The Electronic Structure of the α-Amino Acids of Proteins. II. Dissociation Constants

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(Received February 21, 1964)

In a previous paper[1] we obtained a satisfactory correlation between the charge distribution in the α-amino acids, as determined by a semi-empirical procedure derived from the molecular orbital method,[2] and the proton chemical shifts of these compounds. In the present paper we will report some approximate results concerning the dissociation constants of the same compounds.

The simplest α-amino acids dissociate according to the schemes:

$$RNH_3{}^+COOH \leftrightharpoons RNH_3{}^+COO^- + H^+ \qquad (I)$$

and

$$RNH_3{}^+COO^- \rightleftharpoons RNH^2COO^- + H^+ \qquad (II)$$

The p*K*'s corresponding to these two dissociation processes are conventionally denoted as pK_1 and pK_2. Only these processes will be discussed in the present paper.

The Method

Simple electrostatic models[3] have already led to the conclusion that the dissociation constant of an acidic group is affected by the electrostatic effect of the neighbouring groups. We have assumed, therefore, that the energy variation involved in the dissociation process is given by two essential contributions: a) the change in the sum of the bond energies of the amino acid molecule in passing from the non-dissociated form to the dissociated form; and b) the change in the electrostatic energy of the proton because, in the final dissociated state, it is no longer subject to the attractions or repulsion of the other atoms of the molecule.

Several theoretical chemists have attempted to interpret dissociation constants on the basis of more or less refined calculations. They have, however, studied conjugated compounds almost exclusively and based their considerations on calculations for the π systems of such molecules. Therefore, their procedures cannot be directly applied to our problem. However, some of their considerations have, of course, a general significance. Thus, the basicity of conjugated molecules has been related to the chang in π energy during the dissociation[4] and to the value of the π charge of the protonated heteroatom[5]. The use of a combined index taking into account both the change in π energy and the change in the energy due to an additional electrostatic interaction has also been proposed[6] and shown to be particularly successful. Finally, it has been observed that taking the solvation into account leads to useful improvements.[7]

Only a classical electrostatic theory has been proposed,[3] as far as we know, in connection with saturated compounds.

We have adopted a point of view similar to that of Ref. 6. Already in Ref. 2, the method proposed in that paper was used for a semi-qualitative discussion of dissociation constants. In particular, the total σ-electron energy of a given molecule was calculated by adding the σ energies of the various bonds, according to the formulas;

$$E = \sum_{\substack{\text{bonds}\\ \mu\nu}} E_{\mu\nu} \qquad (1)$$

where*

$$E_{\mu\nu} = 2\alpha + \{(\delta_\mu + \delta_\nu) + 2\varepsilon_{\mu\nu}\sqrt{1 + Q^2{}_{\mu\nu}}\}\beta \qquad (2)$$

and

$$Q_{\mu\nu} = \frac{\delta_\mu - \delta_\nu}{2\varepsilon_{\mu\nu}} \qquad (3)$$

(The parameters α and β are the usual standard Coulomb and bond integrals; δ_μ and δ_ν are defined by the condition that $\alpha + \delta_\mu\beta$ and $\alpha + \delta_\nu\beta$ are the Coulomb integrals of the μ-th and ν-th atom respectively; $\varepsilon_{\mu\nu}\beta$ is the bond

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2) G. Del Re, *J. Chem. Soc.*, **1958**, 4031.

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* In Ref. 2 the factor 2 before $\varepsilon_{\mu\nu}$ had been ommitted in the formula for $E_{\mu\nu}$

integral of the $\mu\nu$ bond.)

If formula 1 is applied to a series of protonated molecules and to the corresponding free bases, one gets energy differences which can be expected to a first approximation to be linearly related to the pK's of the corresponding dissociation equilibria (of course, the changes in the solvation and nuclear repulsion energies are assumed to be constant).

These considerations have been taken as a starting point of the present paper. The $\varDelta E$'s, however, have been calculated according to the refined formula:

$$\varDelta E=E_\sigma(\text{final})-E_\sigma(\text{initial})-\sum_\mu\frac{Q_\text{H}Q_\mu e^2}{R_{\text{H}\mu}} \tag{4}$$

Here E_σ(initial) denotes the energy of the protonated compound, and E_σ(final), that of the base. The third term corresponds to the electrostatic interaction between the proton and the other atoms in the protonated compound. Q_H is the net charge (in e units) of the proton; $Q\mu$, the net charge of μ-th atom, and $R_{\text{H}\mu}$, their mutual distance. The calculation of $R_{\text{H}\mu}$ was carried out using a formula proposed by Eyring,[8] and by Kirkwood and Westheimer.[9] This formula gives the root-mean square distance between the hydrogen and the atom located at the end of the n-th bond, assuming free rotation around the bonds. Let $\eta_{m\ m+1}$ be the cosine of the supplement of the angle between the m-th and $m+1$-th bond and l_k, the length of the k-th bond; then,

$$\overline{R}_n{}^2=\sum_{k=1}^{n}l_k{}^2+2\sum_{k=1}^{n}\sum_{s=1}^{k-1}l_kl_s\prod_{m=s}^{k-1}\eta_{m\ m+1} \tag{5}$$

A few remarks are important in connection with Eq. 4:

1) The net charge, Q_μ, of the μ-th atom must be calculated by adding the charge, $Q_\mu{}^\sigma$, due to the σ electrons to $Q_\mu{}^\pi$, the net charge due to the π electrons, if present:

$$Q_\mu=Q_\mu{}^\sigma+Q_\mu{}^\pi \tag{6}$$

The net charges, $Q_\mu{}^\pi$, calculated for our compounds by the ordinary Hückel method are shown in Fig. 1.

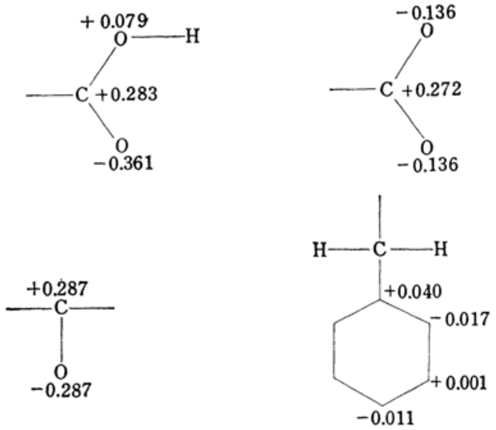

Fig. 1 The net charge of π-electrons in the conjugated parts of the amionacids.

2) $\varDelta E$ should be given by the difference between the total energy of the final and initial molecules in Eqs. I and II the total energy of a molecule being:

$$E_\pi+E_\sigma+\sum_{\mu>\nu}\frac{Q_\mu Q_\nu e^2}{R_{\mu\nu}} \tag{7}$$

(E_π and E_σ denote the total π and σ energies, and the third term denotes the core repulsion energy in the molecule). Equation 4 represents this $\varDelta E$ only if the variation of the total π-electron energy is assumed to be constant, and only if one may reasonably assume that the variation of the third term can be written as:

$$\varDelta\sum_{\mu>\nu}\frac{Q_\mu Q_\nu e^2}{R_{\mu\nu}}=C-\sum_\mu\frac{Q_\text{H}Q_\mu e^2}{R_{\text{H}\mu}} \tag{8}$$

where C is a constant.

3) The last term in Eq. 4 corresponds to the dielectric constant of the solvent being equal to 1. Different values have been adopted in classical theory,[3] but no serious modification could arise if we introduced the same kind of correction, as the change in the dielectric constant has always been taken to be small.

4) The contribution of the solvation energy is not taken into account.

5) The hyperconjugation effects, which may take place in certain amino acids, are ignored throughout all the calculations.

Results and Discussion

The results of the calculation are listed in Tables I and II, together with the experimental values of pK_1 and pK_2 for the various compounds. In the second columns of these tables, the variations of the total σ energies in the course of reactions I and II are given in units of $-\beta$. In the fourth columns the calculated values of the $\varDelta E$'s (Eq. 4) are listed in units of eV.'s. The calculation of the $\varDelta E$'s has required the determination of a value for $-\beta$. We have chosen 5.2 electron volts, a value not unreasonable for this type of property judging from other investigations of saturated compounds[10] and corresponding to a least-square

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TABLE I. THE DISSOCIATION CONSTANT $\mathrm{p}K_1'$ AND CALCULATED RESULTS

Compound	ΔE_σ (in units of $-\beta$)	$\sum_\mu \frac{Q_H Q_\mu}{R_{H\mu}} e^2$ (in units of eV.)	$\Delta E_\sigma - \sum_\mu \frac{Q_H Q_\mu}{R_{H\mu}} e^2$ (in units of eV.)	Apparent dissociation constants $\mathrm{p}K_1'$*
Glycine	1.852	−0.037	9.527	2.34
Alanine	1.821	−0.014	9.483	2.34
Valine	1.815	−0.031	9.469	2.32
Serine	1.815	−0.010	9.448	2.21
Proline	1.803	−0.062	9.437	1.99
β-Alanine	1.818	−0.235	9.689	3.60
Leucine	1.815	−0.094	9.548	2.36
Methionine	1.821	−0.024	9.493	2.28
Aspartic acid	1.821	+0.057	9.412	1.88
Asparagine	1.815	+0.017	9.421	2.02
Phenylalanine	1.811	−0.032	9.449	1.83

* These values are quoted from Tables 4-11 of J. P. Greenstein and M. Winitz, "Chemistry of the Aminoacids," Vol. 1, John Wiley and Sons (1961).

TABLE II. THE DISSOCIATION CONSTANT $\mathrm{p}K_2'$ AND CALCULATED RESULTS

Compound	ΔE_σ (in units of $-\beta$)	$\sum_\mu \frac{Q_H Q_\mu}{R_{H\mu}} e^2$ (in units of eV.)	$\Delta E_\sigma - \sum_\mu \frac{Q_H Q_\mu}{R_{H\mu}} e^2$ (in units of eV.)	Apparent dissociation constants $\mathrm{p}K_2'$*
Glycine	3.367	+0.799	16.709	9.60
Alanine	3.363	+0.794	16.694	9.69
Valine	3.357	+0.782	16.674	9.62
Serine	3.367	+0.791	16.717	9.15
Methionine	3.363	+0.891	16.597	9.21
Proline	3.603	+0.871	17.784	10.60
β-Alanine	3.387	+0.877	16.735	10.19
Asparagine	3.363	+0.850	16.638	8.80
Leucine	3.371	+0.811	16.718	9.60
Phenylalanine	3.366	+0.789	16.714	9.13

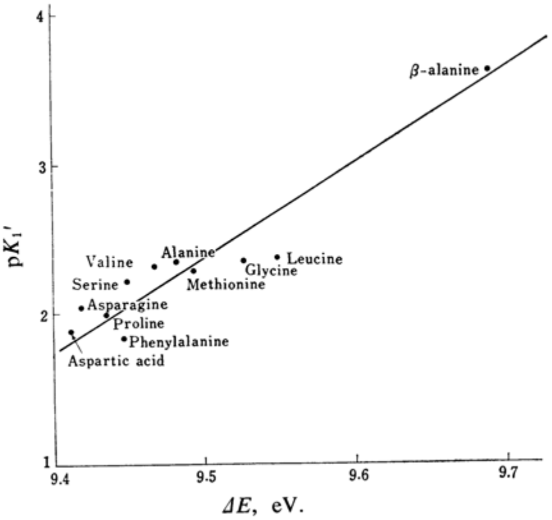

Fig. 2 The dissociation constant $\mathrm{p}K_1'$ vs. $\Delta E\left(\Delta E_\sigma - \sum_\mu \frac{Q_H Q_\mu}{R_{H\mu}} e^2\right)$

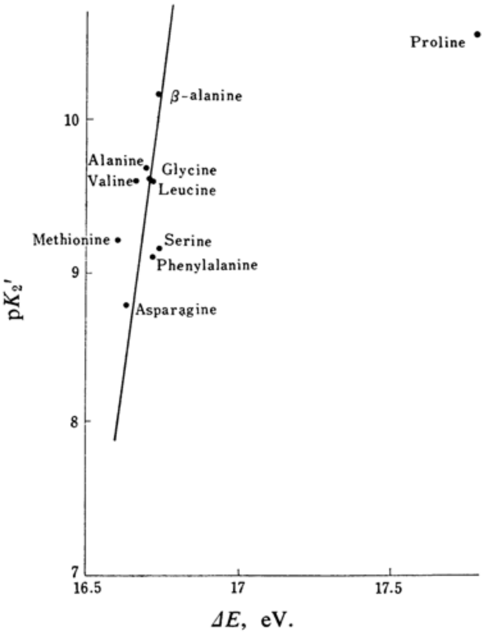

Fig. 3 The dissociation constant $\mathrm{p}K_2$' vs. ΔE.

TABLE III. STATIC REPULSION ENERGY

Dipolar ion	Free rotation $-\sum_{\mu}\frac{Q_{\mu}Q_{\mathrm{H}}}{R_{\mathrm{H}\mu}}e^2$	Hindered rotation $-\sum_{\mu}\frac{Q_{\mathrm{H}}Q_{\mu}}{R_{\mathrm{H}\mu}}e^2$	Hinderd rotation $\varDelta\sum_{\nu>\mu}\frac{Q_{\mathrm{H}}Q_{\mu}}{R_{\mathrm{H}\mu}}e^2$	$+C$
Glycine	−0.799	−0.247	−2.006	−1.759
Alanine	−0.794	−0.363	−2.105	−1.742

fitting of the $\varDelta E$ vs. pK_1 plot by a straight line.

As regards the relationship between the $\varDelta E$'s and the pK_1's, Table I and Fig. 2 show that it corresponds to a straight line as far as could be expected with such drastic simplifications as have been introduced. In Table I, a remarkable contribution of the repulsion term is observed, for instance, in the case of β-alanine. This confirms that one cannot ignore the contributions of the third term of Eq. 4.

In the case of the pK_2's, the agreement between the experimental and theoretical results is less satisfactory (Table II and Fig. 3). For proline, which has the largest pK_2 value, we do obtain the largest calculated value of $\varDelta E$: this value, however, deviates to a large extent from the rough linear relationship observed for the other pK_2's.

It may be useful to consider the influence of some possible refinements in the calculations on these results. Thus, e. g., the present treatment introduces two kinds of approximations in connection with interatomic distances. First of all, the interatomic distances are calculated by means of Eq. 5, assuming that there is free rotation around the bonds; second, the repulsion term is assumed to consist only of those repulsions or attractions which take place between the hydrogen atom which is involved in the dissociation and the other atoms of the initial molecule, to the exclusion of the changes in other repulsion energies (Eq. 8).

As to the first approximation, one may note that, especially in reaction I, the Lewis acid from which the proton dissociates probably has a special steric conformation, so that the free rotation around the various bonds is hindered to some exten. In order to evaluate the changes brought about in the repulsion energy by the assumption of a fixed configuration, let us assign to the dipolar ion of reaction II the configuration shown in Fig. 4. The static repulsion terms, $\sum_{\mu}\frac{Q_{\mathrm{H}}Q_{\mu}e^2}{R_{\mathrm{H}\mu}}$, for this conformation of the glycine and alanine dipolar ions are given in Table III.

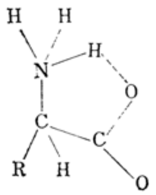

Fig. 4 Assumed conformation in $RNH_3^+COO^-$.

To check the validity of our second assumption, we have also evaluated the variation in the core repulsion energy, $\sum_{\mu>\nu}\frac{Q_{\mu}Q_{\nu}e^2}{R_{\mu\nu}}$, for the same molecules in a similar conformation of the anions, i. e., with a hydrogen bond between the $-COO^-$ group and the NH_2 group. Its values are also given in Table III, together with the values of $\sum_{\mu}\frac{Q_{\mathrm{H}}Q_{\mu}e^2}{R_{\mu\mathrm{H}}}$ corresponding to the freely rotating structure. Table III shows that the values of C (Eq. 8) become −1.759 and −1.742 for glycine and alanine respectively, they are thus approximately the same. These results strongly suggest that the above assumptions do not cause serious errors in connection with dissociation constants, therefore, e. g., the disagreement we have found between calculated and experimental values of the pK_2's may very probably be explained in a different way.

Another dubious point concerns the contribution of the solvation energy to $\varDelta E$, since this contribution is not taken into account in our calculation. No suitable method for estimating the extent of this contribution is available; therefore, we can only estimate it roughly with the help of the Hush-Blackledge and Daudel formula:[7,11]

$$E_s \simeq -\sum_{\mu} Q_{\mu}^2 \tag{9}$$

where the solvation energy, E_s, is considered to be proportional to the sum of the squares of the net charges in the molecule. Formula 9 leads to nearly the same values of $\varDelta E_s$ for all the aminoacids, so that no important improvement of the results can be expected for this correction.

Conclusion

The results reported in the present note seem to make possible another encouraging stop forward in the difficult process of correlating, by means of semi-empirical quantum

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11) N. S. Hush and J. Blackledge, *J. Chem. Phys.*, 23, 514 (1955).

theoretical methods, the properties of α-amino acids. The particularly severe difficulties connected with the treatment of dissociation constants make the deviations from a linear relationship quite expected; therefore, it is gratifying that the overall correlation, especially of the pK_1's, is quite satisfactory.

Improvements should probably be looked for through refinements concerning the solvation effects and possibly through the inclusion of long-range exchange effects.

This work was sponsored by the Convention N° 61-FR-134 of the Délégation Générale à la Recherche Scientifique et Technique of France, and Grant CY-3073 of the United States Public Health Service.

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