s-Cis and s-Trans Isomerism of the His-Pro Peptide Bond in Angiotensin and Thyroliberin Analogues†

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ABSTRACT: The dipeptide His-Pro isomerizes from all-s-trans to partly s-cis when titrated in D_2O from acidic to neutral pD as observed by ^{13}C and ^1H nuclear magnetic resonance of the proline side chain. This isomerization is reported by the His C-2 and C-4 protons and carbons which show distinct, well-resolved resonances for each isomer. The influence of the His-Pro peptide bond rotational state on the histidine protons far removed from the bond has not been previously observed in model compounds or peptides. The peptides thyroliberin (TRH), [3-MeHis^2]-TRH, and [3-MeHis^6]-, [Sar^1,Ala^8]-, and N^α-acetylangiotensin II were found to similarly isomerize from all-s-trans to partly s-cis as reported by their His C-2 and C-4 proton resonances. The His C-2 and C-4 protons in the peptides [1,3-diMeHis^2]-TRH and [1-MeHis^6]-, and [homoHis^6]-angiotensin do not report this isomerization. Angiotensin II has previously been found to exhibit the same isomerization. The reporting of the s-trans to s-cis isomerization by the His C-2 proton appears to be correlated with the known potencies of the five angiotensin peptides in rat uterine strips and of the three TRH peptides by radioimmunoassay of released thyrotropin.

The solution conformation of angiotensin II (Asp^1-Arg^2-Val^3-Tyr^4-Ile^5-His^6-Pro^7-Phe^8) has been intensively studied by proton and ^{13}C NMR[1] (Weinkam & Jorgensen, 1971; Printz et al., 1972; Marshall et al., 1973; Deslauriers et al., 1975; Bleich et al., 1973, 1978; Galardy et al., 1976). These and numerous other studies by sedimentation, optical rotatory dispersion, circular dichroism, infrared, thin film dialysis, hydrogen exchange (see review by Marshall et al., 1973), and chemical modification (Juliano et al., 1974) have not led to an accepted solution conformation (or class of conformations) for angiotensin II, although a pH-dependent conformational change has long been recognized (Franze de Fernandez et al., 1968; Ferriera et al., 1969; Craig et al., 1964; Glickson et al., 1973, 1974; Marshall et al., 1973; Vine et al., 1973). Glickson et al. (1973) and Vine et al. (1973) found pKs of 6.6 and 6.1, respectively, for the conformational change in H_2O. In addition, the mechanism of interaction of angiotensin with its biologically significant binding sites or receptors is not known. Although many primary structural (sequence) requirements for activity have been established (Khosla et al., 1974), few secondary (backbone) or side-chain structural requirements for activity have been determined.

Angiotensin analogues with restricted backbone conformational freedom have been prepared and tested for potency (Marshall et al., 1972; Pena et al., 1974; Turk et al., 1976) and have suggested the tentative assignment of backbone torsional angles ϕ and ψ (describing rotation about the N–C$^\alpha$ and C$^\alpha$–C bonds, respectively) at position 4. In the absence of a method for the direct measurement of the receptor bound conformation, such conformation–activity studies may allow conclusions about the mechanism of interaction of angiotensin with its receptor. We have approached the problem of establishing conformation–activity relationships in angiotensin II by focusing on the torsional angle ω describing rotation about the C–N peptide bond of His-Pro.

Proton and ^{13}C NMR studies have shown that the His-Pro peptide bond in [Asn^1,Val^5]-, [Asp^1,Ile^5]-, and [Phe^4,Tyr^8]-angiotensin II isomerizes from all-s-trans (ω = 180°) to partly s-cis (ω = 0°) in D_2O upon titration from low to neutral pD. The pK of this isomerization is about 6.5 in D_2O and the histidine C-2 and C-4 protons report the isomerization (Bleich et al., 1978; Galardy et al., 1976). Therefore, the change in the conformation of angiotensin around neutral pH (Vine et al., 1973; Glickson et al., 1973; Marshall et al., 1974) involves, at least in part, all-s-trans to partly s-cis isomerization of proline.

We show here that the His C-2 protons in the angiotensin peptides [3-MeHis^6]-, [Sar^1,Ala^8]-, and N^α-acetylangiotensin II report a similar isomerization of the His^6-Pro^7 peptide bond, while the His C-2 protons in the peptides [1-MeHis^6]-, [Asp^1,Ala^7]-, and [homoHis^6]-angiotensin do not. In TRH (<Glu^1-His^2-Pro^3-NH$_2$) and [3-MeHis^2]-TRH, the His-Pro peptide bond also shows a pH-dependent isomerization which is reported by the His C-2 and C-4 protons, while the His imidazole protons of [1,3-diMeHis^2]-TRH do not. In both native TRH and the dipeptide His-Pro, the reporting of the isomerization by the His-C-2 proton has been confirmed by ^1H and ^{13}C NMR of the proline side chain.

Materials and Methods

The following peptides were generously provided by other investigators: [Asp^1,Ile^5,Ala^7]-angiotensin II, J. M. Stewart, University of Colorado Medical School; [Asp^1,Ile^5,3-MeHis^6]-angiotensin, G. R. Marshall, Washington University Medical School; [Asp^1,Ile^5,1-MeHis^6]-angiotensin, J. E. Rivier, Salk Institute. [Sar^1,Ile^5,Ala^8]-angiotensin was a gift of Eaton Laboratories, Norwich Pharmaceuticals. [3-MeHis^2]- and

[1] Abbreviations used: the designations s-cis and s-trans proposed by Mulliken (1942) for single bond rotations are used for the isomerization about the His-Pro peptide bond (Detar & Luthra, 1977). Other abbreviations are c, cis; t, trans; ppm, parts per million; R_f, relative migration; DSS, sodium 2,2-dimethyl-2-silapentane-5-sulfonate; Me_4Si, tetramethylsilane; NMR, nuclear magnetic resonance; TRH, thyroliberin. The drawing shows trans and cis His-Pro and the numbering system employed for the imidazole ring.

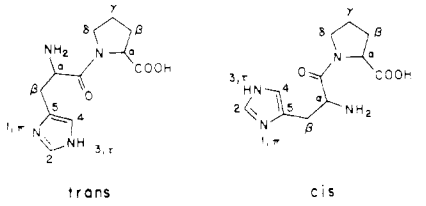

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Table I: pD Dependence of the Proton Chemical Shifts (ppm) of His-Pro at 18 °C

		αCH		βCH$_2$		C-2			C-4	
	pD	trans	cis	trans	cis	trans	cis	% cis	trans	cis
His	1.9	4.70		3.45		8.75		0	7.53	
	4.4	4.70	4.20	3.45		8.66	8.56	11	7.47	7.37
	5.4	4.65	4.14	3.40	3.24	8.56	8.18	22	7.43	7.21
	6.4	4.54	4.075	3.35	3.15	8.29	7.89	35	7.31	7.08
	7.4	4.42	4.02	3.24	3.10	8.04	7.80	50	7.20	7.05
	7.9	4.33	3.94	3.18	3.05	7.93	7.77	47	7.15	7.01
	8.4	4.23	3.85	3.14	2.99	7.86	7.75	44	7.10	6.92

		αCH		βCH$_2$		γCH$_2$		δCH$_2$	
	pD	trans	cis	trans	cis	trans	cis	trans	cis
Pro	1.9	4.52		2.36		2.04		3.76	
	4.4	4.41	3.99	2.35	2.16	2.00	2.00	3.75	3.58-3.33
	5.4	4.40	3.85	2.36	2.00	2.00	1.80	3.75	3.65
	6.4	4.36	3.52-3.33	2.33	1.99	1.99	1.78	3.75	3.59-3.33
	7.4	4.35	3.40	2.30	1.99	1.99	1.77	3.75	3.59
	7.9	4.21	3.40	2.29	1.97	1.97	1.75	3.73	3.408
	8.4	4.34	3.40	2.30	2.00	2.00	1.78	3.75	3.54

[1,3-diMeHis^2]-TRH were provided by Dr. E. L. Woroch and Dr. A. O. Geiszler, Abbott Laboratories, North Chicago, IL. His-Pro from Chemical Dynamics Inc. and TRH from Sigma Chemical Co. were found to be homogeneous by thin-layer chromatography and paper electrophoresis. All peptides were judged to be at least 90% pure from their NMR spectra.

N^{α}-Acetyl-[Asn^1,Val^5]-angiotensin II was prepared as follows. To 20.4 mg of [Asn^1,Val^5]-angiotensin II dissolved in 400 μL of pyridine–water (1:1, v/v) cooled to 0 °C was added 20 μL of acetic anhydride. The mixture was allowed to stand overnight at 4 °C after which time additional 20-μL aliquots of acetic anhydride were added until the acetylation was 100% complete as judged by paper electrophoresis at pH 2.0. The product was homogeneous by paper electrophoresis at pH 2.0, R_f 0.45 (R_f histidine 1.0, R_f angiotensin 0.70), ninhydrin negative, Pauly reagent positive, ϵ_{273} 1450, 0.1 N HCl ($\epsilon_{274.5}$(Ac-Tyr-OMe) 1420 in water; Fasman, 1976).

The ^{1}H NMR spectra were recorded by pulsed Fourier transform on the Bruker HX-270 spectrometer of the Southern New England high-field NMR facility (Yale University) at 18 °C. Sixty to 400 scans were required for each spectrum at peptide concentrations of 20-2 mM. The natural abundance ^{13}C NMR spectra of the His-Pro dipeptide were recorded on the same instrument. The ^{13}C spectra of TRH were recorded on the JEOL PFT-100 spectrometer of the New England Area Research Facility at the University of Connecticut Health Center. The rate of isomerization from all-trans to partly cis in His-Pro was determined at 37 °C by adjusting the pD of a 135 mM pD 1.1 solution (all-trans) to pD 6.9 at 20 °C and then recording the appearance of the cis C-2 and C-4 resonances and the loss of the trans resonances as a function of time at 37 °C in a Varian T-60 spectrometer. A graph of the logarithm of the percentage of trans isomer remaining as a function of the time was linear at early time points and the slope of this linear region was used to calculate $k_{t\rightarrow c}$, the rate constant for the trans→cis isomerization, and $\tau_t = (k_{t\rightarrow c})^{-1}$, the lifetime of the trans rotamer.

Proton chemical shifts are ppm downfield from an internal reference of DSS. All ^{13}C chemical shifts are ppm downfield from an external reference of Me_4Si. pDs were adjusted approximately with pH paper and then measured with a pH meter using the relation pD = pH reading + 0.4 (Glasoe & Long, 1960).

The assignments for the s-cis and s-trans isomers of N^{α}-acetyl-[Asn^1,Val^5]-, [Sar^1,Ala^8]-, and [3-MeHis^6]-angiotensin II and [3-MeHis^2]-TRH are based on the His C-2 proton (and the His C-4 proton when it was resolved from the Phe and Tyr aromatic protons). The percentage of s-cis isomer present at each pD was calculated from either the integrals or the peak heights of the cis and trans resonances of either the His C-2 or C-4 proton. The pKs of the isomerizations were estimated from a graph of the percentage of cis isomer as a function of pD. The assignment of the s-cis and s-trans His C-2 and C-4 protons is based on our previous studies (Galardy et al., 1976; Bleich et al., 1978) and on the ^{1}H and ^{13}C NMR spectra of His-Pro and TRH. The assignment of s-cis and s-trans isomers in His-Pro and TRH was based on ^{1}H and ^{13}C NMR of the proline side chain.

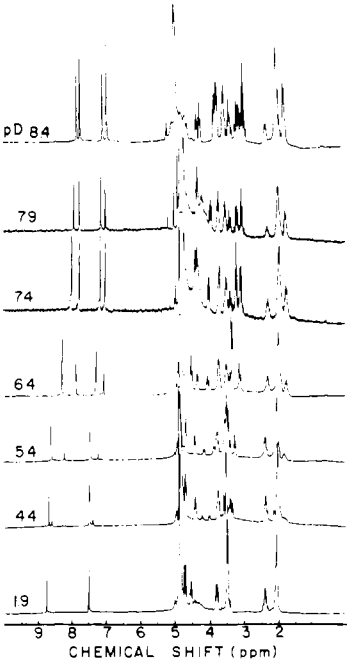

FIGURE 1: The pD dependence of the proton spectrum of His-Pro at 18 °C.

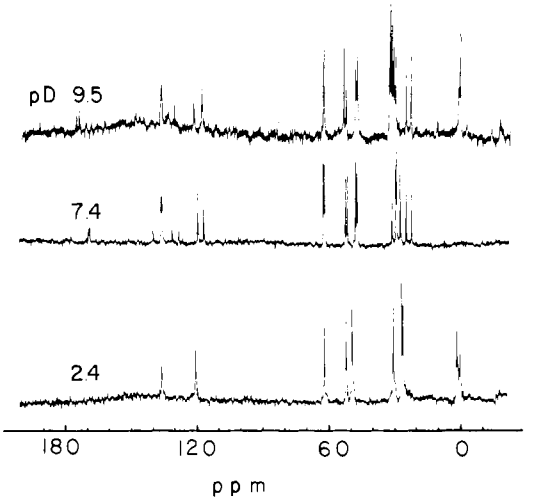

FIGURE 2: The pD dependence of the ^{13}C spectrum of His-Pro at 25 °C.

Results

Figure 1 shows the pD dependence of the proton NMR spectrum of His-Pro. Table I gives the proton chemical shifts for His-Pro in D_2O as a function of pD. The s-trans and s-cis isomers are both present at and above pD 4.4 and are reported

Table II: pD Dependence of the ^{13}C Chemical Shifts (ppm) of His-Pro at 25 °C[a]

		αCH		βCH$_2$		C-2		C-4		C-5	
	pD	trans	cis	trans	cis	trans	cis	trans	cis	trans	cis
His	2.4	51.96		26.05		136.05		120.50	*b*	*b*	
	7.4	51.50, 52.22		27.59, 29.04		136.50, 136.90		117.30, 120.15		128.6, 131.3	
	9.5	53.10, 51.98		29.38, 30.40		136.00, 136.60		117.76, 120.00		130.36, 132.90	

		αCH		βCH$_2$		γCH$_2$		δCH$_2$	
	pD	trans	cis	trans	cis	trans	cis	trans	cis
Pro	2.4	62.01		29.02		25.20		48.70	
	7.4	62.37, 62.82		29.60	31.27	24.71	22.42	47.13, 47.71	
	9.5	62.00	62.00	31.25, 33.03		24.63	22.42	46.97, 47.63	

[a] Pairs of chemical shifts separated by a comma indicate that trans and cis resonances are not unambiguously assignable. [b] The C-5 resonance is either not present or overlaps with either the C-2 or C-4 resonance.

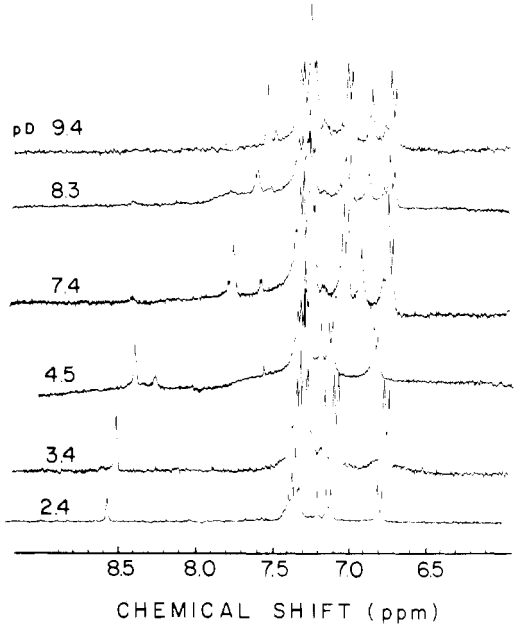

FIGURE 3: The pD dependence of the aromatic region of the proton spectrum of [3-MeHis^6]-angiotensin at 18 °C. (The pD 4.5 spectrum was recorded at a different sweep width (1000 Hz) than the other spectra (900 Hz).) The correct chemical shifts of the C-2 and C-4 protons at pD 4.5 are given in Table III.

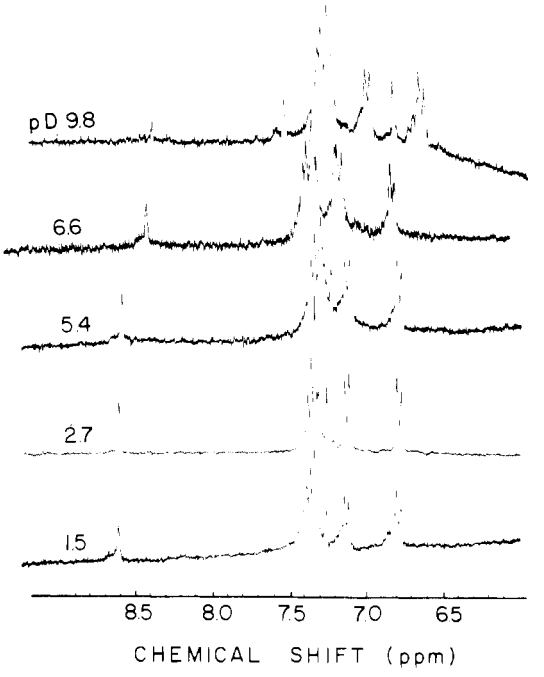

FIGURE 4: The pD dependence of the aromatic region of the proton spectrum of [1-MeHis^6]-angiotensin at 18 °C. The resonance at 8.40 ppm at pD 9.8 is an artifact not associated with the peptide.

by both the His C-2 and C-4 protons as well as the protons of proline. The pK of the isomerization in D_2O is 5.6 ± 0.5 pD units. Figure 2 shows the pD dependence of the ^{13}C NMR spectrum of His-Pro. Table II gives the ^{13}C chemical shifts of His-Pro as a function of pD. The isomerization of the His-Pro peptide bond from all-s-trans to partly s-cis is unambiguously reported by the cis C-γ carbon resonance of proline at 22.42 ppm. Since the proton NMR spectra of even the simplest proline derivatives are ambiguous, ^{13}C NMR spectra must be used to assign the s-cis and s-trans isomers of proline (Thomas & Williams, 1972). The percentage of the cis isomer measured by the proline C-γ carbon is 50% at pD 7.4.

Figures 3 and 4 show, respectively, the pD dependence of the aromatic region of the ^1H NMR spectra of [3-MeHis^6]- and [1-MeHis^6]-angiotensin II. Table III gives the proton chemical shifts and the fractions of cis resonances of the His C-2 and C-4 protons for [3-MeHis^6]- and [1-MeHis^6]-angiotensin II. At and above pD 4.5, two resonances are observed for the His C-2 proton of [3-MeHis^6]-angiotensin while only one resonance, presumably that of the s-trans isomer from its chemical shift, is observed for [1-MeHis^6]-angiotensin II at all pDs. The results for the [1-MeHis^6] peptide do not distinguish between the complete absence of the trans to cis isomerization and the occurrence of the isomerization without the reporting of it by the His side chain. The maximum percentage of s-cis isomer reported by the His C-2 proton in [3-MeHis^6]-angiotensin is ~25% compared with 16% for [Asp^1,Ile^5]-angiotensin and 17% for the [Phe^4,Tyr^8]-angiotensin II (Bleich et al., 1978). Table III also shows the pD dependence of the proton chemical shifts of the His C-2 and C-4 protons for N^α-acetyl-, [Sar^1,Ala^8]-, and [homoHis^6]-angiotensin II. The first two peptides do show the isomerization reported by the His C-2 proton. The maximum percentage of the cis isomer is ~30% for [Sar^1,Ala^8]-angiotensin and N^α-acetylangiotensin II. The cis isomer was not observed and is, of course, not expected for [Ala^7]-angiotensin II.

The rate constant for the trans to cis isomerization of His-Pro at pD 6.9 at 37 °C was found to be $k_{t\rightarrow c} = 2.2 \times 10^{-3}$ s^{-1}, giving a lifetime for the trans rotamer of 5.4 min. From the equilibrium constant of the isomerization, $K = k_{t\rightarrow c}/k_{c\rightarrow t}$, and the cis/trans ratio of 0.78 at 37 °C, $k_{c\rightarrow t} = 2.8 \times 10^{-3}$ s^{-1} and the lifetime of the cis rotamer is 4.1 min. Roques et al. (1977) report lifetimes of the trans and cis rotamers of prolyl-4-hydroxyproline to be about 150 and 47 min, respectively, at 15 °C. The cis–trans exchange rates have not

Table III: pD Dependence of the Chemical Shifts (ppm) of the His C-2H and C-4H for [3-MeHis^6]-, [1-MeHis^6]-, [homoHis^6]-, N^{α}-Acetyl-, and [Sar,Ala^8]-angiotensin II

[3-MeHis^6]-angiotensin					[1-MeHis^6]-angiotensin[b]		
	C-2H		C-4H[a]			C-2H	C-4H
pD	trans	cis	trans	% cis	pD	trans	trans
2.4	8.58		7.17	0	1.5	8.62	7.24
3.4	8.52		7.13	0	2.7	8.63	7.24
4.5	8.52	8.37	7.14	25	5.4	8.60	7.21
7.4	7.75	7.59	6.90	25	6.6	8.45	7.21
8.3	7.60	7.52	6.87	25	9.8	7.57	6.82
9.4	7.53	7.47	6.82	26			

[homoHis^6]-angiotensin II[b]			N^{α}-acetylangiotensin II						[Sar^1,Ala^8]-angiotensin II					
	C-2H	C-4H		C-2H		C-4H				C-2H		C-4H		
pD	trans	trans	pD	trans	cis	trans	cis	% cis	pD	trans	cis	trans	cis	% cis
5.1	8.69	7.01	3.7	8.50		7.07		0	2.1	8.77		7.48		0
6.3	8.36	7.10	5.3	8.38		7.11		0	3.5	8.78		7.48		0
7.6	7.93	6.54	6.5	7.93		7.09		0	5.0	8.71	8.67	7.42	7.36	25
8.6	7.81	6.54	7.4	7.65	7.58	7.01	6.93	24	6.5	8.30	8.15	7.33	7.31	27
9.8	7.74	6.53	8.7	7.61	7.56	6.99	6.94	29	7.5	7.86	7.69	6.97		29
10.1	7.72	6.53	10.4	7.61	7.54	6.97	6.93	28	8.0	7.75	7.69	7.06	6.98	33
11.7	7.71	6.53	11.5	7.57	7.57	6.97	6.93		8.5	7.73	7.68	7.05	6.80	28
									9.5	7.70	7.67	7.03	6.99	26
									10.5	7.61	7.60	6.94	6.90	25

[a] Cis resonances could not be observed due to overlap with the Tyr protons. [b] The cis isomer was not present in these peptides.

Table IV: pD Dependence of the Chemical Shifts (ppm) of the His C-2H and C-4H for TRH and [3-MeHis^2]- and [1,3-diMeHis^2]-TRH

TRH						[3-MeHis^2]-TRH					[1,3-diMeHis^2]-TRH	
	C-2H		C-4H				C-2H	C-4H				C-4H[a]
pD	trans	cis	trans	cis	% cis	pD	trans	trans	cis	% cis	pD	trans
1.45	8.65		7.36		0	1.7	8.62	7.33		0	1.7	7.32
2.40	8.64		7.35		0	3.4	8.61	7.33		0	3.0	7.31
4.0	8.64	8.62	7.35	7.31	10	4.3	8.62	7.33	7.31	11	4.8	7.31
6.6	8.24	8.14	7.21	7.13	16	6.4	8.15	7.16	7.08	15	6.2	7.33
8.4	7.73		7.02	6.97	18	7.7	7.61	6.97	6.92	18	7.2	7.31
9.4	7.71		7.03	6.95	18	10.2	7.55	6.96	6.91	16	9.9	7.31

[a] No cis resonances were observed.

been determined for angiotensin or TRH peptides. However, the cis and trans C-2 proton resonances in [Asp^1,Ile^5]-angiotensin II at pD 9.1 do not coalesce at temperatures up to 60 °C (Bleich et al., 1978), indicating an exchange rate not likely to be faster than that of His-Pro.

Figure 5 shows the pD dependence of the His C-2 and C-4 proton resonances of [3-MeHis^2]- and [1,3-diMeHis^2]-TRH. The His C-2 proton resonance in [1,3-diMeHis^2]-TRH is not present because of facile deuterium exchange at this position, $t_{1/2}$ = 4.5 min for exchange at pH 8.92 for 1,3-dimethylimidazole at 31 °C (Olofson et al., 1964). The His C-4 proton does not report the s-trans to s-cis isomerization in [1,3-diMeHis^2]-TRH, while it does in [3-MeHis^2]-TRH. The His C-2 proton in [3-MeHis^2]-TRH does not report the isomerization. The assignments of ^{1}H and ^{13}C resonances for His-Pro and TRH are in agreement with those of Fermandjian et al. (1972) and Deslauriers et al. (1973) for TRH except that we report in addition the His β, C-2, and C-4 proton cis resonances. Table IV gives the chemical shifts of the C-2 and C-4 proton resonances for the three TRH peptides. Table V shows the known relative affinities for the angiotensin and TRH analogues where the reporting of the s-cis isomerization has been determined and the estimated pKs of the isomerizations.

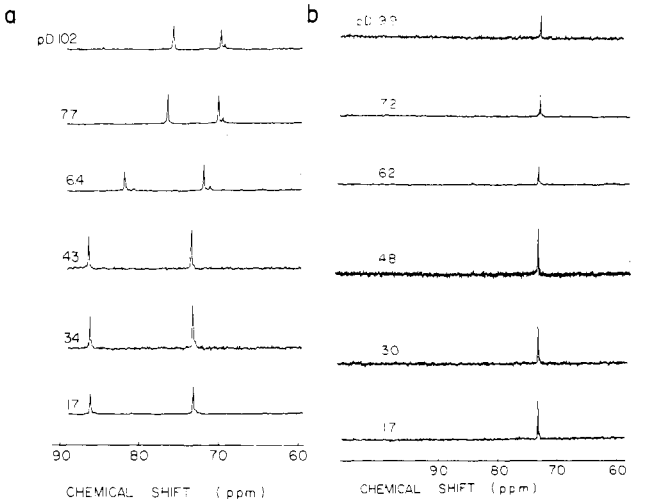

FIGURE 5: The pD dependence of the His C-2 and C-4 proton resonances of [3-MeHis^2]-TRH (a) and [1,3-diMeHis^2]-TRH (b) at 18 °C.

Discussion

The reporting of the s-trans to s-cis isomerization by the His C-2 and C-4 protons in His-Pro implies that the histidine side chain "sees" the conformational state of its following peptide bond. Although the mechanism of this interaction is not known, it seems likely that the imidazole ring must lie over or near either the peptide bond or proline ring in one or both of the isomers. Restricted rotation about ϕ and Ψ backbone angles in glycine containing di- and tripeptides has been observed in organic solvents (Bystrov et al., 1969) and in D_2O

Table V: Biological Activities of Angiotensin and TRH Peptides for Which the Cis/Trans Isomerism Has Been Determined

peptide hormone	isomerism reported by His C-2H	pK of isomerism ± 0.5 pD unit	myotropic act. % in rat uterus
[Asp^1, Ile^5]-angiotensin	yes	6.5	100 (Kosla et al., 1974)
[Phe^4, Tyr^8]-angiotensin	yes	7.0	antagonist (Marshall, 1976)
[Sar^1, Ala^8]-angiotensin	yes	4.3	antagonist (Pals et al., 1971; Brunner et al., 1971)
N^{α}-acetyl-angiotensin	yes	6.3	71 (Nouailhetas et al., 1977)
[3-MeHis^6]-angiotensin	yes	4.1	5 (Needleman et al., 1973)
[1-MeHis^6]-angiotensin	no		0.05 (Needleman et al., 1973)
[homoHis^6]-angiotensin	no		0.01 (Bloemhoff et al., 1973)
			TSH releasing hormonal act. %
TRH	yes	4.0	100[a]
[3-MeHis^2]-TRH	yes	4.2	800[a]
[1,3-diMeHis^2]-TRH	no		~2[b]

[a] TSH measured by radioimmunoassay in vivo in mice (Vale et al., 1972). [b] TSH measured by radioimmunoassay in vivo in cultures of pig pituitary cells (Hopkins, 1977).

(Mandel, 1965; Nakamura & Jardetsky, 1967; Morlino & Martin, 1967). For the glycine peptides in D_2O, the observation of nonequivalent glycine α protons by NMR suggested restricted rotation about backbone single bonds. Distinct rotamer populations were not observed, but rather a range of probable and less probable rotamers was suggested. Mandel (1965) suggested that nonbonded interactions of the amino acid side chain of the nonglycyl residue(s) with atoms of the planar s-trans peptide bond restricted the number of allowed backbone rotamers. In the case of His-Pro, we propose that nonbonded or steric interactions of the imidazole side chain with the s-cis and s-trans peptide bond or with the proline ring cause the reporting of the rotational state of the peptide bond by the C-2 and C-4 protons and carbons.

The conformation or class of conformations responsible for this reporting of the isomerization in His-Pro are maintained in native angiotensin (Galardy et al., 1976; Bleich et al., 1978) and in TRH. The existence of this His-Pro structure appears to be correlated with the biological activity of both of these hormones. Specifically, the absence of the conformation reporting the s-cis isomer in the angiotensin analogues [1-MeHis^6]- and [homoHis^6]-angiotensin and the TRH analogue [1,3-diMeHis^2]-TRH appears to be correlated with very low biological activity. The remaining analogues in Table V which exhibit the reporting of the cis isomer maintain high relative affinity for the angiotensin receptor (as either agonists or potent antagonists) or TRH receptor. It should be noted here that [*N*-MeAla^7]-angiotensin retains 22% of the pressor activity of native angiotensin (Andreatta & Scheraga, 1971). Acyl-*N*-methylalanine has been shown by NMR to exhibit cis–trans isomerization (Goodman et al., 1974). [Ala^7]-angiotensin which is 100% s-trans is inactive (Page & Bumpus, 1961). We have no explanation for the observation that the p*K*s of the isomerizations of [3-MeHis^6]- and [Sar^1,Ala^8]-angiotensin, TRH, and [3-MeHis^2]-TRH are depressed by nearly two pD units compared with the p*K*s of their imidazole rings, 6.2 and 6.09 for TRH and [3-MeHis^2]-TRH, respectively, in H_2O (Paiva et al., 1976).

The high potency of [3-MeHis^2]-TRH compared with [1-MeHis^2]-TRH (and presumably [1,3-diMeHis^2]-TRH) has been ascribed to the mimicking of the biologically active native [3-protio-His^2] tautomer by the [3-MeHis^2] peptide (Deslauriers et al., 1974). We further suggest that specific secondary and tertiary structure or structures of His-Pro in the 3-protio-tautomer of TRH are involved in the hormone's biological activity. The results in Table V suggest that the structure (or the restricted rotamer population) responsible for the reporting of the cis isomer by the His imidazole protons may be important for interaction (binding) with the respective hormone receptors. Alternatively, the cis isomer itself rather than the histidine conformation associated with it may be important for high affinity receptor interaction, as suggested previously (Bleich et al., 1978). If either of these correlations can be proven, possibly by synthesizing conformationally restricted peptides as Marshall et al. (1972), Pena et al. (1974), and Turk et al. (1976) have proposed, then secondary and side chain structural requirements for biological activity in the His-Pro region could be definitely established for both hormones.

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Prothrombin Domains: Circular Dichroic Evidence for a Lack of Cooperativity†

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ABSTRACT: The far-ultraviolet circular dichroism spectra of bovine and human prothrombin, prothrombin fragment 1, prethrombin 1, prothrombin fragment 2, and prethrombin 2 (prethrombin $2_{des(1-13)}$) were determined and the method of Chen et al. [Chen, Y. H., Yang, J. T., & Martinez, H. M. (1972) *Biochemistry 11*, 4120–4131; Chen, Y. H., Yang, J. T., & Chau, K. H. (1974) *Biochemistry 13*, 3350–3359] was used to calculate the apparent α-helix, β-sheet, and random-coil contents of each protein. Prothrombin and its activation components were found to contain a large amount of aperiodic secondary structure and there was little species difference between the spectra and, thus, secondary structures. The hypothesis that the prothrombin activation components exist as relatively noncooperative "domains" within the prothrombin molecule was tested by comparing the circular dichroism spectrum of prothrombin with the sum of the spectra of the components. In support of the hypothesis, no gross alterations in the spectra and, hence, secondary structures of the components were found to have occurred upon activation.

Thrombin, a serine protease, catalyzes the conversion of fibrinogen to fibrin in the blood coagulation process. It also participates in the activation of several coagulation factors (V, VIII, and XIII), as well as in the aggregation of platelets.

During hemostasis, the formation of thrombin is believed to occur via the catalytic conversion of prothrombin by factor Xa. In this reaction, substrate (prothrombin), enzyme (factor Xa), and cofactor (factor Va) are complexed by virtue of mutual adherence to phospholipid via calcium bridges (Papahadjopoulos & Hanahan, 1964; Cole et al., 1965). Upon factor Xa activation, prothrombin undergoes proteolytic cleavage to release the "pro" portion of the molecule and the two-chain thrombin molecule. In addition, the thrombin produced upon activation can cleave prothrombin at one or two sites. The result of the two factor Xa cleavages and the thrombin cleavage(s) is that many activation components of prothrombin are produced (Mann, 1976).

The most readily isolated activation components of bovine and human prothrombin are[1] (Mann, 1976) prothrombin

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