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Random-Coiled Conformation of Polypeptide Chains II. Experimentally Evaluated Characteristic Ratio of Poly(N^5-2-hydroxyethyl-L-glutamine) and Theoretical Conformational Analysis of Poly(L-glutamine) and Poly(L-glutamic acid)

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ABSTRACT: Random-coiled conformations of fractionated poly(N^5-2-hydroxyethyl-L-glutamine) were experimentally investigated and the characteristic ratio was obtained by the Stockmayer–Fixman equation with the experimental results for intrinsic viscosity and weight-averaged molecular weight. Moreover, random-coiled conformations of poly(L-glutamine) and poly(L-glutamic acid) were theoretically analyzed by conformational energy calculations based on intra-residue interactions, and the calculated characteristic ratio was in good agreement with the experimental results.

KEY WORDS Random-Coiled Conformation / Characteristic Ratio / Intrinsic Viscosity / Ultracentrifugation / ECEPP / Conformational Energy Calculation / Poly(N^5-2-hydroxyethyl-L-glutamine) / Poly(L-glutamine) / Poly(L-glutamic acid) /

The characteristic ratio, a basic property of random-coiled polymer chains in dilute solutions, provides information on local conformations of polymer chains. The values of side-chain derivatives of poly(L-glutamic acid) and poly(L-lysine), treated as alanine-type polypeptide chains,[1,2] were experimentally investigated.[3-13] The experimental values of 6 to 10 distribute over a somewhat wide range, due to experimental difficulties, polydispersity of the samples and uncertainty of the universal constant Φ_0[14,15] to calculate the characteristic ratio from viscosity data. Experimental difficulties are concerned with the following points. 1) Synthetic polypeptides have the tendency to take helical conformations stabilized by favorable intra-molecular hydrogen bonds and to cause molecular aggregations with intramolecular hydrogen bonds in common solvents which are good solvents for normal synthetic polymers. 2) A random-coiled conformation can exist only in a limited solvent system[16,17] in which intra- and inter-molecular hydrogen bonds of polypeptides are broken. 3) The Θ-state[14] of synthetic polypeptides has not been found yet because of helical conformations due to favorable short-range interactions such as backbone/backbone hydrogen bond; *i.e.*, short-range interactions are favorable compared to long-range interactions and polypeptide/solvent interactions in the Θ-state.

Such relations as the Stockmayer–Fixman,[18] Orofino–Flory,[19,20] and Flory–Fox,[21] used for evaluation of the characteristic ratio from viscosity data are derived for the monodisperse polymer systems. However, many characteristics ratio[3,7,9,10,13] have been evaluated from data on unfractionated samples. Moreover, several values [(2.1—2.6)×

10^{21}] have been used as the universal constant Φ_0[14] in the Stockmayer–Fixman, Orofino–Flory, and Flory–Fox equations. This ambiguity causes uncertainty in the characteristic ratio by as much as 5% to 20%.

Using the β-methylene group approximation, the characteristic ratio of polypeptide chains composed of alanine-type residues were theoretically investigated,[1,2,22] and 9.27[2] and 8.38[22] were obtained. These values are slightly higher than those experimentally obtained.[4-11] As shown in the previous paper,[23] a more precise analysis in which all intraresidue interactions including side-chain/backbone interactions and freedom of internal rotations of the side chain are considered, gives 11.24 and 12.33 for poly(L-phenylalanine) and poly(L-tyrosine), respectively, and the latter value was very close to that 12.3[24] of poly(*O*-benzyloxycarbonyl-L-tyrosine). These results indicate that side-chain/backbone interactions are very important to stabilize local informations of polypeptide chains even though they are not composed of β-branched residues. It should be of interest to make a precise analysis of alanine-type polypeptide chains such as poly(L-glutamine) and poly(L-glutamic acid), considering the dependence of side-chain/backbone interactions on backbone conformations.

In this paper, poly(N^5-2-hydroxyethyl-L-glutamine), which takes on a random-coil conformation[12] in water at room temperature, was synthesized as a model polypeptide of poly(L-glutamine), and its characteristic ratio was evaluated. Moreover, theoretical analyses of poly(L-glutamic acid) and poly(L-glutamine) were made, considering the side-chain/backbone interactions and freedom of χ^1, χ^2, and χ^3 in the side-chain conformations.

EXPERIMENTAL

Materials

The N-carboxy anhydrides (NCA) of γ-benzyl-L-glutamate (BLG) were prepared[25] by introducing phosgene gas into suspensions of BLG in tetrahydrofuran (THF). BLG-NCA was purified by repeated recrystallization from ethyl acetate solution with the addition of petroleum benzene. BLG-NCA was dissolved in the mixture of dry methylene dichloride and dioxane (volume ratio 1:2), and polymerized at room temperature in the presence of triethylamine as the initiator ([M]/[I] = 50). After polymerization for 3 days at room temperature, poly(γ-benzyl-L-glutamate) (PBLG) was precipitated in excess cold methyl alcohol, filtered and dried under reduced pressure.

PBLG was aminolyzed through a reaction with 2-amino-1-ethanol for 24 h at 55°C,[26] and dialyzed against five times the volume water of the reactant solution using a CU-8000 tube with a 1 mμ pore. After dialysis, poly(N^5-2-hydroxyethyl-L-glutamine) (PHEG) was precipitated from an aqueous solution into acetone. The precipitated PHEG sample was separated into 6 fractions with the water–dioxane system, and each was freeze-dried. Completion of aminolysis was confirmed by the disappearence of absorption spectra of the benzyl-group at 257 nm, using a Hitachi Model ESP-3T Spectrometer.

Measurements

Viscosity measurements were performed in 0.05 M sodium phosphate buffer at pH 7.4 and 25.0°C with an imposed Ubbelohde-type viscometer having a flow time for the solvent longer than 100 s.

The molecular weight of the samples was determined from equilibrium runs on polymer solutions by a MOM Type-3170 B ultracentrifuge. As the solvent, 0.05 M sodium phosphate buffer was used. Solution concentrations from 0.05 to 0.20 (g dl^{-1}) were used in the sedimentation equilibrium runs.

Optical rotatory dispersion (ORD) measurements were carried out with a JASCO J-20 Recording Spectropolarimeter at 25°C. The concentration of the polymer solution was 1.0 g dl^{-1} throughout the measurements to

cancel out the effects of polymer concentration on optical properties. The Moffit–Yang parameter $-b_0$ was determined by the Moffit–Yang procedure.[27]

THEORETICAL

The nomenclature and conventions adopted were those recommended by an IUPAC-IUB nomenclature commission.[28] Assumptions and definitions used in this work corresponded to those in the previous work.[23] Conformational energy $E_i(\phi_i, \psi_i, \chi_i)$ of residue i was calculated for a model single residue peptide with two blocking end groups, acetyl- and *N*-methylamide- (*i.e.*, Ac-X-NHMe). All interactions in this model peptide are referred to as intra-residue interactions. The validity of this treatment is discussed in the paper I.[23]

Conformational energy calculations were carried out for Ac-L-Gln-NHMe and Ac-L-Glu-NHMe with ECEPP[29] using the standard geometry for bond lengths and bond angles, except that the C^{α}–H bond length was increased from 1.00 to 1.09 Å.[30,31] The backbone conformations ϕ and ψ, and two side-chain dihedral angles χ^1 and χ^2 were changed at 15° intervals. χ^3 was changed at 30° intervals, and χ^4 of Gln and $\chi^{4.2}$ of Glu were fixed at 180°. As shown by Zimmerman *et al.*,[32] Ac-Gln-NHMe has 69 energy minima with $\Delta E < 3$ kcal mol^{-1} and all their χ^4 are nearly 180° ± 4°; also, Ac-L-Glu-NHMe has 60 energy minima with $\Delta E < 3$ kcal mol^{-1} and all their $\chi^{4.2}$ are almost 180° ± 2° except that only two energy minima have $\chi^{4.2} = 24°$ ($\Delta E = 1.82$ kcal mol^{-1}) and $-26°$ ($\Delta E = 2.14$ kcal mol^{-1}). Their results suggest that fixing χ^4 of Gln and $\chi^{4.2}$ of Glu at 180° has minor effects on the stability of backbone conformations with intra-residue interactions, since the contributions of these conformations to the partition function (eq I-1[33]) are negligible. All other dihedral angles of backbone were fixed at 180°.

Table I. Molecular characterization of fractionated PHEG samples

Sample code	$M_w \times 10^{-4}$ [a]	M_z/M_w [a]	$[\eta]$ / dl g^{-1} [b]	$-b_0$ [b]
PHEG1	0.86	1.24	0.093	
PHEG2	1.36	1.16	0.125	
PHEG3	2.53	1.15	0.184	0
PHEG4	4.47	1.18	0.264	
PHEG5	6.69	1.45	0.355	
PHEG6	14.3	1.80	0.572	0

[a] Determined by a MOM ultracentrifuge 3170-b.
[b] 0.05 M sodium phosphate buffer, pH 7.4, 25°C.

RESULTS

Experimental Evaluation of the Characteristic Ratio

The weight-average molecular weight M_w, degree of polydispersity M_z/M_w, intrinsic viscosity $[\eta]$, and Moffit–Yang parameter $-b_0$ are summarized in Table I. The degree of polydispersity M_z/M_w is relatively small, except for the last two fractions, PHEG5 and PHEG6. The value $-b_0 = 0$ indicates that PHEG takes on a random-coil conformation in 0.05 M sodium-phosphate buffer at pH 7.4 and 25°C. Figure 1 shows a double logarithmic plot of $[\eta]$ *versus* M_w for PHEG in 0.05 M sodium-phosphate buffer at pH 7.4 and 25°C.

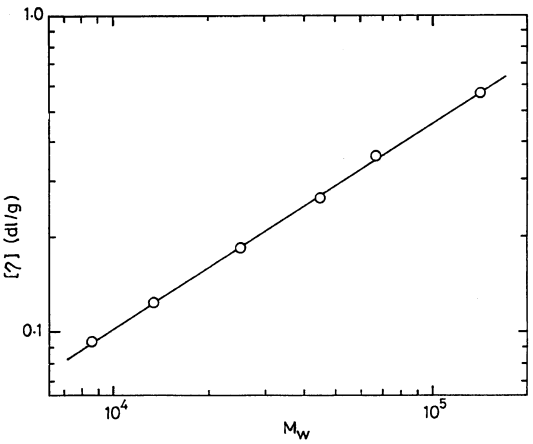


Figure 1. Double-logarithmic plot of $[\eta]$ *vs.* M_w for PHEG in 0.05 M sodium phosphate buffer at pH 7.4 and 25°C.

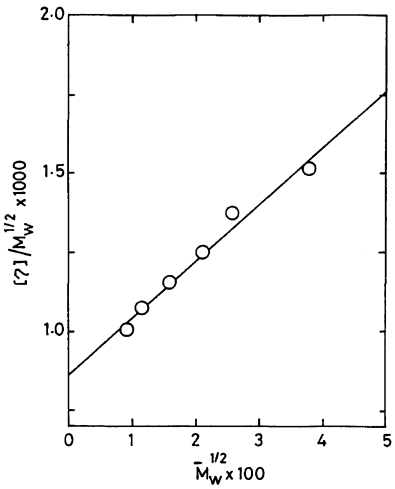


Figure 2. $[\eta]/M_w^{1/2}$ plotted against $M_w^{1/2}$ for PHEG.

The slope of the plot 0.65 also indicates that PHEG takes on a random-coil conformation in this medium. The straight line in Figure 1 leads to the following Mark–Houwink–Sakurada equation.

$$[\eta]=2.62\times10^{-4}M_w^{0.65}$$
$$0.86\times10^4<M_w<14.3\times10^4 \qquad (1)$$

The characteristic ratio was estimated in accordance with the Stockmayer–Fixman equation[18]

$$[\eta]/M_w^{1/2}=K+(3/2)\Pi^{3/2}C\Phi_0BM_w^{1/2} \qquad (2)$$

where M_w is the weight-averaged molecular weight, Φ_0, a hydrodynamic constant assigned as $\Phi_0=2.5\times10^{21.15}$, B, a parameter related to excluded volume, and C, a constant. $[\eta]/M_w^{1/2}$ plotted against $M_w^{1/2}$, the intercept of straight line on the ordinate gives K, from which the characteristic ratio $\langle R^2\rangle_{0,\infty}/nl^2$, is estimated by the equation

$$\langle R^2\rangle_{0,\infty}/nl^2=(K/\Phi_0)^{2/3}(M_0/l^2) \qquad (3)$$

where, $\langle R^2\rangle_{0,\infty}$ is the mean square end-to-end length (cm^2), l ($=3.8\times10^{-8}$ cm),[34] the distance between successive α-carbon atom, n, the degree of polymerization, and M_0 ($=192$), the molecular weight of monomer residue. In Figure 2, $[\eta]/M_w^{1/2}$ is plotted against $M_w^{1/2}$. Thus,

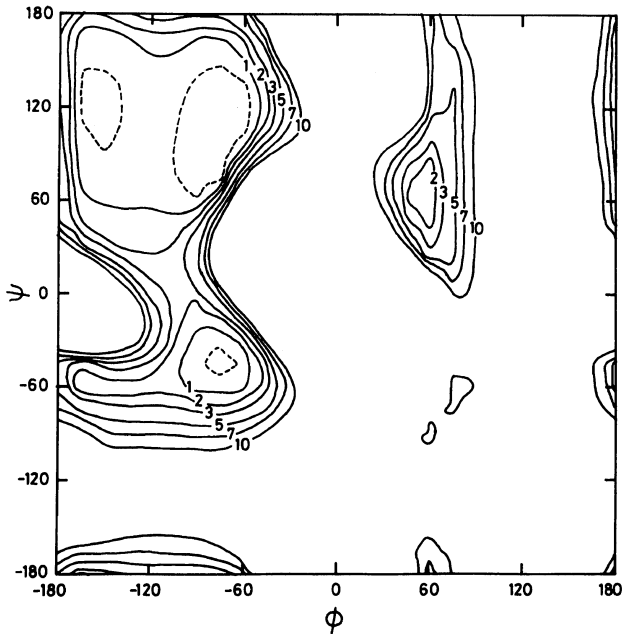
$$K=0.865\times10^{-3}$$
$$\langle R^2\rangle_{0,\infty}/nl^2=6.6$$

are obtained.

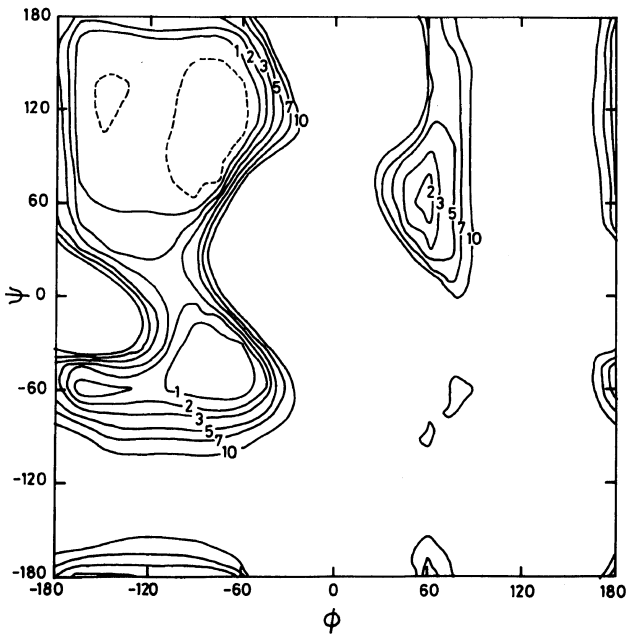
Theoretical Evaluation of the Characteristics Ratio

As shown in paper I, the effects of side-chain conformations (χ^1 and χ^2) on the stability of backbone conformations are very significant (see Figure 4 in paper I). The side chains of L-Gln and L-Glu residues have two more freedom of internal rotation than those of the L-Phe residue treated in paper I, *i.e.*, χ^3 and χ^4 for L-Gln, and χ^3 and $\chi^{4.2}$ for L-Glu. As mentioned in Theoretical section, it is reasonable to fix χ^4 and $\chi^{4.2}$ at 180°. The theoretical results for single residue minima[32] of L-Gln and L-Glu suggest that to the broad distribution of χ^3, it should not be fixed at a specified value. Typical energy contour (ϕ,ψ) maps of the L-Gln residue averaged over the whole (χ^1,χ^2) space with specified χ^3 are shown in Figure 3, and those of the L-Glu residue in Figure 4. These figures indicate that χ^3 dependency of the backbone conformations is not as significant as the χ^1 and χ^2 dependencies. The contour lines of 1 kcal/mol for the L-Gln and L-Glu residues have nearly the same shape. However, in regions with $\Delta E<0.5$ kcal mol^{-1}, where the major contribution is by the partition function, the contour lines (dashed) show the dependence on χ^3. Table II gives the calculated characteristic ratios $\langle R^2\rangle_{0,\infty}/nl^2$, averaged over the whole $(\phi,\psi,\chi^1,\chi^2)$ space at specified χ^3, and the normalized statistical weights u_j for the jth χ^3 value are obtained by the following equation.

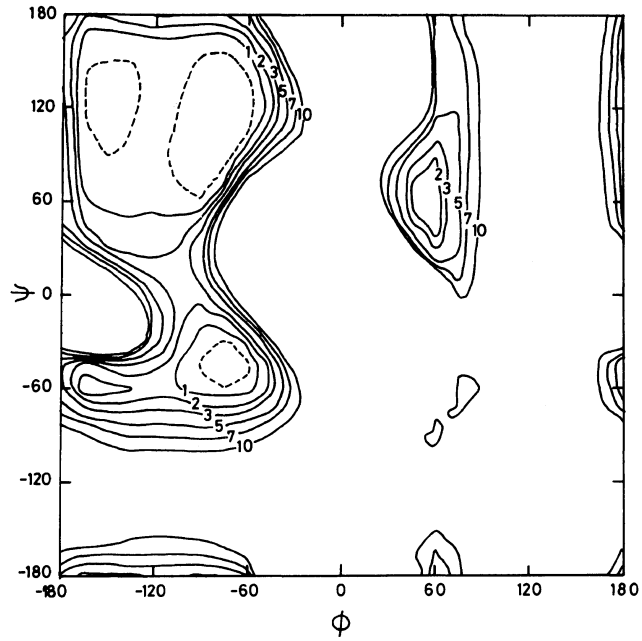
$$u_j=Z_i^{-1}\int\int\int\int\exp(-\beta E_i(\phi,\psi,\chi^1,\chi^2,\chi_j^3))\,d\phi\,d\psi\,d\chi^1\,d\chi^2 \qquad (4)$$



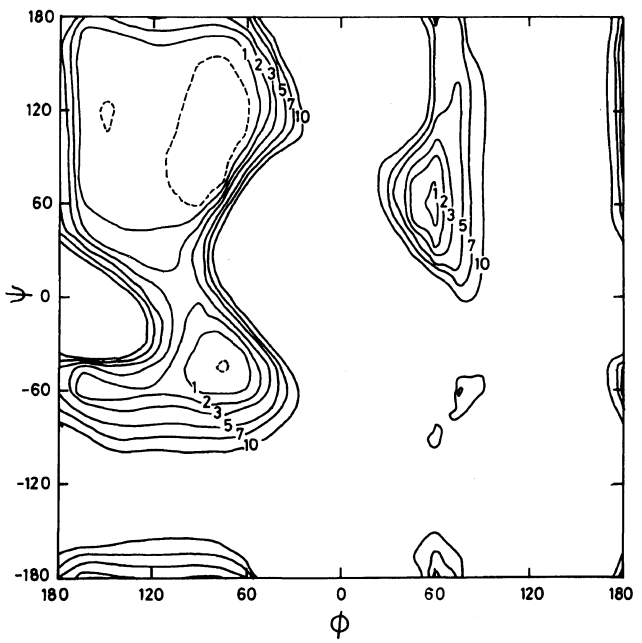
(a) $\chi^3 = -90°$ and $\chi^4 = 180°$



(b) $\chi^3 = 30°$ and $\chi^4 = 180°$

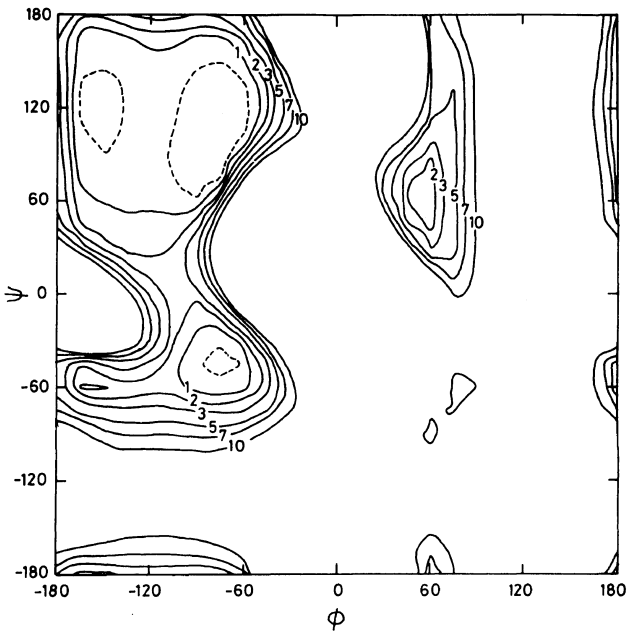


(c) $\chi^3 = 90°$ and $\chi^4 = 180°$

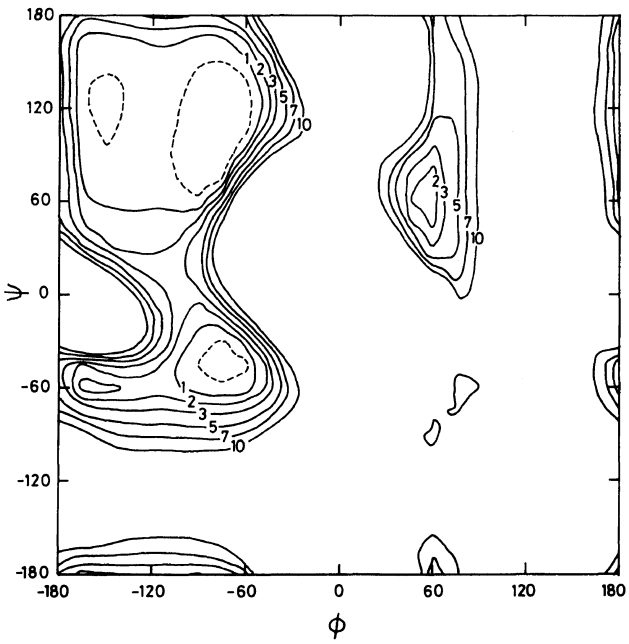


(d) $\chi^3 = 150°$ and $\chi^4 = 180°$

Figure 3. Energy contour (ϕ, ψ) maps of the L-Gln residue averaged over the (χ^1, χ^2) conformational sapce at 15° intervals. The contour lines are labeled as energy in kcal mol^{-1} above the minimum energy point. The dashed lines indicate the 0.5 kcal mol^{-1} energy contour lines.



(a) $\chi^3 = -90°$ and $\chi^{4,2} = 180°$



(b) $\chi^3 = 30°$ and $\chi^{4,2} = 180°$

Figure 4. Energy contour (ϕ, ψ) maps of the L-Glu residue averaged over the (χ^1, χ^2) conformational sapce at 15° intervals.

Table II. Characteristic ratios of poly(L-glutamine) and poly(L-glutamic acid) for a specified side-chain dihedral angle χ^3

χ^3	Poly(L-glutamine)[a]		Poly(L-glutamic acid)[b]	
	u_j	$\langle R^2\rangle_{0,\infty}/nl^2$	u_j	$\langle R^2\rangle_{0,\infty}/nl^2$
−180	0.000	8.32	0.012	7.51
−150	0.000	8.02	0.031	7.48
−120	0.034	8.18	0.126	8.09
−90	0.282	7.72	0.163	7.84
−60	0.062	6.85	0.120	7.40
−30	0.099	6.27	0.054	7.23
0	0.147	5.77	0.032	6.87
30	0.096	5.51	0.055	6.69
60	0.056	5.59	0.127	7.23
90	0.202	6.59	0.149	7.48
120	0.022	7.97	0.104	7.68
150	0.000	8.79	0.027	7.72

[a] χ^4 fixed at 180°.
[b] $\chi^{4,2}$ fixed at 180°.

where Z_i is defined by eq I-1. These results indicate that fixing χ^3 at a specified value does not bring about drastic effects on the characteristic ratio, in contrast to the case of fixing χ^1 and χ^2 (see Table I of paper I), though the difference in the maximum and minimum values for the L-Gln residue (8.79 and 5.51) exceeds the range of experimental uncertainty (±10%). Figure 5 shows the energy contour (ϕ,ψ) maps of L-Gln and L-Glu residues averaged over the whole (χ^1,χ^2,χ^3) space at 15° intervals for χ^1 and χ^2, and 30° intervals for χ^3 with fixed values of $\chi^4=180°$ and $\chi^{4,2}=180°$. Comparing the maps with the (ϕ,ψ) contour map of the L-Ala residue, it is evident that the E and D conformations are destabilized, and the A conformation stabilized by side-chain/backbone interactions. With the results of conformational energies averaged on the side-chain conformations, the transformation matrices of poly(L-glutamine) and poly(L-glutamic acid) are obtained as

$$\langle T\rangle_{\text{L-Gln}}=\begin{bmatrix} 0.328 & -0.112 & 0.610 \\ +0.194 & -0.554 & -0.065 \\ 0.766 & -0.233 & -0.245 \end{bmatrix} \quad (5)$$

$$\langle T\rangle_{\text{L-Glu}}=\begin{bmatrix} 0.329 & -0.112 & 0.654 \\ -0.204 & -0.591 & -0.066 \\ 0.764 & -0.234 & -0.251 \end{bmatrix}$$

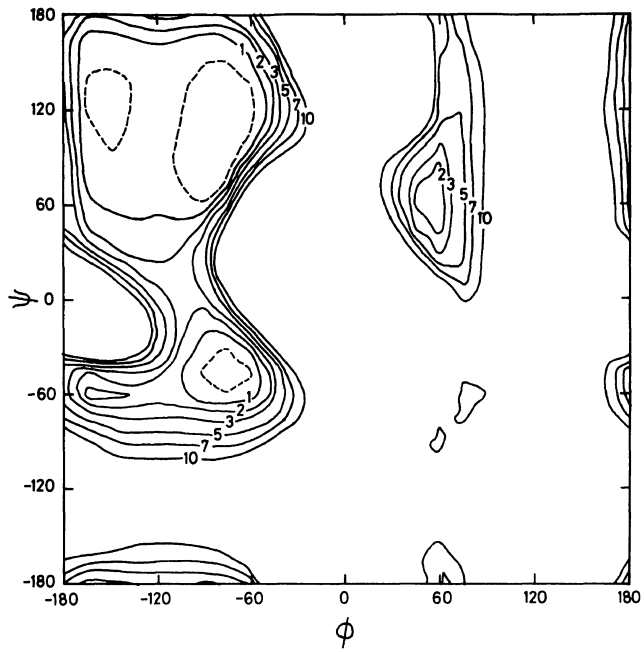
The calculated characteristic ratios of poly(L-glutamine) and poly(L-glutamic acid) are 6.62 and 7.51, respectively.

DISCUSSION

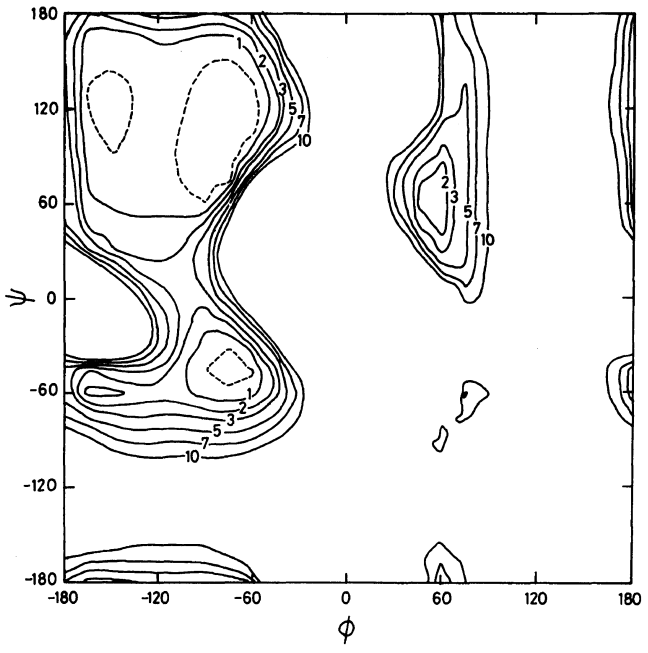
Experimentally and theoretically evaluated characteristic ratios, which have been published, are listed in Tables III and IV, respectively. Our experimental results (6.6) of PHEG correspond to those of Miyake *et al.* (6.2), estimated from the viscosity data[12,35] in H_2O at 25°C, using eq 2 and 3. Mattice and Lo[13] also estimated the characteristic ratio of PHEG (10) experimentally in H_2O at 30°C. The exponent a in the Mark–Houwink–Sakurada equation,

$$[\eta]=KM^a \quad (7)$$

evaluated from Figure 2 in ref 13 is 0.35.[36] This value is much lower than that expected from the dilute polymer solution theory.[15] The PHEG samples may have broad molecular-weight distributions, or molecular weights underestimated or intrinsic viscosities overestimated. Miyake *et al.*'s results, $a=0.8$ in H_2O at 25°C and our results, $a=0.65$ in 0.05 sodium phosphate buffer at pH 7.4 and 25°C are reasonable in the light of the dilute polymer solution theory.[15] Moreover, Miyake *et al.*'s results $\langle R^2\rangle_{0,\infty}/nl^2=6.2$ and ours, $\langle R^2\rangle_{0,\infty}/nl^2=6.6$, were evaluated by eq 2 and 3, but Mattice and Lo's results, $\langle R^2\rangle_{0,\infty}/nl^2=10$ were evaluated by the Orofino–Flory[19,20] and Flory–Fox[20] equations which would be inapplicafle to a sample having broad molecular-weight distribution. Thus, ours and Miyake *et al.*'s results are more reliable than those of Mattice and Lo. The values, 6.6 and 6.2, are compatible to the theoretical result, 6.62, of poly(L-glutamine).



(a)



(b)

Figure 5. Energy contour (ϕ, ψ) maps of L-Gln (a) and L-Glu (b) residues averaged over all side-chain conformations at 15° intervals for χ^1 and χ^2, 30° intervals for χ^3, with $\chi^4 = 180°$ and $\chi^{4,2} = 180°$.

Table III. Experimentally evaluated characteristic ratios

Sample	$\langle R^2 \rangle_{0,\infty}/nl^2$			Reference
PHEG	6.6	SF[a]	f[c]	This work
	6.2	SF	f	12, 35
	10	OFF[b]	u[d]	13
PBLG[e]	8.2	SF	f	8
	7.7	SF	f	11
	7.5	SF	u	6
	5.9	SF	u	3
PMLG[f]	7.7	SF	f	11
	7.7	SF	u	10
PELG[g]	6.9	SF	u	7
PPLG[h]	6.2	SF	u	9
PLG-Na[i]	8.8	OFF	u	4
	6.5	OFF	u	5
	7.6	OFF	u	5

[a] Evaluated by the Stockmayer–Fixman equation.
[b] Evaluated by the Orofino–Flory and Flory–Fox equations.
[c] Fractionated samples.
[d] Unfractionated samples.
[e] Poly(γ-benzyl-L-glutamate).
[f] Poly(γ-methyl-L-glutamate).
[g] Poly(γ-ethyl-L-glutamate).
[h] Poly(γ-phenacyl-L-glutamate).
[i] Na-Poly(L-glutamic acid).

Table IV. Theoretically evaluated characteristic ratios

Polypeptide	Oka *et al.*	Tanaka *et al.*	Flory *et al.*
Gln	6.62		
Glu	7.51		
Ala	8.15[a]	8.38[b]	9.27[c]
Gly	2.15[a]	2.17[b]	2.16[c]
Phe	11.24[a]		
Tyr	12.33[a]		

[a] From ref 23.
[b] From ref 22.
[c] From ref 2.

As shown in Table III, the experimentally evaluated characteristic ratio of various side-chain substituents of poly(L-glutamic acid) are distributed in the range of 5.9 to 8.8. As mentioned in the Introduction section, this wide distribution of characteristic ratios was due to the polydispersity of the polypeptide samples, ambiguity of the universal constant Φ_0, and experimental difficulties. 8 of the values were obtained for unfractionated samples and show broad distribution in the range 5.9 to 8.8. But three values obtained for the well fractionated samples were approximately the same as 8.2[8,37] and 7.7[11] for PBLG, and 7.7[11] for PMLG. The average value was 7.9, and that of all 11 values from Table III, 7.7. These values are close to the theoretical results, 7.51, of poly(L-glutamic acid) within experimental error. The theoretical results for poly(L-glutamine) and poly(L-glutamic acid) were obtained by exact calculations, considering all atom-atom pair interactions in the backbone and side chain. However, the atomic groups of the side-chain derivative were not considered in our theoretical calculations. It is thus considered that the experimental results should deviate from those theoretically obtained if additional interactions between substituent groups and other residue parts are important. Fairly good agreement was obtained between experimental and theoretical results. Moreover, there was no appreciable difference among the five substituents of poly(L-glutamic acid). Thus group substitution in the side chain may not have any significant effect on the characteristic ratios of poly(L-glutamine) and poly(L-glutamic acid).

In our calculations, only the intra-residue interactions were considered and not inter-residue interactions. Both of inter-residue interactions, particularly side-chain/side-chain interactions, and intra-residue interactions were important for the conformational stability of oligopeptides.[38,39] The good agreement between experimental and theoretical results indicates that inter-residue interactions have only minor effects on the characteristic ratios of poly(L-glutamine) and poly(L-glutamic acid). This is consistent with the calculation of the characteristic ratio of poly(L-phenylalanine)[23] and theoretical conformational analysis of di- and tripeptides of L-phenylalanine.[40]

Theoretical results summarized in Table IV indicate that the intra-residue side-chain/backbone interactions of poly(L-glutamine) and poly(L-glutamic acid) lessen the characteristic ratio but those of poly(L-phenylalanine) and poly(L-tyrosine) enhance it; that is, the overall stability of the backbone conformation depends on side-chain groups beyond the β-carbon atom.

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33. Equation 1 in the previous paper (paper I) is abbreviated as eq I-1, and so on.
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36. $a=0.35$ is more compatible to Figure 2 of ref 13 than $a=0.55$ which mentioned in ref 13.
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