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Cu^{2+}/Mn^{2+}存在下白花丹素对人血清白蛋白构象的影响

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摘要:用圆二色和拉曼光谱法表征了 Cu^{2+}或 Mn^{2+}存在下白花丹素对人血清白蛋白构象的影响。结果表明,白花丹素改变了人血清白蛋白的二级结构并降低了它的 α-螺旋的含量。同时白花丹素也引起了二硫键的构象以及色氨酸和酪氨酸微环境的变化。在 Cu^{2+}或 Mn^{2+}存在时这种改变的趋势逐渐增强。

关键词:白花丹素;人血清白蛋白;圆二色;拉曼
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Conformational Changes of Human Serum Albumin by Plumbagin in Presence of Cu^{2+} or Mn^{2+}

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Abstract: Circular dichroism (CD) and Raman spectroscopy were employed to investigate the binding of plumbagin (PLU) to human serum albumin (HSA) in the presence of Cu^{2+} or Mn^{2+}. The results indicate that PLU changes the secondary structure of HSA and reduces the α-helix content. The binding of PLU also causes the conformational changes of disulfide bridges and the microenvironment of Tyr, Trp residues. In the presence of the metal ion (Cu^{2+}or Mn^{2+}), the above changes are gradually strengthened.

Key words: plumbagin; HSA; circular dichroism spectroscopy; raman spectroscopy

0 Introduction

Plumbagin (5-hydroxy-2-methyl-1, 4-naphthoquinone, PLU, $C_{11}H_8O_3$, Scheme 1) is a natural naphthoquinone and a major component of the herb Plumbago indica L. In traditional Chinese medicine, the whole plant is usually used for the treatment of rheumatic pain, menostasis, carbuncle, and injury due to bumping[1]. Some pharmacological effects of PLU have been investigated on anticancer[2], anti-leishmanial[3], anti-bacterial[4], anti-fungal properties[5], anti-immediate allergic reaction[6] and anti-Helicobacter pylori infection[7].

Human serum albumin (HSA) is the most important drug carrier protein. It serves to amplify the capacity of plasma for transporting drugs, fatty acids, bilirubin, colic acid and thyroid hormones, and acts also as a buffer on the free drugs concentration[8]. There are many kinds of metal ions stored in blood plasma. These metal ions can bind with serum albumin and participate in many biochemical processes[9]. It has been proven that the conformation

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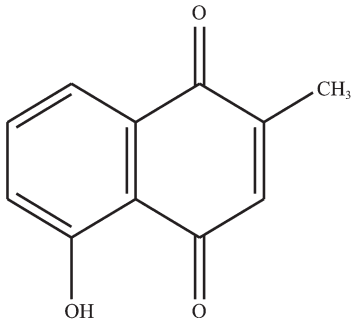


Scheme 1 Chemical structure of PLU

of HSA can be changed after binding with metal ions[10-14]. On the other side, medicine molecules can coordinate with many metal ions. As a result, properties of medicine molecules could also be changed. In the ternary system of drug-protein-metal ion, metal ions would affect the interaction of HSA with medicine molecules. So it is not difficult to deduce that metal ions could influence the distribution, pharmacological property, and metabolism of medicine in blood. Therefore, it is necessary to investigate the interaction of protein-drug in the presence of metal ions[15].

In our previous work, the interaction between PLU and HSA was studied by fluorescence spectroscopy [16]. The thermodynamic parameters, association constant and binding site had been determined. In this work, to explore the influence of Cu^{2+} and Mn^{2+} on the interaction between PLU and HSA, we have studied the ternary system of drug-protein-metal ion by means of CD spectroscopy and Raman spectroscopy.

1 Experimental

1.1 Materials

Human serum albumin was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and used without further purification. PLU was extracted from Plumbago indica L[17].

The HSA stock solution (6.0 μmol·L^{-1}) was prepared in 0.05 mol·L^{-1} phosphate buffer solution (PBS) with pH value of 7.4 and 0.1 mol·L^{-1} NaCl. The stock solutions of PLU (1.0 mmol·L^{-1}) were prepared in PBS. The other chemicals were of analytical reagent grade, and water used in all experiments was doubly distilled water. All stock solutions were stored at 0~4 ℃.

1.2 CD and Raman spectroscopy

The samples were prepared by mixing certain amounts of the corresponding solutions, and then diluted by PBS for spectroscopic study. Circular dichroism (CD) measurements were executed on a Jasco-810 automatic recording spectropolarimeter with a quartz cell of 0.1 cm. The spectra were recorded in the range of 190~250 nm. The Raman spectra were recorded on a Renishaw Invia +Plus FT-Raman spectrometer using an Ar^+ laser excitation with a wavelength of 514 nm. The laser power was 3 mW and the recording range was 300~2 000 nm. Each spectrum should record 0.5 mmol·L^{-1} HSA, 0.5 mmol·L^{-1} HSA+0.5 mmol·L^{-1} PLU, 0.5 mmol·L^{-1} HSA+0.5 mmol·L^{-1} PLU+0.5 mmol·L^{-1} metal ions (Cu^{2+}, Mn^{2+}) mixture solution, respectively. The buffer solution, 0.5 mmol·L^{-1} PLU and 0.5m mmol·L^{-1} metal ions solution were tested.

2 Results and discussion

2.1 Circular dichroism spectra

The circular dichroism spectra in the far ultraviolet region are sensitive to the polypeptide backbone structure of proteins. The negative bands at 208 and 222 nm are rationalized by the n-π^* transition in the peptide bond of α-helix[18].

Fig.1 shows the CD spectra of HSA with PLU in the absence and presence of metal ions. The shape of the CD spectra in presence of PLU and metal ions are similar to that of free HSA, suggesting that the structure of HSA is also predominantly α-helix under our experimental condition [19]. The proportion of α-helix obviously is decreased by adding PLU in the presence of metal ions, indicating the interaction between PLU and HSA, and the metal ions effect on the conformation of HSA.

We can use the following equation to express the CD result (MRE: residue ellipticity):

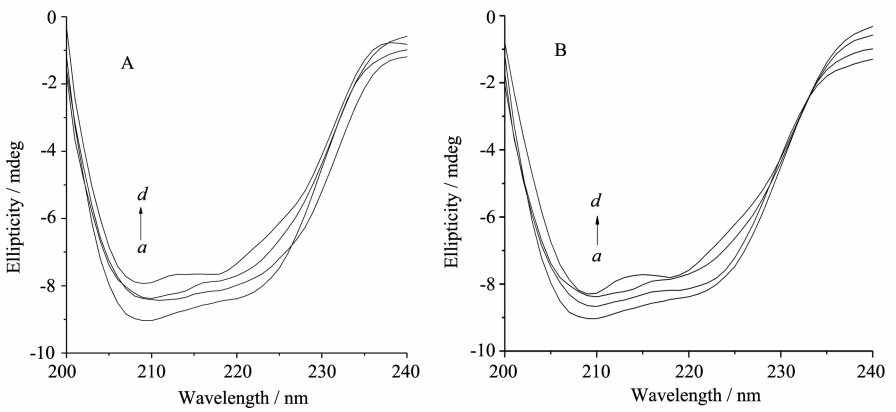
$$\mathrm{MRE} = \theta_{obs}(10nlC_p) \quad (1)$$

where θ_{obs} is the CD in millidegrees, n is the

number of amino acid residues (585), l is the path-length of the cell, and C_p is the molar concentration. The following equation is used to calculate the helical content from the MRE values at 208 nm:

$$W_{\alpha\text{-helix}}=[(MRE_{208}-4\ 000)/(33\ 000-4\ 000)]\times 100\% \quad (2)$$

From the above equation, we can determine the α-helix content in the secondary structure of HSA (Table 1). From Table 1, the content of α-helix of HSA in the presence of metal ions is smaller than the value in the absence of metal ions, indicating that metal ions could promote conformational transition of HSA, i.e. the formation of metal ions-HSA compound leads to the conformational transition of HSA.



A: a: HSA, b: HSA+Cu^{2+}, c: HSA+PLU, d: HSA+PLU+Cu^{2+}; B: a: HSA, b: HSA+Mn^{2+}, c: HSA+PLU, d: HSA+PLU+Mn^{2+}; $c_{HSA}=c_{PLU}=c_{Cu^{2+}}=c_{Mn^{2+}}=8\times10^{-7}$ mol·L^{-1}

Fig.1　CD spectra of HSA with PLU in the absence and presence of metal ions

Table1　The content of α-helix of HSA with PLU, Cu^{2+} and Mn^{2+}

c_{metal}:c_{PLU}:c_{HSA}	Cu^{2+}-HSA-PLU / %	Mn^{2+}-HSA-PLU / %
0:0:1	51.68	51.68
1:0:1	46.81	48.46
0:1:1	46.22	46.22
1:1:1	43.76	45.96

2.2　Raman spectra

The Raman spectra of PLU-HSA-metal ions are showed in Fig.2.

In the Raman spectra, the amide I band of 1 610~1 700 cm^{-1} is assigned to the C=O stretching mode and it is dependent not only on the solvent but also the molecular environment[20]. The spectral region at 1 650~1 600 cm^{-1} can be attributed to α-helix and it is the main content of conformation for HSA [21]. In amide I bands of HSA, the bands near 1 630 cm^{-1} and 1 684 cm^{-1} are assigned to short-segment chains connecting α-helix and β-turn, respectively [22~24]. Fig.3 shows the curve-fitted Raman spectra of HSA, HSA-PLU, HSA- metal ion and HSA-PLU-metal ion system, and Table 2 lists the corresponding curve fitting results. The results indicate that free HSA contain major α-helix and decrease from 57.10% to 50.07% after PLU being added. In presence of Cu^{2+} or Mn^{2+},

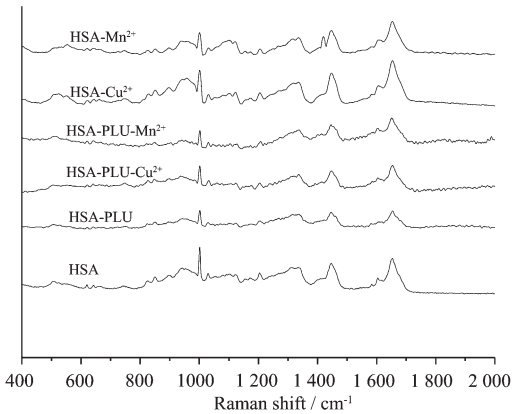


Fig.2　Raman spectra of HSA with PLU-Cu^{2+} or Mn^{2+}

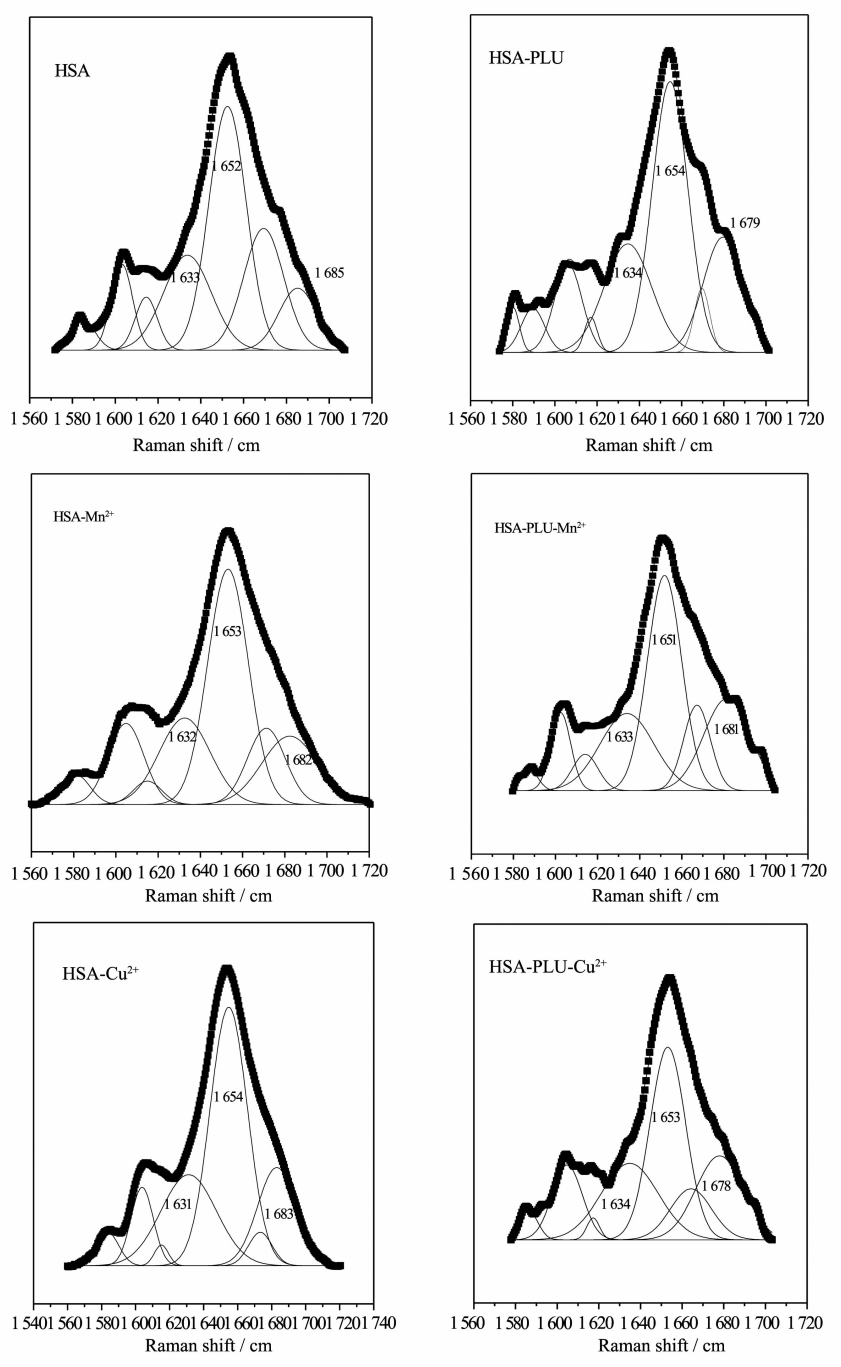


Fig.3 Raman spectra for Amid of HSA

Table 2 Curve fitting results of Raman amide I of HSA

System	Frequency / cm^{-1}	Assignment	Content / %
Free HSA	1 652	α-helix	57.10
	1 633	Short segment	28.42
	1 685	β-turn	14.48
HSA-PLU	1 654	α-helix	50.07
	1 634	Short segment	26.59
	1 679	β-turn	23.34

Continued Table 1

HSA-Cu^{2+}	1 654	α-helix	52.46
	1 631	Short segment	27.15
	1 683	β-turn	20.39
HSA-PLU-Cu^{2+}	1 653	α-helix	45.24
	1 634	Short segment	28.73
	1 678	β-turn	26.03
HSA -Mn^{2+}	1 653	α-helix	53.82
	1 632	Short segment	24.81
	1 682	β-turn	21.37
HSA-PLU-Mn^{2+}	1 651	α-helix	46.97
	1 633	Short segment	26.41
	1 681	β-turn	26.62

the content of α-helix decreases to 52.46% and 53.82%. In HSA-PLU-Cu^{2+} and HSA-PLU-Mn^{2+} system, the content of α-helix further decreases to 45.24% and 46.97%.

HSA contains 17 disulfide bridges. The region of 500~550 cm^{-1} in Raman spectrum is attributed to the stretching vibration mode of S-S bridges [25]. Raman bands at ~510, ~525 and ~540 cm^{-1} due to the S-S stretching mode can be ascribed to gauche-gauche-gauche(g-g-g), gauche-gauche-trans (g-g-t or t-g-g) and trans-gauche- trans(t-g-t) configurations of the Cβ-S-S′-Cβ′ disulfide bridges, respectively [26-27]. The curve fitting of the band could be used to determine the conformation of the 17 disulfide bridges. Fig.4 shows results for the curve fitting in the region of 500~550 cm^{-1} for the Raman spectra. The main conformation of free HSA was g-g-g (511 cm^{-1}). The addition of PLU and metal ions induces prominent changes of the conformations in disulfide bridges. Fig.4 also shows the percentages of three types of disulfide bridges form. We can see that the conformation of HSA is prominently changed.

Fig.5 suggests that about 10 out of 17 disulfide bridges of free HSA take the g-g-g conformation, 6 take the g-g-t or t-g-g, and 1 has the t-g-t conformation. But there are about 7 out of 17 disulfide bridges taking g-g-g conformations, the remaining 8 being the g-g-t or t-g-g conformations, and 2 t-g-t conformers are presented for HSA-PLU system. There are 5 take the g-g-g conformation and the number of g-g-t or t-g-t conformation increase to 8 and 4 after Cu^{2+} joined to HSA. When Cu^{2+} is added to HSA-PLU system, there are 8 with the g-g-g conformation, the number of g-g-t or t-g-g is decreased to 5, while the number of t-g-t conformation is increased to 4. With the addition of Mn^{2+} only, the number of g-g-g or g-g-t conformation decreases to 6 and 1, the remaining 10 being the t-g-t conformations. When Mn^{2+} is added to HSA-PLU system, there are about 10 disulfide bridges with g-g-g conformation while the number of g-g-t or t-g-g conformation changes to 5 and the remaining 2 are the t-g-t conformation. The results indicate that at least 3 disulfide bridges change their conformation due to the interaction between PLU and BSA. When Cu^{2+} or Mn^{2+} join to HSA, there are 5 or 9 disulfide bridges changed respectively. After Cu^{2+} or Mn^{2+} is added to HSA-PLU systerm, both 3 bridges are rearranged.

HSA contains 18 Tyr residues that play an important role in the stability of the protein structure, being involved in many hydrogen bonds. Among the Tyr Raman bands, the 850~830 cm^{-1} components are considered to be useful for determining the environment of the Tyr side chains, as their intensity ratio reflects the hydrogen bonding degree of the phenoxyl OH group("exposed or buried Tyr)[28-29].

The intensity ratio of this doublet (I_{850}/I_{830}) reflects the microenvironment of tyrosine residues. The value of this ratio between 0.3 ~0.5 indicates that the hydroxyl groups of tyrosy1 residues are "buried",

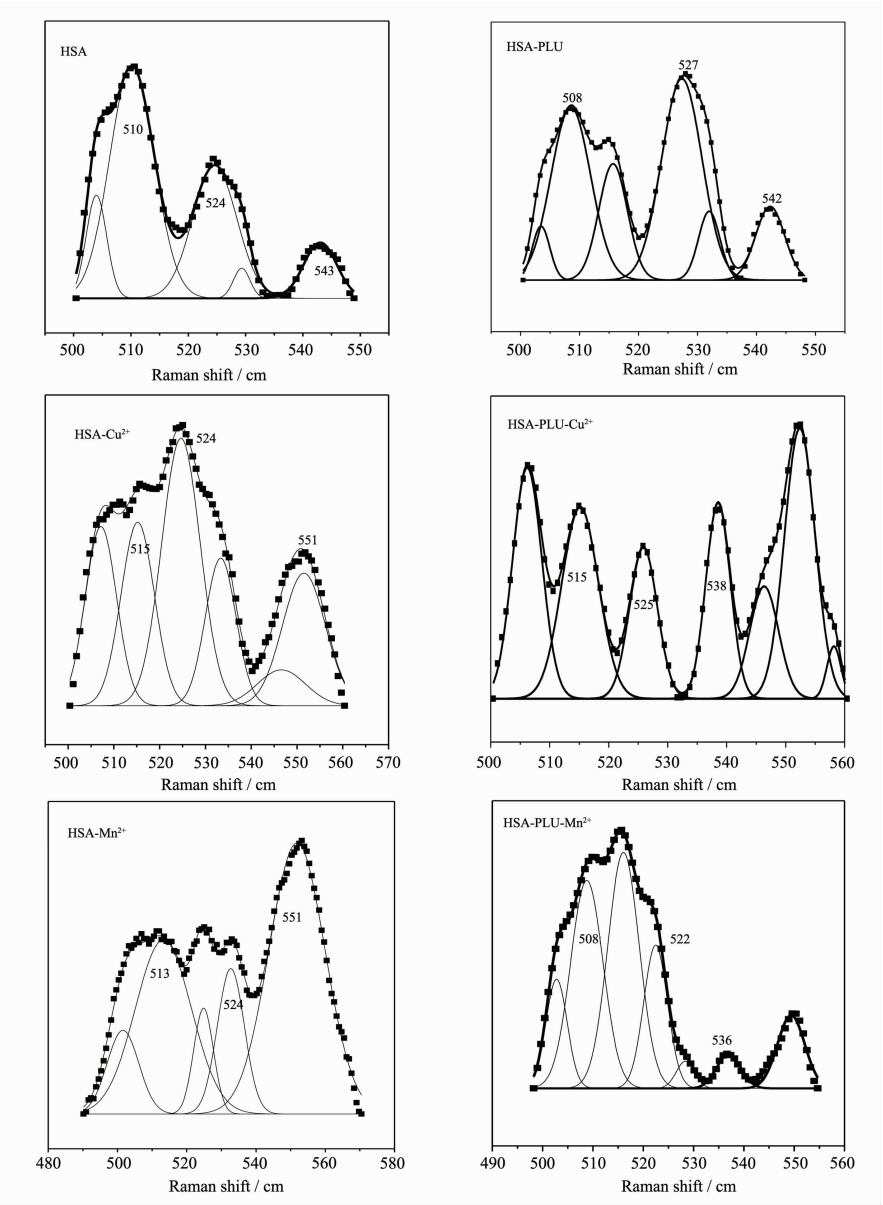


Fig.4 Raman spectra for the disulfide bridge of HSA

while the value of 1.25~1.40 suggests the "exposed" of the hydroxyl groups [30-31]. As shown in Fig.6, the tyrosyl is shifted due to the addition of PLU, Cu^{2+} and Mn^{2+}, meanwhile the intensity ratio of I_{850}/I_{30} is changed from 1.20 to 1.37, 1.11 and 1.93, suggesting that PLU and Mn^{2+} could enhance the process of exposedness while Cu^{2+} has an opposite effect. When Cu^{2+} or Mn^{2+} is added to HSA-PLU system, the ratio is changed to 0.99 and 2.85, respectively, suggesting that the metal ions change the system a lot and Cu^{2+} could hinder the process of exposedness while Mn^{2+} has a promoting effect.

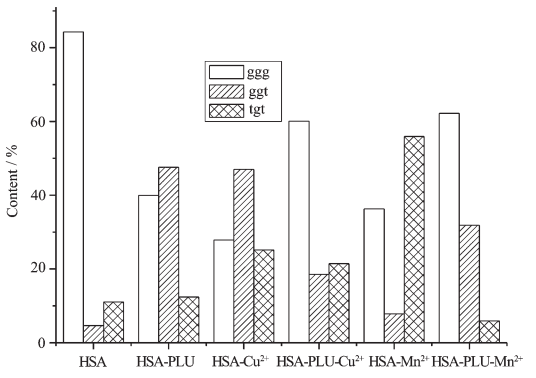


Fig.5 Content of three stretching vibration modes of HSA upon the addition of PLU and metal ions

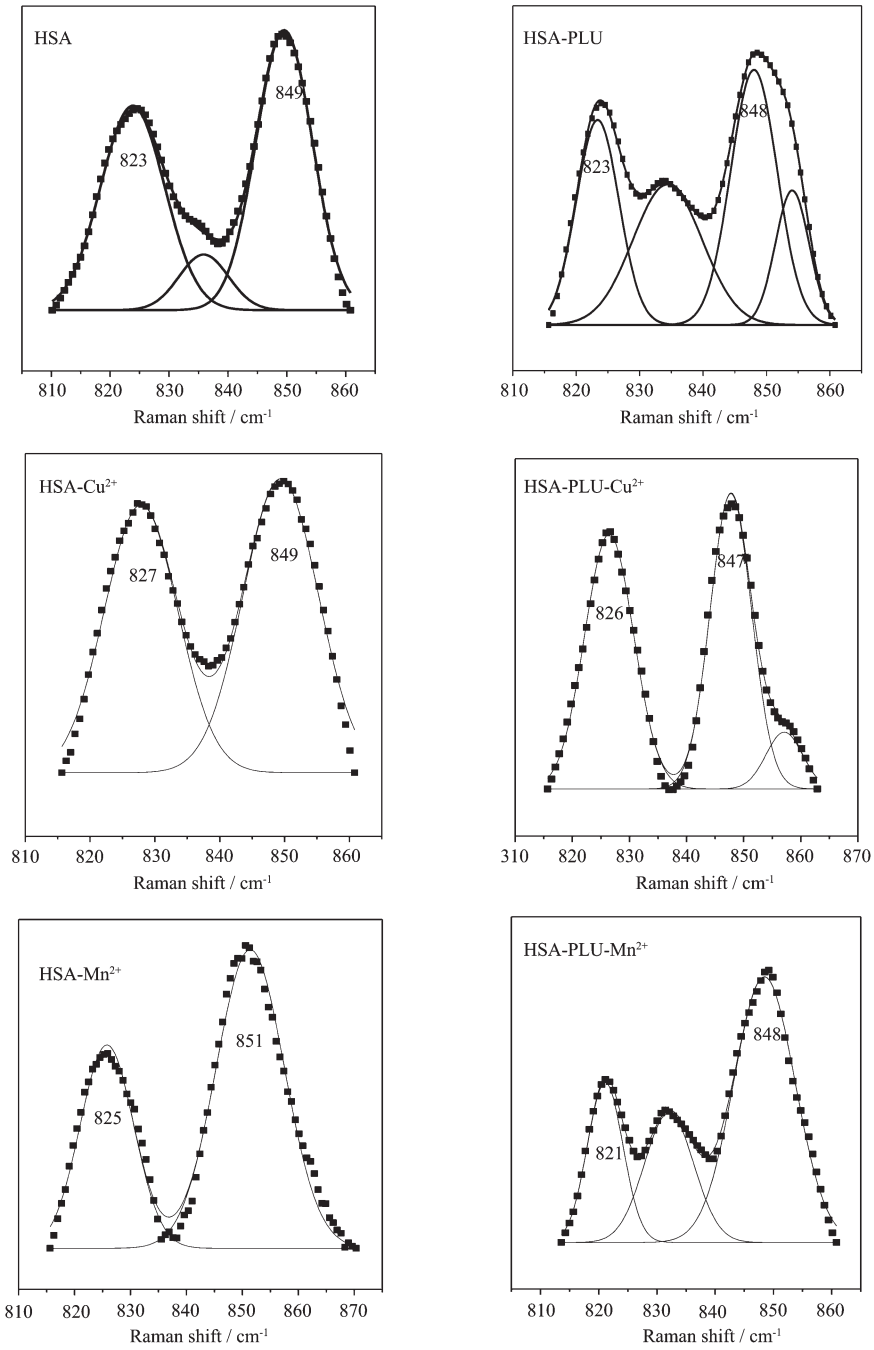


Fig.6　Raman spectra for the Tyr residue of HAS

The value of I_{1360}/I_{1340} could reflect the hydrophobicity of tryptophan residues[31]. Fig.7 displays the analysis for the environment of Trp residues. The value of I_{1360}/I_{1340} changes from 0.061 to 0.052, 0.088 and 0.034 after PLU, Cu^{2+} and Mn^{2+} being added respectively, and changes to 0.076 and 0.031 alone while Cu^{2+} and Mn^{2+} are added to HSA-PLU system. The value reflects that PLU could decrease the hydrophobicity of Trp and Cu^{2+} could promote this trend. And Mn^{2+} has an opposite effect on HSA-PLU system.

3　Conclusions

The confo rmational changes of HSA due to the binding of PLU in absence and presence of Cu^{2+} or Mn^{2+} were characterized by CD and Raman

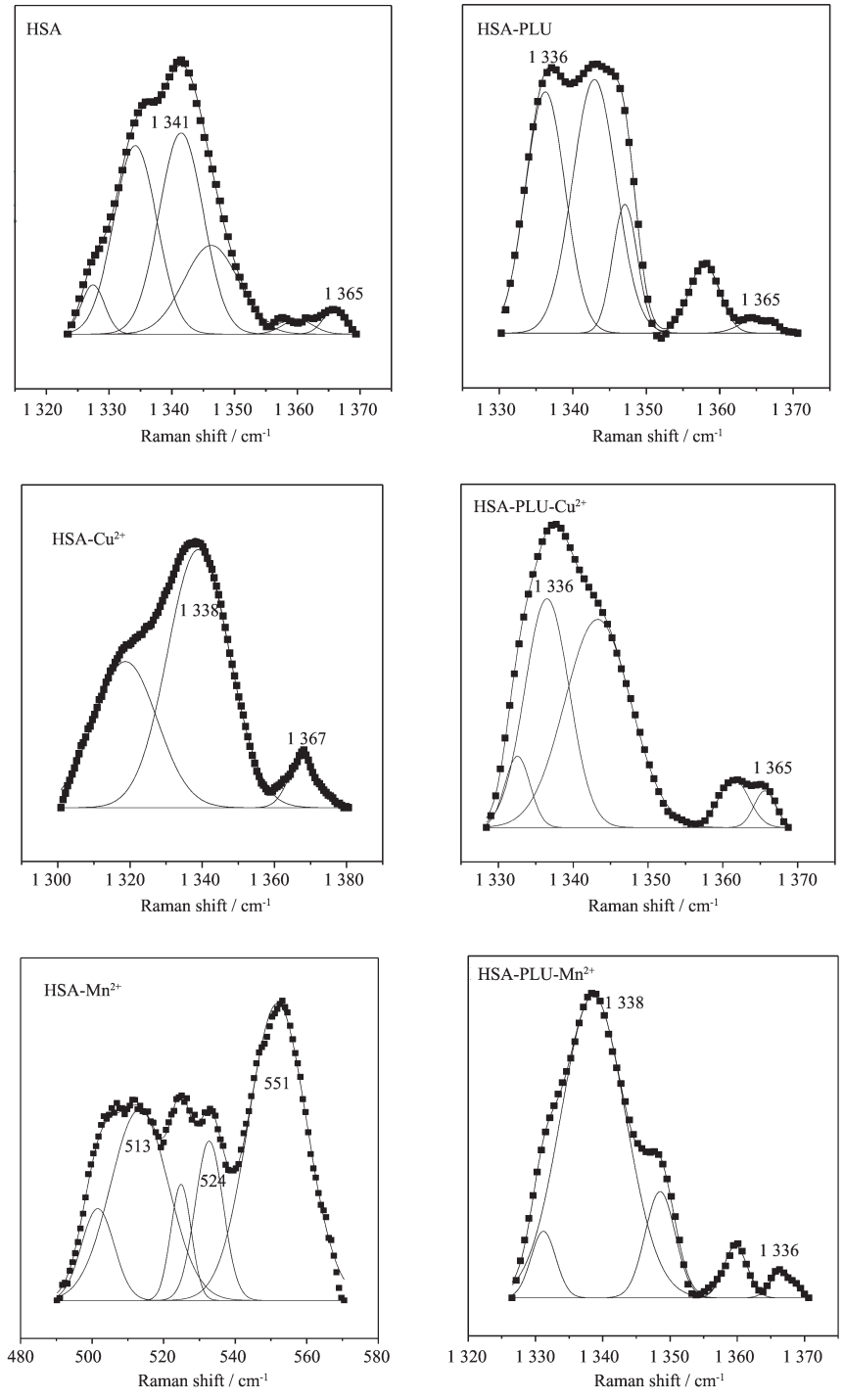


Fig.7 Raman spectra for the Trp residue of HAS

spectroscopy. The analyses of spectroscopic results demonstrate that the binding of PLU or metal ions to HSA alters the conformations of the secondary structure of protein and the microenvironment of amino acid. The binding of drug and metal ions causes the loosening and unfolding of the protein skeleton. Both CD and Raman spectra reveal the similar tendencies on secondary structure analysis: the content of α-helix structure decreases, and the metal ions promote the changes. It can be confirmed from the analysis of the Raman band that the changes occur in the secondary structure of the protein

associate with the conformational change of S-S bridges. The main conformation of disulfide bridges changes from g-g-g to g-g-t with the addition of PLU. The Cu^{2+} has little effect on PLU-HSA system while Mn^{2+} induces prominent changes of the conformations in disulfide bridges from g-g-t to g-g-g. In addition, the binding of PLU and metal ions increases the exposure of Tyr residues and the hydrophobic property of Trp residues in HSA.

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