Conformation of Angiotensin II in Aqueous Solution. Titration of Several Peptide Analogs and Homologs†

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ABSTRACT: Fifteen analogs and homologs of [IIe^5]angiotensin II (AII) were titrated electrometrically in the temperature range 15–40°. A positive linear relationship between $\Delta H°$ and $T\Delta S°$, with slope near unity, was found for the dissociation of carboxyl, imidazole, amino, and phenoxyl groups. The differences in pK_a values for these groups in the series of AII analogs and homologs were interpreted as indicative of intramolecular electrostatic interactions. The evidence indicates that the N- and C-terminal ends of AII, in aqueous solution, are in closer proximity than would be expected in a random coil. It is concluded that presently available evidence does not allow a precise idea of AII conformation, although it seems certain that it tends to assume a folded structure in aqueous solution.

Several different models for the solution conformation of the octapeptide hormone angiotensin II have been proposed. The helical model of Smeby *et al.* (1962) was not supported by the optical rotatory dispersion (ORD), titration, and hydrogen exchange data of Paiva *et al.* (1963), who proposed a random coil. However, evidence from thin-film dialysis (Craig *et al.*, 1964) pointed to a unique compact conformation, while later studies on gel filtration and dialysis kinetics were interpreted as indicative of two slowly interchangeable forms (Ferreira *et al.*, 1969). More recently, mainly as a result of nuclear magnetic resonance (nmr) studies, new contrasting proposals have been advanced. Weinkam and Jorgensen (1971) postulate a stable conformer containing an 8 → 6 hydrogen bond[1] and a carboxylate–imidazole ion–dipole bond. Fermandjian *et al.* (1971, 1972a) propose a preferred antiparallel β conformation in which all ionizable side chains remain free of intramolecular interactions. A different conformation is proposed by Printz *et al.* (1972) and Bleich *et al.* (1973), whose γ-turn model contains two hydrogen bonds (5 → 3 and 3 → 5) and allows for an electrostatic interaction between the C-terminal carboxylate and the guanidinium group of the arginine side chain.

Other authors (Zimmer *et al.*, 1972; Marshall *et al.*, 1973; Vine *et al.*, 1973; Glickson *et al.*, 1973) interpret ^{13}C nmr and proton magnetic resonance (pmr) data as indicating that angiotensin II exists in an equilibrium of rapidly interchanging conformations, some of which are largely disordered. The latter authors also observed signs of conformational transitions associated with the titration of the α-amino and/or the imidazole group(s) and with the titration of the phenol group.

Since the different models proposed for the conformation of angiotensin II would affect in different ways the ionization of its titratable groups, a study of these groups should contribute to a better understanding of that problem. Such a study, involving the titration of several angiotensin analogs and homologs at different temperatures, is presented here.

Materials and Methods

[IIe^5]Angiotensin II (AII)[2] and several analog and homolog peptides were synthesized by the solid phase method (Merrifield, 1963; Stewart and Young, 1969; Paiva *et al.*, 1973, 1974) and were purified by countercurrent distribution and ion exchange chromatography until the following criteria for purity were met: (a) the amino acid analysis of acid hydrolysates yielded a molar ratio within 3% of the theoretical value for each amino acid; (b) the peptide content determined by amino acid analysis, spectrophotometry (ϵ_{275} 1375) and titration agreed within 1%; (c) only one spot was detected with Pauly, ninhydrin, and Sakaguchi reagents after thin-layer chromatography (tlc) of a 0.1-μmol sample with three solvent systems and high-voltage paper electrophoresis with three different buffers (pH 2.8, 4.9, and 9.9).

Of the peptides listed in Table I, [Pro^3,Pro^5]AII has not been previously described, and presented the following physical properties: mp 215° dec; $[\alpha]_D^{25}$ −120.3° (1 N HCl, *c* 0.1); partition coefficient in *n*-butyl alcohol–acetic acid–water (4:1:5), 0.10. Thin-layer chromatography on 0.1-mm silica gel plates (Eastman "Chromagram") yielded the following R_f values: with *n*-butyl alcohol–acetic acid–water (5:1:1), 0.10; with *n*-butyl alcohol–acetic acid–ethanol–water (1:1:1:1), 0.33; with pyridine–acetic acid–water (50:30:15), 0.35. The amino acid analysis of a 72-hr acid hydrolysate, done on a Beckman 120C analyzer, yielded a peptide content of 85% and the following amino acid molar ratios: Asp, 0.99; Arg, 0.98; Pro, 2.96; Tyr, 1.02; His, 1.03; Phe, 1.00.

The electrometric titrations were done as previously described (Paiva *et al.*, 1963). Not less than three independent titrations, with more than 80 data points each, were made in each temperature in the pH range 2.5–11. The pK_a values were calculated by a least-squares method with an iterative program on a Varian 620/L-100 computer. Titrations were performed in at least three temperatures covering a range of 20°, and estimates of $\Delta H°$ and $\Delta S°$ were made from linear

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[1] Hydrogen bonds are indicated according to the IUPAC recommendations for the description of peptide conformations (1970), *Biochemistry 9*, 3471.

[2] Abbreviations used are: AII, [Ile^5]angiotensin II; Suc, succinyl. Peptides were named according to the IUPAC tentative rules for naming synthetic modifications of natural peptides (1967), *Biochemistry 6*, 362.

TABLE I: Peptides Employed in This Study.

No.	Name	Amino Acid Sequence	Pressor Activity[a]
1	AII	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe	100
2	[Asn^1]AII	Asn-Arg-Val-Tyr-Ile-His-Pro-Phe	100[b]
3	[Suc^1]AII	Suc-Arg-Val-Tyr-Ile-His-Pro-Phe	60[b]
4	Ac-AII	Ac-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe	100[c]
5	[Gly^1]AII	Gly-Arg-Val-Tyr-Ile-His-Pro-Phe	42[d]
6	[Arg^1]AII	Arg-Arg-Val-Tyr-Ile-His-Pro-Phe	15[b]
7	[Arg^6]AII	Asp-Arg-Val-Tyr-Ile-Arg-Pro-Phe	0[e]
8	[Leu^6]AII	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe	1.3[f]
9	[Gly^1,Gly^2]AII	Gly-Gly-Val-Tyr-Ile-His-Pro-Phe	10[g]
10	[Pro^3,Pro^5]AII	Asp-Arg-Pro-Tyr-Pro-His-Pro-Phe	0.5[c]
11	[Asn^1]AII-α-amide	Asn-Arg-Val-Tyr-Ile-His-Pro-Phe-NH_2	0[h]
12	AII-(2–8)-heptapeptide	Arg-Val-Tyr-Ile-His-Pro-Phe	35[b]
13	AII-(3–8)-hexapeptide	Val-Tyr-Ile-His-Pro-Phe	1–2[b]
14	AII-(4–8)-pentapeptide	Tyr-Ile-His-Pro-Phe	0[b]
15	AII-(5–8)-tetrapeptide	Ile-His-Pro-Phe	0[b]

[a] The activities on the rat blood pressure are expressed in percentage of AII activity. [b] Schröder and Lübke (1966). [c] T. B. Paiva and M. E. Miyamoto, in preparation. [d] Page and McCubbin (1968). [e] Khosla *et al.* (1972a). [f] Khosla *et al.* (1972b). [g] Jorgensen *et al.* (1971). [h] Bumpus (1972).

equations adjusted by the method of least squares to the dependence of pK_a on $1/T$ (Table II). The standard errors of the $\Delta H°$ values were estimated from the standard errors of the slopes of the pK_a *vs.* $1/T$ plots.

Results

The titrations of all the peptides were reversible and could be fitted to simple sums of Henderson–Hasselbalch terms without corrections for possible electrostatic interactions (Tanford, 1950). No attempt was made to evaluate the dissociation constants for the guanidino groups because of the large errors involved in titrations above pH 11. As an example of the fit obtained between experimental data and calculated pK_a values a typical experiment is illustrated in Figure 1. The errors associated with the pK_a values were very similar for the same titratable group in the different peptides. The maximum value for the 95% fiducial limits was ± 0.05 for the β-carboxyl groups, ± 0.04 for the phenoxyl groups, and ± 0.03 for the α-carboxyl, imidazole, and amino groups. In the case of [Arg^1]- and [Arg^6]AII, however, the errors involved in the phenoxyl titrations were larger because these peptides were contaminated with traces of ammonium chloride that could not be removed by lyophilization.

Tables III–V show the pK_a values obtained at 25°, as well as the $\Delta H°$ and $\Delta S°$ values calculated from the temperature dependence of pK_a in the range 15–40°. In this range none of the pK_a *vs.* $1/T$ plots deviated significantly from linearity, as is exemplified by the data of Figure 2. Ac-AII was titrated only at 25°, because of insufficient amount of pepide, and only its pK_a values at that temperature are shown in Tables III–V.

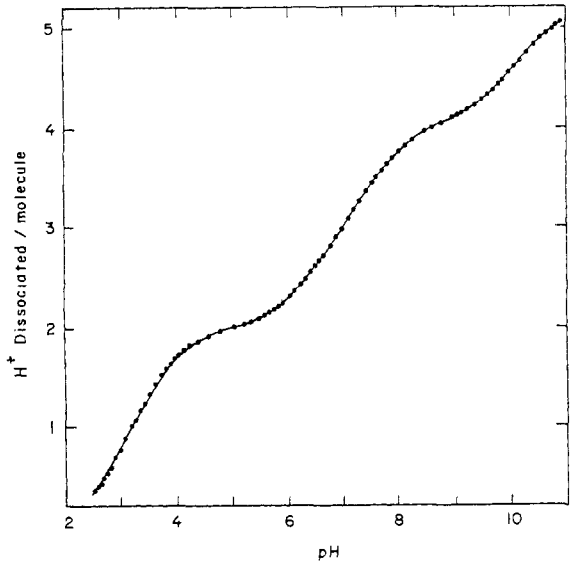

FIGURE 1: Titration curve of AII in 0.15 M KCl at 25°. The points represent experimental data from one titration and the curve is a theoretical one calculated with the following pK_a values: β-carboxyl, 2.96; α-carboxyl, 3.49; imidazole, 6.47; amino, 7.58; phenoxyl, 10.09; guanido, 12.2.

TABLE II: Parameters for the Linear Correlation between $\Delta H°$ and $\Delta S°$ Values for 15 Angiotensin Analogs and Homologs.

Group	Correlation Coefficient	m^a	$s_m{}^b$	b^a (kcal/mol)	$s_b{}^b$ (kcal/mol)
α-Carboxyl	0.96	1.03	0.08	4.8	0.3
β-Carboxyl[c]	1.00	0.91	0.02	3.68	0.001
Imidazole	0.99	0.84	0.03	8.49	0.09
Amino	0.92	1.00	0.01	10.2	0.5
Phenoxyl	1.00	0.98	0.03	13.5	0.1

[a] The data were adjusted to the equation: $\Delta H° = b + mT\Delta S°$ by the method of least squares. [b] s_b and s_m are the standard errors of the intercept (b) and the slope (m), respectively. [c] The data for [Suc^1]AII were not included (see text).

TABLE III: pK_a, $\Delta H°$, and $\Delta S°$ for Carboxyl Groups at 25° in 0.15 M KCl.

	Peptide	α-Carboxyl			β-Carboxyl		
		pK_a	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/°mol)	pK_a	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/° mol)
1	AII	3.49	0.6 ± 0.5	−14.1 ± 1.7	2.95	0.5 ± 0.1	−11.7 ± 3.7
2	[Asn^1]AII	3.36	−1.5 ± 1.1	−20.2 ± 4.0			
3	[Suc^1]AII	3.26	−1.5 ± 0.3	−20.0 ± 1.0	4.45	−0.8 ± 0.3	−23.2 ± 1.0
4	Ac-AII	3.20			4.00		
5	[Gly^1]AII	3.26	0.01 ± 0.4	−14.9 ± 1.2			
6	[Arg^1]AII	3.33	0.1 ± 0.8	−15.0 ± 2.7			
7	[Arg^6]AII	3.43	0.04 ± 0.04	−15.6 ± 0.1	2.95	0.02 ± 0.2	−13.5 ± 0.7
8	[Leu^8]AII	3.78	−0.2 ± 0.5	−18.0 ± 1.7	2.95	−0.02 ± 0.6	−13.6 ± 2.0
9	[Gly^1,Gly^2]AII	3.40	−3.3 ± 0.8	−26.5 ± 2.5			
10	[Pro^3,Pro^5]AII	3.34	−0.8 ± 0.5	−18.0 ± 1.7	3.02	−1.0 ± 0.4	−17.2 ± 1.3
12	AII-(2–8)-heptapeptide	3.34	−0.01 ± 0.8	−15.3 ± 2.7			
13	AII-(3–8)-hexapeptide	3.30	−0.6 ± 0.4	−17.2 ± 1.3			
14	AII-(4–8)-pentapeptide	3.32	0.05 ± 0.1	−15.0 ± 0.2			
15	AII-(5–8)-tetrapeptide	3.24	−0.5 ± 0.4	−16.5 ± 1.4			

TABLE IV: pK_a, $\Delta H°$, and $\Delta S°$ Values for the Imidazole and Amino Groups at 25°, in 0.15 M KCl.

	Peptide	Imidazole			Amino		
		pK_a	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/°mol)	pK_a	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/° mol)
1	AII	6.47	7.1 ± 0.3	−5.7 ± 1.0	7.60	8.8 ± 0.5	−5.2 ± 1.7
2	[Asn^1]AII	6.30	7.0 ± 0.5	−5.4 ± 1.7	6.82	9.7 ± 0.3	1.6 ± 1.0
3	[Suc^1]AII	6.58	6.2 ± 0.3	−9.4 ± 1.0			
4	Ac-AII	6.62					
5	[Gly^1]AII	6.45	7.5 ± 0.3	−4.5 ± 1.0	7.90	10.9 ± 0.4	0.3 ± 1.2
6	[Arg^1]AII	6.38	7.9 ± 0.1	−2.6 ± 0.3	7.22	10.6 ± 0.1	2.4 ± 0.5
7	[Arg^6]AII				7.50	9.3 ± 0.3	−3.0 ± 1.0
8	[Leu^8]AII	6.45	7.5 ± 0.2	−4.3 ± 0.7	7.58	8.3 ± 0.2	−6.8 ± 0.7
9	[Gly^1,Gly^2]AII	6.46	7.4 ± 0.5	−4.7 ± 1.8	7.87	10.1 ± 0.4	−2.3 ± 1.3
10	[Pro^3,Pro^5]AII	6.54	6.4 ± 0.1	−8.5 ± 0.3	7.64	8.1 ± 0.4	−7.7 ± 1.3
11	[Asn^1]AII-α-amide	6.14	8.6 ± 0.4	0.8 ± 1.3	6.77	7.1 ± 0.3	−5.7 ± 1.0
12	AII-(2–8)-heptapeptide	6.38	7.7 ± 0.2	−3.3 ± 0.7	7.33	11.6 ± 0.6	5.4 ± 1.9
13	AII-(3–8)-hexapeptide	6.38	6.7 ± 0.2	−6.7 ± 0.7	7.55	10.8 ± 0.5	0.5 ± 1.7
14	AII-(4–8)-pentapeptide	6.36	7.9 ± 0.4	−2.5 ± 1.5	7.40	9.8 ± 0.6	−1.1 ± 2.0
15	AII-(5–8)-tetrapeptide	6.30	6.9 ± 0.6	−5.8 ± 2.0	7.81	11.9 ± 0.4	4.1 ± 1.3

TABLE V: pK_a, $\Delta H°$, and $\Delta S°$ Values for the Phenoxyl Groups at 25°, in 0.15 M KCl.

	Peptide	pK_a	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/° mol)
1	AII	10.09	6.6 ± 1.0	−23.8 ± 3.0
2	[Asn^1]AII	10.07	8.9 ± 0.5	−15.7 ± 1.7
3	[Suc^1]AII	10.12	7.6 ± 0.9	−20.5 ± 3.0
4	Ac-AII	10.05		
5	[Gly^1]AII	10.03	9.1 ± 1.2	−14.5 ± 4.0
6	[Arg^1]AII	9.93	8.3 ± 2.7	−17.6 ± 9.0
7	[Arg^6]AII	10.03	10.1 ± 3.8	−11.4 ± 12.7
8	[Leu^8]AII	10.08	6.5 ± 0.4	−23.4 ± 1.4
9	[Gly^1,Gly^2]AII	10.10	7.7 ± 0.8	−19.7 ± 2.6
10	[Pro^3,Pro^5]AII	9.92	5.2 ± 0.3	−27.6 ± 1.0
11	[Asn^1]AII-α-amide	10.00	5.5 ± 0.8	−27.4 ± 2.5
12	AII-(2–8)-heptapeptide	10.07	9.3 ± 0.7	−14.5 ± 2.3
13	AII-(3–8)-hexapeptide	10.11	7.2 ± 0.6	−22.3 ± 2.0
14	AII-(4–8)-pentapeptide	10.21	6.5 ± 0.6	−24.4 ± 2.0

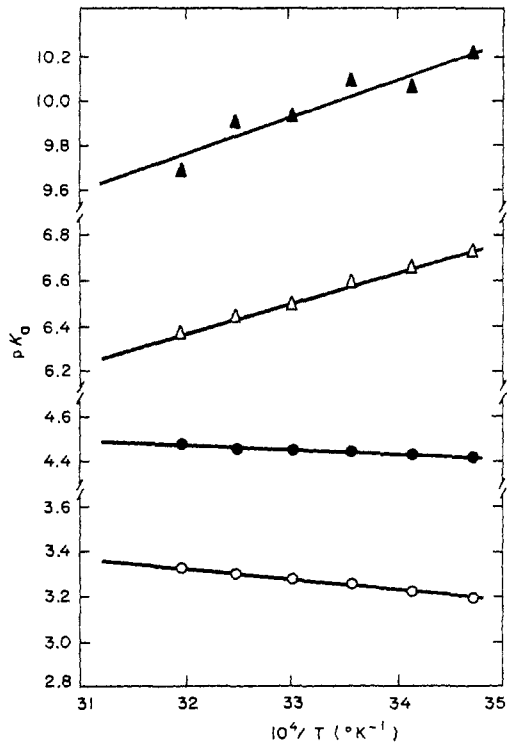

FIGURE 2: Temperature dependence of pK_a values of the α-carboxyl (○), β-carboxyl (●), imidazole (△), and phenoxyl (▲) groups of [Suc^1]AII in 0.15 M KCl. Temperature range: 15–40°.

Discussion

Titration data for the peptides studied here have not been reported before. The pK_a values found for [Asn^1]AII are in good agreement with those found in earlier direct titrations of [Asn^1,Val^5]AII (Paiva *et al.*, 1963) with the exception of that for the amino group (Table III), which is 0.10 unit lower than previously reported. As the replacement of Ile for Val in position 5 of the peptide chain would not be expected to affect the dissociation of the amino group, we attribute the discrepancy to the improved methods of titration curve analysis employed in the present study. [Asn^1,Val^5]AII has also been titrated with ^{13}C nmr (Zimmer *et al.*, 1972) and pmr (Glickson *et al.*, 1973) methods. The agreement between the results of that work and those presented here is good, in view of the differences in experimental error, and the conditions of the experiments, such as ionic strength, peptide concentration, and temperature.

The $\Delta H°$ and $\Delta S°$ values obtained for the peptides that were studied (Tables III–V) were, within the experimental error, in the range reported in the literature for normal carboxyl (Eberson, 1969), imidazole (Cohn and Edsall, 1943; Nozaki *et al.*, 1957; Paiva *et al.*, 1970), amino (Cohn and Edsall, 1943; Smith, 1968), and phenoxyl (Cohn and Edsall, 1943; Fernandez and Hepler, 1959; Chen and Laidler, 1962) groups. A positive linear relationship between $\Delta H°$ and $T\Delta S°$ was found for all the titratable groups with the exception of the data for the β-carboxyl group of [Suc^1]AII. These data were not included in the computation of the parameters for the linear relationships, shown in Table II. When the values for [Suc^1]AII are included, the $\Delta H°$ *vs.* $T\Delta S°$ dependence of the β-carboxyl groups has a correlation coefficient of 0.82, $m = 0.47 \pm 0.25$, and $b = 1.5 \pm 0.4$.

The obtention of slopes near unity (Table II) reveals a compensation between the enthalpic and entropic contributions to the free energy of dissociation which is indicative of the electrostatic origin of the observed differences in $\Delta S°$ and $\Delta H°$ values (Beetlestone and Irvine, 1964, 1965). The above mentioned abnormality in the case of [Suc^1]AII may be attributed to the contribution of an inductive effect of the amino group on the β-carboxyl.

The differences in the pK_a for each titratable group in the series of peptides studied, although small, are significant in relation to the experimental errors involved.

Carboxyl Groups (Table III). The pK_a values found for the lower homologs **12–15** are in the expected range for tetra- to hexapeptides with C-terminal phenylalanine (Cohn and Edsall, 1943; Ellenbogen, 1952). The addition of the N-terminal Asp residue in AII significantly raised the pK_a, suggesting an electrostatic interaction between the N- and C-terminal ends of the octapeptide. This indicates that there is a greater proximity between the extremities of the molecule than would be expected in a random conformation in solution. This interaction appears to be mostly due to the ionized β-carboxylate group, since the α-carboxyl pK_a is significantly lowered in [Asn^1]AII where the β-carboxyl is blocked. A further lowering was observed in [Gly^1]AII, possibly because the absence of the carboxymethyl side chain in this analog permits a closer proximity between the N-terminal ammonium and α-carboxyl groups. Furthermore, removal or blocking of the amino group, in [Suc^1]AII and Ac-AII, raises the pK_a of the β-carboxyl group, which remains mostly un-ionized during the titration of the α-carboxyl, and this is reflected in lower pK_a values for the latter group.

The guanidinium group of the arginine side chain in position 2 also appears to favor the dissociation of the α-carboxyl since [Gly^1,Gly^2]AII, where that side chain is absent, has an α-carboxyl pK_a 0.14 unit higher than [Gly^1]AII. An additional arginine side chain in the N-terminal position also favored the dissociation of the C-terminal carboxyl, since the α-carboxyl pK_a in [Arg^1]AII is significantly lower than in AII.

The pK_a of the α-carboxyl group of [Leu^8]AII was the highest of those shown in Table III. This may be explained by the difference in the C-terminal side chain, since the pK_a values reported for the carboxyl groups of phenylalanine and leucine are 1.83 and 2.36, respectively (Cohn and Edsall, 1943). An interpretation of the difference in α-carboxyl dissociation between AII and [Leu^8]AII, based on a comparison of the pK_a difference with that for the free amino acids, would depend on the assumption of free energy additivity, which may not be justifiable (Laidler, 1959).

The replacement of the valine and isoleucine residues of AII by two prolines lowered the α-carboxyl pK_a from 3.49 to 3.34. This indicates that the proximity between the N- and C-terminal ends postulated for AII does not occur in [Pro^3,-Pro^5]AII.

Regarding the dissociation of the β-carboxyl groups (Table III), the pK_a values found for [Suc^1]AII and Ac-AII are normal for aspartyl side chains in peptides (Cohn and Edsall, 1943). These groups were considerably more acidic in peptides **1**, **7**, **8**, and **10**, because of the influence of the protonated amino group. However, **1**, **7**, and **8** had lower β-carboxyl pK_a than **10**, again suggesting a closer proximity between the extremities of AII, [Arg^6]AII, and [Leu^8]AII. The low pK_a values of the β-carboxyl groups in these peptides may be explained by the stabilization of the dissociated form by an ion-dipole interaction with the α-carboxyl group.

Imidazole Groups (Table IV). The pK_a for the imidazole group of the tetrapeptide **15** was found to be 6.30. Comparison of this value with those of analogous peptides is not possible

because of lack of data in the literature. The removal of the N-terminal ammonium group by one more residue (pentapeptide, **14**) resulted in an increase of 0.06 unit in the pK_a. The value found for the pentapeptide is much lower than the 6.76 obtained for Gly-Gly-His-Gly-Gly by ^{13}C nmr titration (Gurd *et al.*, 1972). This would argue against the carboxylate–imidazole ion–dipole interaction proposed by Weinkam and Jorgensen (1971) for the C-terminal portion of AII. On the other hand, the data do not rule out a hydrogen bond between the *pro-s* nitrogen atom of the imidazole ring and the amide nitrogen of the histidine residue, similar to the bond proposed for the thyrotropin-releasing factor (TRF) (Grant *et al.*, 1972; Fermandjian *et al.*, 1972b).

After the pentapeptide, the lengthening of the peptide chain, in the hexa- and heptapeptides, did not significantly alter the imidazole pK_a. This indicates that in these homologs the ammonium and guanidinium groups are sufficiently removed from the imidazole ring as to have a negligible electrostatic effect on its dissociation. However, the presence of the aspartyl residue in AII increases the imidazole pK_a from 6.38 to 6.47, suggesting an electrostatic effect of the β-carboxylate group, confirmed by the lowering of that pK_a in [Asn^1]AII. It is interesting that the blocking of the β-carboxyl group produces a lowering of 0.17 unit in the pK_a, and is lowered by the further blocking of the α-carboxyl group, in [Asn^1]AII-α-amide. Since the β-carboxyl and α-carboxyl groups are removed from the histidyl residue by four and one residues in the peptide chain, respectively, it may be assumed that the imidazole ring lies in a similar average distance from the two carboxyl groups (Beetlestone and Irvine, 1964).

The increase in imidazole pK_a in [Suc^1]- and in Ac-AII would indicate that the ammonium group also exerts an electrostatic effect on the imidazole dissociation. On the other hand, the absence of the carboxymethyl side chain, in [Gly^1]-AII, does not change the pK_a found in AII. This may be due to balancing of the effects of removal of the carboxylate group and of the increase in distance of the ammonium group, in agreement with the closer proximity between ammonium and α-carboxyl groups proposed for the [Gly^1]AII analog. The further removal of the arginine side chain, in [Gly^1,Gly^2]AII, does not have a measurable effect on the imidazole dissociation while the introduction of an additional guanidinium group, in [Arg^1]AII, lowered the imidazole pK_a (compared with that of AII).

The proximity between the N- and C-terminal portions of [Leu^8]AII, postulated on the basis of the β-carboxyl titrations, is also confirmed by the imidazole dissociation, as its pK_a is very close to that of AII. On the other hand, the extensive peptide chain alteration introduced in [Pro^3,Pro^5]AII appears to allow some effect of the N-terminal carboxylate group on the imidazole dissociation, as its pK_a is increased by 0.07 unit in relation to that of AII.

Amino Groups (Table IV). A valid comparison of the amino group pK_a values can only be made with peptides that have the same N-terminal residues. Of the four peptides of Table IV that have an N-terminal aspartyl residue, there is no significant difference between the amino pK_a values of AII and of [Leu^8]AII, and that of [Pro^3,Pro^5]AII is slightly higher. This corroborates the conclusion that the amino group in AII is not involved in different electrostatic interactions in these peptides. The replacement of His by Arg in position 6 of the peptide chain, however, is felt by the amino group, as indicated by the significantly lower pK_a of [Arg^6]AII.

Comparison of the amino pK_a values for the two analogs with N-terminal asparagine ([Asn^1]AII and [Asn^1]AII-α-amide) shows that the dissociation of the amino group is influenced by the C-terminal carboxylate group. This again points to a proximity of the two ends of the molecule.

The two analogs with N-terminal glycine have similar amino pK_a values, with only a slight effect of the guanidinium group being evidenced by the small difference in the values for [Gly^1]- and [Gly^1,Gly^2]AII.

Phenoxyl Groups. The values for the pK_a of the phenoxyl groups, shown in Table V, lie between 10.00 and 10.12, with two exceptions. The higher pK_a of the pentapeptide may be attributed to the N-terminal position of tyrosine in this peptide. The pK_a of 9.92 found for [Pro^3,Pro^5]AII, where the tyrosyl residue is flanked by two prolines, appears to indicate a greater accessibility of the phenoxyl group to the solvent, in that peptide. The pK_a value of 9.93 found for [Arg^1]AII cannot be considered significantly different from that of AII, because of the large error involved in the phenoxyl titration of that analog.

Conclusions

The analysis of the effects of structural changes of the AII molecule upon the dissociation of its titratable groups has given some information about the conformation of AII in aqueous solution. The following conclusions were drawn from the titration data.

1. The N- and C-terminal ends of AII, in aqueous solution, are in closer proximity than would be expected in a random coil. This proximity also occurs in the other octapeptides that were studied with the exception of [Pro^8,Pro^5]AII, and agrees with both the β-cross (Devynck *et al.*, 1973) and the γ-turn (Printz *et al.*, 1972) models for AII conformation.

2. No evidence was found for carboxylate–imidazole ion–dipole interaction, as proposed by Weinkam and Jorgensen (1971). The imidazole group appears to be similarly affected by both the β-carboxyl group of the Asp residue and the C-terminal carboxyl group.

3. There are indications that the phenoxyl groups of AII, and of most of the analogs and homologs studied, may not be totally free to interact with the solvent, as has been thought by the apparently normal tyrosyl dissociation in AII (Paiva and Paiva, 1962; Paiva *et al.*, 1963).

4. The pH-dependent conformational transitions for which there is nmr evidence (Glickson *et al.*, 1973; Marshall *et al.*, 1973) could not be detected by the titration data.

A review of all the data collected by several authors with various methods leads to the conclusion that no clear picture can be drawn, at present, of the conformation of AII, although it seems certain that it does tend to assume a folded structure in aqueous solution. This structure, however, is probably not rigid, and it is possible that it represents an average of several more or less equally preferred conformations of the molecule. For this reason we have not attempted to analyze our data more quantitatively through calculations based on the Kirkwood–Westheimer model (Tanford, 1957). Such an analysis, however, may be useful in the future for selecting among possible conformations, if new data will justify more detailed rigid models for AII.

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References

Beetlestone, J. G., and Irvine, D. H. (1964), *Proc. Roy. Soc., Ser. A 277*, 414.

Beetlestone, J. G., and Irvine, D. H. (1965), *J. Chem. Soc.*, 3271.

Bleich, H. E., Galardy, R. E., and Printz, M. P. (1973), *J. Amer. Chem. Soc. 95*, 2041.

Bumpus, F. M. (1972), *in* Structure-Activity Relationships of Protein and Polypeptide Hormones, Margoulies, M., and Greenwood, F. C., Ed., Amsterdam, Excerpta Medica, p 18.

Chen, D. T. Y., and Laidler, K. J. (1962), *Trans. Faraday Soc. 58*, 480.

Cohn, E. J., and Edsall, J. T. (1943), Proteins, Amino Acids and Peptides, New York, N. Y., Reinhold, p 89.

Craig, L. C., Harfenist, E. J., and Paladini, A. C. (1964), *Biochemistry 3*, 764.

Devynck, M. A., Pernollet, M. G., Meyer, P., Fermandjian, S., and Fromageot, P. (1973), *Nature (London), New Biol. 245*, 55.

Eberson, L. (1969), *in* The Chemistry of Carboxyl Acids and Esters, Patai, S., Ed., London, Interscience, p 211.

Ellenbogen, E. (1952), *J. Amer. Chem. Soc. 74*, 5198.

Fermandjian, S., Fromageot, P., Tistchenko, A. M., Leicknam, J. P., and Lutz, M. (1972a), *Eur. J. Biochem. 28*, 174.

Fermandjian, S., Morgat, J. L., and Fromageot, P. (1971), *Eur. J. Biochem. 24*, 252.

Fermandjian, S., Pradelles, P., and Fromageot, P. (1972b), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 28*, 156.

Fernandez, L. P., and Hepler, L. G. (1959), *J. Amer. Chem. Soc. 81*, 1783.

Ferreira, A. T., Hampe, O. G., and Paiva, A. C. M. (1969), *Biochemistry 8*, 3483.

Glickson, J. S., Cunningham, W. D., and Marshall, G. R. (1973), *Biochemistry 12*, 3684.

Grant, G., Ling, N., Rivier, J., and Vale, W. (1972), *Biochemistry 11*, 3070.

Gurd, F. R. N., Keim, P., Glushko, V. G., Lowson, P. J., Marshall, R. C., Nigen, A. M., and Vigna, R. A. (1972), *in* Chemistry and Biology of Peptides, Meienhofer, J., Ed., Ann Arbor, Mich., Ann Arbor Publishers, p 45.

Jorgensen, E. C., Windridge, G. C., and Lee, T. C. (1971), *J. Med. Chem. 14*, 631.

Khosla, M. C., Kumar, S., Smeby, R. R., and Bumpus, F. M. (1972a), *J. Med. Chem. 15*, 627.

Khosla, M. C., Leese, R. A., Maloy, W. L., Ferreira, A. T., Smeby, R. R., and Bumpus, F. M. (1972b), *J. Med. Chem. 15*, 792.

Laidler, K. J. (1959), *Trans. Faraday Soc. 55*, 1725.

Marshall, G. R., Bosshard, H. E., Vine, W. H., and Glickson, J. D. (1973), *Nature (London), New Biol. 245*, 125.

Merrifield, R. B. (1963), *J. Amer. Chem. Soc. 85*, 2149.

Nozaki, Y., Gurd, F. R. N., Chen, R. F., and Edsall, J. T. (1957), *J. Amer. Chem. Soc. 79*, 2123.

Page, I. H., and McCubbin, N. C., (1968), Renal Hypertension, Chicago, Ill., Yearbook Medical Publishers, p 80.

Paiva, A. C. M., Nouailhetas, V. L. A., Miyamoto, M. E., Mendes, G. B., and Paiva, T. B. (1973), *J. Med. Chem. 16*, 6.

Paiva, A. C. M., and Paiva, T. B. (1962), *Biochim. Biophys. Acta 56*, 339.

Paiva, T. B., Goissis, G., Juliano, L., Miyamoto, M. E., and Paiva, A. C. M. (1974), *J. Med. Chem. 17*, 238.

Paiva, T. B., Paiva, A. C. M., and Scheraga, H. A. (1963), *Biochemistry 2*, 1327.

Paiva, T. B., Tominaga, M., and Paiva, A. C. M. (1970), *J. Med. Chem. 13*, 689.

Printz, M. P., Nemethy, G., and Bleich, H. (1972), *Nature (London), New Biol. 237*, 135.

Schröder, E., and Lübke, K. (1966), The Peptides, Vol. 2, New York, N. Y., Academic Press, p 52.

Smeby, R. R., Arakawa, K., Bumpus, F. M., and Marsh, M. M. (1962), *Biochim. Biophys. Acta 58*, 550.

Smith, J. W. (1968), *in* The Chemistry of Amino Groups, Patai, S., Ed., London, Interscience, pp 161–204.

Stewart, J. M., and Young, J. (1969), Solid Phase Peptide Synthesis, San Francisco, Calif., W. H. Freeman.

Tanford, C. (1950), *J. Amer. Chem. Soc. 72*, 441.

Tanford, C. (1957), *J. Amer. Chem. Soc. 79*, 5348.

Vine, W. H., Brueckner, D. A., Needleman, P., and Marshall, G. R. (1973), *Biochemistry 12*, 1630.

Weinkam, R. J., and Jorgensen, E. C. (1971), *J. Amer. Chem. Soc. 93*, 7038.

Zimmer, S., Haar, W., Maurer, W., Rüterjans, H., Fermandjian, S., and Fromageot, P. (1972), *Eur. J. Biochem. 29*, 80.