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NMR STUDY OF AMINO ACIDS AND THEIR DERIVATIVES: DISSOCIATION CONSTANT OF EACH ROTATIONAL ISOMER OF AMINO ACIDS

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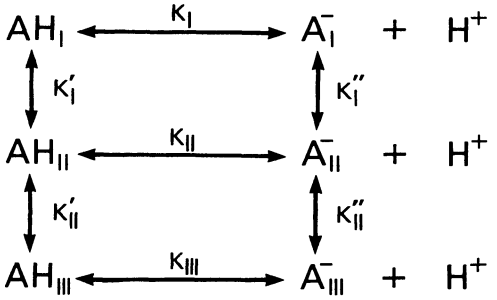
Dissociation constants of each of the three rotational isomers of amino acids, L-serine, L-aspartic acid, L-histidine and L-phenylalanine have been determined by the use of the fractional weights obtained from the NMR coupling constants at several pH values.

Nuclear magnetic resonance (NMR) has been successfully used to determine the fractional weights of the three rotational isomers[1-5] of simple α-amino acids as well as the bulk dissociation constant[6-7], Kd, the dissociation constant of the molecules as a whole. However, no work has ever been done for the evaluation of the dissociation constant of each rotational isomer of molecules. We will report here the result of the determination of the latter constants for several acids. With the consideration of the fact that the dissociation constant is a significant property of molecules, this report will form the first step to study chemical properties of each rotational isomer separately from each other.

The dissociation equilibria of rotational isomers may be written as follows:



Here, K_I, K_{II} and K_{III} are the dissociation constants of the three rotational isomers I, II and III of the staggered forms in Fig. 1, and the K' and K" are the equilibrium constants for the three rotational isomers of AH-form and A^--form, respectively. Whereas, the bulk dissociation constant, Kd, can be presented as follows:

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$$Kd = \frac{([A_I] + [A_{II}] + [A_{III}])\,[H^+]}{[AH_I] + [AH_{II}] + [AH_{III}]}$$

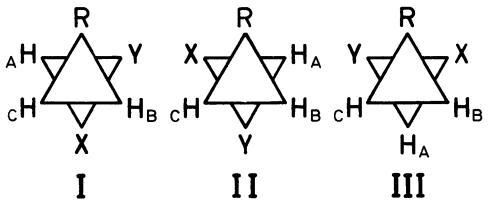

Fig. 1 The three rotational isomers of amino acids. X and Y are the amino and carboxyl groups, respectively. R is the hydroxyl group for serine, the carboxyl group for aspartic acid, the imidazolyl group for histidine and the phenyl group for phenylalanine.

The dissociation constant of each rotational isomer can be evaluated by Kd, K' and K", i.e., each dissociation constant is given by:

$$K_I = \frac{(1 + K'_I + K'_I \cdot K'_{II})}{(1 + K''_I + K''_I \cdot K''_{II})} Kd\,, \quad K_{II} = \frac{(1/(K'_I) + 1 + K'_{II})}{(1/(K''_I) + 1 + K''_{II})} Kd,$$

$$K_{III} = \frac{(1/(K'_I \cdot K'_{II}) + 1/(K'_{II}) + 1)}{(1/(K''_I \cdot K''_{II}) + 1/(K''_{II}) + 1)} Kd.$$

The K' and K" values are calculated from the fractional weights at the extreme ranges of pH, where either AH- or A^--form dominates. Hence, the fractional weights, p_i's, at the extreme ranges of pH are obtained as shown in Table I from the NMR coupling constants as previously described[7-8], where the extreme ranges of pH can be referred to by titration curve of the chemical shift data. Table II shows the difference between the pK value of each rotational isomer and the bulk pK, which is referred to as ΔpK, for L-serine, L-aspartic acid, L-histidine and L-phenylalanine. The negative sign in Table II refers to the fact that the pK value of the rotational isomer is smaller than the bulk pK.

Although the present investigation is preliminary, it still presents some noticeable remarks. For example, in the case when the fractional weight of any one of the rotational isomers increases with the dissociation of bulk molecule ($\Delta p_i/\Delta pH > 0$), the sign of ΔpK is negative. This states that the dissociation constant of the rotational isomer which is more stable in the ionic state than in the neutral form is larger than the bulk Kd.

For example, in the case of L-aspartic acid at pH 8.6, rotational isomer II with two negatively charged carboxyl groups in the trans arrangement is dominant compared with other rotational isomers which hold those two groups in the gauche form. Strong electrostatic repulsion between the two COO^- groups is responsible for the further stabilization of the trans arrangement. Moreover, between the rotational isomers III and I, the former is more stable than the latter as the

result of the electrostatic interaction between the COO^- group and NH_3^+ group. In an alkaline medium (pH 10), however, the electrostatic stabilization of rotational isomer III decreases because of de-protonation of the NH_3^+ group, whereby its fractional weight decreases($\Delta p_{III}/\Delta pH < 0$). Consequently, the dissociation constant of rotational isomer III is smaller than Kd (Table II, ΔpK:0.15).

In the case of L-serine, in an acid medium, rotational isomer III is dominant owing to the intermolecular hydrogen bond formation[8]. In the neutral solution (pH 5.0), however, rotational isomer II is stabilized by the electrostatic interaction between the OH group and NH_3^+ group ($\Delta p_{II}/\Delta pH > 0$), while the fractional weight of rotational isomer III decreases by repulsion between the OH and COO^- groups ($\Delta p_{III}/\Delta pH < 0$). Therefore, K_{II} is larger and K_{III} is smaller than Kd. Thus, an increase of the fractional weight after dissociation gives larger dissociation constants than bulk constants. Contrary, smaller dissociation constants are obtained when $\Delta p_i/\Delta pH < 0$. It may be concluded that the dissociation constant of each rotational isomer closely reflects the change of its fractional weight, i.e., stability of the rotational isomer.

Details of the discussion on the relationship between the dissociation constant of each rotational isomer of amino acids and its conformation in a wider pH range will be published in the near future.

Table I The vicinal spin coupling constants and the fractional wieghts of the three rotational isomers at the extreme range of pH.

	pH	J_{AB}	J_{AC}	p_I	p_{II}	p_{III}
L-Ser [8]	13.9	4.2 Hz	6.1 Hz	0.15	0.32	0.54
	5.0	3.8	5.4	0.11	0.26	0.64
	3M HCl	3.4	4.4	0.07	0.16	0.76
L-Asp [7]	12.2	3.6	9.9	0.09	0.67	0.25
	8.6	3.6	8.8	0.09	0.56	0.35
L-His	11.7	5.2	7.8	0.24	0.47	0.30
	7.7	4.6	8.2	0.18	0.51	0.31
L-Phe	2M NaOH	5.5	7.6	0.27	0.46	0.28
	5.8	5.2	8.0	0.24	0.50	0.27
	2M HCl	5.9	7.0	0.30	0.40	0.30

The accuracy in fractional weight is within $\pm$ 0.02.

Table II Difference of pK value of each rotational isomer from bulk pK value.

		ΔpK*		
		Rotational isomer I	Rotational isomer II	Rotational isomer III
L-Ser	$COOH-COO^-$	-0.18	-0.19	0.08
	$NH_3^+-NH_2$	-0.12	-0.09	0.08
L-Asp	$NH_3^+-NH_2$	0.01	-0.07	0.15
L-His	$NH_3^+-NH_2$	-0.12	0.04	0.02
L-Phe	$COOH-COO^-$	0.05	-0.09	0.06
	$NH_3^+-NH_2$	-0.06	0.03	-0.01

* The accuracy in ΔpK is dependent on that in the fractional weight and varies for the values in Table II between ± 0.05 and ± 0.02.

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