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Estimation of the Molecular Weight of Ions by Isotachophoresis. IV.[1)] Comparison of the Correlations of Mobility with Molecular Volume and with Molecular Weight[2,3)]

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The determination of the molecular volumes of organic substances for which values of specific gravity (d) are not available was investigated. The values of van der Waals volume (V_A: Å^3/molecule) for aliphatic saturated monocarboxylic acids are closer to those of molecular weight per specific gravity (M/d: cm^3/mol) than those of V_W (cm^3/mol). The slope of the correlative equation between V_A and M/d for organic anionic substances is 1.03, but that between V_W and M/d is 0.62.

The values of V_A for organic anionic substances show a good correlation with the absolute ionic mobility (m_0) as well as M/d. The values of V_A for amino acids and dipeptides also showed a better correlation with m_0 than the molecular weight (M). Thus, the values of mobility could be better estimated from the molecular volume than the molecular weight.

Keywords——isotachophoresis; molecular weight estimation; molecular volume; ionic mobility; amino acid isotachophoresis; peptide isotachophoresis; β-lactam antibiotic isotachophoresis

In the previous study,[1)] we reported the correlative equations between the molecular volume, that is, the molecular weight (M) per specific gravity (d), and R_E for organic anionic substances, *i.e.*, $R_E = a + b(M/d)^{2/3}/|Z|$, where R_E is the ratio of the potential gradient of the sample zone to that of the leading zone,[4)] and Z, a and b are the electric charge of ions, and two constants, respectively. According to these equations, the molecular volume or the molecular weight of ions may be estimated from the value of R_E, when the electric charge is known. The values of R_E were more significantly correlated with molecular volume (M/d) than molecular weight (M).[1)]

However, the values of specific gravity (d) of many organic substances are not available in the literature.[5)] Thus, we wished to calculate the values of molecular volume without knowledge of the values of specific gravity (d). This paper describes our studies[2,3)] on the relationship of van der Waals volume (V_A) with the molecular weight per specific gravity (M/d) for organic substances,[2)] on the relationship of the molecular volume (V_A) with the molecular weight (M) for amino acids[2,3)] and dipeptides,[3)] and on the application of these results to β-lactam antibiotics.[3)]

Calculation

The significance of the correlation coefficient (r) was tested by means of the t-test in each case after transformation of r to z. The significance of the difference of r_1 and r_2 was tested by means of the following equation: $z_0 = |z_1 - z_2|/\{1/(N_1 - 3) + 1/(N_2 - 3)\}^{1/2}$.

Table I. The Values of van der Waals Volume (Å^3/molecule)[a] for Some Atoms or Groups

Atom or group	V_A[a]	Atom or group	V_A	Atom or group	V_A
$-OH$	13.35	$-H$[b]	5.73	$-O-$(c.e.)	8.63
$>C=O$	19.43	$>C<$	5.53	$-O-$(a.e.)	6.14
$-COOH$[c]	32.78	$>C<_{H}$	11.26	$-O-$(ph.e.)	5.31
$-NH_2$	17.50	$>CH_2$	16.99	$-S-$	17.93
$>NH$	13.42	$-CH_3$	22.70	$-SH$	24.58
$>N-$	7.19	$=C=$	11.56	$=O$	11.13
$-NO_2$	27.90	$>C=C<$	16.64	$-C\equiv N$	24.41
$\geqslant C-$[d]	7.87	$=C<_{H}$	14.06	$>C=CH_2$	28.15
$\geqslant C-$(alkyl)[e]	9.20	$=CH_2$	19.83	$>C=C<_{H}$	22.40
$\geqslant C-H$[e]	13.38	$-C\equiv$	13.37	$\equiv C-H$	19.18
Phenyl	76.12	Naphthyl	118.64	-F(ph)	9.63
-F(pr)	9.50	-F(s, t)	10.30	-F(p)	9.96
-Cl(pr)	19.30	-Cl(s, t, p)	20.32	-Cl(ph)	19.90
-Br(pr)	23.91	-Br(s, t, p)	24.24	-Br(ph)	25.11
-I(pr)	31.85	-I(s, t, p)	33.79	-I(ph)	32.61

a) The values (cm^3/mol) reported by Bondi[6] were transformed into (Å^3/molecule): 1 cm^3/mol = 1.66 Å^3/molecule. *b)* $(-H)=(>C<_{H})-(>C<)$. *c)* $(-COOH)=(>C=O)+(-OH)$. *d)* Aromatic condensation. *e)* Aromatic. pr, attached to alkane in primary position; s, t, attached to alkane in secondary or tertiary position; p, per- or polyhalide of alkane; ph, attached to phenyl ring. c.e., heterocycloaliphatic ethers; a.e., (poly)alkane ethers; ph.e., (poly)phenyl ethers.

Correlation of van der Waals Volume (V_A) with the Molecular Weight per Specific Gravity (M/d) for Organic Anionic Substances

The values of van der Waals volume (V_A) for organic anionic substances were calculated after transformation of the values (V_W) as cm^3/mol given by Bondi[6] to the values as Å^3/molecule for some atoms or groups, that is, $1\,\text{cm}^3/\text{mol} = 1 \cdot 10^{24}\,\text{Å}^3/6.02 \cdot 10^{23}$ molecules $=$ 1.66 Å^3/molecule. The values of van der Waals volume (Å^3/molecule) for some atoms or groups are listed in Table I. The values of M/d were calculated from those of molecular weight (M) and specific gravity (d) taken from the literature.[5]

Derivation of the Correlative Equations between Molecular Volume (V_A) and the Absolute Mobility (m_0), the Molecular Weight per Specific Gravity (M/d) and m_0, and V_A and R_E for Organic Anionic Substances

The values of V_A were calculated from those in Table I[6] and M/d values were calculated as described previously. Substances of known specific gravity were selected ($N=115$),[5] as described in the previous paper.[1] The values of m_0, R_E and electric charge (Z) at pH_L 10.0 given by Hirokawa *et al.*[4] were used. pH_L is the pH of the leading electrolyte.

Comparison of the Correlative Equation between Molecular Volume (V_A) and the Absolute Mobility (m_0), with That between Molecular Weight (M) and m_0 for Amino Acids

The values of m_0 of amino acids[7] given by Hirokawa *et al.* were used. The values of V_A were calculated from the data in Table I.[6] The values of M were taken from the literature.[5] The mean deviation (MD: %) was calculated by means of the following equation: MD (%) $= 1/N \cdot \Sigma\,|100\{(a+bx)-y\}/y|$, where a and b are the constants of the correlative equations.

Comparison of the Correlative Equation between Molecular Volume (V_A) and the Absolute Mobility (m_0), with That between Molecular Weight (M) and m_0 for Dipeptides

The values of m_0 of dipeptides[8] given by Hirokawa *et al.* were used. The values of V_A were calculated from the data in Table I.[6] The values of M were taken from the literature.[5] The mean deviation (MD: %) was calculated as described previously.

Derivation of the Correlative Equations between Molecular Volume (V_A) and R_E, and V_A and the Absolute Mobility (m_0) for Some β-Lactam Antibiotics

The values of V_A of β-lactam antibiotics were calculated from the data in Table I.[6] The values of R_E were obtained experimentally, as follows. The approximate values of m_0 were calculated from R_E, that is, $R_{E,\min}$ obtained using only one pH_L in the case of maximum electric charge.[1]

Experimental

Values of R_E for Some β-Lactam Antibiotics at pH_L 8.6——Samples and Reagents: The sodium salts of cefalotin (CET, M: molecular weight$=418.41$, $pK_a=3.6\pm0.13$),[9] cefazolin (CEZ, $M=476.49$, $pK_a=2.1$),[9,10] cefapirin (CEPR, $M=445.44$, $pK_a=ca.\ 5.3$),[9,10] cefmetazole (CMZ, $M=493.51$, $pK_a=2.34$)[9,10] and cefotaxime (CTX, $M=477.44$, $pK_a=ca.\ 3.4$)[10] were purchased from Shionogi & Co., Ltd., Fujisawa Pharmaceutical Co., Ltd., Bristol-Myers Co., Sankyo Co., Ltd., and Hoechst Japan Ltd., respectively. Other reagents were purchased from Nakarai Chemicals, Ltd. Hydroxypropyl cellulose (HPC, 1000—4000 cps) was purchased from Tokyo Kasei Kogyo Co., Ltd.

Electrolytes System for Isotachophoresis and Instruments: The leading ion was 0.01 M chloride. The pH of the leading electrolyte (pH_L) was adjusted to 8.6 by using Amediol (2-amino-2-methyl-1,3-propanediol),[4,11] and 0.02% HPC was added as a surfactant in order to suppress electroendosmosis and to increase the sharpeness of the zone boundaries.[7,8] The terminating ion was 0.01 M β-alanine. The pH of the terminating electrolyte (pH 11.4) was adjusted by using $Ba(OH)_2$; Ba^{2+} precipitates with CO_3^{2-}. The migration current was 100 μA. An IP-1B isotachophoretic analyzer with the separating tube of 0.5 mm i.d. and 15 cm length, and a PGD-1 potential gradient detector (Shimadzu Seisakusho Ltd., Kyoto, Japan) were used.[12]

Results and Discussion

Correlation of van der Waals Volume (V_A) with the Molecular Weight per Specific Gravity (M/d) for Organic Anionic Substances

First, the correlation of van der Waals volume (V_A) with the molecular weight per specific gravity (M/d) for aliphatic saturated monocarboxylic acids was investigated. The values of M/d reported by Dorinson *et al.*[13] and those of V_A calculated from van der Waals volume (V_W) by Bondi[6] are listed in Table II. The unit of M/d is cm^3/mol, because the unit of M is g/mol,[14] and the unit of d is g/cm^3.[14] The unit of V_W reported by Bondi[6] is also cm^3/mol. However, as shown in Table II, the values of M/d (cm^3/mol) are different from those of V_W (cm^3/mol), and the values of M/d are fairly close to V_A (Å^3/molecule). The mean deviation MD $(\%)=1/N\cdot\Sigma|100\{V_A-(M/d)\}/(M/d)|=1.75$ (%).

In order to confirm that the values calculated from van der Waals volume (V_A) can

Table II. Molecular Volume for Aliphatic Saturated Monocarboxylic Acids

Acid	M/d[a] (cm^3/mol)	V_W[b] (cm^3/mol)	V_A[c] (Å^3/molecule)	D[d] (%)
Formic	37.71	23.19	38.51	2.12
Acetic	57.21	33.41	55.48	3.02
Propionic	74.55	43.64	72.47	2.79
Butyric	91.93	53.87	89.46	2.69
Valeric	108.69	64.11	106.45	2.06
Caproic	125.04	74.34	123.44	1.28
Enantoic	141.89	84.57	140.43	1.03
Caprylic	158.57	94.80	157.42	0.73
Pelargonic	174.53	105.03	174.41	0.07

a) The values reported by Dorinson *et al.*[13] *b*) The values calculated from van der Waals volumes (V_W) by Bondi.[6] *c*) The values calculated from the values (V_A) in Table I. *d*) The deviation between V_A (Å^3/molecule) and M/d: $D(\%)=|100\{V_A-(M/d)\}/(M/d)|$. The mean deviation MD$=1.75\%$.

substitute for the molecular weight per specific gravity (M/d), the following correlative equation between V_A (A^3/molecule) and M/d (cm^3/mol) for organic anionic substances ($N = 111$)[1)] was derived.

$$V_A = 9.69 + 1.03M/d \quad (r = 0.910, p < 0.001) \tag{1}$$

The significance level (p) shows that the values calculated from van der Waals volume may be used as the molecular volume of organic substances. When the unit of van der Waals volume (V_W) is shown as cm^3/mol, Eq. 1 is as follows ($N = 111$).

$$V_W = 5.84 + 0.62M/d \quad (r = 0.910, p < 0.001) \tag{1'}$$

Of course, the correlation coefficients (r) and the significance level (p) of Eq. 1 and Eq. 1′ are the same, and the constants of Eq. 1′ are 1/1.66 times of those of Eq. 1, because 1 cm^3/mol = 1.66 Å^3/molecule, as mentioned previously. The slope of Eq. 1 is 1.03 and that of Eq. 1′ is 0.62, so Eq. 1 (V_A: Å^3/molecule) is clearly better than Eq. 1′ (V_W: cm^3/mol).

Further, since we are investigating the relationship between the mobility of an ion and the molecular volume of an ion (Å^3/molecule), the unit of Å^3/molecule is suitable.

Derivation of the Correlative Equations between Molecular Volume (V_A) and the Absolute Mobility (m_0), the Molecular Weight per Specific Gravity (M/d) and m_0, and V_A and R_E for Organic Anionic Substances

In the previous study,[1)] we investigated the relationships between the value of R_E, which is defined as the ratio of the potential gradient of the sample zone to that of the leading zone, and the molecular volume (M/d). We used R_E, the ratio of the potential gradients,[4)] the value of which is corrected for the relative step height (h_R) in an isotachopherogram.[15,16)] That is, the molecular volume or molecular weight may be estimated from an isotachopherogram, when the electric charge is known. For example, in the case of $pH_L = 10$ we may employ the maximum electric charge of the sample ion, but in other cases, we should calculate the electric charge (Z) and pH of the sample zone,[1,16)] by using the absolute mobilities (m_0).[4)] The following correlative equations between V_A and m_0 ($10^{-5}\,cm^2\,V^{-1}\,s^{-1}$) and M/d and m_0 for organic anionic substances were derived.

$$m_0 = 8.44 + 572|Z|/V_A{}^{2/3} \quad (r = 0.976, N = 111, p < 0.001) \tag{2}$$

$$m_0 = 12.8 + 452|Z|/(M/d)^{2/3} \quad (r = 0.962, N = 115, p < 0.001) \tag{3}$$

The values of m_0 of divalent and trivalent anionic substances were used in the cases of maximum electric charge, that is, $Z = 2$ and $Z = 3$, respectively.[1,4)]

The exponent of V_A or M/d was 2/3, because the drag ratio is obtained as a function of $V^{2/3}$, that is, 2/3 represents the cross section per the volume of the ion.[1)]

The correlation coefficient (r) in the case of V_A (Eq. 2) is slightly better than that in the case of M/d (Eq. 3). However, the difference was not significant. Thus, the values of the absolute mobility (m_0) may be estimated from either the molecular volume (V_A; Eq. 2) or the molecular weight per specific gravity (M/d; Eq. 3), when the electric charge (Z) is known.

The values of V_A, M/d and m_0 for amino acids for which specific gravity (d) values are available in the literature[5)] are listed in Table III. The values of m_0 calculated from Eq. 2 (V_A) are closer to those reported by Hirokawa *et al.*[7)] than those calculated from Eq. 3 (M/d).

The following correlative equation between V_A and R_E for organic anionic substances was derived.

$$R_E = 0.544 + 0.083V_A{}^{2/3}/|Z| \quad (r = 0.953, N = 111, p < 0.001) \tag{4}$$

When the electric charge is known, the molecular volume for compounds whose specific gravity is not available in the literature[5)] may be estimated from the value of R_E calculated

Table III. Comparison of the Molecular Volume and the Mobility for Some Amino Acids

Amino acid	V_A[a]	M/d[b]	m_0[c]	m_0[d]	m_0[e]
Ala	84.24	62.22	38.2	41.6	32.2
Asn	115.46	85.63	32.6	36.1	31.6
Asp	111.31	80.12	33.2	37.1	30.1
			57.9	61.4	55.4
Glu	128.30	95.66	30.9	34.4	27.0
			53.4	56.0	54.3
Gly	67.27	64.66	43.0	40.9	37.4
Leu	135.19	101.44	30.2	33.6	26.4
Tyr	163.82	124.44	27.5	30.9	20.0
			46.7	49.1	40.0
Val	118.20	95.24	32.2	34.5	28.4

a) The molecular volumes (Å^3/molecule) calculated from the values in Table I. *b*) The molecular volumes (cm^3/mol) calculated from literature values.[5] *c*) The mobilities calculated from Eq. 2: $m_0=8.44+572|Z|/V_A^{2/3}$. *d*) The mobilities calculated from Eq. 3: $m_0=12.8+452|Z|/(M/d)^{2/3}$. *e*) The absolute mobilities reported by Hirokawa *et al.*[7] The units of m_0 (*c*, *d* and *e*) are 10^{-5} cm^2 V^{-1} s^{-1}.

from isotachopherograms. R_E–$\mathrm{pH_L}$ curves for determination of the optimum pH of electrolytes can be obtained according to this equation.[1]

Comparison of the Correlative Equation between Molecular Volume (V_A) and the Absolute Mobility (m_0), with That between Molecular Weight (M) and m_0 for Amino Acids

We reported that molecular volume (M/d) is more significantly correlated with R_E than molecular weight (M) for organic acids.[1,4] In order to confirm that the molecular volume (V_A) also correlates well with the absolute mobility (m_0) for amino acids, the following correlative equations between V_A and m_0 for amino acids[7] were derived. The exponent of V_A was 2/3, as discussed previously.[1]

$$m_0=7.4+530.4|Z|/V_A^{2/3} \quad (r=0.955,\ \mathrm{MD}=6.2\%,\ N=22) \tag{5}$$

$$m_0=6.2+546.7|Z|/V_A^{2/3} \quad (r=0.950,\ \mathrm{MD}=7.0\%,\ N=26) \tag{6}$$

Equation 5 was derived using the values of m_0 of divalent amino acids ($N=4$, Asp, Cys, Glu, Tyr) in the case of $Z=2$, and Eq. 6 was derived using the values of m_0 of those in the case of $Z=1$ and $Z=2$.[7] The difference between these equations was not regarded as significant. However, Hirokawa *et al.* reported that the correlation coefficient (r) between the mobilities and $M^{1/2}$ for amino acids ($N=22$, $Z=1$) was only 0.69,[8] significantly different from that of Eq. 5 (or Eq. 6, $p<0.01$). Hirokawa *et al.* obtained $r=0.82$ except for Cys, Tau and Tyr ($N=19$),[8] but the difference from Eq. 5 (or Eq. 6) is still significant (Eq. 5, $p<0.05$; Eq. 6, $p<0.06$).

Figure 1 shows the relationship between the exponent (n) of molecular weight (M) or molecular volume (V_A) for amino acids, and (A) the values of the correlation coefficient (r), and (B) the mean deviation (MD: %). The best exponent of V_A was not 2/3 in Fig. 1, but we used 2/3 as the exponent of V_A in any case, because it represents the cross section per the volume of the ion, as discussed previously.[1]

On the whole, Fig. 1 shows that the correlation coefficient (r) between the absolute mobility (m_0) and V_A is better than that between m_0 and M, and shows that MD (%) in the case of V_A is smaller than in the case of M.

Comparison of the Correlative Equation between Molecular Volume (V_A) and the Absolute Mobility (m_0), with That between Molecular Weight (M) and m_0 for Dipeptides

The following correlative equations between V_A and m_0 for dipeptides were derived. The

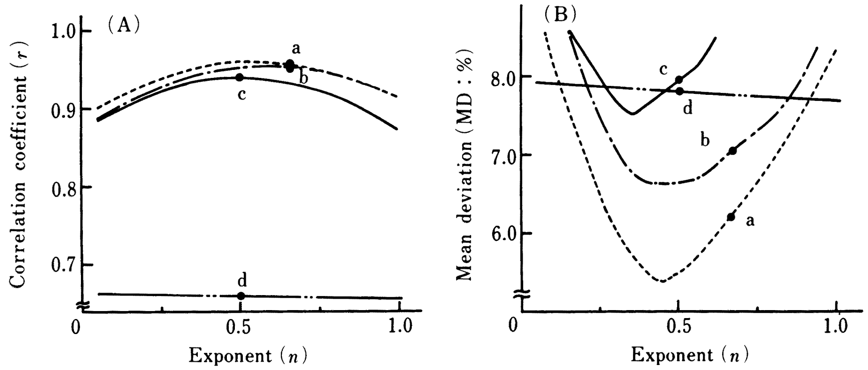

Fig. 1. Relationships between the Exponent (n) of Molecular Weight or Molecular Volume for Amino Acids and (A) the Correlation Coefficient (r), and (B) Mean Deviation (MD: %)

a) $n=2/3$, $m_0=7.4+530.4|Z|/V_A^{2/3}$ ($r=0.955$, MD$=6.2\%$, $N=22$, Eq. 5), b) $n=2/3$, $m_0=6.2+546.7|Z|/V_A^{2/3}$ ($r=0.950$, MD$=7.0\%$, $N=26$, Eq. 6), c) $n=1/2$, $m_0=4.4+278.5|Z|/M^{1/2}$ ($r=0.938$, MD$=8.0\%$, $N=26$), d) $n=1/2$, $m_0=7.5+244.2|Z|/M^{1/2}$ ($r=0.659$, MD$=7.8\%$, $N=22$) where m_0 ($10^{-5}\,\mathrm{cm^2\,V^{-1}\,s^{-1}}$) is the absolute mobility of amino acid[7] and Z is the electric charge. The values of the m_0 of divalent amino acids ($N=4$, Asp, Cys, Glu, Tyr) were used as follows. In the cases of molecular volume (V_A): ---- and a, $Z=2$ ($N=22$); —-— and b, $Z=1$ and $Z=2$ ($N=26$). In the cases of molecular weight (M): —— and c, $Z=1$ and $Z=2$ ($N=26$); —--— and d, $Z=1$ ($N=22$). The difference between a (or b, c) and d is significant ($p<0.01$).

exponent of V_A was taken as 2/3, as described previously.[1]

$$m_0=7.6+570.5|Z|/V_A^{2/3} \qquad (r=0.985,\ \mathrm{MD}=1.9\%,\ N=28) \qquad (7)$$

$$m_0=6.4+602.9|Z|/V_A^{2/3} \qquad (r=0.968,\ \mathrm{MD}=3.0\%,\ N=30) \qquad (8)$$

Equation 7 was derived using the values of m_0 of divalent dipeptides ($N=2$, Gly–Tyr, Ley–Tyr) in the case of $Z=2$, and Eq. 8 was derived using the values of the m_0 in the cases of $Z=1$ and $Z=2$.[8] The difference between these equations was not regarded as significant. Hirokawa *et al.*[8] reported that the correlation coefficient (r) between the mobilities and $M^{1/2}$ for monovalent dipeptides except for Gly–Tyr and Ley–Tyr ($N=26$) was 0.94. The difference between the correlation coefficient (r) reported by Hirokawa *et al.* and that of Eq. 8 was not significant, though in the case of Eq. 7 there was a significant difference ($p<0.03$).

Figure 2 shows the relationship between the exponent (n) of molecular weight (M) or molecular volume (V_A) for dipeptides, and (A) the values of the correlation coefficient (r), and (B) the mean deviation (MD: %). The best exponent of V_A was near 2/3 in Fig. 2, probably because the influence of the drag coefficients (C_D)[1] may be small. The correlation coefficient (r) between the molecular volume ($V_A^{2/3}$) of the dipeptides of Ala and the absolute mobilities (m_0), is better ($r=0.982$) than for other constituent amino acids ($r=0.930$, $N=9$), and r between $V_A^{2/3}$ of the dipeptides of Gly and m_0 is better ($r=0.963$) than that of the other amino acids ($r=0.911$, $N=13$). Further, r between $V_A^{2/3}$ of the dipeptides of Leu and m_0 is better ($r=0.986$) than that of the other constituent amino acids ($r=0.962$, $N=5$).

On the whole, Fig. 2 also shows that the correlation coefficient (r) between the absolute mobility (m_0) and molecular volume (V_A) is better than that between m_0 and molecular weight (M), and shows that MD (%) in the case of V_A is smaller than in the case of M.

When the values of the m_0 of amino acids or dipeptides are taken for $Z=1$, r and MD (%) are almost independent of the exponent (n) of M ($0<n<1$). The values of MD (%) of amino acids are smaller than 7% (Fig. 1), and those of dipeptides are smaller than 4% (Fig. 2).

The absolute mobilities (m_0) for dipeptides[8] are invariably smaller than those of the

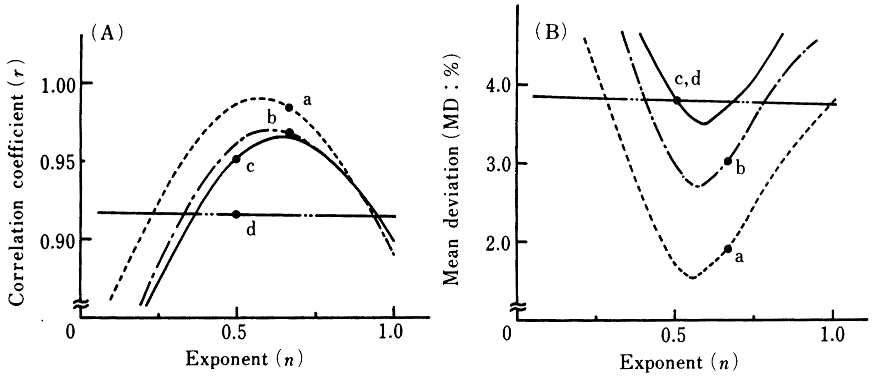

Fig. 2. Relationships between the Exponent (n) of Molecular Weight or Molecular Volume for Dipeptides and (A) the Correlation Coefficient (r), and (B) Mean Deviation (MD: %)

a) $n=2/3$, $m_0=7.6+570.5|Z|/V_A^{2/3}$ ($r=0.985$, MD$=1.9$%, $N=28$, Eq. 7), b) $n=2/3$, $m_0=6.4+602.9|Z|/V_A^{2/3}$ ($r=0.968$, MD$=3.0$%, $N=30$, Eq. 8), c) $n=1/2$, $m_0=5.5+272.9|Z|/M^{1/2}$ ($r=0.952$, MD$=3.8$%, $N=30$), d) $n=1/2$, $m_0=-0.9+362.3|Z|/M^{1/2}$ ($r=0.917$, MD$=3.8$%, $N=28$) where m_0 ($10^{-5}\,\text{cm}^2\,\text{V}^{-1}\,\text{s}^{-1}$) is the absolute mobility of dipeptide8) and Z is the electric charge. The values of m_0 of divalent dipeptides ($N=2$, Gly–Tyr, Ley–Tyr) were used as follows. In the cases of molecular volume (V_A): ---- and a, $Z=2$ ($N=28$); —-— and b, $Z=1$ and $Z=2$ ($N=30$). In the cases of molecular weight (M), —— and c, $Z=1$ and $Z=2$ ($N=30$); —--— and d, $Z=1$ ($N=28$). The differences between a and d ($p<0.01$), and between a and c ($p<0.05$) are significant.

constituent amino acids,7) and the values of molecular volume (V_A) or the molecular weight (M) of dipeptides are naturally larger than those of the constituent amino acids. When the electric charge is constant, M or V_A is inversely proportional to m_0 and proportional to R_E.

Derivation of the Correlative Equation between Molecular Volume (V_A) and R_E, and V_A and the Absolute Mobility (m_0) for Some β-Lactam Antibiotics

Figure 3 shows the observed isotachopherogram of a β-lactam antibiotic, cefotaxime (CTX). The value of R_E of CTX was calculated from the isotachopherogram, and those of other β-lactam antibiotics were obtained similarly. The values of R_E, V_A and m_0 for some β-lactam antibiotics are listed in Table IV. The values of m_0 calculated from R_E are close to those calculated from Eq. 5.

The following correlative equation between V_A and R_E for some β-lactam antibiotics was derived.

$$R_E=-0.08+0.092V_A^{2/3}/|Z| \quad (r=0.939,\ N=5) \tag{9}$$

The optimum pH of electrolytes for the estimation of the molecular volume or for the separation of β-lactam antibiotics may be determined from the R_E-pH$_L$ curves obtained on the basis of this equation.1)

The following correlative equation between V_A and m_0 for some β-lactam antibiotics was derived.

$$m_0=0.1+867.6|Z|/V_A^{2/3} \quad (r=0.932,\ N=5) \tag{10}$$

β-Lactam antibiotics consist of cysteine and valine.17) Then, the values of m_0 of these amino acids were added to those of the β-lactam antibiotics, and the following correlative equations were derived.

$$m_0=7.6+508.6|Z|/V_A^{2/3} \quad (r=0.999,\ N=7) \tag{11}$$

$$m_0=7.4+499.8|Z|/V_A^{2/3} \quad (r=0.994,\ N=8) \tag{12}$$

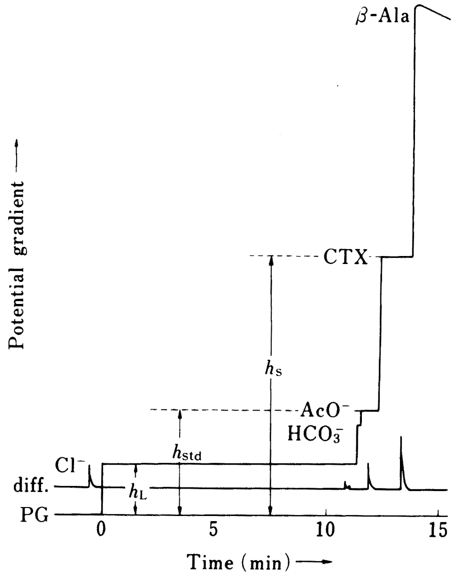

Fig. 3. The Isotachopherogram of a β-Lactam Antibiotic, Cefotaxime (CTX)

The value of R_E of CTX was calculated according to the following equations[4]: $R_E=E_s/E_L=(h_s+\Delta h)/(h_L+\Delta h)$, $\Delta h=[h_{std}-h_L R_E(\mathrm{std})]/[R_E(\mathrm{std})-1]$, $R_E(\mathrm{std})=E_{std}/E_L=m_L/m_{std}=79.08/42.4=1.865$. R_E is the ratio of the potential gradient of the sample zone (E_s) to that of the leading zone (E_L), h_s is the observed step height of the sample zone (CTX), h_L and h_{std} are the apparent step height of the leading zone (Cl^-) and that of the zone of the internal standard (acetic acid), and m is the absolute mobility.[4] The relative step height (h_R) was defined as h_s/h_L.[15,16]

Table IV. Some Values for β-Lactam Antibiotics

Antibiotic	$R_E\pm$S.D.[a]	V_A[b]	m_0[c]	m_0[d]
CET	4.09±0.14 ($N=4$)	308.64	19.3	19.0
CEZ	4.37±0.21 ($N=3$)	326.58	18.1	18.6
CEPR	4.34±0.40 ($N=4$)	329.21	18.2	18.5
CTX	4.38±0.05 ($N=4$)	344.01	18.1	18.2
CMZ	4.55±0.10 ($N=5$)	354.13	17.4	18.0

a) The experimental values. *b*) The molecular volumes calculated from the values in Table I. *c*) The mobilities calculated from R_E: $m_0 \fallingdotseq m_{0,\mathrm{Cl}}/R_{E,\mathrm{min}}=79.08/R_{E,\mathrm{min}}$. *d*) The mobilities calculated from Eq. 5: $m_0=7.4+530.4|Z|/V_A^{2/3}$.

Equation 11 was derived using the value of the m_0 of Cys in the case of $Z=2$, and Eq. 12 was derived using the values of m_0 in the cases of $Z=1$ and $Z=2$.[7] The difference between these equations was not regarded as significant, but there were significant differences between the correlation coefficients of Eq. 10 and Eq. 11 (or Eq. 12, $p<0.01$).

As described previously, the values of m_0 of dipeptides are invariably smaller than those of the constituent amino acids, that is, the zones of dipeptides in the isotachopherogram invariably follow those of the constituent amino acids, when the electric charge is constant. Further studies are in progress.

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