system. Actually his proposition was stated in these terms: "Autrement dit, certains éléments de symétrie peuvent coexister avec certains phénomènes, mais il ne sont pas nécessaires. Ce qui est nécessaires, c'est que certains éléments de symétrie n'existent pas. C'est la dissymétrie qui crée le phénomène."

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Ionization of tyrosyl group in angiotensin II

The thermodynamical quantities for the ionization of tyrosyl group have been used as a basis for speculating on its functional behaviour on the tertiary structure of proteins[1]. Experiments on surface activity of polypeptides of the angiotensin group demonstrated the convenience of knowing beforehand the ionization constant of its tyrosyl group, its temperature dependence, and the thermodynamical parameters ΔF, ΔH, and ΔS in bulk solutions of angiotensin II.

The spectrophotometric method was used, employing a Beckman DK-2 ratio-recording spectrophotometer. Temperatures were measured directly inside the cells and were always within $\pm$ 0.1°, using a special circulation system from an external, thermostatically controlled, water bath. A stream of dry nitrogen was used to prevent water condensation on the cells.

The synthetic angiotensins II used were: (a) a mixture of 90% β-asp[1], val_5-angiotensin II and 10% α-asp[1], val_5-angiotensin II and (b) val_5-angiotensin II β-aspartylamide; both were kindly provided by Drs. R. Schwyzer, F. Gross and B. Iselin from Ciba Ltd. (Basle). 1 mole of acetic acid and 5 moles of water per mole of octapeptide were also present in these preparations. Deionized water freshly distilled in an all-glass apparatus was used throughout. Prior to use it was boiled to

expel carbon dioxide and cooled under a soda lime tube. All reagents were "analytical grade".

The following solutions were used: 0.001 M HCl–0.099 M KCl; 0.05 M $NaHCO_3$–0.1 M NaOH; 0.2 M NaOH–0.2 M KCl.

The ionization constant was determined from spectra obtained at pH values of: ~ 3, 9.70, 10.10, 10.20, 10.30, 10.40, 10.50, 10.90, 12.00 and ~ 13 using the equations:

$$pK = pH - \frac{\alpha}{1-\alpha} - \log\gamma;\ \alpha = \frac{\varepsilon - \varepsilon_{ac}}{\varepsilon_{alk} - \varepsilon_{ac}};\ -\log\gamma = \frac{A\sqrt{I}}{1+\sqrt{I}} + CI$$

where ε = absorption in buffer solution, ε_{ac} = absorption in acid solution, ε_{alk} = absorption in alkaline solution, γ = activity coefficient, I = ionic strength, A and C = constants. The values of A at different temperatures were taken from ROBINSON AND STOKES[2] and the value of C, following DAVIES[3], was taken as $0.1\,|z_1 z_2|$, where z_1 and z_2 are the valencies of the ions. Absorption was corrected in all cases for the absorption of solvent and electrolytes.

The pH values for $NaHCO_3$–NaOH and NaOH–KCl buffer solutions were taken from BATES AND BOWERS[4]. Systematic checking with a Leeds and Northrup 7661-A1 pH-meter always gave the values predicted, within experimental error (± 0.02 pH unit). Table I gives the values of pK obtained at three different temperatures and the calculated values of ΔF, ΔH and ΔS at 25° using the equations:

TABLE I

THERMODYNAMICAL PARAMETERS FOR THE IONIZATION OF TYROSYL GROUP IN ANGIOTENSIN II

Maximum estimated errors: pK = ±0.04, ΔF = ±0.05, ΔS = ±5, ΔH = ±1.2.

	Temperature		
	15°	*25°*	*35°*
pK	10.52	10.37	10.22
ΔF (kcal/mole)		14.14	
ΔH (kcal/mole)		6.3	
ΔS (cal/mole/grad)		−27	

$$\Delta F = 2.303\,RT\mathrm{p}K;\ \Delta H = 2.303\,R\left(\frac{\Delta \mathrm{pH}}{\Delta(1/T)}\right)_{\alpha};\ \Delta F = \Delta H - T\Delta S.$$

The heat of ionization found is of the same order of magnitude as that corresponding to the "normal phenolic hydroxyl" in phenol[5], tyrosine[6], tyrosinamide[7], etc. TANFORD AND ROBERTS[6] demonstrated for albumin that an hydrogen-bonded tyrosyl group has a larger heat of ionization, 5–6 kcal/mole being necessary to break the hydrogen bond and then 5–6 kcal/mole to ionize the phenolic hydroxyl. It seems justified then to conclude that it is highly improbable that the tyrosyl group is hydrogen-bonded to a side chain in the angiotensin II molecule. SMEBY *et al.*[8] have recently arrived at the same conclusion studying the difference spectra in urea solutions of a closely related compound: isoleucyl$_5$-angiotensin II.

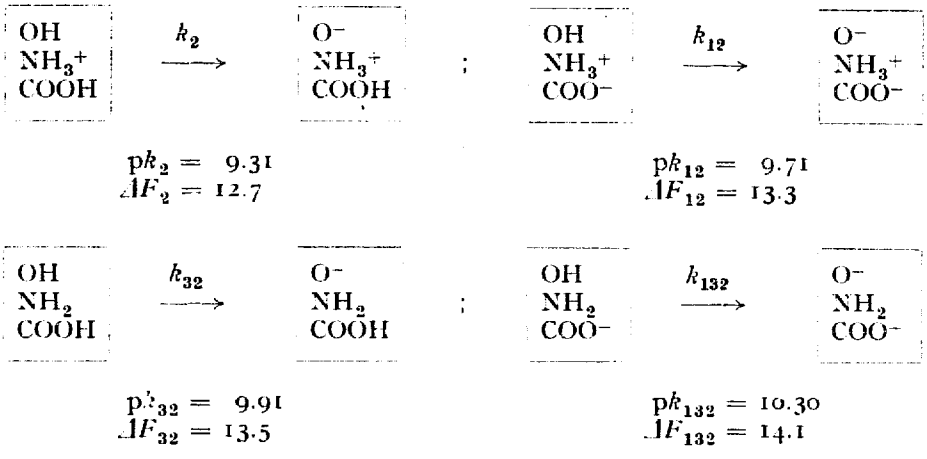
Comparison of pK and ΔF values obtained with angiotensin with those corresponding to free tyrosine must be done very cautiously because blocking amino and

carboxyl groups through peptide linkage introduces a significant difference; it is known that the ionization of the phenolic hydroxyl is influenced by the charge of the neighbouring amino and carboxyl groups. The complete ionization scheme for tyrosine worked out by MARTIN *et al.*[7] was therefore applied. Table II represents the four possibilities for the ionization of phenolic hydroxyl in free tyrosine and the corresponding values of pK and ΔF.

TABLE II

IONIZATION OF PHENOLIC HYDROXYL IN FREE TYROSINE (AFTER MARTIN *et al.*[7])

All values at $I = 0$ and 25°; ΔF in kcal/mole; ΔF values calculated with pK data from MARTIN *et al.*[7].



Tyrosine within the angiotensin molecule is comparable to free tyrosine when the amino and the carboxyl groups are un-ionized. Accordingly one would expect the ΔF value for angiotensin II to be close to the calculated with microconstant k_{32}. This is not the case, however, and the difference between them (0.64 kcal/mole) is considerably greater than the maximum estimated error (± 0.05).

The ΔF found for angiotensin II coincides with that corresponding to the microconstant k_{132} in free tyrosine; in this equilibrium the carboxyl group of free tyrosine is already ionized and consequently a negative charge exists in the tyrosine molecule prior to the ionization of the phenolic hydroxyl.

The coincidence of the ΔF values could be a suggestive indication of the existence of a negative charge near the tyrosyl group of angiotensin.

It is interesting to discuss these results having in mind the model proposed by SMEBY *et al.*[8] for isoleucyl$_5$–angiotensin II. In the configuration of this model the C-terminal phenylalanine and the N-terminal aspartic acid are very close to the tyrosyl group and it is conceivable that the negative charge of one or both nearby carboxyl groups affects the ionization of the phenolic hydroxyl.

With the same method the pK and the corresponding ΔF for val$_5$–angiotensin II β-aspartylamide were determined. The values obtained, 10.23 and 13.96 kcal/mole respectively, do not evidence a significative difference from the free acid. It can be concluded then that, if the above mentioned supposition is true, the only factor responsible for the perturbation in the ionization of the phenolic hydroxyl is the carboxyl group of phenylalanine.

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