Macrocyclization Equilibria of Polypeptides

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Abstract: An elaborated form of the Jacobsen–Stockmayer theory of cyclization equilibria is used to calculate the cyclization constants K_x for the x-membered peptide sequences poly(D-Ala-L-Ala) (I), poly(D-Pro-L-Ala) (II), poly(Gly) (III), poly(L-Pro-L-Ala) (IV), poly(D-Ala-D-Ala-L-Ala-L-Ala) (V), and poly(L-Ala) (VI). Cyclics with 6–20 amino acid residues are considered, the peptide bond being in the trans planar position. According to this theory, $K_x = W(\mathbf{0})[2\Gamma_0(1)]/\sigma_{cx}N_A$, where $W(\mathbf{r})$ is the probability density function for the chain vector $\mathbf{r}$; $\Gamma_0(\gamma)$ is the probability distribution, when $\mathbf{r} = \mathbf{0}$, of $\gamma \equiv \cos \Delta\theta$, $\Delta\theta$ being the angle between a hypothetical bond $(3x + 1)$ and bond 1; σ_{cx} corresponds to the symmetry number of the ring; N_A is Avogadro's number. The evaluation of $W(\mathbf{r})$ is performed in different approximations. The required configurational averages for computing the density distribution $W(\mathbf{r})$ and the angular correlation factor $\Gamma_0(1)$ are obtained by Monte Carlo techniques. The statistical weights for discrete values of φ_i and ψ_i for the individual amino acid residues were obtained by conformational energy calculations using semiempirical potential functions. From this theory, the formation of rings is predicted to be very unfavorable for VI, whereas sequence I has a high ring closure probability. The other sequences have intermediate K_x values and show decreasing tendency to cyclize in going from II to VI. K_x decreases with the number of residues in the ring. Strong deviations of $W(\mathbf{r})$ from Gaussian distribution are indicated, except for the racemic sequences I and II. The deviations depress the value of K_x considerably. Similarly, the angular correlation is found to be unfavorable in nearly all cases. As a general result, for chains which take on extended conformations as expressed in a large characteristic ratio C_x and persistence vector $\mathbf{a}$, the formation of a ring is difficult. By taking poly(Gly) as standard, the cyclization constants K_x for $x = 10$ (20) are found to be 4 (5), 1.02 (1.7), 0.09 (0.08), 0.017 (0.06), and 0.01 (0.01) times the value for poly(Gly) for sequences I, II, IV, V, and VI, respectively. According to these results, the formation of cyclics should be easy for the regularly alternating D,L sequence and for chains containing the flexible Gly residue, whereas sequences of the *all*-L-Ala type are expected to have a negligible tendency to cyclize. These predictions are shown to be in good qualitative agreement with observation. From these results, the density distribution $W(\mathbf{0})$ of the end-to-end distance r and the angular correlation of chain ends are major factors in determining K_x.

Cyclic peptides are of particular interest because many biologically active peptides are cyclic compounds. Furthermore, recent investigations have shown that the formation of cyclic peptides in the polymerization of amino acid N-carboxylic anhydrides is a common feature.[1] Despite the fact that the conformation of several biologically important cyclic peptides has been elucidated by experimental and theoretical methods,[2,3] no systematic investigations have been performed concerning the equilibrium concentration of cyclic species with linear peptide chains. The equilibrium concentration reflects the propensity of linear chain molecules to undergo a cyclization reaction. Those investigations have impact not only in predicting the equilibrium concentration of cyclic rings but also in delineating the structural features of various peptide sequences in respect to the tendency of polypeptides to form a ring, and provide a theoretical basis for the conception of synthesis of cyclic compounds.

In order to undergo a cyclization step, two requirements are necessary. (i) Atom $3x - 1$ in Figure 1 must be situated at a distance from atom 0 equal to the length of the anticipated bond $3x$ (joining atom $3x - 1$ and $3x$), i.e. vector r must be $\mathbf{0}$. (ii) The direction of the bond to be formed, $3x$, must yield acceptable bond angles at atoms $3x - 1$ and 0. The former requirement is taken into account by the classical Jacobson–Stockmayer theory, the latter in recent theory.[4]

In a series of three papers, we calculated the equilibrium constants K_x of poly(dimethylsiloxane)[5] and poly(6-aminocaproamide),[6] applying an elaborated form of the Jacobson–Stockmayer theory of macrocyclization equilibria.[4] The new theory includes the angular correlation of chain ends, expressed as a correlation factor $2\Gamma_0(1)$, and gives special attention to the evaluation of the density distribution for chains with intermediate size, where the deviation from Gaussian distribution can be large. Angular correlations and deviations from Gaussian distribution were major factors in improving the classical theory of Jacobson and Stockmayer.[7]

In this paper, we calculate K_x for polypeptides with different amino acid sequences on the basis of this new theory. Specifically, this includes the calculation of the density $W(\mathbf{0})$ for chains with small end-to-end distances in different approximations and the angular correlation factor $2\Gamma_0(1)$. The amino acid sequences treated in this paper reflect the main structural features of many naturally occurring and synthetic cyclic peptides. Six different sequences are investigated: poly(L-Ala), poly(D-Ala-L-Ala), poly(Gly), poly(D-Ala-D-Ala-L-Ala-L-Ala), poly(L-Ala-L-Pro), and poly(D-Ala-L-Pro). In all cases, the peptide bond is assumed to be in the trans planar position.[8]

Theory of Macrocyclization

The theory of macrocyclization in its elaborated form has recently been published;[4] therefore, only the main equations are given here.

Consider the cyclization process:

$$-\mathrm{M}_{x+y}- \rightleftharpoons -\mathrm{M}_{y}- + \text{c-}\mathrm{M}_{x} \qquad (1)$$

where $-\mathrm{M}_{x+y}-$ and $-\mathrm{M}_{y}-$ are chain molecules comprising sequences of $x + y$ and y repeating units, respectively, and c-M_x is a cyclic compound with x units. The nature of the terminal groups, expressed by dashes, is immaterial; yet, the unspecified terminal groups in the species $-\mathrm{M}_{y}-$ and $-\mathrm{M}_{x+y}-$ must be chemically equivalent.

At equilibrium conditions, the cyclization process is governed by the cyclization constant:

$$K_x = [-\mathrm{M}_y-][\text{c-}\mathrm{M}_x]/[-\mathrm{M}_{x+y}-] \qquad (2)$$

where K_x measures the propensity of cyclization. According to a relationship established by Jacobson and Stockmayer:

$$K_x = [\text{c-}\mathrm{M}_x] \qquad (3)$$

in the limit of high extents of reaction. Hence, K_x measures the propensity for cyclization and equates to the concentration of the cyclic compound when the concentrations of $-\mathrm{M}_y-$ and $-\mathrm{M}_{x+y}-$ are equal.

We consider the cyclization process in Figure 1. By elabo-

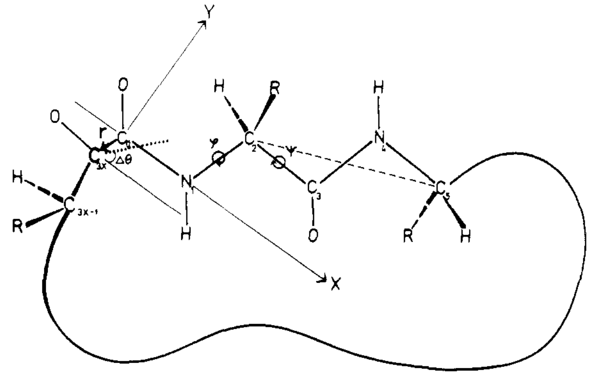

Figure 1. Polypeptide chain in the reference frame of the first amide bond. $\Delta\theta$ denotes angle between the direction of the hypothetical $(3x + 1)$st bond and the first bond.

rating the Jacobson–Stockmayer theory to include angular correlations of chain ends we get the expression:

$$K_x = 2W(\mathbf{0})\Gamma_{\mathbf{0}}(1)/\sigma_{cx}N_A \tag{4}$$

where $W(\mathbf{r})$ is the probability of the value $\mathbf{r}$ for the end-to-end vector per unit volume; $\gamma = \cos \Delta\theta$ corresponds to the bond angle between the first and the hypothetical bond $3x + 1$, dotted in Figure 1; hence, $\Gamma_{\mathbf{0}}(1)$ is the probability that γ assumes the specific value, namely $\gamma = 1$, and therefore $\Delta\theta = 0$ for chains with $\mathbf{r} = \mathbf{0}$. σ_{cx} is the symmetry number and N_A is Avogadro's number. The direction correlation distribution $\Gamma_{\mathbf{r}}(\gamma)$ is expanded in the Legendre polynomials $P_k(\gamma)$. For the circumstances of immediate interest, $\mathbf{r} = \mathbf{0}$ and $\gamma = 1$ (Figure 1) we get:

$$2\Gamma_{\mathbf{0}}(1) = \sum_{k=0}^{\infty} (2k + 1)\langle P_k \rangle_{\mathbf{r}=\mathbf{0}} \tag{5}$$

where $\langle P_k \rangle_{\mathbf{r}}$ are averages of the polynomials over all configurations having the specified value of $\mathbf{r}$. $\langle P_k \rangle_{\mathbf{r}}$ is given by:

$$\langle P_k \rangle_{\mathbf{r}} = \bar{Z}^{-1} \int_{\mathbf{r}} \ldots \int P_k(\gamma) \exp(-E\{\mathbf{l}\}/kT)\, \mathrm{d}\{\mathbf{l}\}/\mathrm{d}\mathbf{r} \tag{6}$$

where $\{\mathbf{l}\}$ is the set of all skeletal bond vectors 1 to $3x + 1$, and the integrations include all configuration space $\{\mathbf{l}\}$ in which the chain vector spanning bond 1 to $3x$ conforms to the value $\mathbf{r}$. The configuration integral subject to the same conditions is:

$$\bar{Z}_{\mathbf{r}} = \int_{\mathbf{r}} \ldots \int \exp(-E\{\mathbf{l}\}/kT)\, \mathrm{d}\{\mathbf{l}\}/\mathrm{d}\mathbf{r} \tag{7}$$

Fourier inversion allows $\langle P_k \rangle_{\mathbf{r}=\mathbf{0}}$ to be expressed as:

$$\langle P_k \rangle_{\mathbf{r}=\mathbf{0}} = (\bar{Z}_{\mathbf{r}=\mathbf{0}}/Z)^{-1}(3/2\pi\langle r^2 \rangle)^{3/2}\left[f_{k;0} - \left(\frac{3}{2}\right)f_{k;2} + \left(\frac{3}{2}\right)^2 \frac{1}{2!} f_{k;4} - \ldots\right] \tag{8}$$

where

$$f_{k;0} = \langle P_k \rangle$$

$$f_{k;2} = \frac{\langle P_k r^2 \rangle}{\langle r^2 \rangle} - \langle P_k \rangle$$

$$f_{k;4} = \frac{\langle P_k r^4 \rangle}{\langle r^2 \rangle^2} - \frac{10}{3}\frac{\langle P_k r^2 \rangle}{\langle r^2 \rangle} + \frac{5}{3}\langle P_k \rangle. \text{ etc.} \tag{9}$$

In eq 9, $\langle P_k \rangle$ denotes P_k averaged over all configurations regardless of $\mathbf{r}$, and Z is the configuration integral likewise without restriction on $\mathbf{r}$.

The density distribution of vector $\mathbf{r}$ is given by $W(\mathbf{r}) = \bar{Z}_{\mathbf{r}}/Z$. In the approximation that $W(\mathbf{r})$ is Gaussian:

$$\bar{Z}_{\mathbf{r}=\mathbf{0}}/Z = \mathrm{W}(\mathbf{0}) = (3/2\pi\langle r^2 \rangle)^{3/2} \tag{10}$$

The density distribution can be approximated to any desired degree by the Hermite series $\mathcal{H}(\mathbf{r})$,[9] which for $\mathbf{r} = \mathbf{0}$ is:

$$\mathcal{H}(0) = 1 + 3 \cdot 5g_4 + 3 \cdot 5 \cdot 7g_6 + \ldots \tag{11}$$

where

$$g_4 = -(1/2^3)(1 - 3\langle r^4 \rangle/5\langle r^2 \rangle^2)$$

$$g_6 = -(1/2^3 \cdot 3!)[3(1 - 3\langle r^4 \rangle/5\langle r^2 \rangle^2) - (1 - 3^2\langle r^6 \rangle/5 \cdot 7\langle r^2 \rangle^3)] \tag{12}$$

Then:

$$\langle P_k \rangle_{\mathbf{r}=\mathbf{0}} = [\mathcal{H}(0)]^{-1}[f_{k;0} - 3/2 f_{k;2} + \ldots] \tag{13}$$

The quantities $\langle P_k r^{2p} \rangle$ are estimated by resorting to Monte Carlo methods[9] using conditional probabilities deduced from a suitable set of rotational states for analysis of the configurational statistics.

Basis of Computations

A polypeptide chain in a conformation approaching the requirements for cyclization is depicted in Figure 1. The coordinate system of reference is defined by the C(O)–N bond (X axis); the Y axis is in the plane of the bonds C(O)–N and N–C$^\alpha$ with the positive direction chosen to form an acute angle with the N–C$^\alpha$ bond. In order to form a ring, the dotted hypothetical bond $3x + 1$ must coalesce with bond 1 (joining atom 0 and 1).

Bond angles and bond lengths are assigned the values used previously.[10,11] The length of the virtual bond of the trans peptide unit, spanning consecutive C$^\alpha$ atoms, is 3.80 Å.[10]

Conformational energy calculations were performed using semiempirical potential functions, including terms corresponding to bond torsional strain, van der Waals repulsion, London attractions, and electrostatic interactions between nonbonded atoms and groups as described previously.[10,12] In the case of X-Pro sequences, the empirical conformational energy program for peptides (ECEPP) was used.[13] Average quantities such as the square of the mean end-to-end distance $\langle r^2 \rangle$ and the persistence vector $\mathbf{a}$[14] calculated with both sets of parameters[10,13] for poly(Gly), poly(L-Ala), and poly(L-Pro) differed by no more than 10%.

All calculations were performed at a temperature of 25 °C for chains with $6 \leq x \leq 20$. The required averages were evaluated by summing over discrete values of φ_i and ψ_i using 30° intervals if not specified otherwise. Conformations for which $E(\varphi_i, \psi_i)$ exceeds its minimum value by more than 4.0 kcal mol^{-1} were omitted from the sums inasmuch as their contributions turned out to be insignificant. The quantities of interest, i.e. the averaged Legendre polynomials $\langle P_k \rangle_{\mathbf{r}=\mathbf{0}}$ and the density $W(\mathbf{0})$ in different approximations, were obtained using Monte Carlo procedures as follows. On the basis of sets of n computer generated random numbers, the state of each interdependent $\varphi_i\psi_i$ pair was identified with an interval of the numerical range corresponding to the Boltzmann factor for this particular conformation.

Peptide sequences containing Pro residues cannot be treated as totally independent units. Owing to the geometry of the pyrrolidine ring, Pro restricts the conformation space of its predecessor. Therefore, in the conformational energy calculations for obtaining the Boltzmann factors for individual conformations of residue X$_i$ in a X$_i$-Pro$_{i+1}$-dipeptide, all interactions within this unit were taken into account, i.e. interactions of atoms within residue X$_i$ with the pyrrolidine ring of Pro$_{i+1}$, precipitated by rotation about φ_i and ψ_i. In contrast to a Pro-Pro unit, the different puckering of the pyrrolidine ring turned out to be of minor importance for the conformational

Table I. Characteristic Ratio $C_n = \langle r^2 \rangle / nl^2$ and Persistence Vector $\mathbf{a} \equiv \langle r \rangle$ for the Amino Acid Sequences I-VI (See Text) with $n = 20$ Residues

Sequence	C_{20}	$\mathbf{a}_{20}$
I: (D-Ala-L-Ala)	0.73	4.08
II: (D-Pro-L-Ala)	1.41	5.9
III: Gly	1.89	6.4
IV: (L-Pro-L-Ala)	3.26	10.7
V: (D-Ala-D-Ala-L-Ala-L-Ala)	3.62	12.0
VI: L-Ala	6.45	21.0

characteristics of its predecessor in an X-Pro unit. Hence, the proline ring was taken in the "down" conformation with φ_{Pro} fixed at $-75°$, which corresponds to the ring conformation with minimal internal energy.[13,15] Because of the independence of the pair φ_i/ψ_i from ψ_{Pro}, the Boltzmann factors for residue X_i preceding Pro_{i+1} were obtained in the customary way from the two-dimensional energy surface as a function of φ_i and ψ_i by including the interactions with the following Pro ring. The summation over the conformation space was done in this case by using 10° intervals for ψ_{Pro} (for the energy map of Pro) and the $\varphi_i.\psi_i$ pair of its predecessor (for the energy map of residue X_i).

Conformational Energy Diagrams

As the propensity for cyclization is intimately related to average chain dimensions, i.e. to the extendedness of the chain molecule in the random conformation, the energy maps of the single residues serve as a basis for the interpretation of the cyclization equilibria constants, computed according to the theory presented above. Therefore, the main features of the relevant energy maps will be shortly discussed. The energy map of Gly obtained by methods described above has four pronounced energy minima, corresponding to $g^{\pm}g^{\mp}$ and $g^{\pm}g^{\pm}$ conformations for φ/ψ. Specifically, the $\pm\mp$ quadrants of the energy surface account for ca. 65% and the $\pm\pm$ quadrants for ca. 35% of the residue partition function. Owing to the relative symmetric energy surface and to the large accessible conformation space, Gly is expected to be very flexible. This is confirmed by a low characteristic ratio C_n and a small value of the persistence vector $\mathbf{a}$, as shown in Table I, for $(\text{Gly})_{20}$. Contrary to Gly, Ala has a much more restricted conformation space. Though there are three domains of low energy, 93% of the residue partition function is comprised by one region, which corresponds to an extended conformation. Consequently, Ala is much stiffer than Gly. This is confirmed by a high value of C_n and $\mathbf{a}$ (see Table I). The energy map of a D residue is obtained by reflecting the L residue map through its center, i.e. the signs of the rotation angles are reversed. Owing to this symmetry of a D and L residue, the racemic sequence D,L-Ala is very compact, as can be realized from the low values of C_n and $\mathbf{a}$.

Due to the five-membered ring, the rotation around φ is restricted in Pro; when the ring is taken in the "down" conformation, φ_{Pro} is fixed at $-75°$.[13] Consequently, the energy map of Pro is one-dimensional, i.e. a function of ψ_{Pro} only. The energy diagram has two minima at $\psi_1 \simeq -50°$ and $\psi_2 \simeq 145°$. The minimum at ψ_2 comprises nearly 70% of the partition function whereas the former one accounts for ca. 30%. Thus, the statistical weight of the more compact conformation (ψ_1) is considerably higher in the case of Pro compared to Ala. Substitution of an L-Ala by an L-Pro residue in a poly(L-Ala) chain should consequently decrease the chain dimensions.

The Probability Density $W(\mathbf{0})$ and Its Influence on K_x

The evaluation of $W(\mathbf{0})$, required for the calculation of $\langle P_k \rangle_{\mathbf{r}=\mathbf{0}}$ as well as for K_x, is difficult for short chains where the deviation from Gaussian distribution is large. In the following, we first calculate $W(\mathbf{0})$ assuming Gaussian distribution; second, $W(\mathbf{0})$ is obtained in higher approximation by scalar Hermite expansion described in detail elsewhere.[9] Third, $W(\mathbf{0})$ is estimated using a direct Monte Carlo procedure.

(1) **Gaussian Distribution.** For $\mathbf{r} = \mathbf{0}$ the Gaussian density distribution equates to:

$$W(\mathbf{0}) = (3/2\pi\langle r^2 \rangle)^{3/2}$$

The square of the average end-to-end vector, $\langle r^2 \rangle$, is obtained by Monte Carlo generation of 30 000 chains. To check the accuracy of the Monte Carlo procedure, $\langle r^2 \rangle$ was evaluated also by exact matrix multiplication methods[10] for poly(L-Ala) and poly(Gly), using averaged transformation matrices obtained from summation over 30° intervals of φ_i and ψ_i. In both cases, $\langle r^2 \rangle$ for chain lengths investigated in this paper, i.e. $x = 6$-20, differed less than 3%, thus confirming the Monte Carlo result.

(2) **Scalar Hermite Expansion.** In applying several higher approximations of $W(\mathbf{0})$ in an earlier study,[5] truncation of the scalar Hermite expansion at g_4 gave best agreement with the other approximations, namely the direct Monte Carlo method and the three-dimensional Hermite expansion. For further improvement of $W(\mathbf{0})$, many more terms would have to be included, making this method impractical. Therefore, in the present investigation, we truncate the series expansion in eq 11 at g_4, as before. The quantities $\langle r^2 \rangle$ and $\langle r^4 \rangle$ are obtained by Monte Carlo generation of 30 000 chains.

(3) **Direct Monte Carlo Method.** Here, the density in the vicinity of $\mathbf{r} = \mathbf{0}$ was calculated by generating 30 000 Monte Carlo chains, the number of chain termini falling within a sphere of radius δr about the origin being divided by the volume of the sphere; r is chosen to be $0.3\langle r^2 \rangle^{1/2}$ if not otherwise specified.

The Angular Correlation Factor $2\Gamma_0(1)$. The angular correlation factor $2\Gamma_0(1)$ was evaluated according to eq 5. The averaged Legendre polynomial $\langle P_k \rangle_{\mathbf{r}=\mathbf{0}}$ with $k = 1, 2, \ldots, 5$ and arguments $\gamma = \cos \Delta\theta$ over all configurations for which $\mathbf{r} = \mathbf{0}$ were obtained from eq 8 and 9 by Monte Carlo generation of the moments $\langle P_k r^{2p} \rangle$ without restriction on r. Again, 30 000 chains were generated for each sequence and chain length. The Hermite series $\mathcal{H}(0)$ required in eq 13 was truncated at g_4.

Results

The equilibrium constant K_x is calculated according to eq 4; K_x is evaluated first on the basis of $W(\mathbf{0})$ in different approximations, neglecting angular correlations, i.e. for $2\Gamma_0(1) = 1$. then, the angular correlation factor is additionally taken into account.

(1) **Poly(Gly).** K_x calculated assuming Gaussian distribution of $W(\mathbf{r})$ is represented by curve 1 in Figure 2. Curves 2 and 3 correspond to the scalar Hermite series expansion truncated at g_4 and to the direct Monte Carlo procedure, respectively. Both higher approximations indicate a departure from Gaussian distribution throughout the investigated range. As a consequence, K_x is lowered by factors of ca. 1.7, 1.4, and 1.2 for $x = 6$, 10, and 20, respectively. Curves 2 and 3 are in excellent agreement for $x \geq 6$. Curve 4 of Figure 2 will be discussed later. In Figure 3 we show $\langle P_1 \rangle_{\mathbf{r}=\mathbf{0}}$ calculated with truncation of eq 13 at the terms $f_{1;2s}$ of the indicated rank $2s$ and plotted against x. The error bars represent twice the standard deviation σ_m of the mean, embracing a confidence limit of ca. 95%. The first term of eq 1, i.e. $f_{1;0}$, is zero for all x. By including the second term, $f_{1;2}$, $\langle P_1 \rangle_{\mathbf{r}=\mathbf{0}}$ is rendered negative throughout; $f_{1;4}$ decreases $\langle P_1 \rangle_{\mathbf{r}=\mathbf{0}}$ further, but its influence on $\langle P_1 \rangle_{\mathbf{r}=\mathbf{0}}$ vanishes beyond $x = 12$. The next term, $f_{1;6}$, has no contribution to $\langle P_1 \rangle_{\mathbf{r}=\mathbf{0}}$ for all x. Hence, terms up to and including $f_{1;4}$ suffice to determine $\langle P_1 \rangle_{\mathbf{r}=\mathbf{0}}$ for $x \geq 6$.

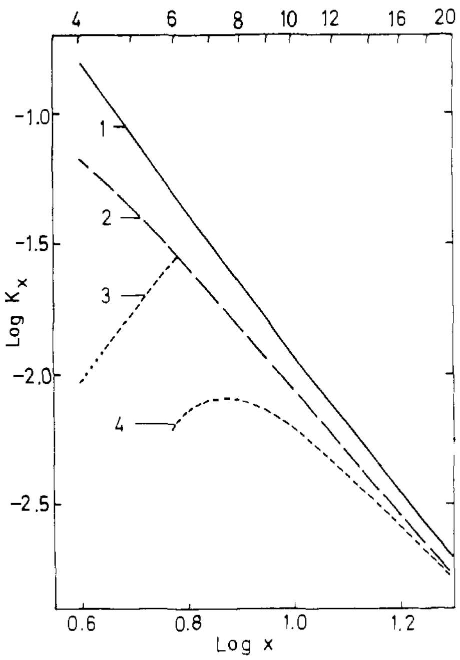

Figure 2. Cyclization equilibrium constant K_x plotted on log scales against the number of amino acid residues x for poly(Gly). Curves 1, 2, and 3 correspond to different approximations for $W(\mathbf{0})$, with neglect of orientational correlations between terminal bonds: curve 1, spherical Gaussian; 2, Hermite series expansion truncated at g_4; 3, direct Monte Carlo method, curve 4 is calculated according to eq 4, including departures from Gaussian distribution and angular correlations (for details see text).

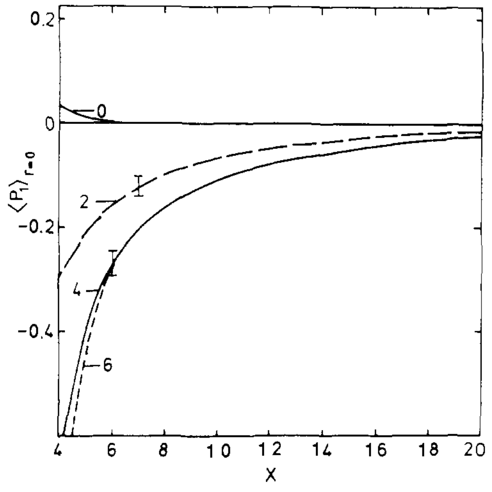

Figure 3. Averaged Legendre polynomial of first-order $\langle P_1 \rangle_{r=0}$ for poly(Gly) calculated with truncation of the series expansion in eq 13 at the term $f_{l;2s}$ of the indicated rank 2s. Error bars denote $2\sigma_m$ limits.

The Legendre polynomials of higher order, $\langle P_k \rangle_{r=0}$ up to $k = 5$, calculated similarly from 30 000 Monte Carlo chains, have no contribution to $2\Gamma_0(1)$, i.e. within the error limit of $2\sigma_m$ they are indistinguishable from zero. Hence, $2\Gamma_0(1)$ is adequately represented by eq 14 for poly(Gly).

$$2\Gamma_0(1) = 1 + 3\langle P_1 \rangle_{r=0} \tag{14}$$

The constants K_x were evaluated according to eq 4, using eq 11 truncated at g_4 and eq 14 for $W(\mathbf{0})$ and $2\Gamma_0(1)$, respectively. The symmetry number σ_{cx} equals x. The result is shown by curve 4 in Figure 2. The inclusion of angular correlations, being negative for all x, leads to a further depression of K_x. Specifically, K_x calculated by ignoring angular correlations is lowered by factors of 4.6, 1.5, 1.4, and 1.2 for x = 6, 8, 10, and 15, respectively. For $x > 15$, the influence of angular correlations is very small. The relative moderate deviations from Gaussian distribution, the high density at r = **0**, and the rapidly decreasing angular correlation with chain length reflect the high flexibility of this polypeptide chain, as was expected from the energy map discussed above. As a consequence, rings containing Gly residues should be easily formed. These predictions will be related to experimental results in a later section of this paper.

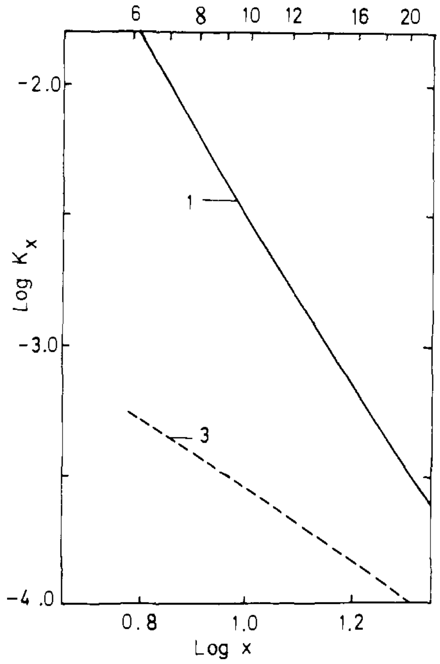

Figure 4. Log K_x for poly(L-Ala) against the number of residues x with neglect of angular correlations: curve 1, spherical Gaussian approximation of $W(\mathbf{0})$; 3, direct Monte Carlo method for $W(\mathbf{0})$.

(2) **Poly(L-Ala).** As before, K_x was first calculated according to eq 4 ignoring angular correlations. The results are represented by curves 1 and 3 in Figure 4. The values of K_x in Gaussian approximation are lower compared to poly(Gly) by factors of ca. 4 and 6 for x = 10 and 20, respectively, indicating a low density at r = **0**. This is a direct consequence of the greater C_x. Furthermore, a strong deviation from the spherical Gaussian distribution is indicated by curve 3, which corresponds to the direct Monte Carlo method. This result is in harmony with recent calculations by Conrad and Flory on the density distribution of poly(L-Ala),[11] where departures from Gaussian behavior were shown to be significant even at x = 40. Because of the low density at r = **0**, which is a consequence of the extendedness of this peptide chain, the moment method to evaluate $W(\mathbf{0})$ must fail in this case for short chain lengths. For the estimation of $W(\mathbf{0})$ we must therefore rely on the Monte Carlo method. To this end, different spheres σ_r were chosen for determinating $W(\mathbf{0})$.

When σ_r was taken to be $0.3\langle r^2 \rangle^{1/2}$ and $0.5\langle r^2 \rangle^{1/2}$, sampling ca. 1 and 5%, respectively, of the total population, both estimates for $W(\mathbf{0})$ were different by less than 10% for $x > 7$. Therefore, curve 3 is believed to be a good approximation. Taking these values, the depression of K_x due to the departure from Gaussian distribution amounts to factors of ca. 17, 8, and 3 for x = 8, 12, and 20, respectively. The results for the evaluation of the angular correlation factor $2\Gamma_0(1)$ are shown in Figure 5.

The first term in $\langle P_1 \rangle_{r=0}$, $f_{1;0}$ (Figure 5), is positive for all x. The following terms, $f_{1;2}$, $f_{1;4}$, and $f_{1;6}$, render $\langle P_1 \rangle_{r=0}$ continuously more negative and all of them have a significant contribution to $\langle P_1 \rangle_{r=0}$ throughout the range of x. Alternating

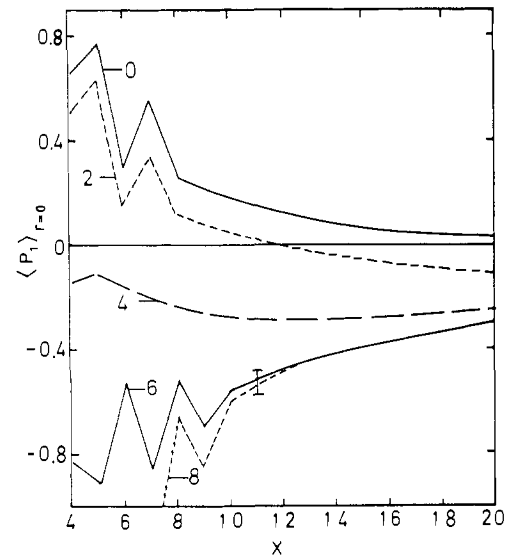

Figure 5. $\langle P_1 \rangle_{r=0}$ for poly(L-Ala) with truncation of eq 13 as indicated. The Hermite expansion $\mathcal{H}(0)$ in eq 13 was substituted in this case by the direct Monte Carlo approximation for evaluating $W(\mathbf{0})$.

lower and higher values of $\langle P_1 \rangle_{r=0}$ are obtained for small x. The next term, $f_{1;8}$, contributes to $\langle P_1 \rangle_{r=0}$ only for $x < 10$ and virtually vanishes for $x \geq 10$. $f_{1;10}$, not shown in Figure 5, still is significant for $x < 10$, $\langle P_1 \rangle_{r=0}$ being rendered even more negative. For fully determining $\langle P_1 \rangle_{r=0}$, more terms in $f_{1;2s}$ would have to be included; the evaluation at these higher terms is outside the scope of the present calculations. Hence, for $x > 10$, $\langle P_1 \rangle_{r=0}$ is determined by terms up to $f_{1;6}$, whereas for $x < 10$, $\langle P_1 \rangle_{r=0}$ does not converge satisfactorily. Nevertheless, a strongly negative value of $\langle P_1 \rangle_{r=0}$ for $x < 10$ is clearly indicated.

Legendre polynomials of higher order, i.e. $\langle P_k \rangle_{r=0}$ with k up to 5, have also been computed. All higher polynomials were positive throughout the range of x, their magnitudes being close to the error limit of $2\sigma_m$.

For $\langle P_2 \rangle_{r=0}$ and $\langle P_3 \rangle_{r=0}$, a medium value of ca. 0.03 ± 0.02 is indicated, individual points showing considerable scattering. The higher terms, $\langle P_4 \rangle_{r=0}$ and $\langle P_5 \rangle_{r=0}$, were practically indistinguishable from zero for $x > 15$. The addition of the higher terms in $\langle P_k \rangle_{r=0}$ to $\langle P_1 \rangle_{r=0}$ leads to a value of $2\Gamma_0(1)$ very close to zero. For $x = 6$, the higher polynomials of $\langle P_k \rangle_{r=0}$ are consistently more positive than for other chain lengths indicating much higher values for $2\Gamma_0(1)$. Yet, the Legendre polynomials did not converge satisfactorily for this small chain length, making a reliable estimate for $2\Gamma_0(1)$, and hence for K_6, impossible. Due to the relatively large errors in the higher polynomials, $\langle P_k \rangle_{r=0}$, a quantitative evaluation of $2\Gamma_0(1)$ (and consequently of K_x) is impossible. Nevertheless, a maximum value of K_x can be estimated. For example, taking the maximum values of $\langle P_k \rangle_{r=0}$ for $k > 1$ within the probable error including terms up to $k = 5$, $2\Gamma_0(1)$ takes on values between 0 and 0.2 throughout the range of $10 \leq x \leq 20$.

In contrast to poly(Gly) and the polymers treated earlier, higher terms in $\langle P_k \rangle_{r=0}$ are important for the determination of the angular correlation factor $2\Gamma_0(1)$.

For reasons mentioned above, only maximum values for the cyclization constants K_x are calculated. The negative angular correlation depresses the K_x values of poly(L-Ala) very drastically. Specifically, K_x taken from curve 3 in Figure 4 is reduced by a factor that does not exceed 0.20 when angular correlations are included. The very low K_x values of poly(L-Ala) are a consequence of the pronounced tendency of this residue to adopt extended conformations, which makes a cyclization reaction very unfavorable due to a low density $W(\mathbf{0})$.

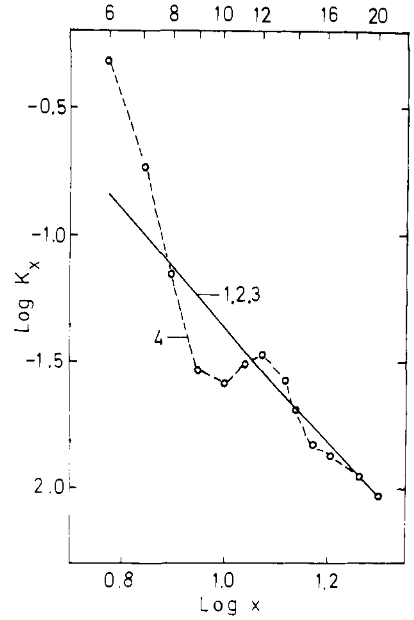

Figure 6. Log K_x for poly(D-Ala-L-Ala) against the number of residues x. The different approximations for $W(\mathbf{0})$ are shown by the straight line labeled 1, 2, 3, as before. Curve 4 corresponds to log K_x calculated from eq 4.

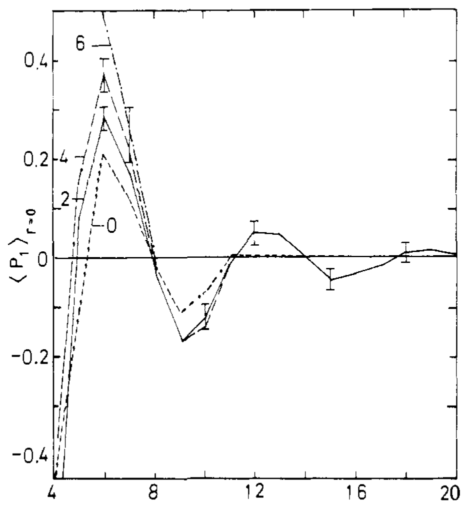

Figure 7. $\langle P_1 \rangle_{r=0}$ for poly(D-Ala-L-Ala). The labeling of the curves has the same meaning as in Figure 3.

The large value of the positive X component of the persistence vector **a** of ca. 16 is a further indication that the angular correlation factor $2\Gamma_0(1)$ is expected to be strongly negative. This has been rationalized in our previous paper.[4] Hence, the present calculations indicate that the tendency of poly(L-Ala) chains to cyclize is extremely low.

(3) **Poly(D-Ala-L-Ala)**. The constants K_x calculated from eq 4 for the different approximations of $W(\mathbf{0})$ by setting $2\Gamma_0(1) = 1$ are plotted against x in Figure 6. All three approximations gave identical results, i.e. the alternating D,L-polypeptide chain obeys Gaussian distribution for all $x \geq 6$. The K_x values are still considerably higher than those obtained for poly(Gly). This result can be explained by the very low characteristic ratio and the small persistence vector **a**, both values being only ca. one-tenth of the corresponding quantity for the stereoregular peptide chain (see Table I).

Figure 7 shows $\langle P_1 \rangle_{r=0}$ calculated according to eq 13 with terms $f_{1;2s}$ truncated at $2s$, plotted against x. The first term,

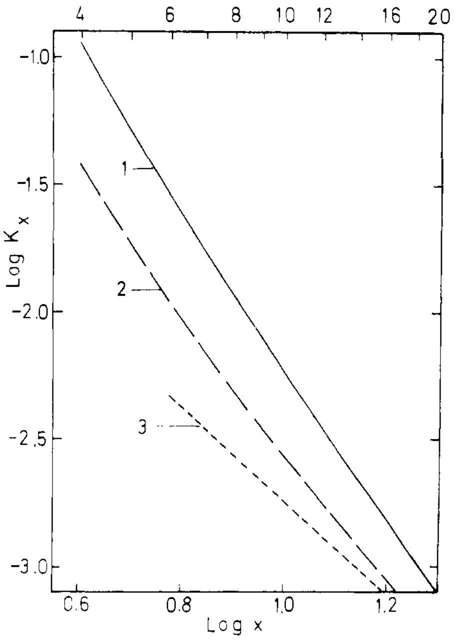

Figure 8. Log K_x for poly(D-Ala-D-Ala-L-Ala-L-Ala) against x. For details see Figure 2.

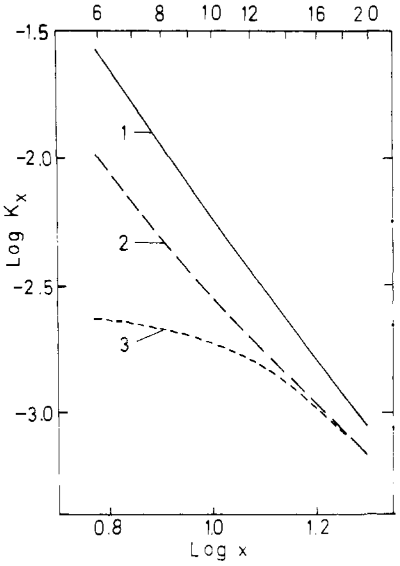

Figure 9. Log K_x for poly(L-Ala-L-Pro$_d$) against x. For details, see Figure 2.

Table II. Log K_x for the Peptide Sequences I–VI (Compare Table I) for Different Chain Lengths x, Calculated According to Eq 4[a]

Sequence	$x =$ 6	10	20
I	-0.33 (75)	-1.60 (4)	-2.04 (5)
II	-1.61 (4)	-2.19 (1.02)	-2.55 (1.7)
III	-2.21 (1)	-2.20 (1)	-2.78 (1)
IV	-2.69_{max} (0.33)	-3.24_{max} (0.09)	-3.86_{max} (0.08)
V	-3.02_{max} (0.15)	-3.44_{max} (0.017)	-3.98_{max} (0.06)
VI	-3.95_{max} (0.006)	-4.19_{max} (0.01)	-4.67_{max} (0.01)

[a] The numbers in parentheses refer to the propensity of cyclization, when Gly is taken as standard. Numbers labeled "max" correspond to estimated maximal values of K_x. (For details see text.)

$f_{l:0}$, has a maximum at $x = 6$ and a minimum at $x = 9$. For $x > 10$, the term converges to zero. By inclusion of $f_{l:2}$, the maximum and minimum get more pronounced, $\langle P_l \rangle_{r=0}$ adopting positive and negative values, alternating in steps of three residues. The next term, $f_{l:4}$, contributes to $\langle P_l \rangle_{r=0}$ only for $x = 6$ and $x = 7$ and vanishes for $x > 7$. $f_{l:6}$ is significant for $x = 6$. Hence, terms up to and including $f_{l:2}$ suffice to determine $\langle P_l \rangle_{r=0}$ for $x > 7$, whereas for $x = 7$ and $x = 6$ one or two, respectively, more terms must be included.

The Legendre polynomials $\langle P_k \rangle_{r=0}$ of higher order up to $k = 5$ have no contribution to $2\Gamma_0(1)$. For $x > 6$, the angular correlation factor is determined by eq 14, whereas for $x = 6$, $\langle P_2 \rangle_{r=0}$ and $\langle P_3 \rangle_{r=0}$, not shown here, contribute significantly to $2\Gamma_0(1)$, being also positive. The values of $2\Gamma_0(1)$ for $x = 6$ are 2.5, 3.14, and 3.32 after truncation of eq 5 at $k = 1, 2$, and 3, respectively. Higher polynomials of $\langle P_k \rangle_{r=0}$ were undistinguishable from zero.

The constants K_x calculated using eq 14 are shown by curve 4 in Figure 6. The inclusion of angular correlations increases K_x for $x = 6$ and $x = 7$ by factors of ca. 3.3 and 1.8, respectively. For $x = 9$ and 10, K_x is lowered by factors of 0.55 and 0.7. For all other chain lengths, $2\Gamma_0(1)$ is unity within the error limits.

With regard to this result, the formation of cyclics of racemic sequences with alternating D,L sequences should be very favorable.

(4) **Poly(D-Ala-D-Ala-L-Ala-L-Ala)**. This sequence shows close similarity to the poly(L-Ala) chain. As could be expected from the large values for the characteristic ratio and the persistence vector (see Table I), a relatively strong deviation from Gaussian distribution is found; as a further consequence of the low density at $W(\mathbf{0})$, the higher approximations are in poor agreement for $x < 20$ (see Figure 8). Again, the direct Monte Carlo estimate of $W(\mathbf{0})$ gave consistent results, when different spheres of σ_r around the origin were taken.

The angular correlation factor $2\Gamma_0(1)$ is dominated by the first polynomial, $\langle P_l \rangle_{r=0}$, which is strongly negative. Higher terms in $\langle P_k \rangle_{r=0}$ were positive throughout and slightly larger than the error limit of $2\sigma_m$, similar to poly(L-Ala). Hence, $2\Gamma_0(1)$ is determined only when higher polynomials of $\langle P_k \rangle_{r=0}$ are included. The exact value of K_x is difficult to obtain because of the large errors involved in the calculation of $\langle P_k \rangle_{r=0}$ when $k > 1$. Very small values of $2\Gamma_0(1)$ are indicated, leading to strong depressions of K_x. Maximum values of K_x are given in Table II.

(5) **Poly(L-Ala-L-Pro)**. The insertion of L-Pro residues in a poly(L-Ala) chain leads to considerably higher values of K_x (see Figure 9). This is a direct consequence of the reduced average chain dimensions (see Table I) due to the larger accessibility of the minima at ψ_l for Pro compared to Ala, as pointed out above. The depression of the K_x values due to deviations from Gaussian distribution amounts to factors of ca. 2.5 and 1.3 for $x = 10$ and 20, respectively. The agreement between the direct Monte Carlo estimate and the Hermite series expansion for getting $W(\mathbf{0})$ is within less than 10% for $x > 12$.

In calculating $2\Gamma_0(1)$, polynomials $\langle P_k \rangle_{r=0}$ up to $k = 3$ had to be included, similar to poly(L-Ala). Again, the angular correlation was negative for all x, $\langle P_l \rangle_{r=0}$ being the dominant term. K_x values calculated from eq 4 indicate that the incorporation of L-Pro residues raises the K_x values of an all poly(L-Ala) chain by a factor of about 6.

(6) **Poly(D-Ala-L-Pro)**. The results for this sequence resemble those of poly(D-Ala-L-Ala). Yet, in this case a significant but small deviation from Gaussian distribution for $x < 20$ is indicated and the values of K_x obtained from eq 4 by setting $2\Gamma_0(1) = 1$ are lower by a factor of ca. 3 for all x investigated (see Figure 10). Again, the angular correlation factor $2\Gamma_0(1)$ is alternating, having positive values for $x = 8$ and 12. For $x = 6, 10$, and 14, minimum (negative) values are obtained. $2\Gamma_0(1)$ is fully determined by eq 14. Values for K_x

are shown in Table II. Though the K_x values are significantly lower compared to the racemic *all*-Ala sequence, the propensity to form a cyclic compound is still very high.

Discussion

The present calculations indicate that the ring closure probabilities, expressed in the equilibrium constant K_x for the different peptide sequences investigated here, span a very large scale. The K_x values obtained on the basis of the density $W(\mathrm{r})$ for $\mathbf{r} = \mathbf{0}$ by neglecting angular correlations, i.e. $2\Gamma_0(1) = 1$, reflect the extendedness of the different sequences as expressed in the characteristic ratio given in Table I. With the values of K_x from curve 3 in Figures 2, 4, 6, 8, 9, and 10, corresponding to the direct Monte Carlo estimates of $W(\mathbf{0})$, the following results are obtained. When poly(Gly) is taken as reference, the propensity for ring closure for poly(D-Ala-L-Ala) is higher by a factor of ca. 5, whereas it is lower for poly(L-Ala) by factors of ca. 50, 30, and 15 for $x = 6$, 10, and 20, respectively. The incorporation of L-Pro residues in a poly(L-Ala) chain raises the K_x values; however, K_x is still lower by a factor of ca. 3 compared to poly(Gly) for the regularly alternating L-Pro-L-Ala sequence. On the other hand, the substitution of the D-Ala residue by a D-Pro residue lowers K_x by a factor of ca. 2.5 for $x = 10$, but the K_x values still are considerably higher than for poly(Gly).

Poly(D-Ala-D-Ala-L-Ala-L-Ala) has unexpectedly low values of K_x, being on the order of those of poly(L-Pro-L-Ala). As a general feature, all chains investigated, with the exception of poly(D-Ala-L-Ala), show significant departures from Gaussian distribution in the range of $6 < x \leq 20$, lowering K_x up to a factor of ca. 30, as in the case of poly(L-Ala). The more extended the chains are, the stronger is the departure from the spherical Gaussian.

The inclusion of the angular correlation factor $2\Gamma_0(1)$ amplifies the pronounced differences in the K_x values for the individual chains even more. Hence, chains with low densities at $\mathrm{r} = \mathbf{0}$ have generally a small value of $2\Gamma_0(1)$, indicating an unfavorable angular correlation, whereas chains with high values in $W(\mathbf{0})$ have values of $2\Gamma_0(1)$ close to 1, in some cases $2\Gamma_0(1) > 1$, corresponding to positive angular correlations. The magnitude and direction of the persistence vector **a** were found to be a qualitative measure for the angular correlation for chain termini in polymers.[4-6] This finding is confirmed by the peptide sequences investigated here. So, the extended poly(L-Ala) chain with an exceptionally large X component of the persistence vector has a value of $2\Gamma_0(1)$ close to zero, i.e. the angular correlation is very unfavorable. The racemic D,L sequence, on the other hand, has an extremely small persistence vector and, consequently, $2\Gamma_0(1)$ is close to 1 and even shows favorable angular correlation ($2\Gamma_0(1) > 1$) for certain chain lengths. Table II contains K_x values for the different chains, calculated according to eq 4. Values labeled as "max" correspond to estimated maximal values of K_x. In reality, these values, which are difficult to calculate accurately for reasons mentioned above, can be smaller by several orders of magnitude. In the last column of Table II, ring closure probabilities are compared relative to Gly as standard. From these data, some predictions for the propensity to undergo a cyclization reaction are made and related to experimental evidence. The cyclization reaction should be most difficult for a poly(L-Ala) type chain. At equilibrium conditions, less than 0.01% are cyclics; consequently, the cyclization step in the synthesis of an *all-trans-all*-L-Ala type compound is expected to proceed in extremely low yields. This prediction from the conformational properties of this chain is in agreement with experimental findings. For example, the exceptional behavior in cyclization experiments of this type of peptide sequence has been observed frequently.[16] As a striking example, the synthesis of an *all*-L-gramicidin S analogue has been unsuccessful despite many efforts.[17] Recently, the synthesis of a cyclic L-hexaalanine has been reported.[18] However, the yield has been exceptionally low. In this respect it is worth mentioning that for $x = 6$ a much higher value for the angular correlation factor is indicated as mentioned before, leading to higher K_x values. This is in general harmony with the fact that hexapeptides give higher yields in cyclization reactions compared to other chain lengths. Hence, the exceptional behavior of chains with six residues is at least qualitatively confirmed in the present calculations. On the opposite scale, the regularly alternating racemic sequence of poly(D-Ala-L-Ala) is expected to undergo a cyclization very readily, K_x being higher by factors of 75, 4, and 5 for $x = 6$, 10, and 20, respectively, compared to poly(Gly). For $x = 20$, the tendency to form a cyclic compound is ca. 500 times higher than for poly(L-Ala). Interestingly, racemic sequences are a very common feature of naturally occurring antibiotics such as enniatin, gramicidin S, and valinomycin. The synthesis of the regularly alternating D,L sequence of the hexapeptide enniatin and its analogues is especially readily accomplished,[19] in harmony with the exceptionally high value of K_x for $x = 6$ due to favorable angular correlations indicated in the present calculations.

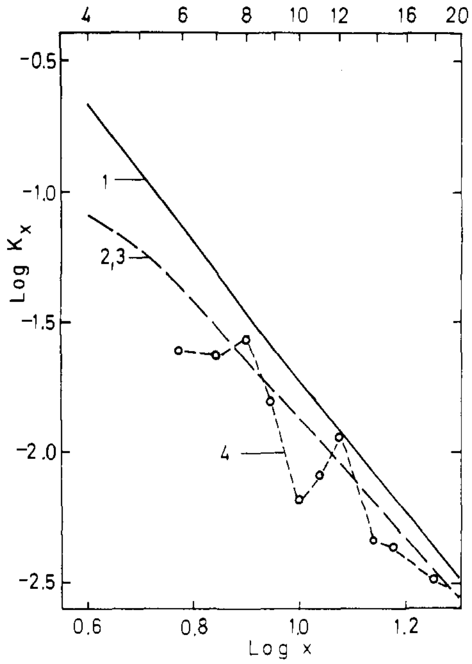

Figure 10. Log K_x for poly(D-Ala-L-Pro$_d$) against x. The numbers have the same meaning as in Figure 2.

Further experimental evidence of the high ring closure probability of racemic sequences are the generally high yields in synthesizing these cyclic compounds; a large number of synthetic cyclics of this interesting class of peptides have therefore been reported.[2,16,19] In agreement with the theoretical results obtained here, the synthesis of *cyclo*-(Gly-L-Leu-D-Leu-Gly-Gly) and *cyclo*-(Gly-L-Leu-L-Leu-Gly-Gly) resulted in considerably higher yields of the D,L compound. Similar observations were made during the synthesis of related sequences.[20] From our results, the synthesis of cyclic peptides of the type (D,D,L,L), as in valinomycin, should be considerably more difficult compared to the (D,L) type. Cyclic peptides containing Gly residues also have high values of K_x, i.e. rings containing Gly should be easily formed. The K_x values for the poly(Gly) chain are considerably lower compared to the D,L chain, but still higher by a factor of ca. 100 than poly(L-Ala). Consequently, a large variety of cyclic peptides containing Gly residues have been synthesized.[16,21] Even the incorporation of only one Gly residue in an (L-Ala) hexapeptide was sufficient to raise the cyclization yield several times.[21]

Despite the fact that Pro restricts the conformation space of its predecessor to extended conformations, its incorporation in an L-Ala-type chain raises K_x significantly due to the accessibility of more compact conformations for Pro. The successful synthesis of the decapeptide antamanid,[22] which contains four Pro residues, is therefore not an unexpected result.

The general agreement between theoretical predictions of the propensity for cyclization and the experimental evidence indicates that the density distribution $W(\mathbf{0})$ of the end-to-end distance r and the angular correlation factor $2\Gamma_0(1)$ are major factors in determining K_x. It should be emphasized that our results were obtained by assuming a random-coil conformation of the peptide chain, precipitated by short-range interactions only. Departures from our predictions for the cyclization tendency of polypeptides are expected when the experimental conditions chosen for the cyclization step are such that long-range interactions, e.g. conditions which enhance the onset of a secondary structure, are no longer negligible.

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References and Notes

(1) M. Rothe and D. Mühlhausen, *Angew. Chem.*, **88**, 338 (1976).

(2) Yu. A. Ovchinikov in "The Chemistry of Polypeptides", Plenum Press, New York, N.Y., 1973.

(3) N. Go and H. A. Scheraga, *Macromolecules*, **3**, 188 (1970): **6**, 525 (1973).

(4) P. J. Flory, U. W. Suter, and M. Mutter, *J. Am. Chem. Soc.*, **98**, 5733 (1976).

(5) U. W. Suter, M. Mutter, and P. J. Flory, *J. Am. Chem. Soc.*, **98**, 5740 (1976).

(6) M. Mutter, U. W. Suter, and P. J. Flory, *J. Am. Chem. Soc.*, **98**, 5745 (1976).

(7) H. Jacobson and W. H. Stockmayer, *J. Chem. Phys.*, **18**, 1600 (1950).

(8) IUPAC–IUB Commission on Biochemical Nomenclature. Abbreviations and Symbols for the Description of the Conformation of Polypeptide Chains. Tentative Rules (1969), *Biochemistry*, **9**, 3471 (1970).

(9) P. J. Flory and D. Y. Yoon, *J. Chem. Phys.*, **61**, 53 (1974).

(10) P. J. Flory, "Statistical Mechanics of Chain Molecules", Interscience, New York, N.Y., 1969.

(11) J. C. Conrad and P. J. Flory, *Macromolecules*, **9**, 41 (1976).

(12) D. A. Brant and P. J. Flory, *J. Am. Chem. Soc.*, **87**, 2791 (1965).

(13) H. A. Scheraga, "Empirical Conformational Energy Program for Peptides (ECEPP)". Quantum Chemistry Program Exchange, QCPE Program No. 286.

(14) P. J. Flory, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 1819 (1973).

(15) F. A. Momany, R. F. McGuire, A. W. Burgess, and H. A. Scheraga, *J. Phys. Chem.*, **79**, 2361 (1975).

(16) Schröder and Lübke, "The Peptides", Vol. I, Academic Press, New York, N.Y., 1965; see especially p 271.

(17) R. Schwyzer, *Amino Acids Pept. Antimetab. Act., Ciba Found. Symp., 1958*, 171 (1958).

(18) V. T. Ivanov, V. V. Shilin, Ya. Bernat, and Yu. A. Ovchinnikov, *J. Gen. Chem. USSR (Engl. Transl.)*, **41**, 2341 (1971).

(19) M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, A. V. Evstratov, I. I. Mikhaleva, and I. D. Ryabova, *J. Gen. Chem. USSR (Engl. Trans.)*, **42**, 2316 (1972).

(20) P. M. Hardy, G. W. Kenner, and R. C. Sheppard, *Tetrahedron*, **19**, 95 (1963).

(21) V. T. Ivanov, V. V. Shilin, and Yu. A. Ovchinnikov, *J. Gen. Chem. USSR (Engl. Transl.)*, **40**, 902 (1970).

(22) Th. Wieland, W. Konz, J. Faesel, and J. Lewalter, U.S. Patent No. 3 705 887 (1973).

Communications to the Editor

Single-Crystal Susceptibilities of an $S = 3/2$ Iron(III), Insulating Ferromagnet

Sir:

Several studies[1,2] have been reported recently on the molecules $Fe(X)(S_2CNR_2)_2$ where X is a halogen and R is an alkyl group. These molecules are of interest because, as a result of their tetragonal pyramidal geometry, with C_{2v} symmetry at the iron site, they are among the few examples of a spin $3/2$ ground state for trivalent iron. The most extensively studied example, $Fe(Cl)[S_2CN(C_2H_5)_2]_2$, hereafter $Fe(dtc)_2Cl$, crystallizes in space group $P2_1/c$ and has a geometry in which the chlorine atom is at the apex of the pyramid and the iron atom is at the centroid, 0.62 Å above the plane of the four sulfur atoms of the dithiocarbamate ligands.[3] This particular example of this series of molecules is especially interesting because it has been reported as ordering ferromagnetically at 2.43 K.[2] Further significance attaches to $Fe(dtc)_2Cl$ in that, on the basis of the present measurements, and measurements in the critical region, this substance appears to be one of the very few examples of a three-dimensional Ising ferromagnet.

We have measured the magnetic susceptibility along the three principal axes in single crystals of $Fe(dtc)_2Cl$ using the near-zero-field mutual inductance method. The crystal structure is monoclinic and therefore the *b* axis, [010], must be the direction of one of the principal axes. Careful measurements along various directions in the *ac* plane revealed that the other two principal axes, of maximum and minimum susceptibility in this plane, were, within an experimental uncertainty estimated to be 2°, respectively, along the [101] direction and along the direction normal to [010] and [101], that is the direction normal to the $(10\bar{1})$ plane. These results, corrected for demagnetization, appear in Figure 1. The susceptibility along the [101] direction increases very rapidly with decreasing temperature until, just below 2.47 K, it attains an

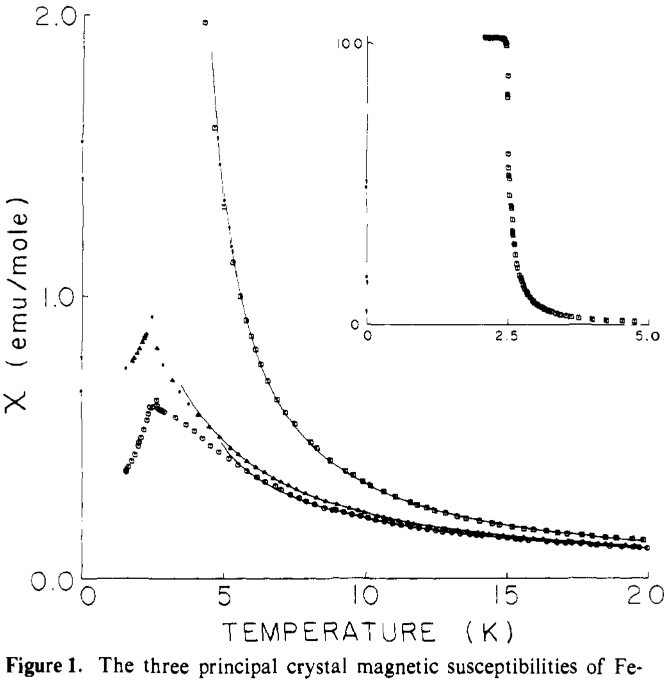

Figure 1. The three principal crystal magnetic susceptibilities of $Fe(dtc)_2Cl$. Circles, triangles, and squares are experimental data along the [010], $(10\bar{1})$, and [101] axes, respectively. The curves are the best fits to the data with the parameters given in the text. The inset shows the susceptibility in the [101] direction at low temperatures; these data were obtained using a 1-mg crystal and have not been corrected for demagnetization.