Asian Journal of Chemistry; Vol. 25, No. 18 (2013), 10569-10571

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.15991

Effect of $Fe^{2+/3+}$ and Cu^{2+} Ions on Spectral Properties of Type I Collagen

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(*Received*: 3 July 2013; *Accepted*: 27 November 2013) AJC-14449

The effects of Fe^{3+}, Fe^{2+} and Cu^{2+} ions on type I collagen were investigated by UV-visible and fluorescence spectra. The UV-visible spectra revealed that the Fe^{3+} ion can cause the 270 nm absorbance peak of type I collagen decreased, but Fe^{2+} and Cu^{2+} enhanced this peak. The fluorescence emission spectrum of the type I collagen show two emission band centered at 307/278 nm and 380/295 nm, adding Fe^{3+} will enhanced the intensity of emission band both 307 and 380 nm. However, adding Fe^{2+} and Cu^{2+} ions can not cause the two fluorescence peak enhanced evidently. The 380 nm fluorescence peak increased in presence of Fe^{3+} suggested the Fe^{3+} could induce pyridinoline crosslink formed in collagen solution.

Key Words: Fluorescence, Spectral properties, Collagen, Inorganic salt, Pyridinoline crosslink.

INTRODUCTION

Collagen, including more than 20 types, is the main component of connective tissues, which also is the abundant protein in mammals. The collagen is one of the most biomaterials with widely use in drug delivery[1], tissue engineering[2], and cosmetic, *etc*.

The type I collagen is the most abundant in the family of collagens. It forms the fiber with the triple helix by three polypeptides[3], which contain the sequence repeat $(G\text{-}X\text{-}Y)_n$, X being frequently proline and Y hydroxyproline[3,4]. The three polypeptide form a triple helix for collagen fibril, the triple helix structure is stabilized by the hydrogen bonds span between glycine residues and proline in X position of an adjacent chain[3,5].

Metal ions play the critical functional roles for stabilization of protein structure, induction of conformational transitions, *etc*. Silver ions can drive the self-assembly of a peptide to form a nanoscale material[6], and the Fe (II) ion is demonstrated as a trigger for the self-assembly of collagen peptide fiber and the ion complex (Fe-THP) can stability to type I collagen remarkably[7,8]. The copper ion was reported the influence on the collagen cross-linking[9,10].

In present work, the interaction of type I collagen with metal irons, included $FeSO_4$, $Fe(NO_3)_3$ and $CuSO_4$, were investigated by UV-visible and fluorescence spectroscopy. The Fe^{2+} and Cu^{2+} irons caused the increase of the peak at 270 nm in UV-visible spectra, but Fe^{3+} caused descent. And the Fe^{3+} ion could enhance the 380 nm fluorescence emission peak in type I collagen solution, but Fe^{2+} and Cu^{2+} did not. The results suggested Fe^{3+} induced pyridinoline (PYD) crosslink formed in collagen solution.

EXPERIMENTAL

Type I collagen was purchased from Sigma-Aldrich Co. LLC., K_2HPO_4, KH_2PO_4, $Fe(NO_3)_3$, $CuSO_4$, $FeSO_4$ was purchased from Sinopharm Chemical Reagent Co. Ltd.. All the agents directly used without further purification. F-7000 fluorescence spectrophotometer (Hitachi, Japan) and TU-1901 UV spectrophotometer (Beijing Purkinje General Instrument Co. Ltd., China) were employed to insight the reaction. The phosphate-buffered saline (PBS) of pH = 7.2 was prepared by using 50 mmol/L KH_2PO_4 and 50 mmol/L K_2HPO_4. The working solutions were prepared shortly before use.

General procedure: 2.5 mL 1 mg mL^{-1} type I collagen PBS solution (pH = 7.2) was taken into a 1 cm quartz cell, the inorganic salts solution was inject into collagen solution and stirred at room temperature. All measurements were detected in same conditions.

UV-visible studies: The UV-visible spectra of the reaction system between collagen and inorganic irons were detected by TU 1901 UV spectrophotometer in solution pH = 7.2.

Fluorescence studies: The emission spectra for collagen and inorganic irons were recorded using Fluorescence spectrophotometer in solution pH = 7.2. The reaction solutions were excited at 278 and 295 nm, the emission at 307 and 380 nm were monitored, respectively.

RESULTS AND DISCUSSION

Effect of UV-visible spectrum of collagen by inorganic ions: The UV-visible spectrophotometer was employed to detect the effect of inorganic salts on the type I collagen, included $Fe(NO_3)_3$, $CuSO_4$, $FeSO_4$. The data showed in Figs. 1 and 2.

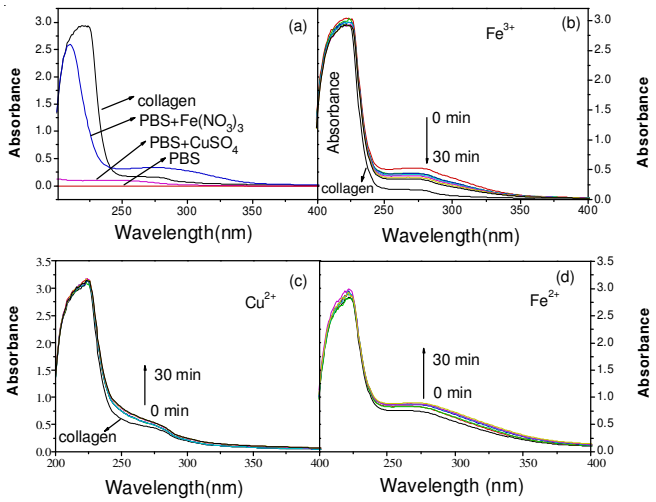

Fig. 1. The UV-visible absorption spectra, (a) include type I collagen solution, PBS, PBS + $Fe(NO_3)_3$, PBS + $CuSO_4$. (b) is the $Fe(NO_3)_3$ react with collagen in PBS solution. (c) is the $CuSO_4$ react with collagen in PBS solution. (d) is the $FeSO_4$ react with collagen in PBS solution

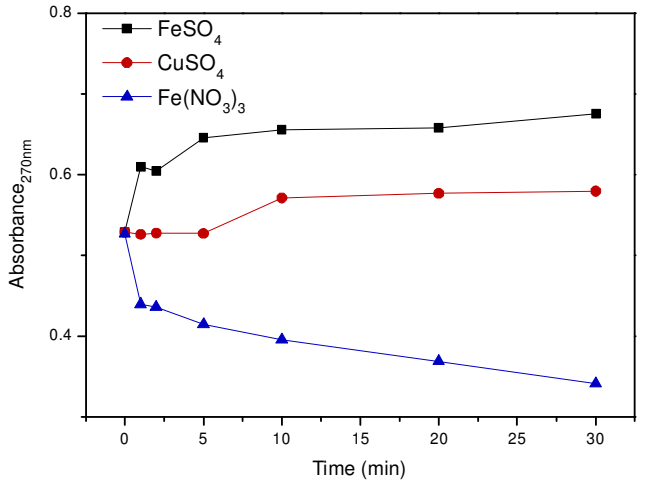

Fig. 2. A plot of intensity of absorb at 270 nm against reaction time in presence of $Fe(NO_3)_3$, $CuSO_4$, $FeSO_4$, respectively

Fig. 1(a) showed that the absorb spectroscopy of type I collagen PBS solution, PBS + $Fe(NO_3)_3$ solution, PBS + $CuSO_4$ solution and PBS solution. Fig. 1 showed the absorb spectroscopy of collagen which shows a strong peak centered at 220 nm and the other around 270 nm, which can be due to the tyrosine[11]. The spectra indicated the $Fe(NO_3)_3$ also has 270 nm absorb peak. Fig. 1(b) showed the absorb peak at 270 nm was enhanced when Fe^{3+} was injected into collagen solution, then decreased with reaction time. The Fig. 1(c) and (d) showed the absorb peak at 270 nm increased with reaction time in presence of $FeSO_4$ and $CuSO_4$. The metal ions induced tyrosine to form the dityrosine formation[11]. The 270 nm peak intensity changed with reaction was recorded in Fig. 2.

Fig. 2 showed the peak at 270 nm descent in presence of Fe^{3+}, but increased when Fe^{2+} and Cu^{2+} present. If there had not any reaction happened, the UV-visible spectroscopy of the reaction system should just is the overlap by collagen and inorganic salt. The result suggested the reaction happened and the collagen stability[8-11] due to Fe^{3+}, Cu^{2+} and Fe^{2+}.

Effect of inorganic ion on tyrosine: The environment can cause the change of the configuration of the protein. Its fluorescence will change with the configuration, herein the fluorescence properties show us the change of the configuration. The Fe^{3+}, Cu^{2+} and Fe^{2+} caused the different change of the UV-visible spectrum, so the fluorescence spectrum of collagen with Fe^{3+}, Cu^{2+} and Fe^{2+} were detected. The 307 nm fluorescence was employed as fluorescence probe to investigate the change of tyrosine of type I collagen in presence of Fe^{3+}, Cu^{2+} and Fe^{2+} ion[11]. All of them were represented in Figs. 3 and 4.

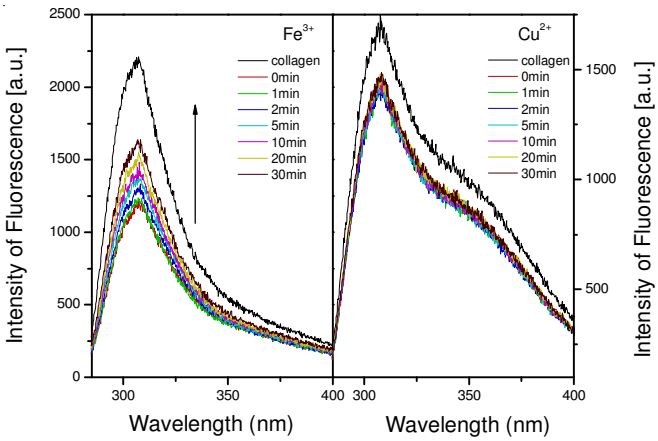

Fig. 3. Change of the fluorescence intensity of collagen with Fe^{3+} and Cu^{2+} with reaction time (λ_{ex} = 278 nm)

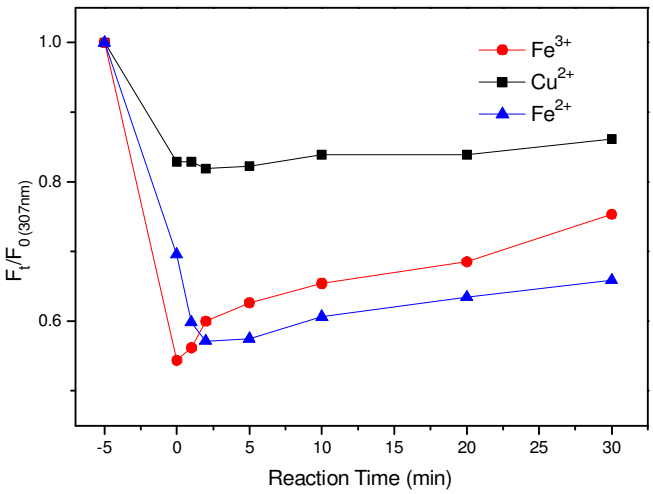

Fig. 4. Ratio of the fluorescence intensity at 307 nm against reaction time in presence of Fe^{3+}, Fe^{2+}, Cu^{2+} ions (λ_{ex} = 278 nm).

Fig. 3 and 4 indicated Fe^{3+}, Cu^{2+} and Fe^{2+} quenched the fluorescence of collagen at 307 nm (excited at 278 nm) at first. The Fe^{3+} iron quenched 45.6 % intensity of fluorescence, the Cu ion only quenched 20.7 % and Fe^{2+} ion quenched 30.4 %. Along prolong the reaction time, the fluorescence intensity at 307 nm increased 38.6 % compared with 0 min when Fe^{3+} present and increased 3 % when Cu^{2+} present within 0.5 h.

The Fe^{2+} caused the intensity fluorescence at 307 nm descent at first and it want to minimum after reaction at 2 min, then it increased but the value was not higher than 0 min. And the fluorescence peak shape was not change, so the data suggested that Fe^{3+}, Fe^{2+} and Cu^{2+} ions did not change the structure of tyrosine reside.

Effect of inorganic ion on type I collagen crosslink: The collagen have many type of crosslink formation, such as pyridinoline (PYD) crosslink, deoxypyridinoline (DPD) crosslink[12,13], PYD crosslink has the fluorescence at 395 nm excited at 295 nm and the intensity fluorescence was identified increase with age[14]. In this experiment, we found fluorescence peak of the PYD crosslink shift to 380 nm, the results were showed in Figs. 5 and 6.

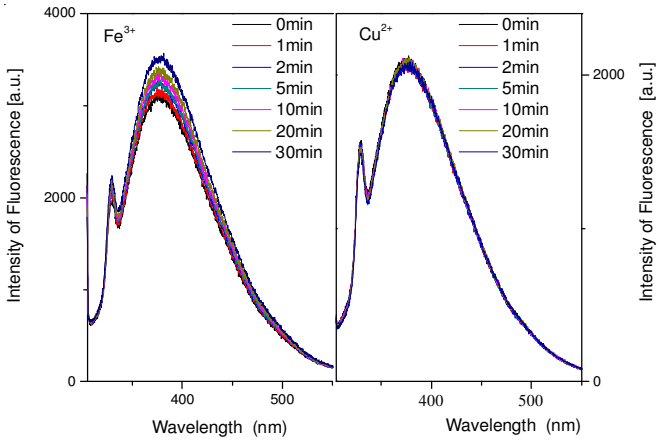

Fig. 5. The change of the fluorescence intensity of collagen with Fe^{3+} and Cu^{2+} with reaction time (λ_{ex} = 295 nm)

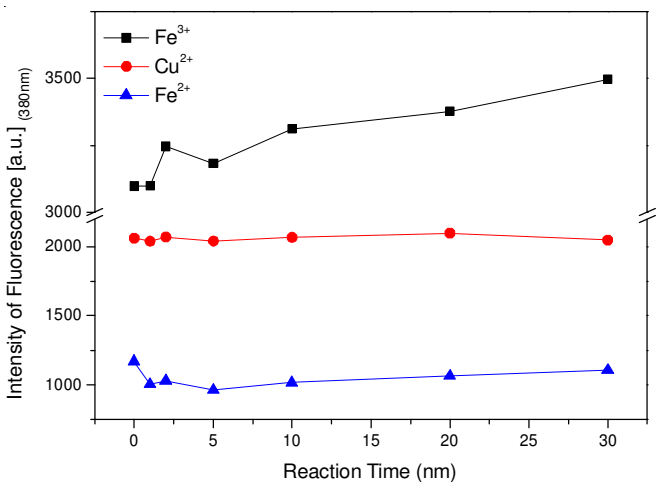

Fig. 6. Fluorescence intensity at 380 nm against reaction time in presence of Fe^{3+}, Fe^{2+}, Cu^{2+} ions (λ_{ex} = 295 nm)

Figs. 5 and 6 showed the fluorescence spectra and fluorescence intensity at 380 nm influenced by Fe^{3+}, Cu^{2+} and Fe^{2+} ions. Fig. 5 showed the fluorescence spectra kept the same shape with native collagen, so the inorganic ions did not affect the structure of the PYD crosslink formation. These figures also showed the fluorescence peak nearly didn't change when Cu^{2+} and Fe^{2+} presence respectively, but the fluorescence peak was enhanced by Fe^{3+}. The result suggested the PYD crosslink contents in collagen increased. The PYD crosslink can't degradation by metal protein enzyme[15]. This mean Fe^{3+} could stability collagen by induced increase the PYD crosslink *in vitro*.

Conclusion

The inorganic ions Fe^{3+}, Cu^{2+} and Fe^{2+} were used to insight the effect to the collagen in PBS (pH = 7.2), the UV-visible spectroscopy and fluorescence spectroscopy were employed to determine the affect. The UV-visible spectra indicated the absorb peak at 270 nm increased in presence of Cu^{2+} and Fe^{2+}, but it descent when Fe^{3+} present. The fluorescence emission spectra showed the fluorescence peak at 307 nm was enhanced by Fe^{3+} and Fe^{2+} and the 380 nm fluorescence peak was only enhanced by Fe^{3+}, the results indicated Fe^{3+} may induce the PYD cross-link formed in collagen solution. The data suggested the collagen self-assembly in solution enhanced by the Fe^{3+} ion. Identification of the mechanism will be the aim of our future investigation. And the Fe^{3+} induced to form the PYD crosslink formation in collagen fiber will be investigated *in vivo*.

ACKNOWLEDGEMENTS

This work was financially supported by National Natural Science Foundation of China (21362010), the Yunnan Natural Science Foundation (2010ZC0153) and Honghe University Science Foundation (XJ1Y0902, 2010PY0106).

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