pH TITRATION EFFECTS ON THE CD SPECTRA OF ANGIOTENSIN II, TRUNCATED PEPTIDES AND OTHER ANALOGUES : AROMATIC REGION

K. LINTNER, S. FERMANDJIAN* and P. FROMAGEOT
Service de Biochimie, Centre d'études nucléaires de Saclay B.P. No 2 - 91190 Gif sur Yvette, France

and

M. C. KHOSLA, R. R. SMEBY and F. M. BUMPUS
The Research Division, Cleveland Clinic Foundation Cleveland Clinic, Ohio 44106, USA

Received 25 June 1975

1. Introduction

The pressorhormone angiotensin II, a linear octapeptide of the sequence Asp–Arg–Val–Tyr–$\frac{\text{Ile}}{\text{Val}}$–His–Pro–Phe has been the subject of numerous conformational analysis studies, most of which have centered on the backbone conformation [1–5]. Among the techniques employed, circular dichroism has advanced our understanding of angiotensin's conformational behaviour the most (see [6] and [7] for reviews on the subject). A universally accepted model of the peptide conformation, however, has not yet emerged. Although NH–C_αH coupling constants measured at pH 2.5 by Marshall et al. [8] were inconclusive regarding the backbone structure, circular dichroism studies by Fermandjian et al. [9], ^{1}H–^{3}H exchange by Printz et al. [10], and ^{1}H and ^{19}F NMR titrations [1,11], pointed nevertheless to the existence of a certain preferred conformation of angiotensin II even in aqueous solution. Circular dichroism is a useful tool to observe conformational changes in peptides. As angiotensin II contains phenylalanine and tyrosine, one can follow the effects of pH in the two spectral regions, the so called peptide region between 250 and 180 nm as well as the aromatic region from 320 to 250 nm. Here we report the preliminary results for the aromatic region obtained during a titration of angiotensin II and several of its structural analogues. It appears that in angiotensin II the tyrosine side chain is preferentially oriented, being subjected to the influences both of the immediately preceding amino acid residue and of the histidine side chain.

2. Materials and methods

The peptides Asn^1-(A II)** and D-His^6-(A II) are a gift from Dr Riniker (Ciba-Geigy, Basel). The peptides Ile^5-(A II), β-Asp^1-(A II), Ala^4-(A II), Ala^6-(A II), Ile^8-(A II), Pro^3-(A II), 4-8 pentapeptide-(A II) and 1-6 hexapeptide-(A II) have been synthesized by solid phase methods. Their preparation and purification has been described elsewhere [12–15].

Solutions were prepared by dissolving the weighed peptide in distilled water to obtain concentrations of 1 to 2 mg/ml. pH adjustments were, after acidification to pH 1 with concentrated HCl, effected with minute amounts of concentrated NaOH. All spectra were recorded on the Jobin-Yvon Dichrograph III model using fused quartz cells of 0.5 and 1.0 cm pathlengths. Circular dichroism results are expressed in units of molar ellipticity [θ].

* To whom correspondence should be adressed.

** *Abbreviations:* (A II): angiotensin II. CD: circular dichroism. NMR: nuclear magnetic resonance.

3. Results

The CD spectrum of angiotensin II in the aromatic wavelength region is strongly pH dependent (fig.1). It consists at acid and neutral pH of a major band attributed to the ^1L_b transition of the tyrosine side chain [16], centered at 275 nm, and the superposed vibronic bands of the phenylalanine side chain transitions at 268, 264, 262, 258 and 254 nm. These latter cannot be followed independently of the tyrosine signal, as they are added on top of the tail of this peak. The $[\theta]$ values at each of these wavelengths reflect only the behaviour found for 175 nm. Yet this tyrosine side chain signal is greatly affected by the titration. At acid pH up to pH 5 the signal at 275 nm is positive with an ellipticity of about +200. From pH 5 to pH 8 the signal intensity decreases, goes through zero and becomes negative with values of $[\theta]$ ~−240. The spectrum changes profoundly between pH 9 and 12

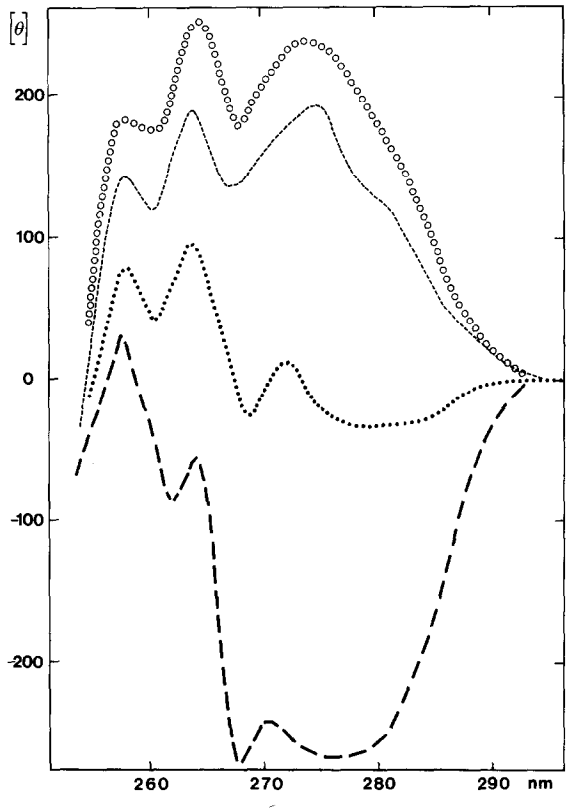

Fig.1. CD spectra of Angiotensin II: aromatic region. (——) pH 1.3. (ooo) pH 4.5. (●●●●●) pH 6.2. (— —) pH 8.0.

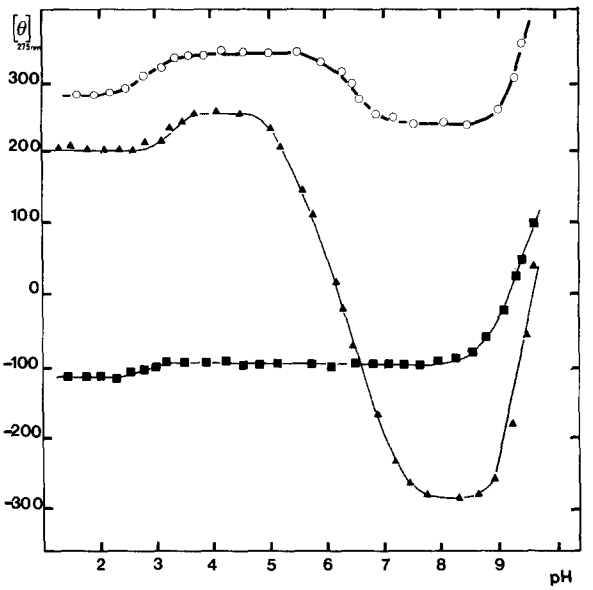

Fig.2. Ellipticities $[\theta]$ at 275 nm plotted as a function of pH. (–▲–▲) Angiotensin II. (–■–) Ala^6-(A II), (–o–o) D-His^6-(A II).

(not presented here), giving rise to a strong positive band at 293 nm.

Plotting the ellipticities at 275 nm as a function of pH we obtain a titration curve with 3 apparent pK values (fig.2). A very small signal variation is observed upon titration of the carboxyl group (pK~3.5), a second pK at 6.4 suggests the influence of the imidazole side chain of histidine, and the pK at 10 reflects the ionization of the tyrosine ring. The spectrum of the Ala^6-(A II) analogue (where histidine has been replaced by alanine) does not change between pH 4 and 8, thus confirming the above suggestion, whereas the small change in ellipticity during the titration of the D-His^6 -(A II) analogue (fig.2) hints at the importance of the specific spatial orientation of histidine. The titration of angiotensin II in aqueous solution by ^{1}H NMR has already shown that the tyrosine ortho and meta protons are somewhat influenced by the deprotonation of the histidine side chain [1]. Although the effects are small and ^{13}C-NMR has shown no such influence [3], this behaviour has been interpreted in terms of conformational changes in the peptide. The inversion of the sign observed in the CD spectra shows that with increasing pH the tyrosine ring is subjected to a new asymetric environment. This might arise from its adoption of a different orientation

Table 1
Ellipticites [θ] at 275 nm (resp. 261 nm) for angiotensin and several analogues at 3 pH values. Differences in ellipticity $\Delta[\theta]$ between pH 4.5 and pH 8.0

	pH 1.0	pH 4.5	pH 8.0	
		[Θ] 275 nm		Δ[Θ]
Angiotensin II	+200	+240	−260	−500
Asn^1 - (A II)	+100	+150	−280	−430
-Asp^1-(A II)	+180	+100	−320	−420
Ile^8-(A II)	+200	+270	−280	−550
Ala^6-(A II)	−120	−100	−100	0
D-His^6-(A II)	+280	+340	+240	−100
Pro^3-(A II)	−110	− 20	−590	−570
1–6-(A II)	+ 60	+ 60	− 40	−100
4–8-(A II)	+300	+250	−120	−370
		[Θ] 261 nm		
Ala^4-(A II)	−140	−120	−120	0

dependent on or independently of the overall peptide conformation. In order to distinguish between the variations of the tyrosine side chain conformation due to spatial interference from histidine and those due to changes in the backbone structure, we examined a series of analogues with substitutions designed to simplify this task. Table 1 gives the spectral parameters for these peptides, revealing the following items: (a) substitution in position 1 (a frequently used substitution for studies of structure–activity relationship) by asparagine or by β-coupled aspartic acid does not change the spectral titration behaviour. (b) Neither does substitution in position 8, replacing phenylalanine by isoleucine. This modification, however, simplifies the CD spectrum to give the pure tyrosine signal at 275 nm. (c) On the other hand, for the Ala^4-(A II) analogue devoid of tyrosine we found no measurable effect of the titration of histidine on the phenylalanine signals. (d) Replacement of histidine by alanine in position 6 leads to a negative CD signal for tyrosine that remains unchanged between pH 4 and pH 8. (e) The spectrum of the D-His^6-(A II) substituted analogue remains positive throughout the titration with only a small perturbation by the imidazole deprotonation. (f) The N-terminal hexapeptide parallels the behaviour of angiotensin II but on a greatly reduced scale. (g) The C-terminal pentapeptide 4–8, however, equals the titration conduct of the complete peptide.

Although a correlation of these results will have to be made with the ones obtained for the peptide region [17], there are certain conclusions that can be drawn already. The presence of histidine and its positive charge in angiotensin II orients the tyrosine ring in such a way as to give rise to a positive CD signal. On the other hand, in Pro^3-(A II) characterized by a conformation similar to angiotensin II, even strengthened [18], the proline ring forces tyrosine to adopt an orientation such as to give a negative signal throughout titration;

this orientation, and thus this signal, is much enhanced with the pH going from 5 to 8 (table 1). The absence of the charged side chain (either after titration or by total elimination: Ala^6-(A II)) gives rise to a negative tendency of varying degree of the CD signal. As the N-terminal hexapeptide (shown to be quite devoid of secondary structure [15]) indicates, the simple proximity of Tyr–X–His alone does not suffice to explain the large signal variations observed. In conclusion it appears that due to the specific backbone structure (primary and secondary) of angiotensin II the orientation of the tyrosine side chain, influenced by the specifically oriented imidazole ring and its positive charge, rather than a major conformational change of the backbone, is responsible for the observed spectral variations. Studies are in progress to determine if these phenomena are compatible with any of the proposed models of angiotensin II.

References

[1] Glickson, J. D., Cunningham, W. D. and Marshall, G. R. (1973) Biochemistry 12, 3684–3692.
[2] Weinkam, R. J. and Jorgensen, E. C. (1971) J. Amer. Chem. Soc. 93, 7038–7044.
[3] Zimmer, S., Haar, W., Maurer, W., Rüterjans, H., Fermandjian, S. and Fromageot, P. (1972) Eur. J. Biochem. 29, 80–87.
[4] Fermandjian, S., Fromageot, P., Tistchenko, A. M., Leicknam, J. P. and Lutz, M. (1972) Eur. J. Biochem. 28, 174–182.
[5] Juliano, L. and Paiva, A. C. M. (1974) Biochemistry 13, 2445–2450.
[6] Hruby, V. J. (1974) in: Chemistry and Biochemistry of Amino Acids, Peptides and Proteins (Weinstein, B. ed.), Vol. 3, pp. 61–66, M. Dekker, New York.
[7] Regoli, D., Park, W. K. and Rioux, F. (1974) Pharmacol. Rev. 26, 69–123.
[8] Marshall, G. R., Bosshard, H. E., Vine, W. V. and Glickson, J. D. (1973) Nature New Biol. 245, 125–127.
[9] Fermandjian, S., Morgat, J. L. and Formageot, P. (1971) Eur. J. Biochem. 24, 252–258.
[10] Printz, M. P., Williams, H. P. and Craig, L. C. (1972) Proc. Natl. Acad. Sci. U.S.A. 69, 378–382.
[11] Vine, W. H., Brueckner, D. A., Needleman, P. and Marshall, G. R. (1973) Biochemistry 12, 1630–1637.
[12] Khosla, M. C., Chaturvedi, N. C., Smeby, R. R. and Bumpus, F. M. (1968) Biochemistry 7, 3417–3421.
[13] Seu, J. H., Smeby, R. R. and Bumpus, F. M. (1962) J. Amer, Chem. Soc. 84, 4948-4950.
[14] Khosla, M. C., Kumar, S., Smeby, R. R. and Bumpus, F. M. (1972) J. Med. Chem. 15, 627–629.
[15] Greff, D., Fermandjian, S., Fromageot, P., Khosla, M. C., Smeby, R. R. and Bumpus, F. M., submitted for publication.
[16] Strickland, E. H. (1974) Crit. Rev. Biochem., 113–175.
[17] Lintner, K., Fermandjian, S., Fromageot, P., Khosla, M. C., Smeby, R. R. and Bumpus, F. M., manuscript in preparation.
[18] Fermandjian, S., Greff, D. and Fromageot, P. (1973) in: Dynamic aspects of Confomation Changes in Biological Macromolecules (Sadron, C. ed.) pp. 493-509. D. Reidel Publ. Comp., Dordrecht-Holland.