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New Insights into the Coordination of Cu(II) by the Amyloid-B 16 Peptide from Fourier Transform IR Spectroscopy and Isotopic Labeling

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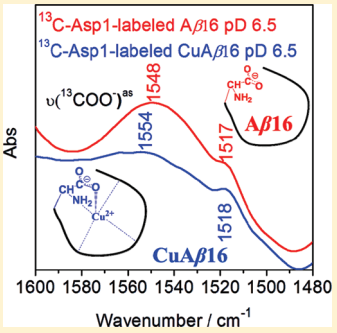
Supporting Information

ABSTRACT: Alzheimer's disease is a neurodegenerative disorder in which the formation of amyloid-β (Aβ) aggregates plays a causative role. There is ample evidence that Cu(II) can bind to Aβ and modulate its aggregation. Moreover, Cu(II) bound to Aβ might be involved in the production of reactive oxygen species, a process supposed to be involved in the Alzheimer's disease. The native Aβ40 contains a high affinity binding site for Cu(II), which is comprised in the N-terminal portion. Thus, Aβ16 (amino acid 1−16 of Aβ) has often been used as a model for Cu(II)-binding to monomeric Aβ. The Cu(II)-binding to Aβ is pH dependent and at pH 7.4, two different type of Cu(II) coordinations exist in equilibrium. These two forms are predominant at pH 6.5 and pH 9.0. In either form, a variety of studies show that the N-terminal Asp and the three His play a key role in the coordination, although the exact binding of these amino acids has not been addressed. Therefore, we studied the coordination modes of Cu(II) at pH 6.5 and 9.0 with the help of Fourier transform infrared (FTIR) spectroscopy. Combined with isotopic labeling of the amino acids involved in the coordination sphere, the data points toward the coordination of Cu(II) via the carboxylate of Asp1 at both pH values in a pseudobridging monovalent fashion. At low pH, His6 binds copper via Nτ, while His13 and His14 are bound via Nπ. At high pH, direct evidence is given on the coordination of Cu(II) via the Nτ atom of His6. Additionally, this study clearly shows the effect of Cu(II) binding on the protonation state of the His residues where a proton displacement takes places on the nitrogen atoms of the imidazole ring.



1. INTRODUCTION

Alzheimer's disease (AD) is a cognitive disorder causing the progressive loss of memory.[1] The brain of AD patients shows extracellular amyloid plaque aggregates consisting mainly of amyloid-beta (Aβ) peptides. According to the amyloid cascade hypothesis, smaller aggregates of Aβ (often called oligomers) are believed to cause the loss of memory. The neurotoxicity of the Aβ oligomers is related to the production of reactive oxygen species (ROS) catalyzed by the redox active transition metals bound to Aβ such as Cu(II),[2−9] which is found in high concentration in amyloid plaques.[10] In the absence of metal ions, Aβ peptides in the monomeric state mostly show a random coil structure, which converts into β-sheet structure in the aggregated, amyloid form. The coordination of Cu or Zn ions modifies the conformation of the Aβ and, thus, the aggregation mode.[3,11−13] The formation of Aβ oligomers can be catalyzed by conformational changes upon binding of Cu(II), but also by dimerization through Tyr radicals.[14,15] The Aβ(1−42) was shown to undergo a dimerization via a cross-linkage of the phenol group of Tyr in the presence of hydrogen peroxide as well as in the presence of peroxidase.[16] Similar behaviors were observed in the presence of Cu(II) cations.[17]

A significant effort has been made in order to determine the binding properties of Cu(II) and Zn(II) by the Aβ peptide, and many aspects are still discussed (reviewed in refs 18−21). Several Cu(II) ions can bind to Aβ, but only the strongest binding mode is likely to be biologically relevant. Small molecules that target the Aβ metal species have been studied.[22,23] The strongest binding site is found in the hydrophilic N-terminal portion,[24−26] and hence the truncated N-terminal portion (amino acids 1−16, called Aβ16) has been used as a valuable model for Cu(II)-binding to the monomeric Aβ. Electron paramagnetic resonance (EPR) and Raman studies of Cu(II)−Aβ did not show differences between Aβ16, monomeric, and aggregated entire forms of Aβ.[27−29] This is also supported by the report that the

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Cu(II)-affinity is very similar for Aβ16 and entire Aβ in different aggregation states.[30]

The coordination of Cu(II) by truncated Aβ peptides has been extensively studied (see, for example, refs 11, 25−28, and 31−36). The resulting Cu(II) complex is very dynamic (ligand exchange in and between Aβ). It was also found that Aβ16 binds Cu(II) in a pH-dependent manner.[35,36] Around pH 7.4, two different type of Cu(II) coordinations exist in equilibrium (often called component I and II). These two components I and II are largely predominant at pH 6.5 and pH 9, respectively.[3]

Electron paramagnetic resonance approaches were used to elucidate the coordination sphere of Cu(II) by the Aβ peptides under physiological pH.[24−26,31,37−39] Recent progress was made due to measurements of pH where the components are well isolated and due to specific isotopic labeling of the potential coordinating amino acids. This allowed the structural characterization of two components of CuAβ16. In component I, Cu(II) is coordinated by two His residues in the equatorial positions (His6 and His13 or His14), the backbone carbonyl, as well as the NH_2 terminus of Asp1.[24,26] The carboxylate side chain of Asp1 contributes to the coordination in the axial position but is in equilibrium with other carboxylate side chains (mainly from Asp7).[27] In component II, two different models have been suggested based on EPR measurements with isotopic labeled Aβ16.[24,26] One model proposes that the equatorial ligands are the NH_2 terminus of Asp1, the deprotonated amide nitrogen of Asp1−Ala2, the carbonyl of Ala2, and one His residue. The carboxylate of Asp1 occupies the axial position alone.[26,27] The other model proposes that the oxygen donor is the carbonyl of Ala2, while the nitrogen donors are the Nτ atoms of His6, -13, and -14.[24]

Despite the fact that EPR spectroscopies in connection with other techniques provided valuable information about the CuAβ16 complex formation, some key information about the main ligands, i.e., His6, -13, -14, and Asp1 are still lacking, particularly which nitrogen is bound in His and their protonation state and the coordination mode of carboxylate. Such detailed information is important, as very subtle changes can have a large impact on the structure and hence on the aggregation behavior.[35] Vibrational spectroscopy was extensively used for the study of the aggregation process (in the absence of metal ions) via the amide I and amide II signals, a spectral feature that provides a unique signature of an aggregated peptide or protein.[40−43] No vibrational study on Cu(II)-binding to Aβ at defined pHs where the components are pure and by using isotopic labeling has been reported so far.

Here, FTIR spectroscopy is used in order to learn more about the coordination of the key residues His6, -13, -14 and Asp1 of Cu(II) within the Aβ16 in components I and II. Vibrational spectroscopy is a powerful tool to visualize the binding mode of metal cations to either nitrogen atoms of His rings.[35,44−46] In addition, FTIR spectroscopy provides information about the involvement of carboxylate moieties in metal coordination. H−D exchange and isotopic labeling of the His residues as well as the Asp1 are crucial for the accurate assignment of the infrared absorption bands of the free and the Cu(II) bound peptide. Concerning the His residues, the isotopic labeling was performed for all the nitrogen atoms, with ^{15}N instead of ^{14}N. Concerning the Asp1 residue, isotopic labeling was performed for all the carbon atoms with ^{13}C instead of ^{12}C. In the Asp1 residue, the nitrogen atom was also labeled with ^{15}N. In a given labeled sample, only one amino acid was labeled.

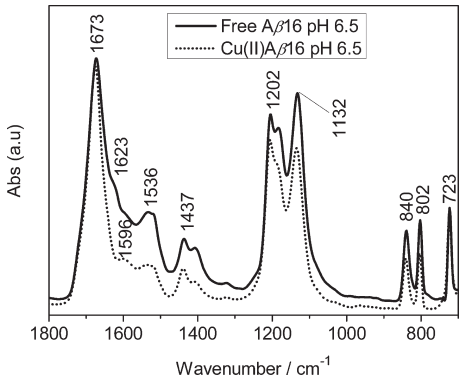

Figure 1. Absorbance spectra of the free and Cu(II) bound Aβ16 at pH 6.5.

The unlabeled and labeled samples were studied by ATR−FTIR spectroscopy. Spectra of the free and Cu(II)−Aβ16 dried films were recorded for pH 6.5 and 9.0 in the infrared spectral range from 1800 to 700 cm^{-1}. H−D exchange was performed for all the samples in order to accurately assign the infrared signals.

2. MATERIALS AND METHODS

Unlabeled Aβ16 peptides were purchased from Genecust (Dudelange, Belgium). The labeled amino acids were purchased from Eurisotop (St-Aubin, France) and sent to GENEPEP (Prades-le-Lez, France) for synthesis of the labeled peptides. The D1- and H6-labeled peptides were 97% pure, and the H13- and H14-labeled ones are 95% pure.

$CuCl_2 \cdot 2H_2O$ (Riedel−de Haën) was dissolved in distilled water or D_2O to a final concentration of 100 mM. The pH (pD) adjustment was done using HCl (DCl) or NaOH (NaOD).

The peptides were dissolved in pure water or in D_2O to a final concentration of 5 mM. Cu(II) was added in equimolar concentration. The pH/pD value was controlled by the addition of small amounts of HCl/ (DCl) or NaOH/ (NaOD). Two microliters of each sample were dried under N_2 flux on the ATR crystal forming a thin film. The final concentration of the peptide was controlled in the UV range by measuring the Tyr absorption at 275 nm (ε = 1410 $Mol^{-1} \cdot cm^{-1}$) of the 700 times diluted sample.

The mid-infrared (MIR) spectra of the CuAβ16 samples were obtained with a vertex 70 spectrometer (Bruker optics, Germany) equipped with a Harrick−Diamond-ATR unit. For each sample, 10 spectra with 256 scans were averaged. The spectral resolution was 4 cm^{-1}. The spectra were normalized on the amide I band.

3. RESULTS AND DISCUSSION

3.1. Absorbance Spectra at pH/pD 6.5. *3.1.1. Amide Bonding Contributions.* The infrared spectra of peptides and proteins involve signals arising from the peptide bonding, the so-called amide bands. The amide I vibration generally occurs in the 1690−1620 cm^{-1} range, including the coordinates of the υ(C═O) vibrational mode, while the amide II signal is expected around 1550 cm^{-1}, including the coupled CN/NH vibrational mode.[47] Other amide vibrations of weaker intensities occur at lower frequencies, such as the amide III vibration, that usually appears in the 1320−1220 cm^{-1} spectral range. The absorption spectra of the free and CuAβ16 at pH 6.5 are presented in

Table 1. Detailed Assignments of the Free Aβ16 Peptide Bands in the MIR[a]

Frequency (cm^{-1})	Assignments
1672	TFA/amide I/υ(C═N) Arg
1645	amide I
1623	$\upsilon(C_4{=}C_4)$ protonated His/$\upsilon(NH_3^+)^{as}$ Arg/υ(C═C) Tyr—OH
1580	$\upsilon(COO^-)^{as}$ acidic residues/amide II/$\upsilon(C_4{=}C_4)$ His
1536	$\upsilon(COO^-)^{as}$ acidic residues/amide II
1437	TFA
1401	$\upsilon(COO^-)^{s}$ acidic residues
1323	amide III/δ(CH) nonspecific
1202	TFA
1132	TFA
1050	δ(ring) N^{π}—protonated His
1010	δ(ring) N^{τ}—protonated His
975	δ(ring) His
840	TFA
802	TFA
723	TFA

[a] Depending on the studied samples, the band positions can be more or less shifted. For more details see text.

Figure 1. In the amide I region, an intense signal can be seen at 1673 cm^{-1}, and a less intense one can be seen at 1623 cm^{-1}. The Aβ16 contains one Arg residue, and the $\upsilon(CN_3H_5^+)^{as}$ vibration is expected in the amide I region around 1675 cm^{-1}.[48,49] The $\upsilon(CN_3H_5^+)^{s}$ vibration is expected around 1635 cm^{-1}.[48,49]

The signal observed at 1623 cm^{-1} can be assigned to the $\upsilon(C_4{=}C_5)$ vibrations of the protonated His residues[50] and may also include the absorption of other amino acids (Tyr, Asp, Glu, Gln).[48,49] The amide II band appears as a broad band at 1536 cm^{-1}. The $\upsilon(C_4{=}C_5)$ vibration of the His side chain as well as of Tyr, Asp, and Glu are expected in this spectral range.[48,49] The shoulder at 1517 cm^{-1} can be assigned to the υ(CC) ring vibrations of the Tyr ring in the neutral state (Tyr—OH).[48,49] In the region below 1450 cm^{-1}, side chain absorptions of His residues are expected to contribute to the spectrum.[50] Vibrations arising from the Lys side chain as well as from the side chain of deprotonated acidic residues are not excluded.[48,49]

However, the signals below 1450 cm^{-1} are too intense to be assigned to the side chain absorptions alone. Therefore something else is contributing to the spectra observed. Indeed, due to the protocol for peptide synthesis (elution with trifluoro-acetic acid (TFA)), it is likely that some TFA salt is present in the samples despite extensive purification. TFA has several contributions in the spectral range studied here (see Supporting Information, Figure S1) and a complete removal was not possible despite extensive purification of the peptide with desalting columns. The amide I band overlaps with an intense absorption of TFA, and in conclusion the secondary structure modifications upon Cu(II) binding cannot be discussed based on the amide I shifts. Furthermore, the region specific of the His ring deformation vibrations (i.e., 1250−1100 cm^{-1}) is masked by the TFA signals. To overcome this problem, we have studied the contribution of TFA in the absence and presence of Cu(II) cations, in order to be able to distinguish it from the amyloid data, and focused on spectral areas where TFA does not contribute, i.e., on the amide II region and the region between 1400 and 1200 cm^{-1}. Because of the signals arising from TFA below 900 cm^{-1}, the spectra of the peptides are limited to the spectral region above 900 cm^{-1}. The broad band of TFA centered at 996 cm^{-1} does not affect the analysis of the data in the spectral region around 1000 cm^{-1}. The overview of the band assignments in the free peptide is presented in Table 1.

3.1.2. Side Chain Contributions of Deprotonated Acidic Residues. Figure 2 shows the spectra of the free and the Cu(II)-bound unlabeled and Asp1 ^{13}C-labeled Aβ16 peptide recorded at pH/pD 6.5 in the region of the $\upsilon(COO^-)$ vibration. The $\upsilon(COO^-)^{as}$ vibrations of acidic residues are expected in the region 1570−1540 cm^{-1}.[48,49] Two signals are present in the amide II region of the unlabeled peptide, one at 1536 cm^{-1}, the other at 1517 cm^{-1}, which can be assigned to the amide II and to the Tyr−OH υ(CC) ring vibrations, respectively.[47,49] These signals are shifted to 1558 cm^{-1} and 1525 cm^{-1}, respectively, in the spectrum of the ^{13}C-labeled Asp1 sample (Figure 2A). The coordination of Cu(II) modifies the spectral features of both, unlabeled and labeled peptides. The spectrum of the former one shows a signal at 1597 cm^{-1}, which includes the $\upsilon(C_4{=}C_5)$ vibrations of the His residues[50] (Figure 2B). The signal observed at 1558 cm^{-1} in the spectrum of the ^{13}C-labeled labeled free peptide shifts toward 1547 cm^{-1} in the spectrum of the coordinated peptide that may be attributed to the $\upsilon(^{13}COO^-)^{as}$ vibration of Asp1. The $\upsilon(^{13}COO^-)^{s}$ vibration shifts from 1366 to 1370 cm^{-1} upon coordination.

Figure 2C,D shows the Asp1-labeled sample after H−D exchange. Here the coupled δ_{op}(NH)/ υ(CN) amide II vibration downshifts from ∼1550 cm^{-1} to ∼1450 cm^{-1} upon uncoupling of the υ(CN) vibration (amide II′). The δ_{op}(ND) vibration is now in the 1070−900 cm^{-1} spectral range[51] (Figure 2C). The spectrum of the unlabeled peptide recorded at pD 6.5 shows a signal at 1582 cm^{-1} that shifts to 1590 cm^{-1} upon Cu(II) binding (Figure 2D). These signals may arise from the $\upsilon(COO^-)^{as}$ vibration of a deprotonated acidic residue.[48,49] The signal observed at 1582 cm^{-1} shifts upon ^{13}C-labeling toward 1548 cm^{-1} in the spectrum of the labeled sample, and it can be assigned to the $\upsilon(^{13}COO^-)^{as}$ vibration of Asp1. In fact, the replacement of an atom by a heavier isotope induces a downshift of the relevant vibration that can be described in a simplified way according to the harmonic oscillator model. For instance, the ^{13}C-labeling of the Asp1 residue is expected to induce a downshift of the signal by about 20 cm^{-1} [52] (See eq 1 in the Supporting Information). The copper binding to the peptide induces an upshift of this signal to 1554 cm^{-1}. Thus the involvement of the N terminus of the Aβ16 (i.e., the carboxylate side chain of Asp1) in the Cu(II) binding at pH/pD 6.5 can be confirmed. The signal observed at 1406 cm^{-1} for the unlabeled sample may arise from the $\upsilon(COO^-)^{s}$ vibrations of an acidic residue.[48,49] For the Asp1-labeled sample, the signal is seen at 1406 cm^{-1}, together with an additional broad signal at 1366 cm^{-1} assigned to the $\upsilon(^{13}COO^-)^{s}$ vibration of the Asp1 residue after ^{13}C labeling (Figure 2A). Upon coordination (Figure 2B), the $\upsilon(COO^-)^{s}$ vibrations of acidic residues remains in position in the spectrum of the unlabeled sample. On the other hand, the $\upsilon(^{13}COO^-)^{s}$ vibration of the Asp1 residue is slightly upshifted to 1370 cm^{-1}.

The direct comparison between the frequencies of the antisymmetric and symmetric stretching vibrations of the free and bound carboxylate side chain is a good indicator for the

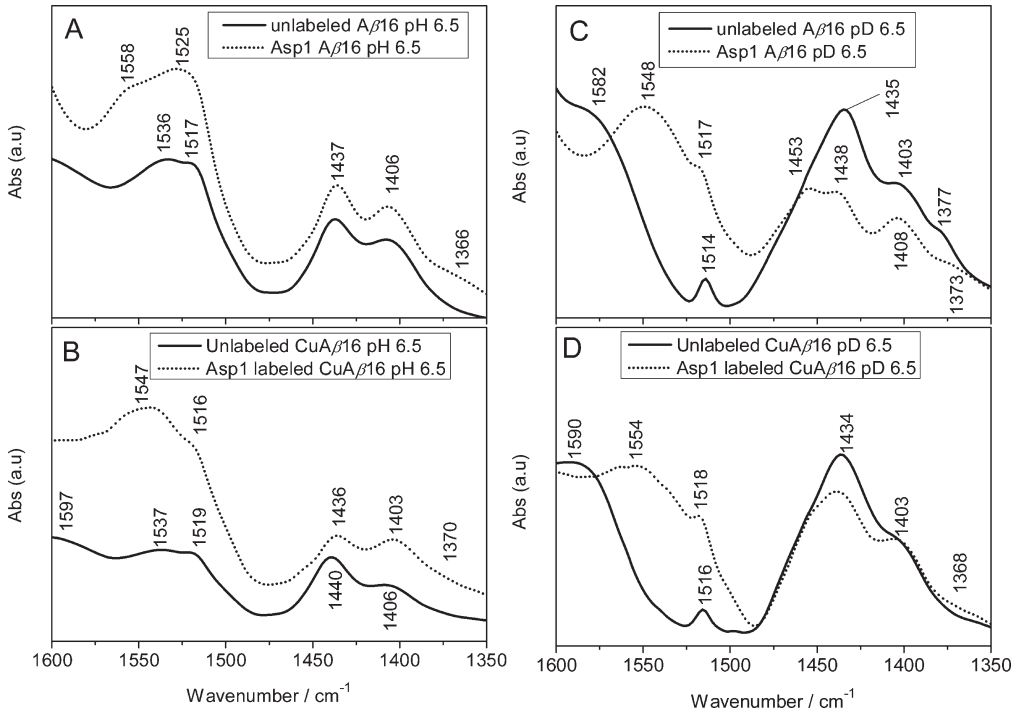

Figure 2. Absorbance spectra of the free unlabeled (solid line) and the free ^{13}C-labeled Asp1 (dashed line) in the 1600−1350 cm^{-1} spectral range recorded at pH 6.5 (A). Spectra of the unlabeled CuAβ16 (solid line) and the ^{13}C-labeled Asp1 CuAβ16 (dashed line) recorded at pH 6.5 (B). Spectra of the free unlabeled (solid line) and the free ^{13}C-labeled Asp1 (dashed line) recorded at pD 6.5 (C). Spectra of the unlabeled CuAβ16 (solid line) and the ^{13}C-labeled Asp1 CuAβ16 (dashed line) recorded at pD 6.5 (D).

coordination mode of the carboxylate, which can bind the metal in a bidentate, monodentate, or in a pseudo-bridging mode. The relationship between the frequencies and the coordination modes are well documented in the milestone review by Deacon and Phillips[46] and can be described as follows:

$$\Delta\upsilon_{as} - \upsilon_s(\text{free}) > \Delta\upsilon_{as} - \upsilon_s(\text{bound}) \quad \text{Bidentate}$$

$$\Delta\upsilon_{as} - \upsilon_s(\text{free}) < \Delta\upsilon_{as} - \upsilon_s(\text{bound}) \quad \text{Monodentate}$$

$$\Delta\upsilon_{as} - \upsilon_s(\text{free}) \approx \Delta\upsilon_{as} - \upsilon_s(\text{bound}) \quad \text{Pseudo} - \text{bridging}$$

It should be noted that the quasi-monodentate or quasi-bidentate forms can be suggested when the difference between the Δ values is greater than ~20 cm^{-1}.

At pD 6.5, the frequencies of the free and bound carboxylate group of the Asp1 residue studied here give the following values:

$$\Delta\upsilon_{as} - \upsilon_s(\text{free}) = 1548 - 1373 = 175 \text{ cm}^{-1}$$

$$\Delta\upsilon_{as} - \upsilon_s(\text{bound}) = 1554 - 1368 = 186 \text{ cm}^{-1}$$

These data confirms the coordination of the Cu(II) by the Asp1 side chain and indicates that Cu(II) is coordinated via a pseudo-bridging mode with a monodentate character.

3.1.3. Side-Chain Contributions of Histidines. The signature of His is expected in the 1600−1500 cm^{-1} spectral range where the $\upsilon(C_4{=}C_5)$ vibration occurs.[35,50] Weaker signals arising from the His ring motions can arise in the 1000 cm^{-1} spectral range.

A comparison between the spectra of the ^{15}N-labebed His residues in the region 1610−1500 cm^{-1} before and after the coordination at pH/pD 6.5 is shown in Figure 3A. The amide II band of the ^{15}N-labeled His is larger than the one of the unlabeled sample and slightly shifted to higher wavenumbers. The upshift reflects the contribution of the $\upsilon(C_4{=}C_5)$ vibrations of the His residues to this large band. For the His13 labeled sample, the shift is more pronounced; a shoulder can be distinguished at 1566 cm^{-1}.

When the coordination of Cu(II) takes place (Figure 3B), a signal appears at 1600 cm^{-1} in the spectrum of the unlabeled, the ^{15}N-His13, and the ^{15}N-His14 samples, which can be assigned to the $\upsilon(C_4{=}C_5)$ vibration of N$^{\pi}$-coordinated His.[53] The observation that this signal is present for both H13 and H14 samples leads us to conclude that this signal could arise from one of these residues or from both residues.

The spectrum of the H6 sample shows a weak shoulder at 1570 cm^{-1}. This signal can be assigned to the N$^{\tau}$ coordinated form.[50] The signature of the His in the amide II region shows that the coordination of Cu(II) by the His residues takes place via N$^{\pi}$ and N$^{\tau}$. These observations are in line with previously reported data suggesting the involvement of the N$^{\tau}$ metal binding in the aggregation of the Aβ peptides.[35]

The signals appearing in the amide II′ region are of higher intensities than the ones obtained before the H−D exchange (Figure 3C). The amide II downshift allows distinguishing the side chain absorptions overlapping with the amide II band in the spectra recorded at pH 6.5. The spectrum of the unlabeled sample shows a signal at 1580 cm^{-1}, which is slightly shifted in the spectra of the ^{15}N-labeled His samples (Figure 3C). The Tyr residue is in the neutral form and gives rise to a signal at 1514 cm^{-1} in all the spectra of the free peptides recorded at pD 6.5. This signal is assigned to the υ(CC) vibration of the Tyr ring.[48,49] At pD 6.5, the $\upsilon(C_4{=}C_5)$ vibration observed at 1590 cm^{-1} for the unlabeled sample remains in the same position in the spectrum of the His13 sample.[50] On the other hand, this

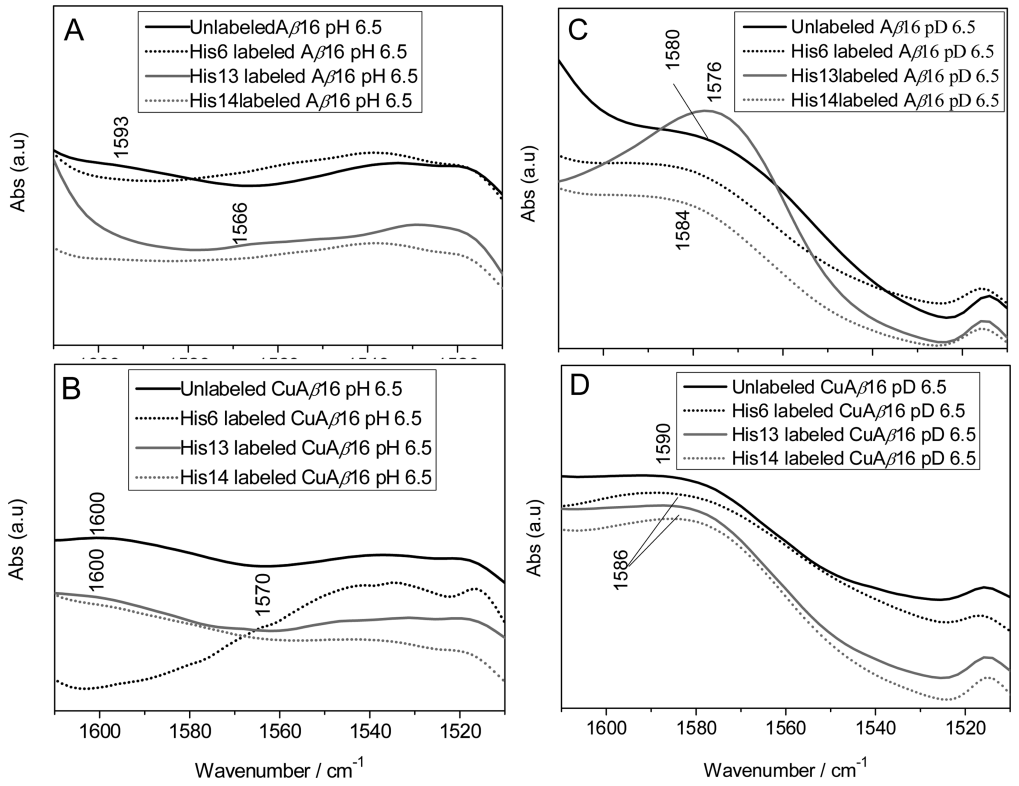

Figure 3. Absorbance spectra of the free unlabeled and the free ^{15}N-labeled His Aβ16 peptides in the $\upsilon(C_4{=}C_5)$ vibrations spectral range recorded at pH 6.5 (A). Spectra of the unlabeled CuAβ16 and the ^{15}N-labeled His CuAβ16 recorded at pH 6.5 (B). Spectra of the free unlabeled and the free ^{15}N-labeled His Aβ16 peptides recorded at pD 6.5 (C), and spectra of the unlabeled CuAβ16 and the ^{15}N-labeled His CuAβ16 recorded at pD 6.5 (D).

signal is downshifted toward 1586 cm^{-1} for the His6 and His14 samples (Figure 3D). In direct comparison to the data of the free peptides, this signal upshifts upon copper coordination, indicating that coordination by a His residue takes place. The lower frequencies found for His6 and His14 indicate that these residues are bound to the copper at pD 6.5. We also note that the $\upsilon(COO^-)^{as}$ vibration may occur in the same spectral range and may overlap with the His motions.

The overlapping of the vibrational modes makes the assignment of the His signals nontrivial in this spectral region, despite the isotopic labeling, and the His signals typically observed around 1000 cm^{-1} can help one better understand the Cu(II) binding to the peptide.[44,50,53] At pH 6.5, the ring breathing mode of the N$^{\pi}$-protonated form of His can be observed at 990 cm^{-1} (Figure 4A).[50] Other signals of weaker intensities can also be observed at lower frequencies. The signal observed at 990 cm^{-1} remains in position upon ^{15}N-labeling of the His residues. Upon Cu(II) coordination (Figure 4B), this signal disappears, and two distinct signals appear: one at 1030 cm^{-1} and the other at 968 cm^{-1}. The first one can also be seen in the spectra of the free peptides as a weak broad signal that can be assigned to the ring deformation mode of the N$^{\tau}$-protonated form of His.[53] Indeed, the coordination of Cu(II) modifies the protonation state of His. Hence, the copper binding is taking place via the N$^{\pi}$ atom of the His ring. The signal observed at 968 cm^{-1} can be assigned to the ring breathing mode of His of both protonated forms.

When the H−D exchange is performed, two signals can be observed at 1010 cm^{-1} and at 940 cm^{-1} (Figure 4C). These signals may arise from the ring deformation vibrations of the His residue in either protonation state.[50] Upon Cu(II) binding, both signals upshift to 1047 cm^{-1} and 972 cm^{-1}, respectively. The signal appearing at 972 cm^{1} is present in all spectra of the bound peptides and arises from the three histidines. It can thus be assigned to the δ(ring) vibration of the copper bound His, where the ligation takes place via the N$^{\pi}$ atom of the imidazole ring[53] and it is independent from isotopic labeling. The spectra of the bound peptides show a signal at 1047 cm^{-1}, which is typical for N$^{\pi}$-deuterated His.[53] The spectrum of the His6 sample shows an additional shoulder at 1030 cm^{-1} (Figure 4D) indicating that the His6 residue coordinates Cu(II) via the N$^{\tau}$ atom, while the N$^{\pi}$ atom is deuterated.[53] The signals seen at around 1005 cm^{-1} in the spectra of the coordinated peptides are assigned to the δ(ring) vibration of the N$^{\tau}$-protonated His.[50,53]

All together, the data recorded at pH/pD 6.5 allows concluding that the carboxylate side chain of the Asp1 participates to the coordination of Cu(II). The three His residues contribute to the coordination sphere of Cu(II): His6 coordinates via the N$^{\tau}$, His13, and/or His14 via the N$^{\pi}$ atoms.

It should be noted that the coordination via the N$^{\tau}$ of a His was previously reported to cause the aggregation of the Aβ peptides.[35] According to the data presented here, this coordination mode is most likely offered by the His6 residue, thus the coordination via the His6 is critical for the aggregation of the Aβ16.

3.2. Absorbance Spectra at pH/pD 9.0. *3.2.1. Amide Bond Contributions.* The coordination of Cu(II) by the Aβ16 for component II was recently studied by EPR spectroscopy.[24,26] Drew et al.[24] worked at pH 8.0 on subtracted spectra to remove the contribution of Component I present at this pH to analyze Component II. They proposed that all three histidines bind

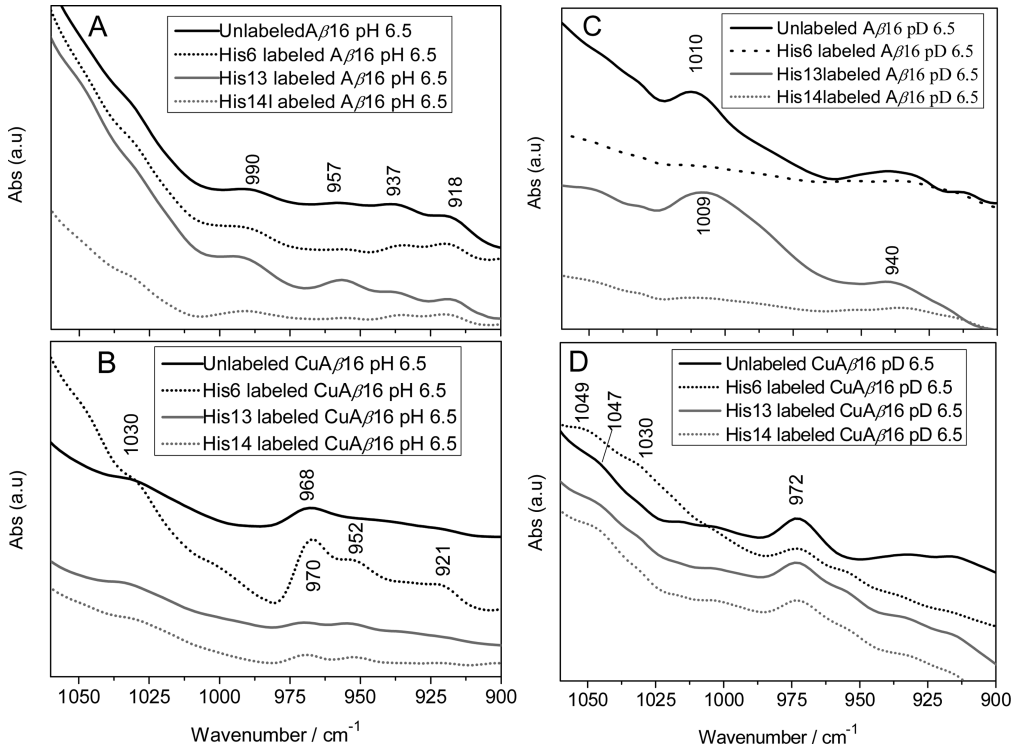

Figure 4. Absorbance spectra of the free unlabeled and the free ^{15}N-labeled His Aβ16 peptides in the His δ(ring) vibrations spectral range recorded at pH 6.5 (A). Spectra of the unlabeled CuAβ16 and the ^{15}N-labeled His CuAβ16 recorded at pH 6.5 (B). Spectra of the free unlabeled and the free ^{15}N-labeled His Aβ16 peptides recorded at pD 6.5 (C), and spectra of the unlabeled CuAβ16 and the ^{15}N-labeled His CuAβ16 recorded at pD 6.5 (D).

simultaneously while the carbonyl of Ala2 was the fourth equatorial ligand. By working at pH 9.0 to isolate Component II, which is predominant at this higher pH, Dorlet et al. showed that only one histidine was bound and could directly detect the binding of the $-NH_2$ terminus and the deprotonated amide of the Asp1−Ala2 peptide bond, the fourth equatorial ligand being the carbonyl of Ala2.[26]

The infrared spectra of the free Aβ16 at pH 9.0 are shown in Figure 5. In the amide II region, two distinct signals are present in all the spectra. For the unlabeled sample, these signals occur at 1584 and 1539 cm^{-1}. The neutral Tyr (Tyr−OH) ring motion occurs at 1517 cm^1. Since the amide II vibration generally occurs between 1575 and 1480 cm^{-1} the involvement of further contributions may be expected.[54]

3.2.2. Side Chain Contributions of Deprotonated Acidic Residues. The signal observed at 1539 cm^{-1} in the spectrum of the unlabeled sample shifts to 1533 cm^{-1} in the spectrum of the Asp1-labeled sample (Figure 5A). Indeed this signal contains coordinates from the amide II band as well as from the $\upsilon(COO^-)^{as}$ vibration of an acidic residue, most likely of the Asp1 residue. In this spectral range, the neutral Tyr (Tyr−OH) ring motion occurs at 1519 cm^{-1}.[48] At lower frequencies than the amide II region, the TFA contribution is seen at 1437 cm^{-1}. The signal appearing at 1406 cm^{-1} can be assigned to the $\upsilon(COO^-)^s$ vibration of acidic residue.[48] The spectrum of the Asp1-labeled sample shows an additional shoulder at 1373 cm^{-1} when compared to the spectrum of the unlabeled sample (Figure 5A). This signal can be assigned to the $\upsilon(^{13}COO^-)^s$ vibration of the Asp1 residue.

The spectrum of the CuAβ16 complex obtained for the Asp1-labeled sample shows an additional shoulder in direct comparison to the other spectra that occurs at 1542 cm^{-1} upon coordination (Figure 5B). This signal can be arising from the $\upsilon(^{13}COO^-)^{as}$ vibrations of the Asp1 residue. On the other hand, this signal overlaps with the amide II band. The signal appearing at 1406 cm^{-1} is mainly arising from the $\upsilon(COO^-)^s$ vibration of acidic residues. The spectrum of the Asp1 sample exhibits an additional shoulder appearing at 1375 cm^{-1}, which can be assigned to the $\upsilon(^{13}COO^-)^s$ vibration. The H−D exchange allows a more accurate assignment of the $\upsilon(^{13}COO^-)^{as}$ vibration in the free and Cu(II) bound form (Figure 5C and D). The $\upsilon(^{13}COO^-)^{as}$ vibration can be observed at 1542 cm^{-1} in the spectrum of the free peptide at this pH, and at 1547 cm^{-1} in the spectrum of the complex. The $\upsilon(^{13}COO^-)^s$ vibration occurs at 1368 cm^{-1} and does not shift upon copper binding.

The relationship between the frequencies and the coordination modes at pD 9.0 is the following:

$$\Delta\upsilon_{as} - \upsilon_s(\text{free}) = 1542 - 1368 = 174\text{cm}^{-1}$$

$$\Delta\upsilon_{as} - \upsilon_s(\text{bound}) = 1547 - 1369 = 178\text{cm}^{-1}$$

Since the values are close to each other, the coordination mode is probably pseudo-bridging with a monodentate character.[46]

3.2.3. Side Chain Contributions of Histidines. The His contribution in the amide II region can be followed using the ^{15}N-His labeling. The spectra of the free and Cu(II) complexes recorded at pH/pD 9.0 are shown in Figure 6. For the unlabeled sample, the signal at 1584 cm^{-1} may be attributed to the $\upsilon(C_4{=}C_5)$ vibration of the His ring. For the His labeled samples, this vibrational mode is involved in the signals at 1582, 1570, and 1575 cm^{-1} for His6, His13, and His14, respectively (Figure 6A).

Upon complex formation, the spectrum of the unlabeled sample shows no significant modification in the region of the

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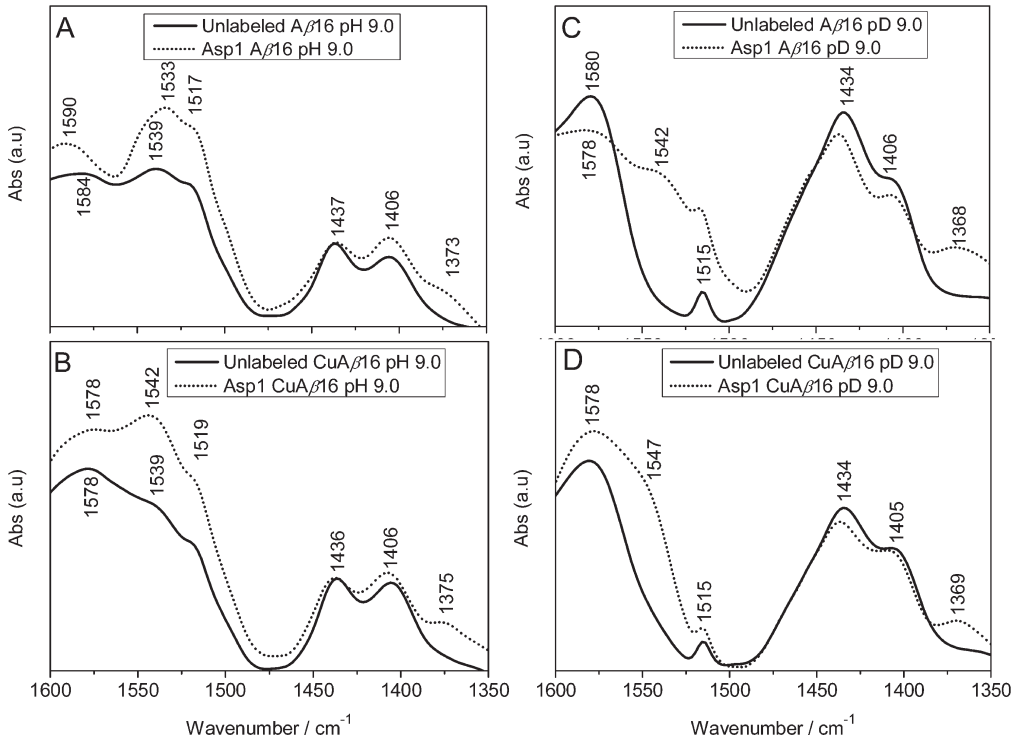

Figure 5. Absorbance spectra of the free unlabeled (solid line) and the free ^{13}C-labeled Asp1 (dashed line) in the 1600−1350 cm^{-1} spectral range recorded at pH 9.0 (A). Spectra of the unlabeled CuAβ16 (solid line) and the ^{13}C-labeled Asp1 CuAβ16 (dashed line) recorded at pH 9.0 (B). Spectra of the free unlabeled (solid line) and the free ^{13}C-labeled Asp1 (dashed line) recorded at pD 9.0 (C). Spectra of the unlabeled CuAβ16 (solid line) and the ^{13}C-labeled Asp1 CuAβ16 (dashed line) recorded at pD 9.0 (D).

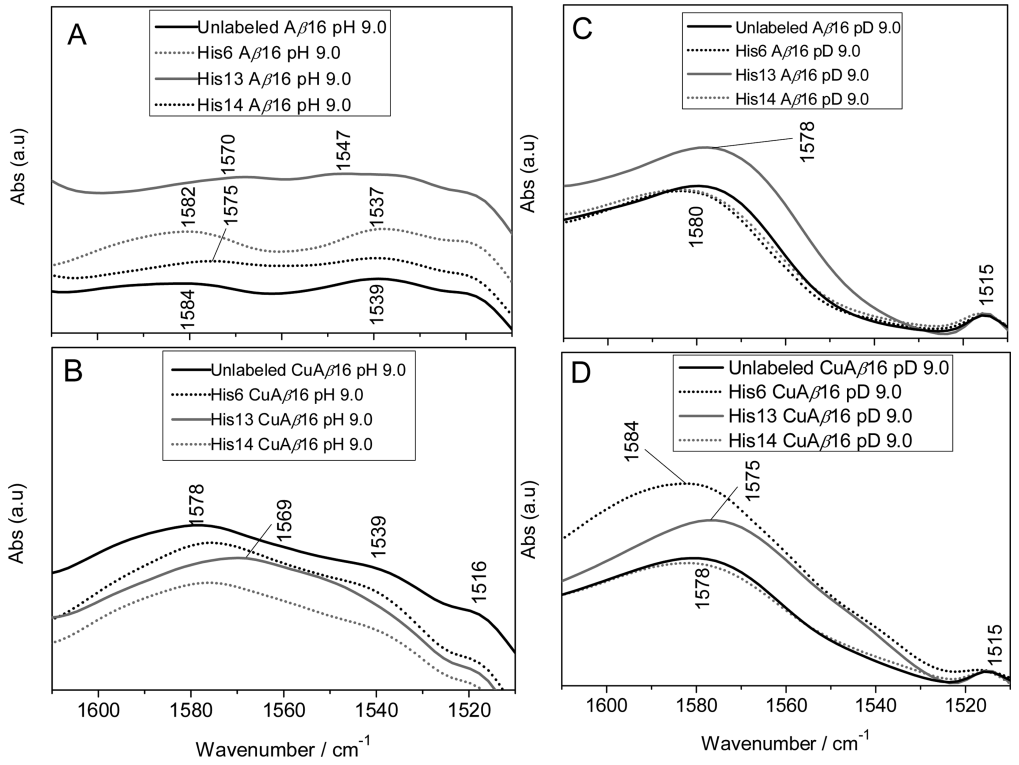

Figure 6. Absorbance spectra of the free unlabeled and the free ^{15}N-labeled His Aβ16 peptides in the $\upsilon(C_4{=}C_5)$ vibrations spectral range recorded at pH 9.0 (A). Spectra of the unlabeled CuAβ16 and the ^{15}N-labeled His CuAβ16 recorded at pH 9.0 (B). Spectra of the free unlabeled and the free ^{15}N-labeled His Aβ16 peptides recorded at pD 9.0 (C), and spectra of the unlabeled CuAβ16 and the ^{15}N-labeled His CuAβ16 recorded at pD 9.0 (D).

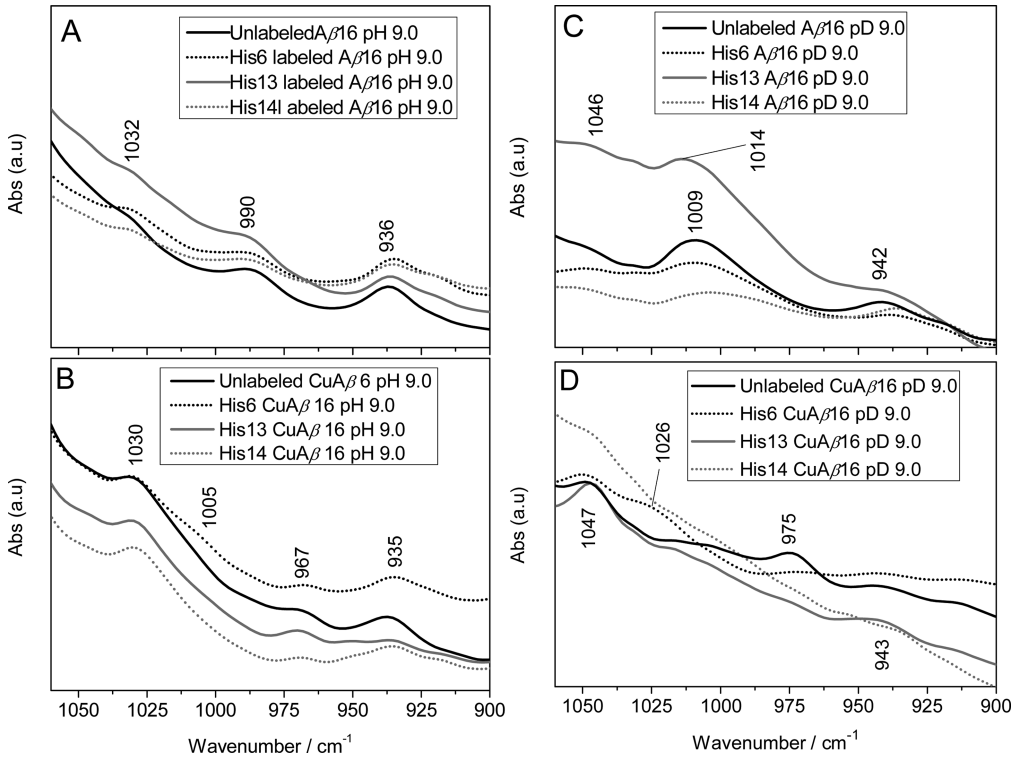

Figure 7. Absorbance spectra of the free unlabeled and the free ^{15}N-labeled His Aβ16 peptides in the His δ(ring) vibration spectral range recorded at pH 9.0 (A). Spectra of the unlabeled CuAβ16 and the ^{15}N-labeled His CuAβ16 recorded at pH 9.0 (B). Spectra of the free unlabeled and the free ^{15}N-labeled His Aβ16 peptides recorded at pD 9.0 (C), and spectra of the unlabeled CuAβ16 and the ^{15}N-labeled His CuAβ16 recorded at pD 9.0 (D).

$\upsilon(C_4{=}C_5)$ vibration domain. This is also the case of the spectra of His6 and His14, where only small shifts occur. Compared to the other CuAβ16 spectra, the spectrum of the His13 sample shows a significant shift where a downshift is observed at 1569 cm^{-1}, whereas the signal does not exhibit a significant shift compared to the free ^{15}N-His13 labeled sample. Upon H-D exchange, the spectra of the unlabeled and ^{15}N-His labeled samples reveals one signal around 1580 cm^{-1}. When copper is bound to the peptide, the signal observed at 1580 cm^{-1} shifts to 1584 cm^{-1} in the spectrum of the His6-labeled sample. The upshift is in line with the coordination of copper by the His6 residue.

The ring deformation vibration of His around 1000 cm^{-1} is shown in Figure 7. The spectra recorded at pH 9.0 shows three main signals at 1032, 990, and 936 cm^1 (Figure 7A). These signals are typical for the N$^\tau$-protonated form of a His residue.[44,50] Upon Cu(II) coordination, a signal appears at 967 cm^1 in all the spectra of the CuAβ16 complexes recorded at pH 9.0 (Figure 7B). This signal can be assigned to the ring deformation vibration of N$^\tau$-bound His while the N$^\pi$ atom is protonated. Indeed, the binding of copper at pH 9.0 modifies the protonation state of at least the imidazole of the binding partner. The H−D exchange affects the spectra of the free Aβ16 (Figure 7C) where a signal appears at 1009 cm^{-1}. This signal is characteristic of the N$^\tau$ deuterated form of His,[50] and it exhibits small shifts upon His labeling. The His13-labeled sample shows a broader signal with a maximum at 1014 cm^{-1}. Furthermore, weak signals appearing at 1046 and 942 cm^{-1} can be also assigned to the ring deformation vibration of the His residues.[44,53]

The His ring deformation vibrations observed in the spectrum of the peptides at around 1010 cm^{-1} is upshifted in the spectra of the coordinated peptides to frequencies higher than 1045 cm^{-1} (Figure 7D). This upshift points toward the coordination of copper by the N$^\tau$ atom of the His residue while the N$^\pi$ atom is protonated.[53] The copper binding seems to displace the hydrogen (or deuterium) atom from the N$^\tau$ to the N$^\pi$ atom.

The ^{15}N-isotopic labeling of the His6 residue induces the splitting of the signal observed at 1047 cm^{-1} in the spectrum of the unlabeled sample into two signals: one remains at the same position, and the second is downshifted toward 1026 cm^{-1}. Thus, the His6 residue binds copper via the N$^\tau$ atom while the N$^\pi$ atom is protonated. The split of the signal may be interpreted in two ways: (1) At least one other His residue binds copper via the N$^\tau$ atom, which would be in line with the model of three His bound (refs 24 and 25). (2) The His6 residue is the only His residue bound to copper, and the N$^\pi$ atoms of the other His residues became protonated (instead of the N$^\tau$) due to environmental modifications caused by the copper binding. This is in line with the model of only one His bound to Cu(II) (ref 26). The observation that the downshift occurs only when the His6 residue is labeled, makes the second suggestion the most plausible one. Otherwise, the spectra of the His13 and/or the His14 samples would show a similar downshift. This indicates that, in our experimental conditions, His6 is the histidine residue preferentially bound to copper. We note that the samples measured here are not in solution, and the ligand exchange suggested by other methods may not take place.[26] The signal observed at 975 cm^{-1} in the spectrum of the unlabeled sample remains at the same position in the spectrum of the Asp1-labeled sample (Figure 7D). This signal can be tentatively assigned to the ring deformation vibration of His residues bound to the copper via the N$^\tau$ atom while the N$^\pi$ is deuterated.[53]

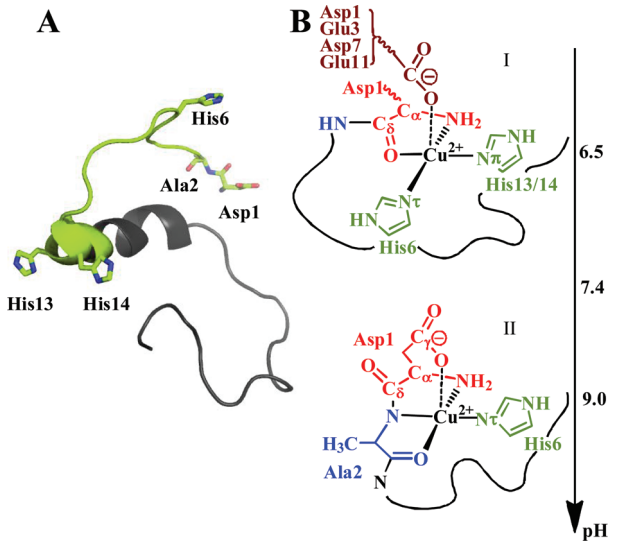

Figure 8. NMR structure of the 1 40 Aβ partially folded peptide at pH 7.3 (PDB code 2FLM). The amino acids 1−16 are colored in green, the amino acids 17−40 are colored in gray. The amino acids involved in the metal coordination are shown as sticks. The figure was made using PYMOL [http://www.pymol.org] (A). Coordination model discussed for pH 6.5 and pH 9.0 (B).

Finally, the coordination of copper by the side chain carboxylate Asp1 residue is taking place at pH (pD) 9.0 by a monodentate mode with a pseudo-bridging character. The isotopic labeling of the His residues revealed the effect of the copper coordination of the protonation state of these residues where copper binds to the N^{τ} atom by displacing the hydrogen to the N^{π} atom.

4. CONCLUSION

An ATR-FTIR spectroscopic study is presented for a better understanding of the copper coordination by the Aβ16 at two pH (pD) values, 6.5 and 9.0. The data obtained by ATR-FTIR spectroscopy support the coordination by the carboxylate side chain of the Asp1 residue at all the pH values used here. We could show that the Cu(II) coordination takes place via a pseudo-bridging mode with a monodentate character. In addition, the suggestion that all the His residues contribute to the coordination at pH 6.5 is also supported. More specifically, the His6 coordinates via the N^{τ}, and His13 and His14 coordinate via the N^{π} atoms. At pH 9.0, the His6 residue was observed to be bound via the N^{τ} atom. The FTIR spectroscopic data shows that the Tyr residue present in the Aβ16 is in the neutral form when copper is bound at pH 6.5 and 9.0, revealing that the Tyr residue remains undoubtedly free in the presence of copper. It is important to underline the proton displacement caused by the copper binding observed here that may be a link in the chain of the aggregation causing the Alzheimer's development. Furthermore, the coordination by His6 and the carboxylate side chain remains the same when the pH is raised from 6.5 to 9.0. However, the pH changing influences the coordination modes by His13 and -14. For clarity, a model is shown in Figure 8. It confirms the coordination described in recent studies (see refs 4, 26, 27, and 55) and additionally shows the coordination with His6 at pH 9.

We note that the data shown here was obtained with the Aβ16 peptide, since the coordination takes place in the N-terminal portion. Despite the influence of the amino acid sequence on the aggregation, this model was found to reflect the coordination in the full length Aβ peptide.[20,29,30,34,36] Metal-binding to Histidines of amyloidogenic peptides play an important role not only in Aβ, but also in several other cases, such as amylin,[56] prion,[5,57] or α-synuclein.[58] Histidines have a pK_a around 6 and can bind metal by N^{τ} or N^{π}, and subtle changes in the coordination can have an important impact on aggregation, thus the exact binding mode of the His in these proteins and their pH dependence in the physiological range are of importance to understand the effect of metal-binding on their structure.

■ ASSOCIATED CONTENT

Supporting Information. Equation 1 and the spectrum of the TFA at pH 14 in the 1800−700 cm^{-1} spectral range. This material is available free of charge via the Internet at http://pubs.acs.org/.

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■ ABBREVIATIONS:

Aβ16, Amyloid-β 16; ATR, attenuated total reflection; FTIR, Fourier transform infrared; δ, bending vibration; υ, stretching vibration; as, antisymmetric vibration; s, symmetric vibration; op, out-of-plane vibration

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