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Zn^{2+}-induced deprotonation of a peptide nitrogen in angiotensin I

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The interaction of Zn^{2+} with angiotensin I, a decapeptide containing two histidyl residues, has been studied by ^{1}H-NMR spectroscopy in both water and dimethylsulfoxide. When Zn^{2+} is added to the peptide in dimethylsulfoxide, binding occurs by coordination of the imidazole rings of both histidines to the metal-ion, enabling the deprotonation of the Phe peptide nitrogen.

^{1}H-NMR; Zinc binding, Deprotonated amide; Angiotensin I; Peptide

1. INTRODUCTION

Zinc has been shown to play an important role in a variety of enzyme-catalysed reactions, particularly those of the zinc hydrolytic enzymes [1,2]. One specific area of recent interest in the study of zinc–peptide interactions is the ability of Zn^{2+} to deprotonate an amide nitrogen in some circumstances [3–6]. To date, Zn^{2+} has been shown to deprotonate a peptide nitrogen only in specific di- and tripeptides that contain a histidyl residue adjacent to the N-terminus [3,4]. In these peptides, in addition to the imidazole nitrogen of the histidine side-chain, the N-terminal amine participates in the coordination of the zinc-ion. While this binding situation is the only reported example of Zn^{2+}-induced peptide nitrogen deprotonation, other model non-peptide amides have been employed to study this type of interaction [5,6]. These model systems also rely on anchoring the metal-ion to at least two coordination sites on the ligand, allowing deprotonation of an amide- or sulfonamide nitrogen.

In an analysis of the X-ray crystal structure of twelve zinc enzymes, Vallee and Auld [1] noted that two of the three protein amino acid side chains that bind to the catalytic zinc ion were separated in the amino acid sequence by one to three residues in all but one case. It was suggested that these closely spaced ligands (predominantly histidines) could 'facilitate the formation of a primary bidentate zinc complex'.

The aim of this study was to determine whether two histidyl residues separated by two other amino acid residues could bind to zinc with the formation of a bidentate complex and subsequent deprotonation of a peptide nitrogen. In this paper we report the results of a ^{1}H-NMR study of zinc complexation of angiotensin I (AngI, Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu), where we have shown that zinc ion binds to the imidazole side chains of both histidine residues and more importantly, to the deprotonated peptide nitrogen of the intervening Phe residue.

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2. EXPERIMENTAL

2.1. *Materials*

Angiotensin I was purchased from Auspep Pty. Ltd. and used without further purification. The 99.8% and 99.99% DMSO were obtained from Aldrich Chemical Company and the 99.96% D_2O was obtained from Varian Pty. Ltd. Peptide samples in aqueous solution were repeatedly lyophilised from 99.8% D_2O and then redissolved in 99.96% D_2O and the pH adjusted by addition of deuterated NaOH. Small aliquots of Zn^{2+} were added to the peptide samples from a stock solution of $ZnCl_2$ (in either H_2O or D_2O) which had been standardised against EDTA.

2.2. *NMR Spectroscopy*

^{1}H-NMR spectra were recorded at 300 MHz on a Varian XL-300 spectrometer. All spectra were recorded at 25°C. Typical one-dimensional spectral acquisition parameters were as follows: spectral width 3000 Hz, 90° rf pulse, 16 000 data points with a total relaxation delay of 2 s. Chemical shifts are expressed as ppm downfield from either 3-(trimethylsilyl)propanesulfonic acid or TMS (for samples in DMSO).

Homonuclear ^{1}H double-quantum filtered phase-sensitive COSY and NOESY spectra were acquired by the use of the hypercomplex method [7] with 2048 points in t_2 for 256 t_1 values. A pulse-repetition delay of 1.7 s was used with a mixing time of 300 ms for the NOESY spectra. The spectra were weighted with an apodization function, zero-filled twice in t_1 and Fourier-transformed in both dimensions.

3. RESULTS

3.1. *Zn^{2+} complexation of angiotensin I in water*

The assignment of most of the non-exchangeable proton resonances of AngI in D_2O has been previously reported [8]. These assignments were confirmed by a

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DQFCOSY experiment and pH-titration. The ^{1}H-NMR spectra of a 1:1 molar mixture of Zn^{2+} and AngI are identical to those of the free peptide at corresponding pH values up to pH 5.7, demonstrating that the metal ion does not bind in detectable amounts to AngI below this pH. Above pH 5.7 the C2-H resonances of His6 and His9 in the 1:1 mixture begin to preferentially broaden and move upfield relative to the chemical shifts of the corresponding resonances in the spectrum of the free peptide (Fig. 1), indicating that Zn^{2+} binds to both histidines, either simultaneously or via exchange. The histidine C2-H resonances of the 1:1 complex become significantly broader with increasing pH, indicating that the AngI– Zn complex is not in fast exchange with free AngI (on the NMR time scale) but exchanges somewhat more slowly. When Zn^{2+} is bound only to the histidine imidazole nitrogen of small histidyl peptides, no exchange broadening is observed because the complexes are in fast exchange with the free peptides [3,4]. The increased kinetic stability of the AngI–Zn complex is consistent with bidentate binding of Zn^{2+} to both histidine residues.

The AngI–Zn complex is not very soluble (<0.1 mM) above pH 7, thus precluding detailed investigation by ^{1}H-NMR of zinc-ion-induced peptide nitrogen deprotonation in water.

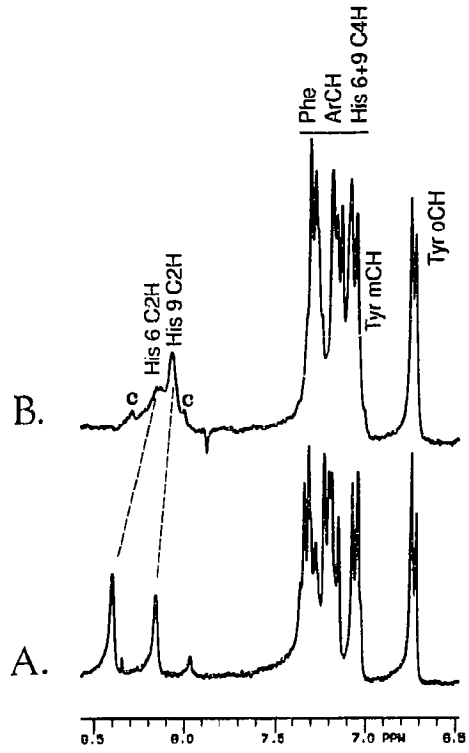

Fig. 1. ^{1}H-NMR spectrum of the aromatic region of (A) free AngI, pH 6.54 and (B) 1:1 molar mixture of Zn^{2+} and AngI, pH 6.56. In spectrum B the small peaks either side of the main His C2-H resonances (denoted c) are due to the *cis* form of AngI (His6-Pro7 peptide bond), while the peak at 8.34 ppm in spectrum (A) is due to a minor impurity (not present in spectrum B).

3.2. *Zn^{2+} binding to angiotensin I in DMSO*

The resonances in the spectrum of AngI in DMSO were assigned by the standard combination of two-dimensional COSY and NOESY experiments [9]. From the SQFCOSY spectrum it was possible to assign the complete spin system of the six aliphatic residues, but it was not possible to unambiguously assign the NH, $C_\alpha H$ and $C_\beta H$ resonances of the aromatic residues due to the overlap of the $C_\beta H$ resonances. The NH and $C_\alpha H$ resonances of the aromatic residues were assigned from the 300 ms mixing time NOESY spectrum (Fig. 2).

Fig. 3 shows the aromatic and NH region of the AngI ^{1}H-NMR spectrum in DMSO in the absence and presence of one equivalent of zinc-ion. In the presence of Zn^{2+}, the NH resonance of Phe (8.86 ppm) is not observed. The disappearance of this resonance and the observation that the Phe $C_\alpha H$ resonance is significantly shifted upon addition of Zn^{2+} indicates zinc-ion-induced Phe-NH deprotonation. The only $C_\alpha H$ resonances to exhibit significant shifts were those of His6, His9 and Phe. Tentative assignments indicate a downfield shift of 0.1 ppm with some exchange broadening for His6 and His9 and an upfield shift of 0.14 ppm with little exchange broadening for Phe $C_\alpha H$. From a NOESY spectrum of the complex in DMSO (where the only cross peaks observed in the NH–$C_\alpha H$ region are the sequential cross peaks for the Arg2–Ile5 region of the molecule), it is noted that the chemical shifts of the NH and $C_\alpha H$ resonances of the N-terminal residues of AngI were not significantly affected by complexation (the greatest shift being 0.03 ppm for Arg2). This fact confirms that the Zn^{2+} binds to the C-terminal region (His6– Leu10) of AngI. Due to the significant resonance

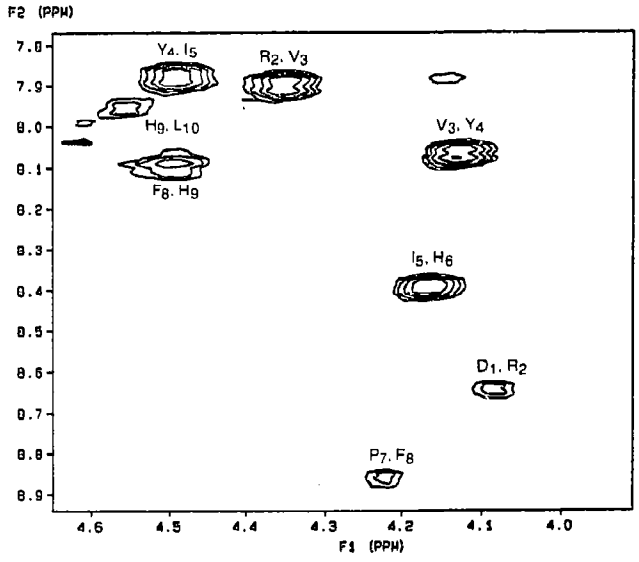

Fig. 2. Expansion of the NOESY spectrum (300 ms mixing time) of free AngI in DMSO, showing NOE connectivities between the $C_\alpha H$ protons (4.0 to 4.6 ppm) of one amino acid to the NH protons (7.8–8.9 ppm) of the next amino acid. One letter amino acid assignments $C_\alpha H_i$ to NH_{i+1} are given next to the contour peaks.

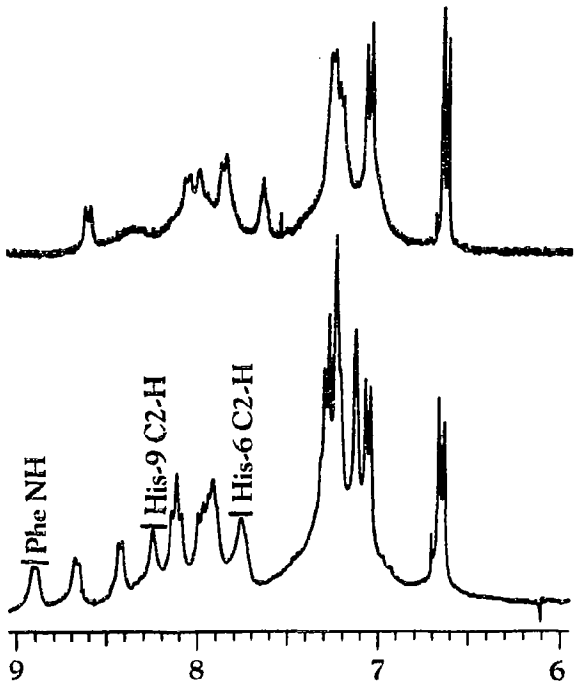

Fig. 3. ^{1}H-NMR spectrum of the NH and aromatic region of free AngI in DMSO (lower spectrum) and a 1:1 molar mixture of Zn^{2+} and AngI in DMSO (upper spectrum). Assignments of the NH resonances of free AngI are given in Fig. 2.

overlap it was not possible to unambiguously assign the His9 C2-H resonance in the spectrum of the AngI–Zn complex, however, the His6 C2-H resonance does move upfield by 0.14 ppm upon complexation, in agreement with the chemical shift movements observed in water.

4. DISCUSSION

Due to the very low solubility of the AngI–Zn complex in water above pH 7 it was not possible to determine whether Zn^{2+} could deprotonate an AngI peptide nitrogen in aqueous solution. However, clear evidence for peptide nitrogen deprotonation is observed in DMSO. This is the first example of zinc-ion induced peptide nitrogen deprotonation in a 'standard' histidyl peptide in which the His residue is not adjacent to the N-terminus (thus allowing the essential anchoring of the zinc ion by the N-terminal amine). Studies of the preferred conformation of biologically active peptides often use DMSO, rather than water, as the solvent to better approximate membrane conditions [10–12], however, the effects of DMSO on the ionization properties of the amino acids are not well known. Amides are considerably less acidic in DMSO than in water. For example, acetamide has a pK_a of 15.1 in water and 25.5 in DMSO [13], suggesting that Zn^{2+}-induced peptide nitrogen deprotonation of AngI may also occur in water, however, other factors such as the relatively poorer solvating ability of DMSO must also be considered.

It is now well established that when Zn^{2+} forms a monodentate complex with peptides it is not capable of deprotonating a peptide nitrogen in neutral or slightly basic aqueous solution. Although it cannot be unambiguously established that the zinc ion binds to both histidyl residues of AngI simultaneously to form a bidentate complex, the ^{1}H-NMR spectra of the AngI–Zn complex in water does indicate greater kinetic stability than would be expected for the zinc-ion in fast exchange between the two histidyl residues. This supports the proposition of Vallee and Auld [1], that two histidyl residues, separated by two amino acids, may anchor the zinc-ion in a bidentate complex, which is then capable of deprotonating a peptide nitrogen.

In summary, this study has shown that if Zn^{2+} can be anchored to a peptide at any position along the peptide chain by multiple coordination then peptide nitrogen deprotonation is possible. Further detailed studies of a variety of peptides are in progress.

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