Bull. Chem. Soc. Jpn., ***68***, *787—790(1995)*

Distribution Study on Electroneutral and Protonated Amino Acids between Water and Nitrobenzene. Determination of the Standard Ion-Transfer Potentials of Protonated Amino Acids

Yasuyuki Takeda,* Tatsuya Ezaki, Yoshihiro Kudo, and Hiroaki Matsuda

Department of Chemistry, Faculty of Science, Chiba University, Yayoi-cho, Inage-ku, Chiba 263

(Received October 3, 1994)

In order to determine the standard ion-transfer potentials ($\Delta\phi_{\mathrm{HA}}^{\ominus\prime}$) of protonated amino acids (HA^+) from water to an organic solvent of moderate dielectric constant, new theoretical equations were derived. Various theoretical predictions were verified experimentally by the solvent extraction of D- and L-amino acids with perchloric and nitric acid from water to nitrobenzene at 25 °C. Furthermore, the distribution constants ($K_{\mathrm{D,A}}$) of the electroneutral D- and L-amino acids (A) between water and nitrobenzene were determined at 25 °C. The amino acids were Ala, Val, Ile, Leu, Met, Phe, Thr, Asn, and Gln. The $K_{\mathrm{D,A}}$ values of the D- and L-form of the same amino acid are equal. The same is true for the $\Delta\phi_{\mathrm{HA}}^{\ominus\prime}$ values. The hydrophilicity of A decreases in the following order: Asn>Thr>Gln>Ala>Val>Met>Ile≥Leu>Phe. The same order of decreasing hydrophilicity was also observed for HA^+. All of the A and HA^+ are much more soluble in water than in nitrobenzene. The A is more lipophilic than the corresponding HA^+. Contributions of a methylene group to the distribution Gibbs-free energies of A were compared with those of HA^+.

The transfer behavior of amino acids from water to organic solvents has been studied in order to clarify the effect of the side chains of the amino acids on the solute–solvent interaction.[1] However, no study has been reported concerning the transfer of protonated amino acids from water to polar organic solvents. In an organic solvent of moderate dielectric constant, the following fundamental equilibria must be considered: (1) the dissociation of the protonated amino acids into neutral ones and protons; (2) the association of the protonated amino acids and protons with anions. The system of the liquid–liquid partition of amino acids under very acidic conditions is complicated. It is thus difficult to determine the standard ion-transfer potentials of the protonated amino acids. Upon complexation with particular racemic protonated amino acids, chiral crown ethers show a high optical-resolution ability. The complex-formation constants are necessary for evaluating the optical resolution ability of the chiral crown ethers. The standard ion-transfer potentials of protonated optically active amino acids are required in order to determine the complex-formation constants of chiral crown ethers with the protonated optically active amino acids by the ion-transfer polarographic method.[2]

In this study, new theoretical equations were derived in order to determine the standard ion-transfer potentials of protonated amino acids from water to an organic solvent of moderate dielectric constant. The theoretical predictions were verified experimentally by the solvent extraction of D- and L-amino acids with perchloric and nitric acid from water to nitrobenzene. Moreover, the partition constants of the electroneutral D- and L-amino acids between water and nitrobenzene were determined. The distribution behavior of the protonated and electroneutral amino acids are discussed.

Experimental

Materials. All of the amino acids were purchased from Peptide Institute, Inc., and were used without further purification. Analytical-grade nitrobenzene (NB), $\mathrm{HClO_4}$, $\mathrm{HNO_3}$, and $\mathrm{CsNO_3}$ were obtained from Kanto Chemical Co., Inc. The NB was purified by distillation under reduced pressure. The distillate was washed three times with deionized water prior to use. Sodium perchlorate was an analytical-grade reagent obtained from Merck Japan Ltd. It was recrystallized twice from an acetone–water mixture (volume ratio 1 : 1) and, before use, dried at 145 °C for 24 h in a vacuum oven. Caesium nitrate was recrystallized three times from deionized water and, prior to use, dried at 150 °C for 24 h under reduced pressure.

Distribution Constants of Electroneutral Amino Acids. A 12—16 $\mathrm{cm^3}$ portion of an aqueous solution of an amino acid (1.5×10^{-2}—3.6×10^{-1} M; 1 M=1 $\mathrm{mol\,dm^{-3}}$) and an equal volume of NB in a stoppered glass tube (volume 30 $\mathrm{cm^3}$) were shaken in a thermostated water bath at 25±0.2 °C for 3 h and centrifuged. Extractions were performed at pH 5.5—6.5. After the amino acid in the NB phase was back extracted into water, the amino acid concentration was determined according to a method of Yemm and Cocking.[3]

**Standard Ion-Transfer Potentials of Protonated

Amino Acids. A 12—15 cm^3 portion of an aqueous solution (pH 0.68—1.89) of an amino acid (8.4×10^{-3}—2.7×10^{-1} M) and $HClO_4$ [$(1.0$—$2.2)\times10^{-1}$ M] or HNO_3 [$(1.0$—$2.2)\times10^{-1}$ M] and an equal volume of NB in a stoppered glass tube were agitated in a thermostated water bath at 25±0.2 °C for 3 h and centrifuged. An 8 cm^3 portion of the aqueous phase was transferred to a 10 cm^3 beaker, and the hydrogen ion concentration was determined by a pH meter. The amino acid and the perchlorate ion or the nitrate ion in the NB phase were back extracted into water. The amino acid concentration was determined according to a method of Yemm and Cocking;[3] the concentrations of the perchlorate ion and the nitrate ion were determined by ion chromatography.

Determination of Acid-Association Constants in NB. A 10—12 cm^3 portion of an aqueous solution of perchloric acid or nitric acid [$(1.0$—$2.2)\times10^{-1}$ M] and an equal volume of NB in a stoppered glass tube were shaken under the same conditions as described above, and then centrifuged. After an 8 cm^3 portion of the aqueous phase was transferred to a 10 cm^3 beaker, the hydrogen-ion concentration was determined using a pH meter. The perchlorate ion, or the nitrate ion, in the NB phase was back extracted into water, and its concentration was determined by ion chromatography.

Determination of Association Constants between Amino Acids and H^+ in Water. The association constants of H^+ with the employed amino acids were determined at 25±0.2 °C by acid-base titration. A 100 cm^3 portion of a 10^{-3}—10^{-2} M solution of amino acid with stirring was titrated with 0.1 M HCl under a nitrogen atmosphere. The pH of the solutions was measured using a pH meter. The ionic strength of all the solutions was kept at 0.1 M with NaCl. The thus-obtained titration curves were analyzed by a method similar to that described elsewhere.[4]

Theory

The electrochemical potential of an ionic species (i) in an aqueous ($\overline{\mu}_i$) and an organic phases ($\overline{\mu}_i^o$) is defined as:

$$\overline{\mu}_i = \mu_i^{\ominus} + RT\ \ln\,(f_i\cdot c_i) + z_iF\phi, \tag{1}$$

$$\overline{\mu}_i^o = \mu_i^{\ominus,o} + RT\ \ln\,(f_i^o\cdot c_i^o) + z_iF\phi^o, \tag{2}$$

where $\mu^{\ominus}$, R, T, f, c, z, F, and ϕ denote the standard chemical potential, gas constant, absolute temperature, activity coefficient, molar concentration, electric charge, Faraday constant, and inner potential, respectively; the subscript "i", the superscript "o", and the lack of a superscript refer to an ionic species, an organic, and an aqueous phases, respectively. When an equilibrium is established between aqueous and organic phases containing an ionic species (i), the following equation holds:

$$\overline{\mu}_i^o = \overline{\mu}_i. \tag{3}$$

The combination of Eqs. 1, 2, and 3 leads to

$$c_i^o = c_i\ \exp\,\zeta_i, \tag{4}$$

where it is assumed that both f_i and f_i^o are nearly equal to 1, $\zeta_i = z_iF(\Delta\phi_{eq} - \Delta\phi_i^{\ominus'})/RT$, $\Delta\phi_{eq} = \phi - \phi^o$, and $\Delta\phi_i^{\ominus'} = (\mu_i^{\ominus,o} - \mu_i^{\ominus})/z_iF$, $\Delta\phi_i^{\ominus'}$ being the formal inner standard potential difference of an ionic species.

In equilibrium between NB having a moderate dielectric constant and a very acidic aqueous solution of an amino acid (A) and an acid (HX), the following fundamental equations are defined:

$$K_{D,A} = [A]_o/[A], \tag{5}$$

$$[H^+]_o = [H^+]\ \exp\zeta_H, \tag{6}$$

$$[HA^+]_o = [HA^+]\ \exp\zeta_{HA}, \tag{7}$$

$$[X^-]_o = [X^-]\ \exp\,(-\zeta_X), \tag{8}$$

$$K_{HA} = [HA^+]/[A][H^+], \tag{9}$$

$$K_{HA}^o = [HA^+]_o/[A]_o[H^+]_o, \tag{10}$$

$$K_{HX}^o = [HX]_o/[H^+]_o[X^-]_o, \tag{11}$$

where the subscript "o" of the brackets and the lack of a subscript designate the organic and aqueous phases, respectively. It is assumed that in the aqueous phase both HX and HAX completely dissociate into ions, and that in the organic phase only HAX does. Equations 12 and 13 were derived based on the electroneutrality condition:

$$[H^+]_o + [HA^+]_o = [X^-]_o, \tag{12}$$

$$[H^+] + [HA^+] = [X^-]. \tag{13}$$

From the mass balances,

$$[A]_{aq,t} = [A] + [HA^+], \tag{14}$$

$$[A]_{o,t} = [A]_o + [HA^+]_o, \tag{15}$$

$$[X^-]_{aq,t} = [X^-], \tag{16}$$

$$[X^-]_{o,t} = [X^-]_o + [HX]_o. \tag{17}$$

Here, subscripts "aq" and "t" denote the aqueous phase and the total concentration, respectively. The K_{HA}^o value is calculated using Eqs. 5, 9, 10, 11, 12, 14, 15, and 17.

The combination of Eqs. 6, 7, 8, and 12 leads to

$$\Delta\phi_{eq} = (RT/2F)\ \ln\ [[X^-]\ \exp\ \{(F/RT)\Delta\phi_X^{\ominus'}\}/([H^+] \times\exp\{(-F/RT)\Delta\phi_H^{\ominus'}\} + [HA^+]\exp\{(-F/RT)\Delta\phi_{HA}^{\ominus'}\})]. \tag{18}$$

For the NB/water system at 25 °C, $\Delta\phi_H^{\ominus'} = 0.33_7$ V,[5] $\Delta\phi_{ClO_4}^{\ominus'} = -0.082_8$ V,[5] and $\Delta\phi_{NO_3}^{\ominus'} = -0.28_9$ V.[6] The distribution ratio (D) of the amino acid is represented by

$$D = [A]_{o,t}/[A]_{aq,t}. \tag{19}$$

The combination of Eqs. 5, 7, 9, 13, 14, 15, 16, 18, and 19 leads to

$$a\ [\exp\ \{(-F/RT)\Delta\phi_{HA}^{\ominus'}\}]^2 - b\ \exp\ \{(-F/RT)\Delta\phi_{HA}^{\ominus'}\} - c = 0, \tag{20}$$

Table 1. Equilibrium Constants for Amino Acids and Standard Ion-Transfer Potentials of Protonated Amino Acids at 25 °C[a)]

Amino acid	$\log K_{HA}$	$\log K^{o}_{HA}$ [b)]	$\log K_{D,A}$	$\Delta\phi^{\ominus'}_{HA}$ [b)] / 10^2 mV	$\log K^{o}_{HX}$ [b)]
L-Ala	2.32±0.01	7.04±0.07	−4.200±0.007	3.07±0.04	4.3±0.3
D-Ala	2.32±0.01	7.04±0.03	−4.210±0.006	3.06±0.02	4.3±0.3
L-Val	2.26[d)]	7.03±0.03	−3.67±0.01	2.72±0.01	5.0±0.4
D-Val	2.26±0.01	7.07±0.06	−3.67±0.01	2.70±0.03	4.8±0.3
L-Ile	2.32±0.01	7.19±0.04	−3.10±0.07	2.32±0.02	4.5±0.4
		7.15±0.09[c)]		2.35±0.05[c)]	8.0±0.1[c)]
L-Leu	2.37±0.01	7.18±0.02	−3.01±0.03	2.31±0.01	4.3±0.6
		7.14±0.06[c)]		2.33±0.04[c)]	8.1±0.1[c)]
D-Leu	2.36±0.01	7.16±0.02	−3.01±0.01	2.31±0.01	4.6±0.1
L-Met	2.28±0.01	7.05±0.03	−3.35±0.01	2.53±0.02	4.7±0.1
		7.09±0.04[c)]		2.51±0.03[c)]	8.1±0.3[c)]
D-Met	2.27±0.01	7.05±0.04	−3.35±0.01	2.52±0.02	4.7±0.1
L-Phe	2.25±0.01	7.36±0.02	−2.89±0.01	2.07±0.01	4.8±0.4
		7.44±0.07[c)]		2.02±0.04[c)]	8.2±0.2[c)]
D-Phe	2.26±0.01	7.35±0.02	−2.895±0.006	2.07±0.01	4.8±0.2
L-Thr	2.21[d)]	6.99±0.03	−4.39±0.01	3.14±0.02	4.4±0.2
D-Thr	2.21±0.01	7.00±0.02	−4.398±0.007	3.14±0.01	4.5±0.2
L-Asn	2.14[d)]	6.81±0.03	−4.59±0.01	3.32±0.02	4.3±0.2
D-Asn	2.14±0.01	6.83±0.03	−4.59±0.02	3.32±0.02	4.2±0.1
L-Gln	2.17[d)]	6.85±0.03	−4.27±0.03	3.13±0.02	4.2±0.2
D-Gln	2.17±0.01	6.87±0.03	−4.32±0.01	3.13±0.02	4.1±0.1

a) Each equilibrium-constant value is the average of 18—25 measurements. The uncertainties are the standard deviations. b) Determined in the presence of $HClO_4$. $\Delta\phi^{\ominus'}_{HA}$/V vs. TPh(As/B)E. c) Determined in the presence of HNO_3. $\Delta\phi^{\ominus'}_{HA}$/V vs. TPh(As/B)E. d) L. G. Sillen and A. E. Martell, "Stability Constants of Metal-Ion Complex," Special Publication No. 17, the Chemical Society, London (1964).

where $a=[X^-]_{aq,t} \exp\{(F/RT)\Delta\phi^{\ominus'}_{X}\}$, $b=([X^-]_{aq,t}-[H^+])[\{(1+K_{HA}[H^+])D-K_{D,A}\}/K_{HA}[H^+]]^2$, and $c=[H^+]^{-1}\exp\{(-F/RT)\Delta\phi^{\ominus'}_{H}\}[\{(1+K_{HA}[H^+])D-K_{D,A}\}/K_{HA}]^2$. The $\Delta\phi^{\ominus'}_{HA}$ value is obtained by Eq. 20.

K^{o}_{HX} Determination. In the absence of an amino acid, Eqs. 12 and 13 lead to

$$[H^+]_o=[X^-]_o, \tag{21}$$

$$[H^+]=[X^-]. \tag{22}$$

The combination of Eqs. 6, 8, 21, and 22 leads to

$$\Delta\phi_{eq}=(\Delta\phi^{\ominus'}_{H}+\Delta\phi^{\ominus'}_{X})/2. \tag{23}$$

From the mass balance,

$$[HX]_t=[X^-]+[X^-]_o+[HX]_o. \tag{24}$$

The K^{o}_{HX} value is obtained by Eqs. 6, 8, 11, 22, 23, and 24.

Results and Discussion

For every system in the presence of an amino acid, the experimental $[X^-]_{o,t}$ value was larger than the $[X^-]_o$ value calculated using Eq. 8. This is attributed to the formation of HX in the NB phase.[7)] The K^{o}_{HX} value for each extraction system in the presence of an amino acid was calculated using Eqs. 6, 8, 11, 13, 16, 17, and 18. The $\log K^{o}_{HX}$ values are summarized in Table 1. The $\log K^{o}_{HClO_4}$ and $\log K^{o}_{HNO_3}$ values in Table 1 are nearly equal to $\log K^{o}_{HClO_4}=4.36\pm0.07$ and $\log K^{o}_{HNO_3}=8.08\pm0.06$, respectively, determined in the absence of an amino acid.[8)] The $\log K^{o}_{HA}$ value of the ClO_4^- system is nearly identical with the corresponding $\log K^{o}_{HA}$ value of the NO_3^- system. The same is true for the $\Delta\phi^{\ominus'}_{HA}$ values. This shows that in the aqueous phase HX and HAX completely dissociate into ions, and that in the NB phase the formation of HAX is negligible. The $\log K^{o}_{HX}$ value of NO_3^- is much larger than that of ClO_4^-. This is due to the smaller ionic size of NO_3^- compared with ClO_4^- and the different structure between NO_3^- and ClO_4^-.

The $\log K_{HA}$ value of a D-amino acid is identical to that of the corresponding L-amino acid. The same holds true for the $\log K^{o}_{HA}$ values. Although the $\log K_{HA}$ values were measured directly by acid-base titration, the $\log K^{o}_{HA}$ values were not. It thus appears that the standard deviations for the $\log K_{HA}$ values are smaller than those for the $\log K^{o}_{HA}$ values. Table 1 shows that the $\log K_{HA}$ and $\log K^{o}_{HA}$ values are slightly influenced by the side chains. The association constants of Thr, Asn, and Gln with H^+ are somewhat smaller than those of the others in both water and NB. The range of the association-constant value of A with H^+ slightly increases from water (amphiprotic solvent) to NB (polar aprotic

Table 2. Standard Molar Gibbs Free Energies of Transfer of A ($\Delta G^{\circ}_{\mathrm{D,A}}$) and HA^+ ($\Delta G^{\circ}_{\mathrm{t,HA}}$) from Water to NB at 25 °C

Amino acid	$\Delta G^{\circ}_{\mathrm{D,A}}$ [a] kJ mol^{-1}	$\Delta G^{\circ}_{\mathrm{t,HA}}$ [a] kJ mol^{-1}	$\Delta G^{\circ}_{\mathrm{t,HA}}-\Delta G^{\circ}_{\mathrm{D,A}}$ kJ mol^{-1}
L-Ala	24.0	29.6	5.6
D-Ala	24.0	29.5	5.5
L-Val	20.9	26.2	5.3
D-Val	20.9	26.1	5.2
L-Ile	17.7	22.4	4.7
L-Leu	17.2	22.3	5.1
D-Leu	17.2	22.3	5.1
L-Met	19.1	24.4	5.3
D-Met	19.1	24.3	5.2
L-Phe	16.5	20.0	3.5
D-Phe	16.5	20.0	3.5
L-Thr	25.1	30.3	5.2
D-Thr	25.1	30.3	5.2
L-Asn	26.2	32.0	5.8
D-Asn	26.2	32.0	5.8
L-Gln	24.4	30.2	5.8
D-Gln	24.7	30.2	5.5

a) $\Delta G^{\circ}_{\mathrm{t,HA}}$ and $\Delta G^{\circ}_{\mathrm{D,A}}$ values were calculated according to the equations $\Delta G^{\circ}_{\mathrm{t,HA}}=F\Delta\phi^{\ominus\prime}_{\mathrm{HA}}$ and $\Delta G^{\circ}_{\mathrm{D,A}}=-RT \ln K_{\mathrm{D,A}}$, respectively, F and R being Faraday and gas constants, respectively.

Table 3. Contributions of a Methylene Group to $\Delta G^{\circ}_{\mathrm{D,A}}$ [$\Delta G^{\circ}_{\mathrm{t,CH_2}}$(A)] and $\Delta G^{\circ}_{\mathrm{t,HA}}$ [$\Delta G^{\circ}_{\mathrm{t,CH_2}}$(HA)] at 25 °C

Combination of Amino acids A$_1$	A$_2$	$\Delta G^{\circ}_{\mathrm{t,CH_2}}$ (A) [a] kJ mol^{-1}	$\Delta G^{\circ}_{\mathrm{t,CH_2}}$ (HA) [b] kJ mol^{-1}
L-Leu	L-Val	−3.7	−3.9
D-Leu	D-Val	−3.7	−3.8
L-Ile	L-Val	−3.2	−3.8
L-Gln	L-Asn	−1.8	−1.8
D-Gln	D-Asn	−1.5	−1.8

a) $\Delta G^{\circ}_{\mathrm{t,CH_2}}(\mathrm{A})=\Delta G^{\circ}_{\mathrm{D,A_1}}-\Delta G^{\circ}_{\mathrm{D,A_2}}$. b) $\Delta G^{\circ}_{\mathrm{t,CH_2}}(\mathrm{HA})=\Delta G^{\circ}_{\mathrm{t,HA_1}}-\Delta G^{\circ}_{\mathrm{t,HA_2}}$.

solvent). The $\log K^{\circ}_{\mathrm{HA}}$ value is much larger than the corresponding $\log K_{\mathrm{HA}}$ values. This is due to the much higher dielectric constant of water and the stronger interaction of water with the carboxylate ion and proton compared with NB. The $\log K^{\circ}_{\mathrm{HA}}$ values are smaller than the $\log K^{\circ}_{\mathrm{HNO_3}}$ value. No clear explanation for this can be provided at present.

Distribution Behavior of A and HA^+. The $\log K_{\mathrm{D,A}}$ values of the D- and L-form of the same amino acid are equal. The same is true for the $\Delta\phi^{\ominus\prime}_{\mathrm{HA}}$ values. Tables 1 and 2 show that all A and HA^+ are much more soluble in water than in NB, and that A is more lipophilic than the corresponding HA^+. The $\Delta G^{\circ}_{\mathrm{t,HA}}-\Delta G^{\circ}_{\mathrm{D,A}}$ values given in Table 2 are nearly equal, except for Phe; the values of Phe are somewhat smaller than the others. The hydrophilicity of A decreases in the order Asn>Thr>Gln>Ala>Val>Met>Ile≥Leu>Phe. The same order of decreasing hydrophilicity is also observed for HA^+. This indicates that the carboxylate ion group of A receives a proton and that protons have no effect on the side chain.

The contributions of a methylene group to $\Delta G^{\circ}_{\mathrm{D,A}}$ and $\Delta G^{\circ}_{\mathrm{t,HA}}$ at 25 °C are compiled in Table 3. For combinations of Leu–Val and Ile–Val, the $\Delta G^{\circ}_{\mathrm{t,CH_2}}$(A) and $\Delta G^{\circ}_{\mathrm{t,CH_2}}$(HA) values are nearly equal. The same is true for combinations of Gln–Asn. The $\Delta G^{\circ}_{\mathrm{t,CH_2}}$(A) and $\Delta G^{\circ}_{\mathrm{t,CH_2}}$(HA) values of the former group are about twice as large as those of the latter group. The contribution of a methylene group to the extraction-free energies of tetraalkylammonium iodides from water to NB has been reported to be −2.6 kJ mol^{-1}.[12] This is close to the average value of the former and latter groups.

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