ScienceAsia 29 (2003): **45-49**

Determination of Acidity and Basicity Constants of Polyaspartic Acids by Potentiometric Titrations

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Received 6 Dec 2001
Accepted 1 Jul 2002

ABSTRACT The acidity and basicity constants of asp-asp-asp, asp-asp-asp-asp, asp-asp-asp-asp-asp and asp-asp-asp-asp-asp-asp polypeptides were determined in 0.1 M potassium nitrate at 25 °C by potentiometric titrations. Logarithm of the basicity constants of the asp-asp-asp, asp-asp-asp-asp, asp-asp-asp-asp-asp and asp-asp-asp-asp-asp-asp are 8.34, 8.50, 8.56 and 8.99 respectively. The number of the acidity constants of the polyaspartic acids is equivalent to the number of carboxylic acids in their molecules. The pH at the isoelectric points of the asp-asp-asp, asp-asp-asp-asp, asp-asp-asp-asp-asp and asp-asp-asp-asp-asp-asp in aqueous solution, evaluated from their corresponding acidity constants and the titration data are 3.1, 2.8, 2.7 and 2.4, respectively.

KEYWORDS: polyaspartic acid, potentiometric titration, acidity constant, basicity constant.

INTRODUCTION

The acidity and basicity constants of numerous amino acids have been recorded in many books.[1-3] Accelerated racemization of aspartic acid was studied using quantum chemical calculations[4] and conformational equilibrium of its zwitterions in water was determined by theoretical method.[5] Structural geometry of D,L-aspartic acid in crystal was determined by means of x-ray crystallographic method and *ab initio* calculations.[6] Protonation constants of L-aspartic acid at different ionic strengths were determined using potentiometric techniques.[7] Self-association behavior of polyaspartic acid in water was studied by fluorescence and dynamic light scattering techniques.[8] Various types of polyaspartic acids were prepared, and their biodegradabilities were estimated using the OECD 301C method. Calcium-chelating abilities were measured to clarify the relationship[9] between the structure of polyaspartic acids and these properties.

Determinations of the acidity and basicity constants of many dipeptides were carried out,[10-15] in order to gain better understanding of their biological behavior and to be used in the study of their complexes with interesting metal cations, Furthermore, proteins or peptides and their complexes with a variety of transition-metal cations have been widely studied for their role in biological systems.[16-17] Novel amino acids, which are mostly synthetic compounds and have not been found in nature, were investigated in both aqueous and non-aqueous systems for their physico-chemical properties. In most cases, the terminal nitrogen of the amino acid can be easily protonated and its acidic proton released to form zwitterions in aqueous solution. In contrast, the amino-nitrogen of N-acetyl amino acid can not be protonated in any solution.[10] Aspartic acid contains two acidic protons: one at the end of backbone and the other at the end of the side chain. Aspartylaspartic acid, on the other hand, contains one basicity and three acidity constants.[17]

Our objective was to determine the acidity and basicity constants of the polyaspartic acids and to investigate their physical properties in aqueous solution. The following polyaspartic acids have been selected for this study : asp-asp-asp, asp-asp-asp-asp, asp-asp-asp-asp-asp and asp-asp-asp-asp-asp-asp. Determination of the equilibrium constants of the polyaspartic acids, the sequence of dissociation of their acidic protons and investigation of their structures would enhance our knowledge on their biological behaviors in aqueous system.

EXPERIMENTAL

Chemicals: The asp-asp-asp, asp-asp-asp-asp, asp-asp-asp-asp-asp and asp-asp-asp-asp-asp-asp polypeptides used in the experiments were of HPLC grade, (Sigma). 0.1 M KNO_3 (AR grade, Fluka) was used as a background electrolyte. In the titrations, 0.05 M NaOH in 0.1 M KNO_3, standardized against potassium hydrogen phthalate, was used as a titrant. The standard solutions of 0.05 M HCl in 0.1 M KNO_3 and the titrant of 0.05 M NaOH were used to adjust the pH of the working solution.

Potentiometric Measurements: The automatic titrator, *Mettler DL25* including combined pH electrode of *Mettler*

DG111-SC, was used in the titration. The pH electrode was calibrated with standard pH buffers of pH 4.00 and pH 7.00. Accuracy of the pH measurement was indicated by the slope exceeding 99% based on the isopotential point of pH 7.00 = 0.0 mV. The calibration of pH electrode and all the potentiometric titrations were carried out at 25 ± 0.1°C. The initial volume of each titration was 10 ml. The titrations were handled under ultrapure argon gas, saturated by 0.1 M potassium nitrate vapour, through the titration chamber. Alkali was added from 5 ml dispenser of the automatic titrator which was calibrated by automatic mode based on the standard method. The Initial concentration of the polyaspartic acids in the titration chamber were 5.7 x 10^{-3} M. The ionic strength of the working solution was kept constant at 0.1 M by KNO_3. In the titrations, at least 50 titration data points were recorded. Each titration was repeated at least three times.

Calculations: The acidity and basicity constants of the polyaspartic acids were refined by using the SUPERQUAD program [18] which was performed on a microcomputer. The titration a data obtained from the measurements were used in the optimizing process of SUPERQUAD. The computer refinement of the acidity and basicity constants for each polyaspartic acid, the dissociation constant of water (K_w) was included in the calculations.

Results and Discussion

The equilibrium constants were exhibited from the $(asp)_n$, designated term of the polyaspartic acids of *n* aspartic units, where *n* = 3 to 6, as one of the protonation (basicity) constant and (*n*+1) of the proton dissociation (acidity) constants (Table 1). Therefore, the chemical equilibria of the polyaspartic acids in aqueous solution can be written as following:

For the asp-asp-asp (LH_4),

K_1: $LH_5^+ \rightleftharpoons LH_4 + H^+$ (1)
K_2: $LH_4 \rightleftharpoons LH_3^- + H^+$ (2)
K_3: $LH_3^- \rightleftharpoons LH_2^{2-} + H^+$ (3)
K_4: $LH_2^{2-} \rightleftharpoons LH^{3-} + H^+$ (4)
K_b: $L^{4-} + H^+ \rightleftharpoons LH^{3-}$ (5)

the asp-asp-asp-asp (LH_5),

K_1: $LH_6^+ \rightleftharpoons LH_5 + H^+$ (6)
K_2: $LH_5 \rightleftharpoons LH_4^- + H^+$ (7)
K_3: $LH_4^- \rightleftharpoons LH_3^{2-} + H^+$ (8)
K_4: $LH_3^{2-} \rightleftharpoons LH_2^{3-} + H^+$ (9)
K_5: $LH_2^{3-} \rightleftharpoons LH^{4-} + H^+$ (10)
K_b: $L^{5-} + H^+ \rightleftharpoons LH^{4-}$ (11)

the asp-asp-asp-asp-asp (LH_6),

K_1: $LH_7^+ \rightleftharpoons LH_6 + H^+$ (12)
K_2: $LH_6 \rightleftharpoons LH_5^- + H^+$ (13)
K_3: $LH_5^- \rightleftharpoons LH_4^{2-} + H^+$ (14)
K_4: $LH_4^{2-} \rightleftharpoons LH_3^{3-} + H^+$ (15)
K_5: $LH_3^{2-} \rightleftharpoons LH_2^{4-} + H^+$ (16)
K_6: $LH_2^{4-} \rightleftharpoons LH^{5-} + H^+$ (17)
K_b: $L^{6-} + H^+ \rightleftharpoons LH^{5-}$ (18)

and the asp-asp-asp-asp-asp-asp (LH_7),

K_1: $LH_8^+ \rightleftharpoons LH_7 + H^+$ (19)
K_2: $LH_7 \rightleftharpoons LH_6^- + H^+$ (20)
K_3: $LH_6^- \rightleftharpoons LH_5^{2-} + H^+$ (21)
K_4: $LH_5^{2-} \rightleftharpoons LH_4^{3-} + H^+$ (22)
K_5: $LH_4^{3-} \rightleftharpoons LH_3^{4-} + H^+$ (23)
K_6: $LH_3^{4-} \rightleftharpoons LH_2^{5-} + H^+$ (24)
K_7: $LH_2^{5-} \rightleftharpoons LH^{6-} + H^+$ (25)
K_b: $L^{7-} + H^+ \rightleftharpoons LH^{6-}$ (26)

where K_1, K_2,.....K_{n+1} represent the acidity constants and K_b is the basicity constant of $(asp)_n$. The acidity and basicity constants of the polyaspartic acids obtained in this work and the N-acetylaspartic acid, aspartic acid and aspartylaspartic acid are listed in Table 1.

It is well known that the basicity of the aspartic acids arises from the amino nitrogen of the N-terminal. According to the previous study of the acidity and basicity of N-acetylaspartic acid, aspartic acid and aspartylaspartic acid, it was concluded that the magnitude of the acidity constants of the aspartylaspartic acid is in the order of $K_1 > K_2 > K_3$, corresponding to the order of release of the acidic proton at the positions A, B and C, respectively (Fig 1).

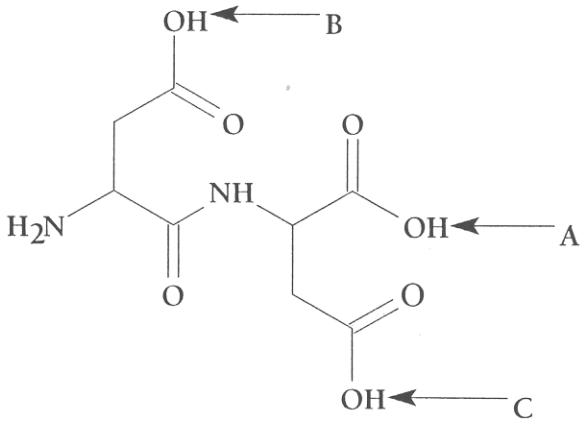


Fig 1. The structural formulae of the aspartylaspartic acid and its acid protons' positions. The order of proton released at the positions A, B and C correspond to the acidity constants of K_1, K_2 and K_3, respectively.

Table 1. Logarithm of the acidity and basicity constants of the N-acetylaspartic acid, aspartic acid, aspartylaspartic acid and polyaspartic acids in 0.1 M KNO_3 at 25 °C.

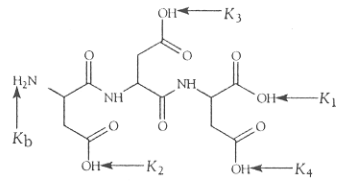
Acids	log K_b	log K_1	log K_2	log K_3	log K_4	log K_5	log K_6	log K_7
N-acetyl asp	-	-3.41 (0.09) [a]	-5.13 (0.04) [a]	-	-	-	-	-
asp	9.80 (0.01) [a]	-2.38 (0.02) [a]	-3.80 (0.01) [a]	-	-	-	-	-
$(asp)_2$	8.79 (0.05) [a]	-3.2 (0.2) [a]	-3.5 (0.2) [a]	-5.4 (0.1) [a]	-	-	-	-
$(asp)_3$	8.34 (0.02)	-3.0 (0.2)	-3.4 (0.1)	-3.86 (0.08)	-5.09 (0.05)	-	-	-
$(asp)_4$	8.50 (0.01)	-3.0 (0.2)	-3.1 (0.1)	-3.9 (0.1)	-4.42 (0.08)	-5.37 (0.05)	-	-
$(asp)_5$	8.56 (0.06)	-3.0 (0.2)	-3.0 (0.2)	-3.5 (0.3)	-4.7 (0.2)	-4.7 (0.1)	-6.1 (0.2)	-
$(asp)_6$	8.99 (0.06)	-3.0 (0.2)	-3.0 (0.2)	-3.0 (0.2)	-3.0 (0.2)	-3.9 (0.2)	-4.5 (0.2)	-6.0 (0.1)

[a] from ref 18.

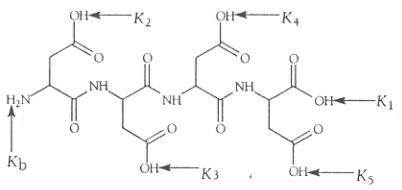
Using the same principle of the assignment of the acidic protons' positions to the acidity constants of the aspartylaspartic acid (Fig 1), the positions of the acidic protons of the $(asp)_3$, $(asp)_4$, $(asp)_5$ and $(asp)_6$ and their corresponding acidity constants have been proposed as depicted in Fig 2.

The number of the acidity constants of the polyaspartic acids of *n* aspartic units, $(asp)_n$ is equal to *n+1* which is equivalent to the number of the carboxylic groups in their molecules. The p*I*, designated term of the pH at the isoelectric point, of the aspartic acid, aspartylaspartic acid, asp-asp-asp, asp-asp-asp-asp, asp-asp-asp-asp-asp and asp-asp-asp-asp-asp-asp and in 0.1M KNO_3 at 25°C are 3.1, 3.3, 3.1, 2.8, 2.7 and 2.4 respectively. We can conclude that the number of the existing species of $(asp)_n$ is *n*+3 and the corresponding species is represented by $LH_{(n+3-i)}^{(i-2)-}$; where i = 1, 2, to (*n*+3) and *n* is the number of the aspartic units. The order of the existing species of $(asp)_n$ with increasing pH, can be written as $LH_{(n+2)}^{+}$, $LH_{(n+1)}$, $LH_{(n)}^{-}$,...$L^{(n+1)}$.

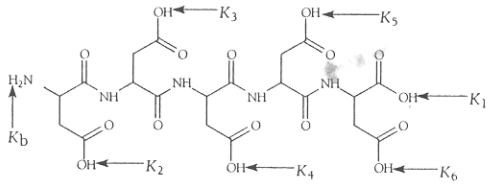
The species distribution of asp-asp-asp (LH_4) in 0.1M KNO_3 at 25°C (Fig 3) shows that the species LH_4, LH_3^- and LH_2^{2-} exist within the pH ranges of 2 to 4.5,



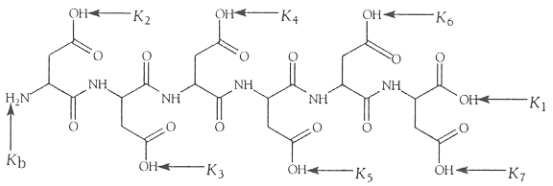
$(asp)_3$



$(asp)_4$



$(asp)_5$



$(asp)_6$

Fig 2. The basicity of the terminal nitrogen and acidity of the carboxylic acids of the $(asp)_3$, $(asp)_4$, $(asp)_5$ and $(asp)_6$ and the proposed sites of their corresponding protons. The equilibrium constants of the $(asp)_3$, $(asp)_4$, $(asp)_5$ and $(asp)_6$ are given in eqs. 1 to 5, 6 to 11, 12 to 18 and 19 to 26, respectively.

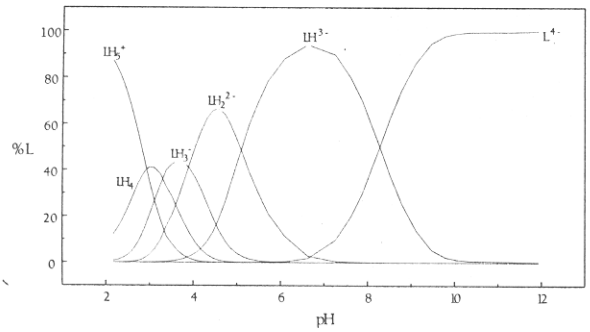


Fig 3. Species distribution curves of $(asp)_3$ in 0.1 M KNO_3 at 25°C with initial concentration of 8.54 x 10^{-4} M

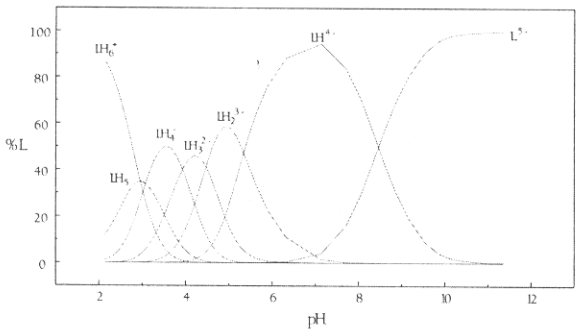


Fig 4. Species distribution curves of $(asp)_4$ in 0.1 M KNO_3 at 25°C with initial concentration of 6.52 x 10^{-4} M.

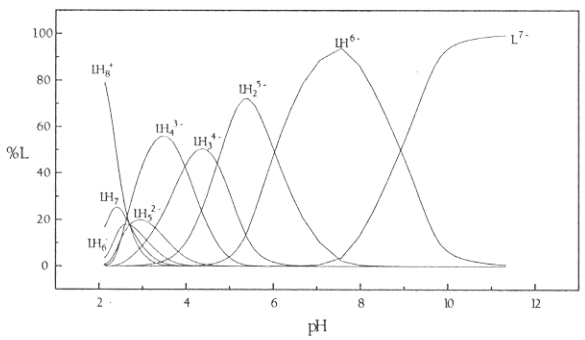


Fig 5. Species distribution curves of $(asp)_5$ in 0.1 M KNO_3 at 25°C with initial concentration of 6.91 x 10^{-4} M.

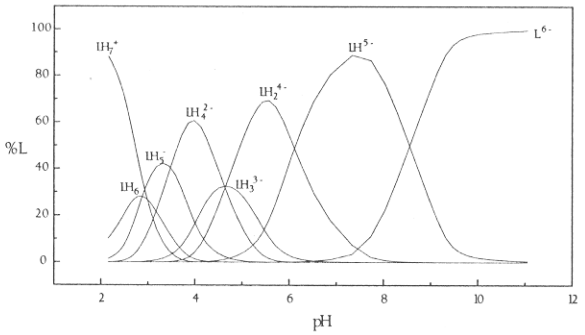


Fig 6. Species distribution curves of $(asp)_6$ in 0.1 M KNO_3 at 25°C with initial concentration of 5.75 x 10^{-4} M.

2.2 to 5.2 and 2.8 to 7.2, respectively. The maximum population of the species LH_4, LH_3^- and LH_2^{2-} located at the pH 3.0, 3.5 and 4.5 are ca. 40 %, 45 % and 65 %, respectively. More than 30% of the compound is present as LH_5^+ at pH below 3. The species LH^{3-} exists within a wide pH range of 4 to 10, and is a predominant species at pH 7. At pH above 10.5, only the species L^{4-} can exist in the solution.

The species distribution of the asp-asp-asp-asp 0.1M KNO_3 in at 25° C (Fig 4) shows that the species LH_5, LH_4^-, LH_3^{2-} and LH_2^{3-} are present within the pH range of 2 to 4.5, 2.2 to 5.1, 2.5 to 5.6 and 3 to 7, respectively. The maximum population of the species LH_5, LH_4^-, LH_3^{2-} and LH_2^{3-} located at the pH 2.9, 3.4, 4.2 and 4.8 are ca. 35%, 50%, 45% and 58%, respectively. More than 30 % of the compound is present as LH_6^+ at pH below 3. The species LH^{4-} exists within a wide pH range of 4 to 10, and is a predominant species at pH 7. At the pH above 10.5, only the species L^{5-} can exist in the solution.

The species distribution of the asp-asp-asp-asp-asp in 0.1M KNO_3 at 25°C (Fig 5) shows that the species LH_6, LH_5^-, LH_4^{2-} and LH_3^{3-} are present within the pH range of 2 to 4.1, 2.0 to 5.0, 2.5 to 5.6 and 3.0 to 6.4, respectively. The maximum population of the species LH_6, LH_5^-, LH_4^{2-} and LH_3^{3-} located at the pH 2.8, 3.3, 4.0 and 4.6 are ca. 30%, 42 %, 61% and 32%, respectively.

More than 80% of the compound is present as LH_7^+ at pH below 2. The species LH_2^{4-} exists within the pH range of 3.5 to 8.1 and constitutes more than 70% at pH 5.5. The species LH^{5-} is present within the pH range of pH 4.5 to 10.5 and its maximum population is located at pH 7.4. The species L^{6-} can exist in the solution at pH above 6.7.

The species distribution of the asp-asp-asp-asp-asp-asp in 0.1M KNO_3 at 25°C (Fig 6) shows that the species LH_7, LH_6^- and LH_5^{2-} are present within the narrow pH range of 2 to 4. The species LH_4^{3-}, LH_3^{4-} and LH_2^{5-} distribute within the pH ranges of 2.0 to 5.5, 2.5 to 6.5 and 3.5 to 8.0, respectively. The maximum peaks of the species LH_4^{3-}, LH_3^{4-} and LH_2^{5-} are located at the pH 3.5, 4.5 and 5.5 at ca. 55%, 53% and 73%, respectively. The species LH_8^+ is present in the solution at pH below 3.4. The species LH^{6-} exists within the pH range of 4.5 to 11.0 and constitutes more than 90 % at pH 7.5. The species L^{7-} can exist in the solution at pH above 7.0.

The relation between the basicity constants (expressed as log K_b), and the number of their aspartic units (n) is plotted (Fig 7). The basicity constants of the aspartic acid (asp) and polyaspartic acids $(asp)_n$, where n = 2 to 6, are 9.80, 8.79, 8.34, 8.50, 8.56 and 8.99, respectively. Note that the log K_b of the $(asp)_3$ is the smallest magnitude.

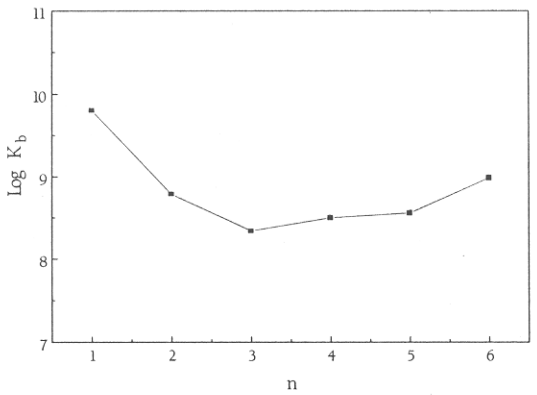


Fig 7. Plot of log K_b of the polyaspartic acids against the number of the aspartic units (n).

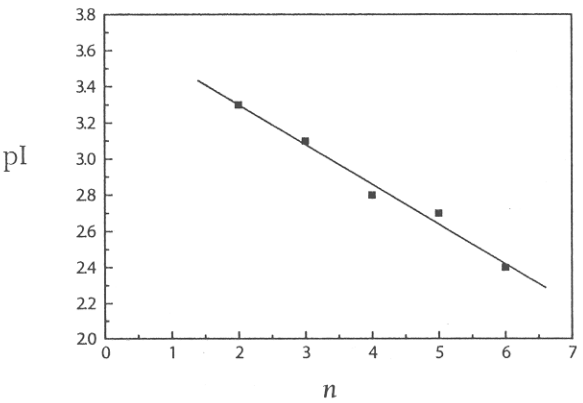


Fig 8. Plot of pI of the polyaspartic acids against the number of the aspartic units (n).

The p*I* of the aqueous solution of the aspartic acid, aspartylaspartic acid , asp-asp-asp, asp-asp-asp-asp, asp-asp-asp-asp-asp and asp-asp-asp-asp-asp-asp in 0.1 M KNO_3 at 25 °C, evaluated from their corresponding acidity constants and their titration data, are 3.1, 3.3, 3.1, 2.8, 2.7 and 2.4 respectively. The plot between the p*I* of the polyaspartic aicds and their corresponding number of the aspartic units is shown in Fig 8.

According to the species distribution curves of all the polyaspartic acids, most of their protonated species are present in the acidic range at the pH below 7. The possibility of complex formation of many amino acids [10-15] is dependent on their binding atoms, charges, molecular structure and the number of the protonated species. Therefore, the polyaspartic acids of the high number of the aspartic units are expected to form stable complex(es) with suitable cations, and exhibit the cation selectivity. The basicity of the polyaspartic acids depends on the number of the aspartic units as indicated by the basicity curve shown in Fig. 7.

The p*I* of the aqueous solution of the aspartic acids in 0.1 M KNO_3 at 25 °C can be calculated using the following equation.

$$pI = 3.75 - 0.225n \quad (27)$$

where *n* is the number of the aspartic units. Equation 27 represents the *pI* of the polyaspartic acids, $(asp)_n$. The standard deviation between the calculated *pI* and the observed values, and its correlation coefficient are 0.0516 and–0.9918, respectively. We can conclude that the number of the aspartic units of the polyaspartic acids affects the number of the exis-ting species. The pH at which the polyaspartic acid is neutral (at the pH = *pI*) decreases as the number of the aspartic units of the polyaspartic acids is increased.

Acknowledgements

This work was supported by the Research Affairs of Chulalongkorn University. We are indebted to the referees of this article for their valuable suggestions.

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