of collagen is observed in NaCl solution. The intrinsic viscosity of collagen in $Cr(OH)SO_4$ solution at pH4.0 is found to be increased first and then decreased with an increase in salt concentration, whereas an opposite trend is observed in same solution at pH2.0. We suggest that the complicated viscosity behaviour of collagen solution is attributed to the comprehensive effects of shielding, overcharging and crosslinking caused by the introduction of the different counterions. In addition, the viscosity result is also confirmed by dynamic light scattering(DLS) experiment.

摘要

利用光电粘度计研究了pH值、盐的种类和盐浓度等因素对胶原溶液粘度行为的影响。结果表明，通过调节胶原溶液的pH值可以改变多肽链上的电荷分布,使胶原溶液的粘度行为表现出聚电解质或两性聚电解质的特性。当pH2.0时, 胶原在Na_2SO_4溶液中的特性粘度随盐的浓度提高先减小后增加，而在NaCl溶液中呈现单调递减的趋势。当pH4.0时,胶原在碱式硫酸铬溶液中的特性粘度随盐浓度的升高先增加后减小，而在pH2.0 的溶液中呈现出相反的变化趋势。胶原分子在盐溶液中的复杂粘度行为可以归因于盐离子的屏蔽、电荷过剩以及交联效应。此外，动态光散射实验也证实了粘度的结果。

1 INTRODUCTION

Collagen is a fibrous protein, which is a major component of skin and bone. Type I collagen, the most abundant protein found in tissues, is a right-hand helix composed of two identical α1(I) chains and one α2(I) chain, which are held together by interchain hydrogen bonds. Each polypeptide chain has a repeating triplet of amino acids (Gly-X-Y), where X and Y are always proline (Pro) and hydroxyproline (Hyp), respectively.[1] Collagen, a long slender molecule about 300nm in length and 1.5nm in width, adopts a triple helical conformation which has been characterized by X-ray diffraction and molecular dynamics studies of collagen like peptides.[2-5]

Collagen has a broad variety of industrial and biomedical applications due to its distinctive advantages – biodegradability, weak antigenicity and superior biocompatibility. The primary reason for the usefulness of a biopolymer in these applications is that collagen molecule can form fibres through its self-aggregation and cross-linking.[6] As a polyampholyte, the aggregation behaviour of collagen molecules in solutions is easily altered by salt addition and variation of pH values of polymer solution. Generally, the addition of a monovalent salt merely results in an increase of ionic strength, and there is no specific interaction between the counterions and charged polymer chains. The screening of the electrostatic interactions between charges on the polymer backbone allows the chain to collapse and assume a more compact conformation, leading to a decrease of solution viscosity.[7-9] However, the situation is different for multivalent salts. In addition to the non-specific electrostatic effects, they can interact specifically with charged groups on the polymer chains.[10] The binding of multivalent counterions to a polyelectrolyte was generally attributed to the electrostatic attractions,[11-13] whereas some researchers proposed that this condensation was driven by the release of waters from the hydration shell of the counterion upon binding.[14] The nature of interaction between multivalent counterion and polyelectrolyte is still under debate.

Although much attention has been paid to studies of the interaction of multivalent salt with collagen fibres,[15-19] few efforts have been made to investigate this interaction between multivalent salt and collagen molecules in solutions.[20] In this work, we have investigated the viscosity behaviour of calf skin collagen (type I) in the presence of different salts with various valency by use of a photoelectric viscometer. The effects of pH and counterion valency on the viscosity behaviour of collagen solutions were discussed in detail. In addition, dynamic light scattering

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(DLS) was also applied to explore Cr^{3+} – collagen interactions. Knowledge of the viscosity behaviours of collagen in salt solutions is crucial for elucidating the mechanism of leather tanning, which is directly related to the interaction between collagen molecules and multivalent metal ions.

2 EXPERIMENTAL PROCEDURES

2.1 Materials

Collagen (type I, from calf skin), NaCl, Na_2SO_4, $Cr(OH)SO_4$ and acetic acid were purchased from Sigma-Aldrich Co. LLC. USA. All aqueous solutions were prepared using ultrapure water (18 MO.cm) from a Milli-Q system (Millipore). All of the chemicals were of analytical grade.

Collagen solution was prepared as follows. A certain amount of collagen sample was dissolved in 0.1M acetic acid solution at 4°C and stirred for 10 hours, and then the mixture was centrifuged at 3000r.p.m at room temperature for 30 minutes, the supernatant fluid was filtered through 0.45μm pore-size PTFE membrane filters to remove impurities. Then it was taken for preparation of collagen solutions with different salt concentrations. The concentration of collagen solutions ranges from 0.01 to 1mg/mL.

2.2 Viscosity measurements

A horizontal gravitational capillary viscometer with a capillary radius of around 0.05cm (Shanghai Liangjing Glass Instrument Factory) was used to determine the intrinsic viscosities of collagen in various salt solutions. Viscosity measurements were carried out in a constant temperature bath, and the temperature near the capillary was determined by a thermometer with an accuracy of ± 0.1°C. The shear rates of this horizontal gravitational capillary viscometer are about 50-80 s^{-1}. The constancy of the solvent flow time was applied for judging the cleanliness of the capillary of viscometer and the consistency of the experimental conditions. In this section, we determined the intrinsic viscosity of collagen in various salt solutions by an isoionic dilution method. It should be noted that the adsorption of collagen molecules on the viscometer capillary walls was avoided, as a result of hydrophobic treatment of the surface of the capillary in this work. A detailed description of hydrophobic treatment of the capillary can be found elsewhere.[21]

2.3 Measurement of Dynamic Light Scattering (DLS)

Dynamic light scattering (DLS) measurements were performed on a DynaPro NanoStar (Wyatt Technology Corp., USA). The light source was a 100mW He-Ne laser (the wavelength $\lambda = 658$nm). The concentration of collagen in trivalent chromium salts solution is 0.05mg/mL. The temperature was kept constant at 15 ± 0.1°C using a Pieter controlling system. Measurements were conducted at a scattering angle of 90°. The data were processed in the Dynamics V6 software (Wyatt Technology Corporation). The hydrodynamic radius (R_h) was calculated with the regularization algorithm provided by the software. The final results were the average of three runs.

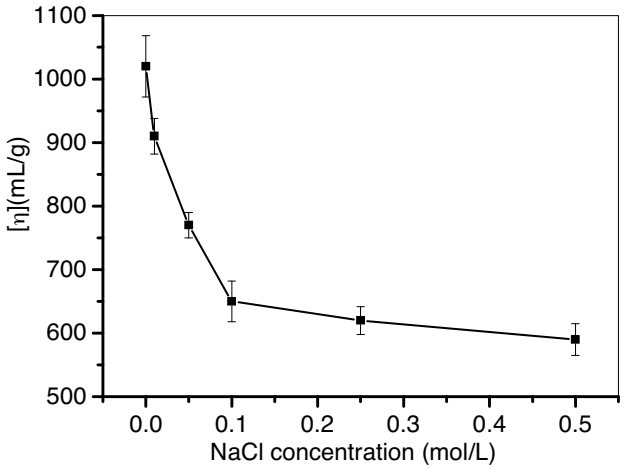

Figure 1. Salt concentration dependence of the intrinsic viscosity of collagen in NaCl solutions (T=15°C, pH2.0).

3 RESULTS AND DISCUSSION

3.1 Effect of counterion valency on the intrinsic viscosity of collagen in salt solutions

Collagen is a typical amphoteric biopolymer since it contains both positive and negative charges due to the functional groups present on the polypeptide chains. Generally, collagen is positively charged in acetic acid solution. In this case, the electrostatic interaction between the positively charged amine functional group has an important effect on the viscosity of collagen solution. Figure 1 shows the salt concentration dependence of the intrinsic viscosity of collagen in NaCl solutions at pH2.0. The results showed that the intrinsic viscosity of collagen decreased monotonously with an increase in NaCl concentration. This phenomenon is very common in polyelectrolyte solution with the addition of simple salt.[22] The conformation of the macromolecules in dilute solutions was readily affected by the intramolecular electrostatic forces. Collagen is positively charged in acetic acid solution at pH2.0, and the counterion is Cl^-. The addition of NaCl only screened this interaction, which led to a monotonic decrease of the intrinsic viscosity of collagen.

However, the situation is different when the multivalent salts are added to the collagen solution. Figure 2 represents the salt concentration dependence of the intrinsic viscosity of collagen in Na_2SO_4 solutions. The results indicated that the intrinsic viscosity of collagen first decreased and then increased with an increase of Na_2SO_4 concentrations. For collagen solution in the presence of Na_2SO_4 at pH2.0, the counterion is SO_4^{2-}. At low Na_2SO_4 concentration, the counterion SO_4^{2-} shielded the intramolecular electrostatic force of collagen and the intrinsic viscosity of collagen decreased. When the concentration of SO_4^{2-} is high enough to neutralize the bare charge NH_3^+, a so-called 'charge inversion effect' may set in as a result of further increase of SO_4^{2-} concentration and the

repulsion between the charges makes the polypeptide chains reexpand.[23] A similar phenomenon has been observed in gelatin/$Cr(OH)SO_4$ system.[24] The result suggests that the addition of SO_4^{2-} can improve the conformational stability of collagen in solutions.

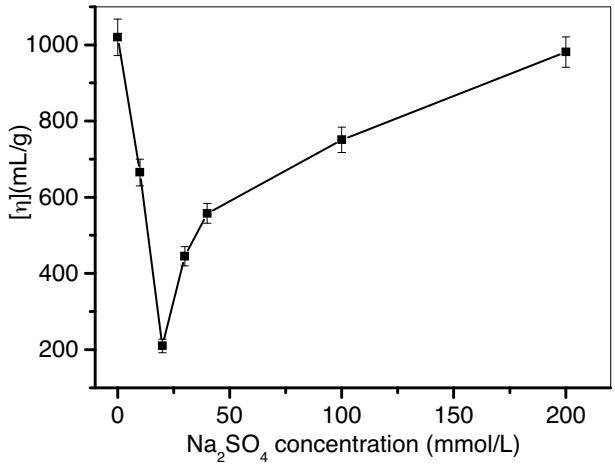

Figure 2. Salt concentration dependence of the intrinsic viscosity of collagen in Na_2SO_4 solutions (T=15°C, pH2.0).

3.2 Effect of pH on the intrinsic viscosity of collagen in $Cr(OH)SO_4$ solutions

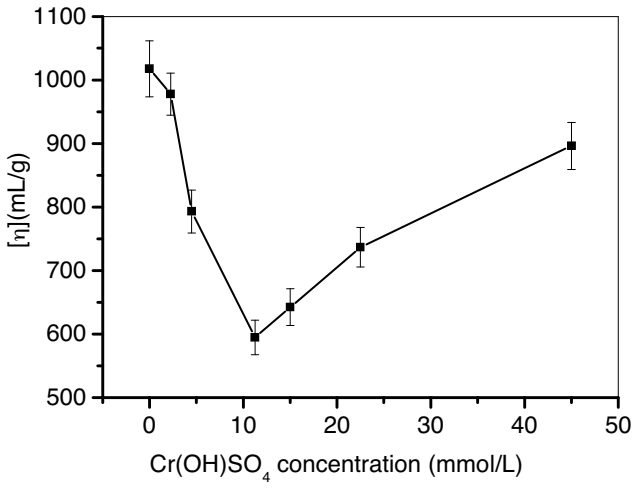

Figure 3. Salt concentration dependence of the intrinsic viscosity of collagen in $Cr(OH)SO_4$ solutions (T=15°C, pH2.0).

Figure 3 shows the salt concentration dependence of the intrinsic viscosity of collagen in $Cr(OH)SO_4$ solutions at pH2.0. Similar to Na_2SO_4, the intrinsic viscosity of collagen first decreased and then increased with an increase in $Cr(OH)SO_4$ concentrations. However, the situation was different when the solution pH values varied from 2.0 to 4.0. As shown in Figure 4, the intrinsic viscosity of collagen first increased and then decreased with an increase in $Cr(OH)SO_4$ concentrations. At pH4.0, -COOH can ionize into COO^-, and both positive and negative charges presented on polypeptide chains. Thus collagen behaves as a polyampholyte, and the individual collagen molecule collapses in salt-free solution, due to the electrostatic attraction between the positively charged amine groups and negatively charged carboxyl groups on polypeptide chains. At low $Cr(OH)SO_4$ concentrations, the polypeptide chains expand due to the weakened electrostatic interactions, which leads to an increase of the intrinsic viscosity of collagen. With a further increase of salt concentration, the individual collagen molecule contracted due to the intrachain complexation of Cr^{3+}/COO^-. Thus the intrinsic viscosity of collagen was found to be decreased at higher salt concentration. In addition, the intramolecular cross-linking effect between SO_4^{2-} and NH^{3+} also contributes to the decrease of the intrinsic viscosity of collagen. It is proposed that the presence of $Cr(OH)SO_4$ facilitates the conformational stability of collagen in solutions.

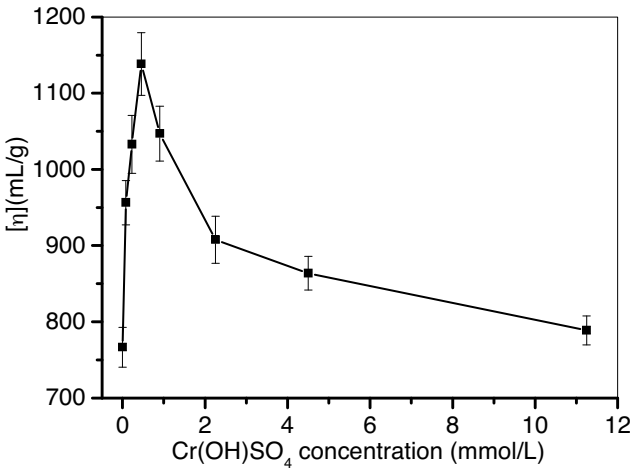

Figure 4. Salt concentration dependence of the intrinsic viscosity of collagen in $Cr(OH)SO_4$ solutions (T=15°C, pH4.0).

3.3 Effect of pH on the average hydrodynamic radius (R_h) of collagen in $Cr(OH)SO_4$ solutions

Generally, the intrinsic viscosity is a molecular parameter which is related to the size of the macromolecule in the solution. Thus the change trend of with salt addition should be the same as the change of intrinsic viscosity. Figure 5 represents the salt concentration dependence of the average

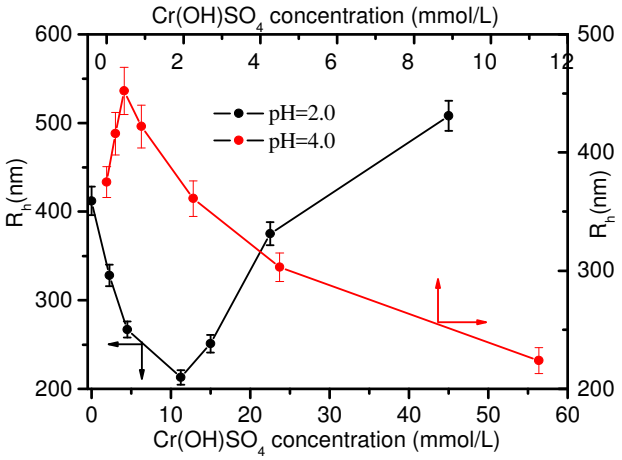

Figure 5. Salt concentration dependence of average hydrodynamic radius (R_h) of collagen in $Cr(OH)SO_4$ solutions at different pH, (the concentration of collagen is 0.05mg/mL, T=15.0°C).

hydrodynamic radius of collagen in $Cr(OH)SO_4$ solutions at different pH values. At pH2.0, theof collagen first decreased and then increased with an increase in $Cr(OH)SO_4$ concentrations, whereas an opposite trend is observed for polymer solutions at pH4.0. The results agree well with that of intrinsic viscosity of collagen in $Cr(OH)SO_4$ solutions.

4. CONCLUSIONS

The intrinsic viscosity of collagen at pH2.0 decreased monotonically with an increase in NaCl concentrations. While it decreased first and then increased with the increasing of Na_2SO_4 as well as $Cr(OH)SO_4$ concentrations. This enhanced intrinsic viscosity may be due to the overcharging effect of the multivalent counterion SO_4^{2-}. The salt concentration dependence of is the same as the dependence of intrinsic viscosity.

The intrinsic viscosity of collagen at pH4.0 increased at low $Cr(OH)SO_4$ concentration, and then decreased with further addition of $Cr(OH)SO_4$. At low salt concentration, the remarkable increase in size of collagen molecules due to the weakened electrostatic interactions. With a further increase of Cr^{3+} concentration, the size of collagen molecules decreased, owing to the intrachain complexation of Cr^{3+}/COO^-, as well as the intramolecular crosslinking effect between SO_4^{2-} and NH_3^+. The addition of $Cr(OH)SO_4$ contributes to the conformational stability of collagen in solutions.

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