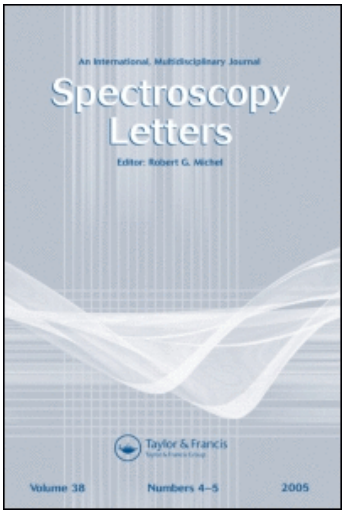

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HISTIDYL TAGS AND STRUCTURAL STABILIZATION OF LINEAR PEPTIDES

Ottavia Spiga[a]; Andrea Bernini[a]; Maria Scarselli[a]; Leonardo Giovannoni[a]; Franco Laschi[a]; Paolo Neri[a]; Luisa Bracci[a]; Luisa Lozzi[a]; Neri Niccolai[a]
[a] Centro per lo Studio Strutturale di Sistemi Biomolecolari, Dipartimento di Biologia Molecolare, Dipartimento di Chimica, Università di Siena, Siena, Italy

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HISTIDYL TAGS AND STRUCTURAL STABILIZATION OF LINEAR PEPTIDES

Ottavia Spiga, Andrea Bernini, Maria Scarselli, Leonardo Giovannoni, Franco Laschi,* Paolo Neri, Luisa Bracci, Luisa Lozzi, and Neri Niccolai

Dipartimento di Biologia Molecolare, Centro per lo Studio Strutturale di Sistemi Biomolecolari and Dipartimento di Chimica, Università di Siena, Via A. Fiorentina 1, 53100 Siena, Italy

ABSTRACT

Histidyl tags of variable length at the carboxy and amino termini of the ASYQDL sequence have been added. The hexapeptide has been chosen for its predicted low propensity to assume a preferred conformation in solution. The NMR data indicate the presence in solution of different folded conformations for two histidyl derivatives of the hexapeptide upon additions of the Ni(II) ion. Thus, presence of four or five histidines located at the extremities of an unfolded peptide seems not to be suitable for a structural ordering inducible by the metal ion to trigger specific biological functions.

Key Words: Histidyl tags; Peptides; Conformational studies

*Corresponding author.

INTRODUCTION

Histidyl tags are routinely used to purify recombinant proteins, due to the high affinity of the imidazyl ring of histidine towards the Ni(II) metal ion. The histidine avidity for this metal ion can be exploited to induce particular folding patterns to specific peptide sequences for a rationale drug design. This goal is already achieved in various ways, e.g., with cystine bridge formation of cyclic analogs or with the covalent bonding in the sequence of templates and modified amino acids[1]. The fact that linear peptides exhibit both a high capability of metal ion complexation[2] and a low folding propensity[3] suggests a possible strategy for the reversible and controllable induction of a particular structure in the presence of specific ions.

In the present report the high affinity of the histidyl residue towards the nickel(II) ion[4] has been exploited as a possible peptide folding engine.

MATERIALS AND METHODS

The sequences: H H H H A S Y Q D L H H H H and H H H H H A S Y Q D L H H H H H, henceforth called respectively lgh4 and lgh5, were synthesized with a solid phase automatic synthesizer (Syro, Multi-SynTech, Bochum, Germany) using standard reaction cycles with FMOC chemistry and DIC/HOBT activation. The FMOC group was cleaved with a 40% (v/v) piperidine solution in DMF. The peptides were cleaved from the resin and deblocked using a cleavage cocktail consisting of 88% (v/v) TFA, 6% phenol, 2% triisopropylsilane and 4% water then purified by gradient RP-HPLC on a Vydac semi-prep C18 column (1.0 × 25 cm). The mobile phase solvents were water containing 0.1% (v/v) TFA and methanol. A linear gradient from 0% to 100% of methanol in 30 min was applied. Peptide identity was confirmed by amino acid analysis using PICO TAG model 510 (Waters, Milford, MA) and by NMR spectroscopy. 2 mg of each peptide were dissolved in 0.5 ml of DMSO-d_6 and the proton chemical shifts were referred to the solvent isotopic impurity (2.5 ppm). The additions of nickel were carried out mixing a solution (1M) of $NiCl_2$ in DMSO-d_6 up to a peptide:nickel ratio of 1:5. All the ^{1}H NMR spectra were acquired at 300°K using a Bruker AMX 600 equipped with SGI workstation.

All the NMR data were processed and analyzed with SwaN-MR 3.4.8[5]. For each peptide a 1D proton NMR spectrum was recorded using a spectral width of 10 ppm and 32K data points. For the various peptides two-dimensional TOCSY experiments with a mixing time of 70 ms were

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carried out to assign the resonances within each spin systems. NOESY spectra with a mixing time of 120 ms were then acquired on the peptide sample for the sequence specific assignment and the detection of dipolar couplings. A total of 512 block were collected in t_1 with 1024 data points and 64 scans in t_2, over a spectral width of 7 KHz in both dimensions. A

Table 1. ^{1}H Chemical Shifts (ppm) of lgh4 Measured at 600 MHz in DMSO and at 300°K

Res	NH	αH	βH	Others
H1	b	4.15	3.09	εH a δH a
	(b)	(4.31)	(3.25)	(εH a δH 9.09)
H2	8.91	4.67	a	εH a δH a
	(9.11)	(4.68)	(3.09, 3.17)	(εH 7.44 δH 9.00)
H3	8.43	4.57	a	εH a δH a
	(8.86)	(4.63)	(3.05, 3.14)	(εH 7.39 δH 9.00)
H4	8.40	4.59	a	εH a δH a
	(8.53)	(4.63)	(3.06, 3.15)	(εH a δH 9.00)
A5	8.28	4.34	1.20	
	(8.31)	(4.34)	(1.28)	
S6	8.18	4.32	3.57	
	(8.16)	(4.32)	(3.55, 3.61)	
Y7	7.91	4.48	2.71, 2.95	δH 6.99 εH 6.62
	(7.93)	(4.56)	(2.74, 2.97)	(δH 6.97 εH 6.62)
Q8	8.22	4.24	1.72, 1.87	γCH 2.10, 2.10 εNH2 7.26, 6.83
	(8.16)	(4.23)	(1.75, 1.88)	(γCH 2.13, 2.13) (εNH2 7.28, 6.84)
D9	8.19	4.57	2.54, 2.72	
	(8.23)	(4.57)	(2.57, 2.75)	
L10	7.88	4.20	1.35, 1.43	γH 1.35 δCH3 0.77, 0.84
	(7.83)	(4.22)	(1.38, 1.54)	(γH 1.38 δCH3 0.76, 0.82)
H11	8.14	4.51	a	εH a δHa
	(8.18)	(4.57)	(3.05, 3.14)	(εH 7.33 δH 9.00)
H12	8.25	4.58	a	εH a δH a
	(8.37)	(4.59)	(3.01, 3.13)	(εH 7.38 δH 9.00)
H13	8.43	4.56	a	εH a δH a
	(8.57)	(4.60)	(3.06, 3.15)	(εH 7.38 δH 9.00)
H14	8.44	4.50	a	εH a δHa
	(8.52)	(4.53)	(3.03, 3.16)	(εH 7.28 δH 9.00)

Chemical shifts are given from the DMSO signal at 2.5 ppm; the resonance positions of the peptide protons in the presence of nickel are given in parenthesis. a: overlapped signal; b: unassigned signal.

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Table 2. ^{1}H Chemical Shifts (ppm) of lgh5 Measured at 600 MHz in DMSO and at 300°K

Res	NH	αH	βH	Others
H1	b	4.18	a	εH a δH a
	b	(4.26)	(a)	(εH a δH) a
H2	8.92	4.65	2.97, 3.10	εH 7.28 δH 8.97
	(9.02)	(4.66)	(3.04, 3.15)	(εH 7.40 δH 8.97)
H3	8.77	4.63	2.95, 3.09	εH 7.28 δH
	(8.82)	(4.62)	(3.03, 3.13)	(εH a δH 8.97)
H4	8.53	a	a	εH 7.29 δH a
	(8.53)	(4.63)	(3.01, 3.10)	(εH a δH 8.97)
H5	8.44	4.59	3.00, 3.09	εH a δH a
	(8.47)	(4.59)	(3.02, 3.11)	(εH a δH 8.97)
A6	8.31	4.32	1.19	
	(8.29)	(4.31)	(1.20)	
S7	8.20	4.31	3.54	
	(8.17)	(4.30)	(3.54, 3.59)	
Y8	7.95	4.45	2.68, 2.93	δH 6.99 εH 6.59
	(7.91)	(4.46)	(2.70, 2.95)	(δH 6.97 εH 6.61)
Q9	8.24	4.22	1.72, 1.85	γCH 2.09, 2.09 εNH2 7.28, 6.81
	(8.18)	(4.22)	(1.74, 1.87)	(γCH 2.12, 2.12) (εNH2 7.26, 6.83)
D10	8.25	4.54	2.52, 2.69	
	(8.22)	(4.56)	(2.56, 2.71)	
L11	7.87	4.19	1.37, 1.52	γH 1.37 δCH3 0.75, 0.80
	(7.84)	(4.19)	(1.40, 1.54)	(γH 1.40 δCH3 0.76, 0.81)
H12	8.15	4.51	2.91, 3.06	εH 7.26 δH
	(8.15)	(4.54)	(2.97, 3.11)	(εH 7.31 δH 8.95)
H13	a	a	a	εH a δH a
	(8.28)	(4.59)	(3.00, 3.12)	(εH 7.33 δH 8.97)
H14	a	a	a	εH a δH a
	(8.54)	(4.59)	(3.01, 3.10)	(εH a δH 8.97)
H15	a	a	a	εH a δH a
	(8.58)	(4.59)	(3.03, 3.11)	(εH a δH 8.97)
H16	8.47	4.51	2.96, 3.10	εH 7.29 δH a
	(8.52)	(4.50)	(3.01, 3.14)	(εH 7.31 δH 9.8.97)

See comments of Table 1.

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90-degree shifted sinebell function was applied using 1024 points in the t_2 dimension. In the t_1 dimension the same function was imposed on 512 points with a shift of 90 degrees. Zero filling was applied before the 2D Fourier transform to end up with a final matrix size of 2048×2048 real points. Interproton distance constraints for structural calculation were derived from the NOESY experiments. For the temperature dependence study of chemical shifts, COSY magnitude spectra were recorded. Distance restraints were defined from NOE intensities using the intra-residue H_β-H_β dipolar coupling of the aspartyl residue as a calibration interproton distance.

A total of 100 structures for each peptide was generated using the DYANA 1.5 distance geometry protocol[6] and the experimental constraints, followed by 10,000 cycles of energy minimization. The conformational contribution of the metal ion was incorporated in the DYANA calculation by modifying the software library with the insertion of a histidyl residue where the Nε is coordinated with a Ni(II) at an internuclear distance of 2Å[2–4]. For both peptides, a converged group of 30 refined structures was identified, on the base of their lowest NOE violations. MOLMOL 2.5.1 was used as graphic program[7].

RESULTS AND DISCUSSION

The lggh4 and lggh5 sequences were submitted to two remote servers[8,9], to check for possible intrinsic folding properties of the peptides. This feature, indeed, could interfere with the efforts of obtaining folded conformations only due to the addition of histidyl residues at the N and C termini. In both cases the obtained Chou-Fasman and GOR IV predictions indicated very low probabilities for helices, strands and turns, suggesting an extensive conformational equilibrium in solution for the two peptides.

Then, a structural study of lggh4 and lggh5 was performed with NMR techniques in DMSO, in the presence and in the absence of the Ni(II) ion.

Table 3. The Temperature Coefficients of NH Protons of the Central Fragment of lgh4 and lgh5 (Values Are Given in ppb)

	A	S	Y	Q	D	L
lgh4	4.7	5.7	2.3	5.7	5.5	4.8
lgh5	3.5	4.5	1.3	4.2	4.2	3.2

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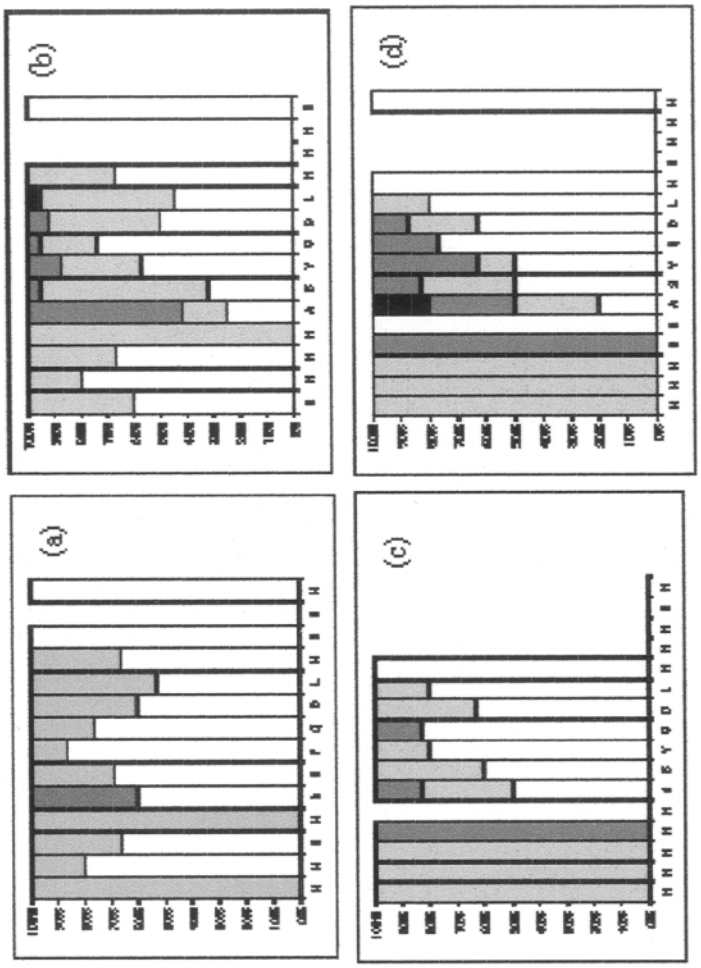

Figure 1. The observed Overhauser effects measured for lgh4 and lgh5: long-, medium-, short-range and intra-residue NOEs vs. sequence position measured for the two ASYQDL analogs. Short, medium, and long range NOE's refer respectively to inter-residue distances of 1, < 5, and $= 5$. (a) and (b) show the NOE obtained respectively for lgh4 residues in the presence and in the absence of Ni(II). (c) and (d) show the same kind of data obtained for lgh5.

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The organic solvent was preferred to water as it ensured suitable peptide solubility.

As a first remark, the possibility of obtaining NMR spectra of the five peptides in the presence of the metal ion totally depended on the pH of the water solution before the peptide lyophilization. In facts, a memory of the protonation, which was reached in the aqueous medium, is maintained in the organic solvent. From peptides lyophilized from neutral or basic solutions no proton resonance can be observed, due to the formation of paramagnetic complexes with a stable octahedral coordination of the Ni(II) ion[10]. Thus, a pH equal to 5.5 is the highest reachable one, before the NMR signals disappearance. In these experimental conditions, where the presence of the metal ion determines only small non-selective broadening of the proton signals, the conformational changes, due to the formation of diamagnetic metal complexes, are investigated.

In Tables 1 and 2, the proton chemical shifts of the two peptides, in the absence and in the presence of the metal ion, are reported. Scalar coupling constants could not be measured due to the broadening induced by the presence of Ni(II).

A conformational equilibrium for lgh4 and lgh5 seems to be suggested by the small chemical shift variations induced by the presence of the metal ion. The similar temperature coefficients of amide proton chemical shifts measured for the two peptides, also do not indicate the finite H-bonding pattern, which should be expected for stable folded structures. As reported in Table 3, indeed, only for the tyrosyl amide protons some solvent shielding could be invoked.

Proton NOESY spectra were recorded for the two analogs at Ni(II)/peptide concentration ratios of 0, 1, 2 and 5. Since the structurally relevant medium- and long-range Overhauser effects, shown in Fig. 1, exhibit a linear dependence with the metal concentration, all the discussion of the NOEs refers to the data measured at the highest [Ni^{+2}]. It should be noted that this feature implies a rather weak Ni(II) complexation by both peptides in the selected experimental conditions. However, the fact that many medium-range and several long-range NOEs only in the presence of the metal ion can be measured, is diagnostic of some induced peptide folding.

The NOE data were used as constraints for the molecular dynamic calculations. The results can be summarized in the following way: i) extensive conformational equilibrium may be suggested for the two peptides in the absence of the metal ion, in agreement with the results of the predictive methods, ii) the presence in solution of Ni(II) reduces significantly the conformational space available for lgh4 and lgh5, but a poor convergence towards a few predominant structures of is obtained. This finding is most likely due to the abundance of potential metal ligands in both peptides, i.e. 8

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or 10 histidyl residues, which allows many alternative ways of metal complexation.

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