Research Article

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Silver-induced conformational changes of polypeptides: a CD study

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The role of silver ions in various pathologies, as well as their effect on peptide conformation and properties are less understood. Consequently, we synthesized several peptides with various residues in their sequence to investigate silver-induced conformational changes at various pH values by Circular Dichroism spectroscopy. Uniquely, the glycine-based, histidine-containing peptide showed a severe change from a random coil and β-turn conformation to large α-helices during silver binding. When comparing the effect of silver ions on the conformation of bradykinin a similar tendency was found. Besides, silver ions reduced the amyloid-β peptide tendency to aggregation. Our results suggest a specific and protective role for silver ions in brain pathologies, which is related to their high affinity toward physiologically and pharmacologically active peptides. Fourier transform infrared spectroscopy studies as well as the mass spectrometric ones support our conclusions.

Keywords: peptide conformation; CD spectroscopy; silver binding; bradykinin; amyloid-β peptide

Introduction

Polypeptides are largely used to create new silver-based inorganic nanostructures and the assembly of soft biomaterials [1]. Silver ions also form aqueous phase complexes with both sulfur and nonsulfur containing peptides and proteins [2]. The structure of silver ion complexes of polypeptides is depended on the sites of the silver ion attachment [3]. Instead, mercury ions in low concentration are able to cause all nerve cell changes, which are typical for Alzheimer's disease (AD) [4]. Although silver is a xenobiotic element and a heavy metal, no recognized toxicity was found to the peripheral nervous system [5]. Several biologically relevant ligands ranging from single amino acids to linear peptides have been used in the stabilization of a variety of noble metal surfaces, such as silver, copper, and gold [6]. Consequently, the complexes of silver with small peptides are currently investigated [7]. However, most biological properties of silver are less understood. For example, although silver compounds are less toxic to the nervous system, they have an important antimicrobial activity, which is most often attributed to the dissolved cation [8]. Histidine is the amino acid with the highest affinity toward silver ions. However, some peptides containing this residue were shown not to be involved in silver binding [9]. Therefore, further research is indicated to evaluate the role of metal binding proteins including metallothioneins as cytoprotectants for neurological tissue [5]. We hypothesize and bring here some evidence that the toxicity degree of silver ions can be modulated by their ability to change peptide and protein conformations. Several techniques such as circular dichroism (CD) and vibrational (infrared and RAMAN) spectroscopy have been developed to provide structural information on proteins [10]. Previously, we investigated metal ion binding to peptides by electrospray ionization mass spectrometry (ESI-MS) and CD and showed by atomic force microscopy (AFM) that peptide films change dramatically upon metal ion binding [11–15].

Therefore, our study aims at revealing some unexpected CD spectra collected from silver-treated peptides under various environmental conditions. We used a very flexible glycine-rich peptide and two synthetic peptides containing histidine and cysteine residues. The nature of silver binding to peptides and its impact on the peptide conformation will be discussed in light of results of CD spectroscopy. We have also investigated bradykinin, a physiologically and pharmacologically active peptide. Because amyloid-β peptide involved in AD contains in its sequence both histidine and glycine residues, we also investigated the silver binding to Aβ to bring the evidence for any protective role of silver ions against neurodegeneration. In addition, starting from the synergic effect of silver ions and sodium dodecyl sulfate (SDS) on peptide conformation and aggregation, we tested some other tensioactive agents such as sodium stearate, which are less noxious being also present in the cells.

Experimental

Materials

All chemicals were of analytical reagent grade. The solvents for peptide synthesis were commercially analytical grade and were redistilled before use. Metal salts and trifluoroethanol (TFE) were purchased from Merck, SDS from Loba-Chemie (Wien, Austria), whereas sodium stearate was from Sigma-Chemie (Deisenhofen,

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Germany). Aβ1-40 peptide was provided by GenicBio (BioTech Co., Shanghai, China), solved in 1 : 1 TFE : water and dry freezed before use. Bradykinin acetate was purchased from Sigma–Aldrich (St Louis, MO, USA).

Synthesis of Oligopeptides and Peptide Complexes

The following peptides were used in this study: Bradykinin (Brd), a 9-amino acid peptide chain, RPPGFSPFR, a nonapeptide designed here as P9 (CHQYHHNRE), a decapeptide, P10 (RCHQYHHNRE), a glycine-rich peptide, H_2N-GGGGHGGGGHGGGGHGGGG-COOH (P19), and the amyloid-β peptide (Aβ1-40 or Aβ). Fluorenyl-methoxycarbonyl (Fmoc) methods were applied for the synthesis of P9 [13], P10 [14], and P19 [15] peptides. Peptides were purified by reverse-phase HPLC and characterized by MALDI-ToF mass spectrometry. Peptide complexes with silver ions were prepared by mixing peptide solutions (1 mg/ml peptide) for 2 h with silver nitrate solutions in the molar ratios 1 : 1, 1 : 2, 1 : 5, and 1 : 10, respectively, at pH 7.4. Corrections of pH were made with ammonium bicarbonate.

Instruments

CD spectra were recorded using a Jasco-715 spectropolarimeter (Labor and Datentechnik GmbH, Germany). A Shimadzu Model 8400S FTIR spectrophotometer was used to collect Fourier transform infrared (FTIR) spectra. The masses of peptides and their metal complexes were measured with an ion trap Esquire3000Plus spectrometer (Bruker Daltonics, Bremen, Germany). The pH values were measured with a HANNA PH 211 microprocessor pH meter. A BIO-RAD Model 2700Elite RP-HPLC system (Bio-Rad Laboratories GmbH, Germany), using a Spherisorb (ODS) C18 reversed-phase analytical column, was used for the purification of peptides.

CD Spectropolarimetry

The measurements were performed in quartz cells with a path length of 0.5 mm, in the range from 260 to 180 nm. The measurements were done under the following parameters: resolution 0.5 nm, band width 1.0 nm, sensitivity 50 mdeg, the response 8 s at a speed of 20 nm/min. Typically, peptide concentration was 0.2 mM and peptide/metal ion ratios were 1 : 1, 1 : 2, 1 : 5, and 1 : 10, respectively. A minimum of four scans were recorded and baseline spectra were subtracted from each spectrum. The measurements were carried out at 25.0 ($\pm$0.2 °C). The direct CD measurements (θ, in mdeg) were converted to molar ellipticity, $\Delta\varepsilon$ ($M^{-1}\cdot cm^{-1}$). Proportions of each secondary structure type were obtained by spectral deconvolution of the peptide CD spectrum as a linear sum of predetermined basis spectra, using the software on the instrument [16]. In addition, DICHROWEB (http://www.ogic.ca/projects/k2d2/) was also used for the deconvolution of spectra because it provides a user-friendly interface to the existing programs and databases and enables a wide range of input formats and limits the need for pre-analysis processing and conversion programs [17].

FTIR Spectroscopy

The FTIR spectra of peptides, silver nitrate, and their complexes were recorded in the solid state (KBr), in the mid infrared range (500–4 000 cm^{-1}), under a resolution of 2 cm^{-1} and with a scanning speed of 2 mm/s. Typically, 200 scans were signal-averaged for a single spectrum. The collected FTIR spectra were compared with the standard spectra of the functional groups. Amide I, II, and III vibrations were investigated for the conformational changes of peptides on metal binding.

MS Measurements

The electrospray ionization-ion trap mass spectra (ESI-MS) were acquired in the 200–2000 *m/z* range. Samples were dissolved in 1 : 1 (v/v) acetonitrile–water solvent mixture, containing 0.1% acetic acid. Binding of metal ions to β-amyloid peptides was studied by ESI-ion trap mass spectrometry (MS) using 5 mM ammonium acetate, pH 7.4. Peptide concentration was 10 pmol/µl and the ratios peptide : metal ion were 1 : 1, 1 : 2, and 1 : 10. Aβ1-40 peptide and silver nitrate were first dissolved in 5 mM ammonium acetate and mixed prior to MS analysis.

Results and Discussion

CD Spectra of CHQYHHNRE Peptide

First, we investigated the effect of silver ions on the conformation of a synthetic peptide containing various amino acid residues (Figure 1). With the two negative maxima at 184 and 189 nm (−12.50 and −12.70 $M^{-1}\cdot cm^{-1}$) and a positive band at 227.5 nm (+2.96 $M^{-1}\cdot cm^{-1}$), the spectrum of P9 in 50% TFE had several characteristics of a mixture of β-turn and unordered populations. A similar spectrum was collected for P9 in aqueous solutions at pH 7.4, denoting a weak interaction with water molecules. Almost similar but less intensive bands were found at 183 nm and 189 nm (−10.04 and −11.18 $M^{-1}\cdot cm^{-1}$), whereas the large band in the positive region had a maximum at 227 nm (+1.96 $M^{-1}\cdot cm^{-1}$). Silver ions severely changed the peptide conformation, supporting the idea that the β-turn proportion of conformers decreased with an increase in the α-helix one. A very sharp band at 197 nm was found in the negative region of absorption spectrum (−11.61 $M^{-1}\cdot cm^{-1}$), which seems to be a characteristic for a combination of both α-helix and random coil spectra, with a diminished contribution from β-turn populations. Besides, the relatively intense peak at 183.5 nm (+2.98 $M^{-1}\cdot cm^{-1}$) confirmed the tendency of silver ions to increase α-helical proportions when bound to peptides under the 1 : 1 molar ratio. We supposed that silver ions bound first to amino terminal group, SH one and a nitrogen atom in the histidine moiety, being wrapped by more than two atoms in the peptide backbone. The next silver ions bound also to peptide backbone, each one at a different site, generating a conformation similar to that in TFE solution. However, the high affinity of polypeptides toward silver ions is also related to the metal-ligand binding, which occurs through the amino nitrogen and carboxyl oxygen. As a result, the proportion of unordered populations increased much. In the case of 1 : 10 P9 : Ag^+ complex, the two bands in the negative region were found to be shifted to 186 and 192.5 nm (−10.77 and −11.83 $M^{-1}\cdot cm^{-1}$).

CD Spectra of RCHQYHHNRE Peptide

The addition of an arginine residue to amino terminus of P9 changed dramatically the shape of CD peptide spectrum. The freshly prepared P10 had an absorption band at 195 nm with a negative maximum of −12.69 $M^{-1}\cdot cm^{-1}$, comparable to that of P9 in water. Besides, the large band in the positive region had a maximum at 225.5 nm (+2.33 $M^{-1}\cdot cm^{-1}$). Conformationally, P10 in the fresh water solutions was found as a mixture of random

Table 1. Conformational changes of peptides in the presence of silver ions (Ag^+) and sodium dodecyl sulfate (SDS) at pH 7.4

Conformation	P10–H_2O	P10–Ag^+, 1 : 2	P10–Ag^+, 1 : 5	Brd–H_2O	Brd–SDS, 1 : 10	Brd–SDS–Ag^+, 1 : 5 : 5
α-helix	0.0	18.3	6.2	0.0	9.3	16.1
β-sheet	40.5	37.2	29.0	59.5	33.6	13.8
β-turn	13.4	12.8	12.9	0.0	16.5	29.4
random coil	46.1	31.7	51.9	40.5	40.6	40.7
Conformation	P19-H_2O	P19-Ag^+, 1 : 1	P19-Ag^+, 1 : 10	Aβ	Aβ-Ag^+, 1 : 10	Aβ-SDS, 10 : 1
α-helix	0.0	34.8	45.0	0.0	17.7	28.0
β-sheet	0.0	0.0	0.0	33.7	0.0	0.0
β-turn	55.7	65.2	55.0	17.3	35.4	35.0
Random coil	44.3	0.0	0.0	49.0	47.0	36.9

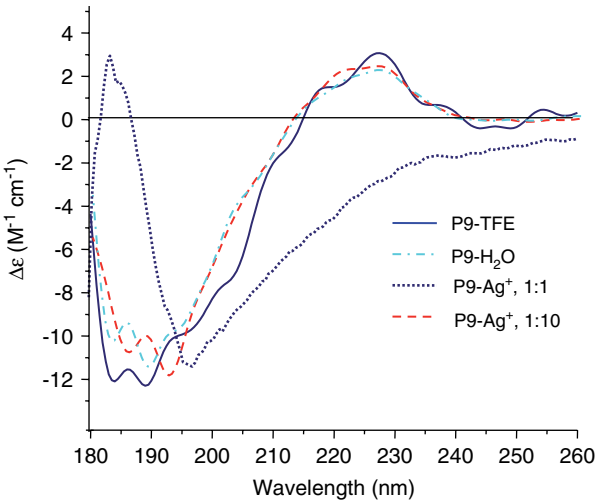

Figure 1. CD spectra of peptide P9 in 50% trifluoroethanol (P9-TFE), water (P9-H_2O), and in the presence of 1 : 1 and 1 : 10 molar ratios of silver ions (P9-Ag^+, 1 : 1 and P9-Ag^+, 1 : 10). This figure is available in colour online at wileyonlinelibrary.com/journal/jpepsci.

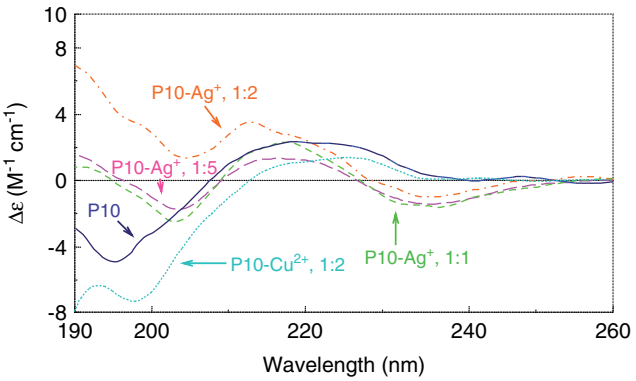

Figure 2. CD spectra of P10 and its complexes with silver and copper ions.

coil (46.1%) and β-sheet (40.5%) forms (Table 1). In solid state, P10 undergoes conformational changes as a function of time, with an increase in the β-sheet proportion. Thus, the negative band shifted from 195 to 196 nm and decreased from −12.69 to −4.68 $M^{-1}\cdot cm^{-1}$, whereas the maximum in the positive region was found at 222 nm (+2.94 $M^{-1}\cdot cm^{-1}$). Silver ions induced an increased absorption in the positive region around 193 nm as well as in the negative one at 222 nm (Figure 2). Hence, the 196 nm negative minimum of pure peptide shifted toward 203 nm (−2.34 $M^{-1}\cdot cm^{-1}$) in the case of 1 : 1 P10–Ag^+ complex. The 1 : 2 P10–Ag^+ had a minimum in the positive region at 204.5 nm (+1.12 $M^{-1}\cdot cm^{-1}$). Such minima were assigned to a mixture of α-helical and unordered conformers, with an increased proportion of α-helical conformers in the case of 1 : 2 molar ratio P10 : Ag^+. A large proportion of α-helical conformers was calculated for 1 : 2 molar ratio adduct of P10 with silver ions, whereas an increase in the concentration of Ag^+ resulted in the accumulation of unordered populations. We supposed that the first silver ion bound to the guanidine group of N-terminal arginine residue, increasing the proportion of unordered conformers. The second one bound to SH group of cysteine and nitrogen atoms in amino groups and imidazol moiety to form a chelate with silver ion wrapped by several atoms of peptide backbone. The next silver ions interfere one to another inducing a random coil conformation for P10.

On the contrary, copper ion complexation has previously been shown to be a strong trigger for a secondary structure switch from β-turn to β-sheet [14]. SDS enhanced conformational changes from β-turn and random coil to β-sheet. On adding copper ions (1 : 10 peptide : Cu^{2+} molar ratio), β-turn proportion decreased from 42.3 to 32.9%, whereas the β-sheet one increased up to 10.1%. The addition of SDS to the solution of P10–Cu^{2+} complex (P10 : Cu^{2+} : SDS, 1 : 10 : 10) resulted in the transformation of the unordered conformers into β-sheet ones (53.8% β-sheet; 6.8% β-turn; and only 39.4% unordered populations). Copper ions enhanced the absorption in the negative region around 198 nm, increasing the proportion of unordered conformers.

CD Spectra of a Histidine-containing Peptide

The CD spectra of peptide P19 (0.2 mM) in aqueous solutions in the presence of TFE at pH 7.4 are shown in Figure 3. P19, a glycine-rich peptide, was found to contain almost only β-turn populations in a 50% TFE solution. Glycine is frequently found in beta turns because, with the smallest side chain of all the amino acids, it is the most sterically flexible. In aqueous solutions, P19 content of β-turn conformers decreased to 55.7%, whereas the unordered populations were 44.3%. The two spectra had a similar shape, although both negative and positive maxima appeared at rather different values. Thus, the aqueous solution of P19 had a negative band at 186.5 nm of −6.64 $M^{-1}\cdot cm^{-1}$ and a large positive one between 203

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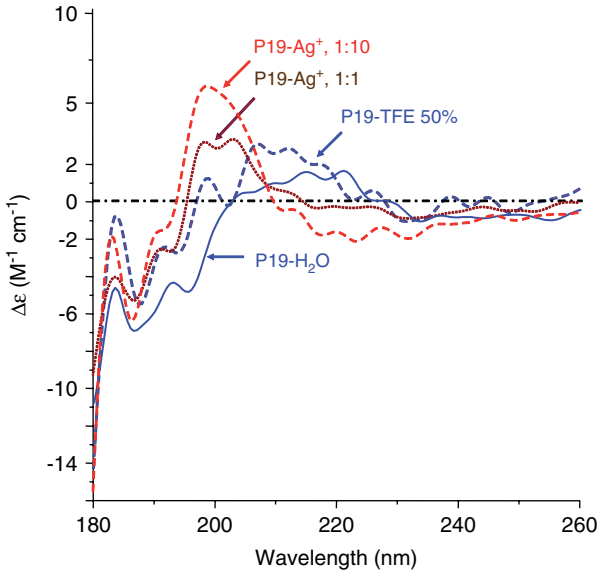

Figure 3. Effect of silver ions on the secondary structure of peptide P19.

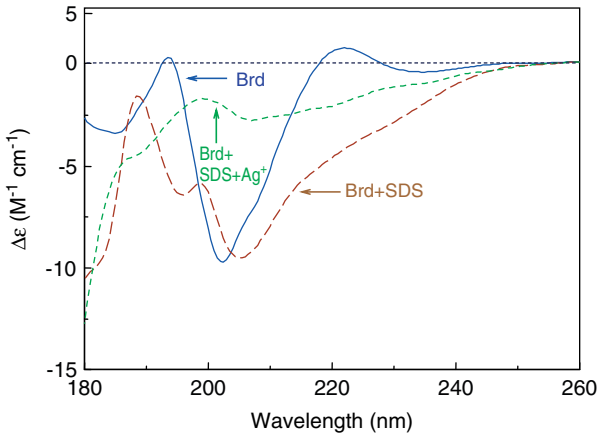

Figure 4. Conformational changes of bradykinin (Brd) in the presence of SDS (Brd+SDS, 1 : 10) and silver ions (Brd+SDS+Ag^+, 1 : 5 : 5). This figure is available in colour online at wileyonlinelibrary.com/journal/jpepsci.

and 228 nm, with two maxima at 215 nm (+1.48 $M^{-1}\cdot cm^{-1}$) and 221 nm (+1.55 $M^{-1}\cdot cm^{-1}$). Another negative maximum was found at 195.5 nm (−4.64 $M^{-1}\cdot cm^{-1}$). The negative absorptions around 190 nm could be correlated to an increased proportion of β-turn populations. TFE induced an increase in the positive bands (between 197.0 nm and 201.0 nm, and from 203.0 nm to 227.5 nm, with a maximum of +2.86 $M^{-1}\cdot cm^{-1}$ at 212 nm). The two negative peaks were found less intensive than those of P19 in water environment (197 nm; −5.60 $M^{-1}\cdot cm^{-1}$, and 194 nm; −2.74 $M^{-1}\cdot cm^{-1}$).

On adding silver ions to P19 in aqueous solutions at pH 7.4, the peptide conformation changed severely (Figure 3). Interestingly, silver ions transformed unstructured peptide molecules into α-helical conformers (from 0% to 34.8% and from 0% to 45%, depending on peptide : silver ions ratio) and stabilized β-turn structures (Table 1). In contrast, silver ions behaved differently within the binding process at pH 7.4. Thus, the 1 : 1 molar ratio P19-Ag^+ spectrum had two negative bands at 186.5 and 192.5 nm (−5.18 and −3.70 $M^{-1}\cdot cm^{-1}$) and a positive region between 196 and 214 nm with two maxima at 198.5 and 203 nm (+2.88 and 3.03 $M^{-1}\cdot cm^{-1}$). A negative minimum of −0.48 $M^{-1}\cdot cm^{-1}$ was also observed at 222.5 nm, suggesting the presence of α-helical conformers. On increasing the silver-peptide ratio up to 10, the shape of spectrum changed dramatically, being similar to that of α-helical peptides. The deconvoluted CD spectrum for the 1 : 10 P19-Ag^+ complex was characterized by a positive peak at 197.5 nm (+5.90 $M^{-1}\cdot cm^{-1}$), with the intensity of the negative band at 208 nm being less negative than that of the 222 nm band (−1.59 and −5.42 $M^{-1}\cdot cm^{-1}$, respectively) in comparison with the regular α-helix, with a positive band at 190 nm and two negative bands at 208 and 222 nm.

CD Spectra of Bradykinin

Brd is produced in the brain, being a proinflammatory and vasoactive peptide, which is released during tissue damage and may contribute to neuronal degeneration, inflammation, and edema formation after brain injury by acting on discrete bradykinin receptors, B1R and B2R [18–20]. We investigated the effect of silver binding on Brd conformation and observed severe conformational changes in the presence of both metal ions and tensioactive agents (Figure 4). With its large content of proline residues, Brd was expected to have an important proportion of β-turn conformers. Proline is well known as a β-sheet breaker amino acid. However, Brd was found to form β-sheet aggregates of molecule associated by hydrogen bonds (59.5% β-sheet). The CD spectrum of Brd also contains an important band at 202.5 nm (−9.85 $M^{-1}\cdot cm^{-1}$) in the negative region and two bands in the positive region at 193.5 nm and 222 nm (+0.21 and +0.66 $M^{-1}\cdot cm^{-1}$) characteristic to α-helical conformers. On adding SDS to form a micellar solution of Brd, the negative band shifted from 202.5 nm toward 208 nm, where α-helical conformers absorb. The maximum was found at 205 nm (−9.60 $M^{-1}\cdot cm^{-1}$), together with another and smaller one at 196 nm (−6.51 $M^{-1}\cdot cm^{-1}$). The peak shifting from 193.5 nm toward lower wavelengths (188.5 nm, −1.67 $M^{-1}\cdot cm^{-1}$), as well as the important absorption below 186 nm suggest the presence of β-turn secondary structures (16.5% β-turn). Hence, SDS induced a small increase in α-helical proportion (9.3% α-helix based on a decrease in β-sheet proportion to 33.7%). While the proportion of unordered conformers remained unchanged, a combination of SDS and silver ions had a synergic effect on the formation of both α-helical (16.1%) and β-turn (29.4%) conformers. SDS and silver ions diminished β-sheet proportion and increased the other two conformational forms. However, SDS is toxic to cells and other tensioactive agents could be of biomedical interest.

CD Spectra of Amyloid-β Peptide

The Aβ peptides are mainly α-helical in their native conformation, but undergo an α-helix to β-strand conversion before or during fibril formation. Moreover, copper ions are known to enhance the formation of β-sheet structures [14]. On adding copper ions, Aβ conformation slightly changed to increase the proportion of β-sheet and β-turn conformers (55.8%). The β-sheet conformers are characteristic to β-amyloid plaques involved in AD. On the contrary, silver ions may interact with amyloid plaques and Aβ aggregates to change the peptide conformation and atomize them. Figure 5 shows the CD spectrum of an aqueous solution of Aβ1-40 (0.2 mM, pH 7.4) with a large negative absorption band having a maximum at 197.5 nm (−22.31 $M^{-1}\cdot cm^{-1}$). Aβ1–40 in the fresh aqueous solutions was found as a mixture of random

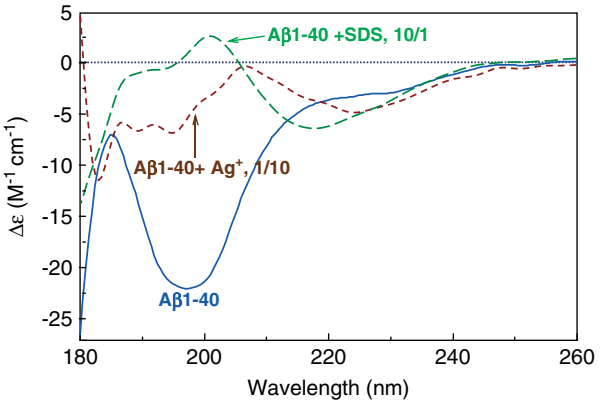

Figure 5. CD spectra of amyloid-β peptide 1–40 (Aβ, 0.2 mM) and in the presence of silver ions and SDS (1 : 10 and 10 : 1 molar ratio). This figure is available in colour online at wileyonlinelibrary.com/journal/jpepsci.

coil (49.01%), β-sheet (33.7%), and β-turn (17.3%) forms (Figure 5). On adding silver ions, the peptide conformation changed severely from β-sheet to α-helix. Even a large molar ratio Ag^+ : Aβ1-40 (10 : 1) induced a significant decrease in the negative absorption around 198.0 nm, from $-22.7\ M^{-1}\cdot cm^{-1}$ to $-4.22\ M^{-1}\cdot cm^{-1}$, suggesting an increase in the proportion of α-helical conformers. Because β-turn conformers absorb significantly at 202.0 nm and 225.0 nm, the characteristic absorption of α-helical populations at 222.0 nm was relatively low. The relatively large molecule of Aβ accommodated easier silver ions than the other oligopeptides investigated here and silver ions interacted with more atoms in the peptide backbone to induce an α-helix-like structure. On adding SDS to Aβ in the SDS : Aβ proportion range from 0.1 to 10.0, the conformational equilibrium changed from β-sheet and unordered to α-helical and β-turn structures. SDS induced important conformational changes even at 1 : 10 molar ratio (α-helix: 28.0%, β-turn: 35.0%, and unordered: 36.9%). In the presence of SDS, the proportion of unordered populations decreased from 49.0% to 36.9%, whereas the β-sheet structures were not found. Therefore, both silver ions and SDS induced the abolition of β-sheet structures of Aβ1-40 peptide, which are related to Aβ neurotoxicity in AD. Because SDS is toxic, sodium stearate was also used to diminish β-sheet proportion of Aβ1-40 peptide with rather similar results (not shown).

FTIR Study

Conformational changes on binding silver ions to various peptides were also observed from FTIR spectra. In the case of P19, two intense peaks at 1379 cm^{-1} and 1356 cm^{-1}, respectively, appeared in the spectrum of silver-peptide complex, whereas some other smaller peaks were found at 1107 cm^{-1}, 1153 cm^{-1}, and 1753 cm^{-1}, respectively (Figure 6). We suspected that the peaks at 1356 cm^{-1} and 1379 cm^{-1} are related to the presence of silver salt. Hence, we showed here that the metal salts used to produce metal-peptide complexes interfere with FT-IR measurements. The spectra of metal salts have to be subtracted from those of complexes, especially when higher proportion of salts are used. In addition, several peaks in the peptide spectrum such as those at 1132 cm^{-1}, 1204 cm^{-1}, 1242 cm^{-1}, and 1420 cm^{-1} were not observed in the spectrum of silver-peptide complex.

The exact position of amide I band is determined by the backbone conformation and the hydrogen bonding pattern.

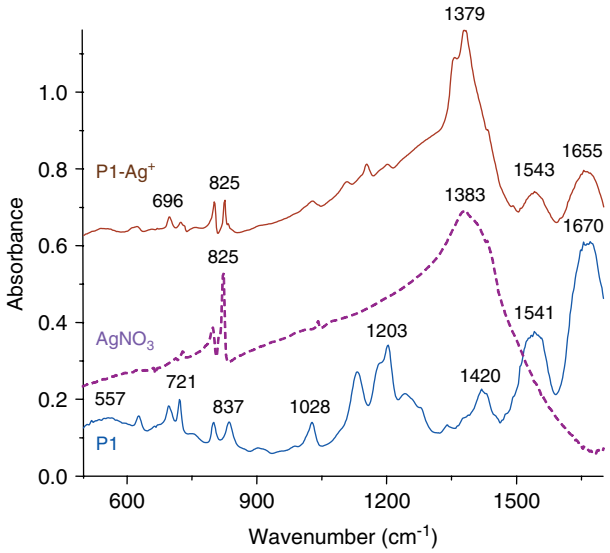

Figure 6. FTIR spectra of peptide P19 and its complex with silver ions. This figure is available in colour online at wileyonlinelibrary.com/journal/jpepsci.

The extract frequency of this vibration band depends on the nature of hydrogen bonding involving the C═O and NH moieties, which is determined by the secondary structure adopted by the polypeptide chain, reflecting the backbone conformation and hydrogen-bonding pattern [21]. The shape of the amide I band of histidine-containing peptides is characteristic of their secondary structure [22]. The band amide II (1510 and 1580 cm^{-1}) is conformationally sensitive and results from the N–H bending vibration as well as from the C–N stretching vibration. The intensity ratio of the two bands, amide I and amide II, was almost the same for the two FT-IR spectra. Instead, the amide I band changed much upon silver binding. The main peaks of amide I band of peptide at 1649 cm^{-1} and 1670 cm^{-1} joined together to form a large band with a maximum at 1655 cm^{-1} (Figure 6).

The intensity of the peak at 1653 cm^{-1} increased much upon silver binding, denoting an increase in the proportion of α-helical conformers. We proposed the assignment of bands around 1670 and 1695 cm^{-1} to β-turns populations of peptide, which were less present in the case of silver complex. The absorption near 1680 cm^{-1} is now clearly assigned to beta turns [23]. It was suggested that glycine residue in the position 3 may induce the formation of β-turn structures. Instead, the peaks around 1608 cm^{-1} could be assigned to peptide-metal aggregates. The percentage of β-sheets as shown by the relative area near 1623 cm^{-1}, decreased much on adding silver ions.

From these data, the results from FTIR and CD methods match well. However, FTIR spectra provided a more complex information than CD spectra did, whereas metal salts may interfere with the measurements.

MS Measurements

Generally, silver ions bound strongly to peptides, whereas this process can be monitored by ESI-ion trap MS [2]. Figure 7 shows the electrospray mass spectra of a pH 7.4 solution of P10 (H-RCHQYHHNRE-NH_2; MW 1377.5) and the same peptide treated with silver(I). Characteristic signals at *m/z* 276.7 (5+), 345.6 (4+), 460.2 (3+), and 689.7 (2+) were observed for P10,

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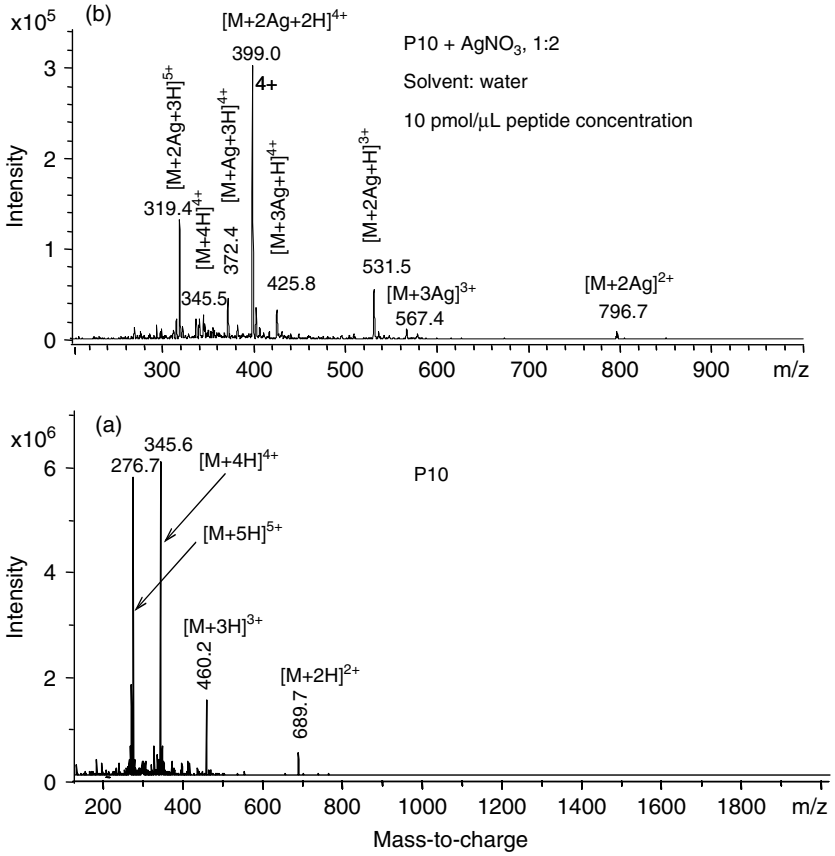

Figure 7. ESI ion trap MS spectra of (A) peptide P10 and (B) its complexes with silver ions.

while its mixture with silver nitrate in 2 : 1 molar ratio led to the formation of complexes with one to three silver ions bound to a single molecule of P10. Silver ions bound strongly to P10 to form coordination compounds containing only one molecule of decapeptide and two silver ions, without favoring oligomerization. The signals for the unbound molecules were the lowest in the spectrum, whereas that for $[M+2Ag+2H]^{4+}$ was the highest. The peaks for P10 coordination compounds with a single silver ion were also observed at *m/z* 298.2, 372.5, and 496.2, respectively ($[M+Ag+4H]^{5+}$, $[M+Ag+3H]^{4+}$, and $[M+Ag+2H]^{3+}$). P10 showed the highest binding capacity toward silver ions, followed by that for nickel and copper ones [14]. In the case of copper affinities the most abundant species corresponded to the attachment of a single Cu^{2+} ion by two molecules of peptide, denoting the property of copper ions to induce the oligomerization.

Each molecule of Aβ also bound one or two silver ions to form multi charged molecular ions such as $[M+Ag+6H]^{7+}$, $[M+Ag+5H]^{6+}$, $[M+Ag+4H]^{5+}$, and $[M+Ag+3H]^{4+}$, which correspond to *m/z* 634.6, 740.1, 888.0, and 1109.9, respectively (Figure 8). Interestingly, some other signals were assigned to adducts of Aβ with two silver ions, in which sodium ions stabilized the molecular ions $[M+2Ag+Na+3H]^{6+}$ and $[M+2Ag+Na+2H]^{5+}$ (*m/z* 762.0 and 914.2).

Discussion

Our investigation revealed unexpected properties of silver ions related to the conformation of biologically active peptides. Because of their tendency to induce α-helical conformations these ions might display a low toxicity toward the nervous system. The most intensively studied examples of conformational diseases are AD, Parkinson's disease, Huntington's disease, and spongiform encephalopathies. The Aß conformation is dependent on pH and TFE and the binding of metals to Aß was found to be dependent on the Aß conformation [24]. The addition of SDS to Aβ-40 fibrils, formed in the presence of copper ions, caused their atomization [14,25]. However, the proportion of α-helical populations was only 22.8%, whereas β-sheet structures reached 46.0%. As adduct reagents for peptides, silver ions have received much attention lately because their binding chemistry tends to be different from that of most transition-metal ions. Silver ions bind strongly to amino acids and oligopeptides even in the gas phase [23]. The peptide wraps around the silver ion and chelates it by using its nitrogen atoms on the peptide backbone. The silver adopts higher coordination, tri-coordinate in the case of the dipeptides and tri- and tetra-coordinate in the case of GPA peptide [8]. This observation may be of paramount importance to understand the behaviors of several peptides and proteins involved in the neurodegenerative pathologies such as prion protein or amyloid-β peptide. Prion-infected cells accumulate a heterogeneous population of aberrantly folded PrP conformers, including the disease-causing isoform (PrP^{Sc}). Positively charged polyamidoamines can modulate the levels of various PrP conformers in cultured prion-infected cells and form benign, insoluble aggregates [22]. The binding of metals to biopolymers and peptides, such as amyloid-ß (Aß) peptide, was found to be dependent on the Aß conformation [26]. Nevertheless, our results showed that silver ions affect much the peptide

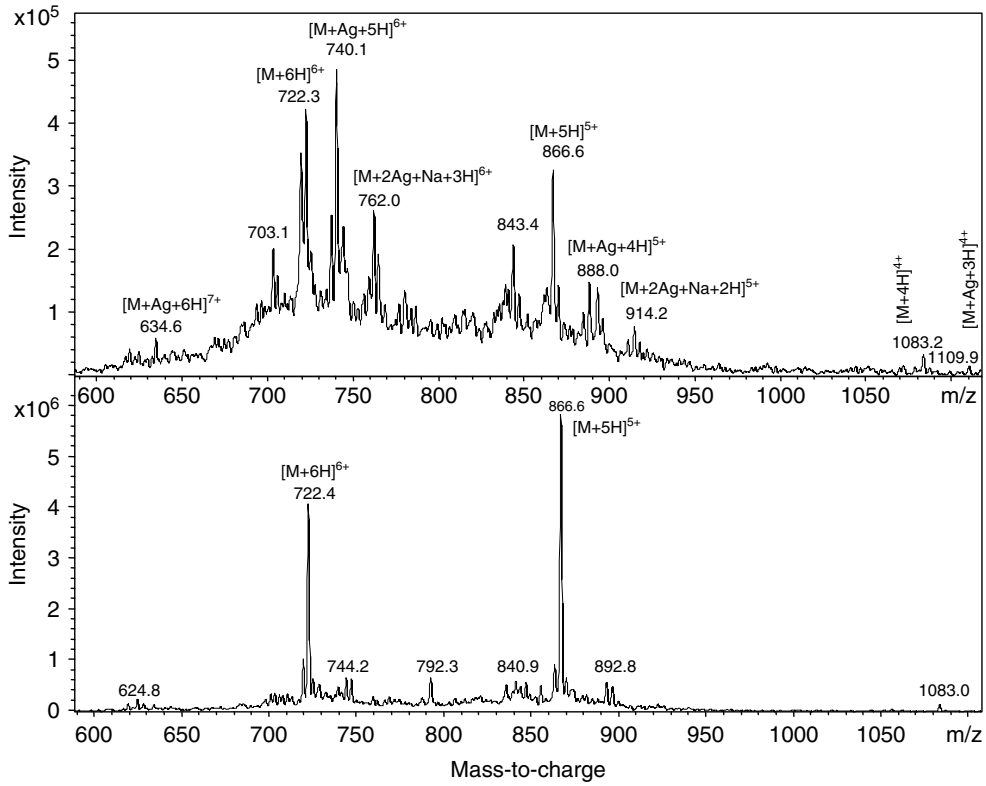

Figure 8. ESI ion trap MS spectra of Aβ peptide and its complexes with silver ions.

conformation, depending also on the peptide : silver ion molar ratio. Therefore, we suspect that silver ions may have both a toxic effect on binding to SH groups of biologically active proteins and peptides and a protective role due to their folding capability.

Conclusions

This study shows that silver binding by peptides is often assisted by a dramatically conformational change. Glycine-rich peptides undergo severe conformational changes depending on the environmental conditions and especially the presence of heavy metal ions such as silver ions. Silver ions severely change the peptide conformation by decreasing the β-turn proportion of conformers and increasing the α-helical one. Contrary to other metal ions such as copper, iron, nickel, or mercury ones, silver binding may reduce β-sheet or β-turn conformations, which is important from the biomedical point of view. Both silver ions and the tensioactive agents such as SDS induce the abolition of β-sheet structures of Aβ1-40 peptide. Silver-induced conformational changes of biological active peptides may be related to the low neurotoxicity of these ions. Owing to the complexity of the interplay between peptide and silver ions, further studies are needed to clarify this aspect.

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