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Coordination Ability of the Thyrotropin Releasing Factor. II. Nickel(II) Complexes with TRF

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The interactions of Ni(II) ions with Pyr-His-Pro-NH_2(TRF) and Pyr-His dipeptide analogue have been studied with use of absorption and CD spectroscopy as well as the ^1H NMR spectra. The formation of two planar complexes characterized by different CD spectra has been found in basic solutions. In both planar species the coordination sites are three nitrogen donors i.e. *N3 of imidazole, N^- of the peptide linkage between Pyr and His residues and N^- of Pyr residue. The difference in CD spectra of two planar complexes has been explained in terms of chelate ring conformation change due to the deprotonation of N1 imidazole nitrogen. The N1 deprotonation is promoted by the Ni(II) coordination to N3 nitrogen of imidazole ring.*

Introduction

As a part of our studies on metal ion interactions with naturally occurring small peptides [1–5] we have begun the investigation of the chelating ability of the thyrotropin releasing factor (TRF, L-pyroglutamyl-L-histidyl-L-prolinamide) and its analogue (Pyr-His, L-pyroglutamyl-L-histidine). The data obtained for the Cu(II)TRF system [6] have shown that only two amino acid residues, L-pyroglutamyl (Pyr) and L-histidyl (His), are able to interact with cupric ions through their nitrogen donors. No major mutual interaction between the metal ion and L-prolinamide (Pro-NH_2) has been found.

This paper reports an extension to the Ni(II) ion of these studies on the chelating ability of TRF.

Experimental

The TRF and Pyr-His peptides were synthesized as described earlier [7]. $Ni(ClO_4)_2 \cdot 6H_2O$ was used as obtained from Fluka AG Buchs SG. (Switzerland). Solutions with a molar ratio of Ni(II): peptide of 1:5 were used for the CD and absorption spectra studies

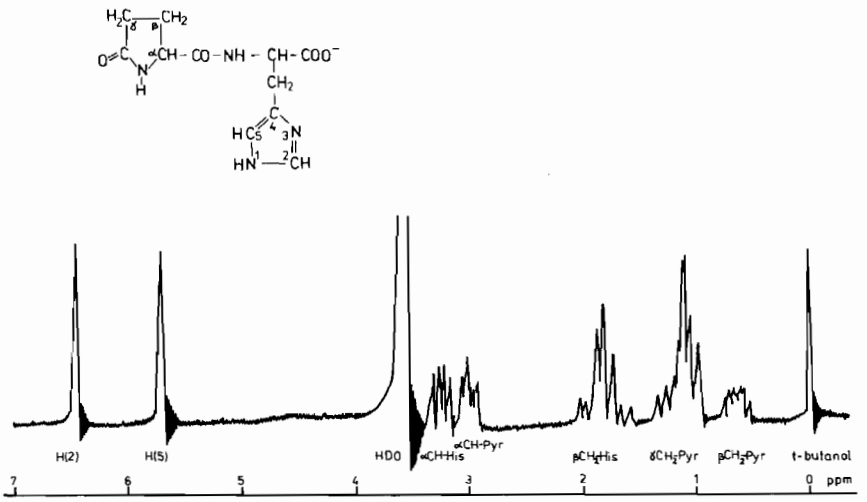

Fig. 1. ^1H NMR spectrum of Pyr-His peptide at pH = 11.53.

TABLE. I. The ^{1}H NMR Data for TRF and Pyr-His Metal-free Peptides. Chemical Shifts are given in ppm relative to t-butanol (0 ppm).

Peptide	pH	Chemical Shifts of Imidazole Protons	
		H(2)	H(5)
TRF	3.87	7.40	6.14
	11.07	6.45	5.79
Pyr-His	3.52	7.40	6.06
	6.52	7.26	6.00
	11.53	6.47	5.71

Peptide	pH	Chemical Shifts of $\alpha CH\beta CH_2$ His Protons			Coupling Constants in Hz			Rotamer Populations		
		ν_A	ν_B	ν_C	$\lvert J_{AB}\rvert$	J_{AC}	J_{BC}	p_1	p_2	p_3
TRF	0.5–4.0	1.93	2.02	3.81	16.0	8.8	5.2	0.63 (0.56)	0.29 (0.24)	0.08* (0.20)**
	>6.0	1.87	1.95	3.74	16.0	8.0	7.5	0.50 (0.49)	0.56 (0.45)	0*** (0.06)
Pyr-His	3.52	1.88	2.07	3.36	15.6	9.3	4.6	0.69 (0.61)	0.24 (0.19)	0.07 (0.20)
	6.52	1.85	2.03	3.30	15.6	9.7	4.6	0.74 (0.65)	0.26 (0.19)	0.00 (0.16)
	11.53	1.72	1.89	3.24	15.6	9.8	4.5	0.75 (0.66)	0.24 (0.17)	0.01 (0.17)

*Feeney approximation [12], **Pachler approximation [13], ***For this pH the Feeney approximation gives $p_3 < 0$, as $p_1 + p_2 > 1$.

with metal ion concentrations of 0.01 *M*. Solutions of molar ratio 1:2 ($[Ni^{+2}] = 0.05$ *M*) were prepared for ^{1}H NMR studies. Excess of the peptide was always necessary to prevent metal ion hydrolysis.

The magnetic moment in solution was established by the Evans method [8]. The absorption spectra were measured on a Beckman UV 5240 spectrophotometer. CD spectra were recorded on an automatic recording spectropolarimeter JASCO-J-20. All CD spectra are expressed in terms of $\Delta\epsilon(\epsilon_l - \epsilon_r)$. The ^{1}H NMR spectra were carried out on a Jeol JNM-PS-100 spectrometer at 25 ± 1 °C with tert-butanol as an internal standard. Chemical shifts can be converted to TMS scale by adding 1.12 ppm. The histidine residue $\alpha CH\beta CH_2$ proton spectra were analyzed as ABC spectra on a JEC-6 computer.

Results and Discussion

^{1}H NMR Characterization of Metal-free Ligands

^{1}H NMR spectra of metal-free TRF have been reported previoulsy [9, 10]. Primary analysis of the spectrum of the histidine $\alpha CH\beta CH_2$ protons has been used to evaluate the rotational isomer conformation

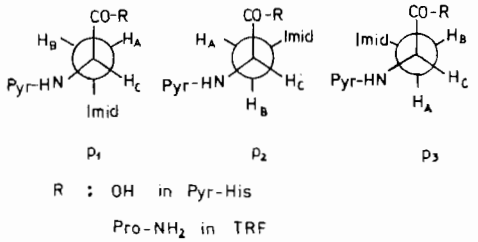

Fig. 2. The rotamer notation for histidine residue in TRF and Pyr-His peptides.

of this residue. We have done a detailed analysis of the ABC histidine spectra at several pH values for TRF as well as for the Pyr-His analogue (Fig. 1) and the data are given in Table I.

The conformation of the histidine side chain is largely independent of pH for Pyr-His and, if the notation is assumed as in Fig. 2, in the most stable rotamer the imidazole ring is in a *trans* position to the carboxyl group (isomer 1). The minor pH dependence of the histidine residue rotational isomerism was also found in the Gly-His dipeptide [11], although in the latter case population of isomer 1 is smaller by 0.2 than that in Pyr-His. The increase of rotamer

TABLE II. Absorption Spectra and Magnetic Moment Values for Ni(II)TRF and Ni(II)Pyr-His 1:5 Molar Ratio Solutions.

pH	λ, nm	ϵ, M^{-1} cm^{-1}	μ, BM
Ni(II)TRF			
7.53	1100	2.4	3.06
	709sh		
	645	4.2	
	383	11.2	
8.35	1040	6.0	3.00
	700sh	~9.0	
	635	11.6	
	425sh	~27.0	
	380sh	~35.0	
8.80	1020	10.0	2.67
	620sh	~26.0	
	420sh	~65.0	
9.00	1030	3.0	
	610	8.9	
	440	53.5	
	300	2000.0	
9.51	1020	1.2	1.50
	600sh		
	446	77.0	
	303	2200.0	
10.80	458	105.4	0.30
11.20	458	93.0	0.00
	303	2600.0	
12.00	460	89.1	0.00
	303	2500.0	
Ni(II)Pyr-His			
9.53	440sh		
10.65	458	85.5	
11.40	457	84.0	
12.00	460	88.5	

1 population in Pyr-His solutions derives mainly from the destabilization of the gauche (3) rotamer in which the vicinity of the carboxyl group and the possible close contact with the bulky Pyr residue is extremely unfavorable.

Distribution of rotamers of the histidine residue in solutions containing TRF at pH values up to about 4 is close to that of Pyr-His (Table I). Increasing the pH to above 6 (N3 deprotonation), however, causes a quite distinct increase in the population of rotamer 2 at the cost of rotamers 1 and 3 (Table I). This suggests some interactions between imidazole and the C-terminal groups of the TRF molecule, which would stabilize rotamer 2, *e.g.* hydrogen bond formation between the N3 nitrogen and amide NH_2 group, or between the N1 nitrogen and the CO His group. The presented data, however, do not preclude some intermolecular interactions which could control the side chain conformation discussed above.

Absorption and CD Spectra of the Ni(II): TRF System

Absorption spectra as well as the magnetic moment values of solutions containing Ni(II) TRF are given in Table II.

At pH about 7 the Ni(II) ion forms only octahedral species. At the pH range 8–10 there is an equilibrium between octahedral and square planar species. The characteristic d–d transition band for a planar complex [20] appears at 440 nm and the magnetic moment decreases considerably. The solutions at pH values above 11 contain only the square planar complex with the d–d transition shifted to lower energy, *i.e.* to 460 nm, compared to the former square planar complex value of 440 nm. The difference in the d–d transition energy between two square planar species of 1000 cm^{-1} may suggest some changes in the metal ion environment, as the pH increases from 9 to 11.

TABLE III. Circular Dichroism of Ni(II) Complexes with TRF and Pyr-His.

Ni(II)TRF							
pH = 9.00–9.85[a]		pH = 10.00		pH = 10.78		pH = 10.40–12.00	
λ, nm	Δε	λ, nm	Δε	λ, nm	Δε	λ, nm	Δε
		542	+0.06	529	+0.20	520sh	+
494	–0.32	495	–0.05				
426	+1.10	428	+0.74	431	+1.40	461	+1.49
				298	+0.18	307	+0.18
		271	–0.24	272	–0.23	272	–0.88
248	+1.92*	248	+2.23*	247	+2.06*	248	+2.80*
	(+0.38)**		(+0.44)**		(+0.42)**		(+0.57)**
231	–0.24**	228	–0.45**	224	–0.82**	221	–1.00**
209	+1.51**	206	+1.06**	205	+1.06**	205	+1.32**

(continued overleaf)

TABLE III. *(Continued)*

Ni(II) Pyr-His							
pH = 9.34		pH = 10.00		pH = 10.99		pH = 12.00	
λ, nm	Δε	λ, nm	Δε	λ, nm	Δε	λ, nm	Δε
		541	+0.05			517sh	+
496	−0.07	495	−0.05				
428	+0.35	426	+0.43	465[b]	+0.77	474	+1.66
280sh	+0.05					309	+0.09
				276	−0.08	274	−0.18
244	+1.04*	247	+1.72*	248	+3.81*	249	+3.68*
	(+0.21)**		(+0.34)**		(+0.75)**		(+0.73)**
208	+2.85**	208	+2.18**	208	+2.27**	206	+2.36**

*Calculated for the metal concentration (as all d–d and CT peaks). **Calculated for the peptide concentration. [a]A similar CD has already been observed for pH ~ 7.50, but with very low Δε values. [b]Very broad.

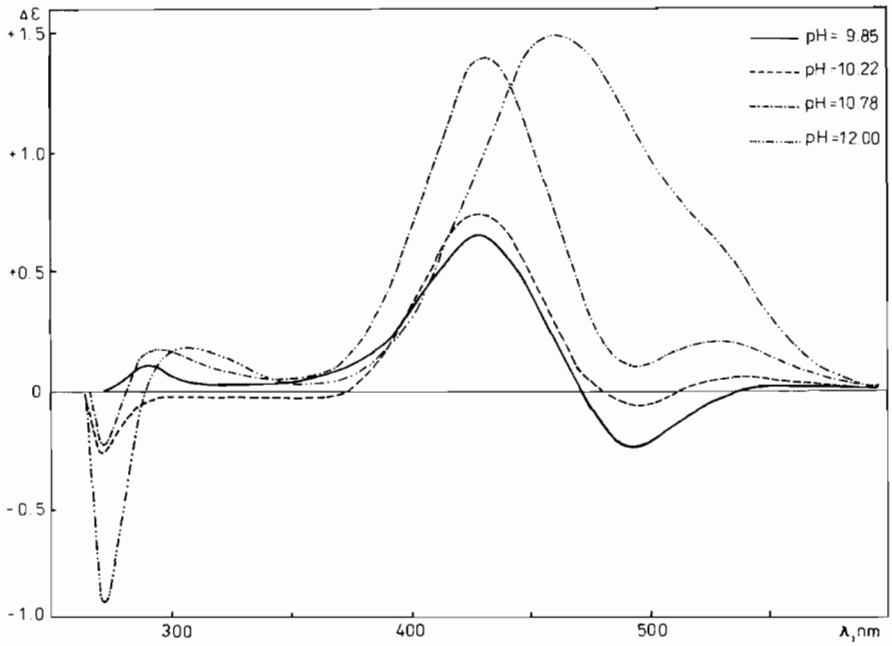

Fig. 3. The CD spectra for Ni(II)TRF 1:5 molar ratio solutions at different pH.

The CD spectra (Table III, Fig. 3) show quite univocally that indeed two distinct square planar complexes are formed. At pH below 10 one observes two CD extrema: the positive at 426 nm and the negative at 494 nm. They may be assigned to $A_{1g} \rightarrow E_g(E)$ and $A_{1g} \rightarrow A_{2g}(A)$ d–d transitions. A similar pattern of the CD peaks was observed for planar Ni(II) complexes with tripeptides containing a bulky amino acid residue on N-terminal [14].

In the pH range 10 to 11 the CD peaks at 494 nm and 426 nm disappear slowly, while new bands are created (Fig. 3). They remain as the pH increases above 11. The latter set of the CD peaks is quite different from that for the planar complex formed at pH below 10. Two positive bands at 461 nm and 520 nm (shoulder) due to the A and E d–d transitions are observed. There are also two other CD extrema in the charge transfer region at 272 and 307 nm (Table III). Both these bands derive most probably from the imidazole nitrogen–Ni(II) CT transitions.

A shift of the two 'd–d' CD peaks towards a lower energy for the planar complex formed at high pH (pH > 10), in comparison to the planar complex created at pH below 10, as well as the change in the sign of the Cotton effect of the A transition suggest a variation in the conformation of the chelate ring. It

TABLE IV. The Upfield Chemical Shifts (in ppm) of ^1H NMR Resonances of Pyr-His Dipeptide Protons upon the Ni-(II) Ion Coordination, at pH = 11.5.

H(2)Imid	0.58
H(5)Imid	0.23
αCH His	0.46
βCH_2 His	0.16
αCH Pyr	0.55
βCH_2 Pyr	0.32
γCH_2 Pyr	0.18

could derive from possible deprotonation of the pyrrole nitrogen (N1) [15–17, 19], which is promoted by the metal ion coordinated to N3 nitrogen of the imidazole ring.

In the CD spectra of both planar complexes one observes also a positive band at 248 nm due to the coordination of the nickel ion to a deprotonated peptide linkage nitrogen [18].

The absorption and CD spectra of the solutions containing the Ni(II) Pyr-His system are exactly the same as for the Ni(II) TRF solutions, as far as the d–d and CT transitions are concerned (Table III). Thus the Pro-NH_2 residue is not involved in any major interaction with the Ni(II) ions, as was also the case in the system containing Cu(II) ions [6].

^1H NMR Studies of Ni(II) Peptide Complexes

More precise data on the coordination sites of Ni(II) ions in the planar diamagnetic complexes may be obtained from proton NMR spectra. The planar complex formed at pH < 10 exists in equilibrium with the paramagnetic octahedral species and a high resolution spectrum could not be obtained. At pH > 11 only diamagnetic species exist in solution and ^1H NMR spectra are more informative. All mutliplets in the spectrum of the Ni(II) complex studied were broadened, but the chemical shift analysis is rather easy.

As the spectrum of metal-free TRF is very complicated, it is more convenient to examine the Ni(II)-Pyr-His system. Table IV presents the upfield chemical shifts of proton resonances of Pyr-His due to metal ion coordination to the dipeptide molecule. The large shifts of αCH Pyr, αCH His and H(2,5) of the imidazole ring upon complexation to Ni(II) strongly suggests coordination of the metal ion to three nitrogen atoms, as shwon in Fig. 4. The resonances of H(2) and H(5) protons of imidazole in the Ni(II) complex at pH > 11 are strongly upfield shifted by 0.58 and 0.23 ppm compared to the metal-free dipeptide with N3 deprotonated or by 1.53 and 0.60 ppm compared to metal-free ligand with N3 protonated. These large upfield shifts may provide quite reasonable proof for deprotonation of the N1 nitrogen atom in the sqare planar complex (Fig. 4),

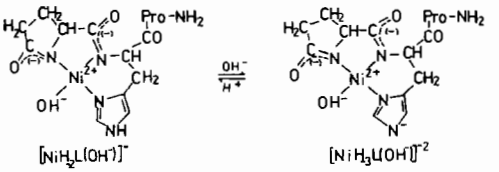

Fig. 4. Proposed coordination in planar Ni(II) complexes with TRF.

because coordination of Ni(II) [23] or Pd(II) [11] to the imidazole of histidine causes a much smaller chemical shifts variation.

Thus the ^1H NMR studies fully support the conclusions derived from the CD and absorption spectra.

Conclusions

In both systems studied – Ni(II)TRF and Ni(II)-Pyr-His – at least two square planar complexes are formed with the metal coordination sites at N3 imidazole, N^- of the peptide linkage and the deprotonated nitrogen of Pyr. The difference between these complexes is the pyrrole type nitrogen (N1) of imidazole, which may be protonated (pH < 10) or deprotonated (pH > 10). The drastic variations in CD spectra in the d–d transition region due to deprotonation of the N1 imidazole, result most probably from a change in the chelate ring conformation. The N1 deprotonation of the imidazole ring changes its aromatic character and the coordinated N3 site alters its π acceptor character. It may change the Ni(II)–N3 bond length slightly. This bond length is very sensitive to the geometry of metal imidazole interaction [21, 22].

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