Scattering functions of poly(amino acid) in helix-coil transition

Y. Ishimuro, H. Hayashi, F. Hamada* and A. Nakajima
Department of Polymer Chemistry, Kyoto University, Kyoto 606, Japan
(Received 30 June 1983; revised 6 October 1983)

Scattering functions of poly(amino acid) in the helix–coil transition region have been calculated on the basis of the Zimm–Bragg theory, to examine the effects of the helical content on the scattering function and on the cross-section factor, from which the radius of gyration of the cross section and the mass per unit length of the helical sequence can be obtained. The molecule is assumed to contain only one α-helical sequence of any length, leaving unfolded coils at the chain ends. The statistical mechanical averages of the interference among scattering units, apart from the interference within the helical sequence, are approximated by the expansion in terms of the even moments up to the eighth moment, which are calculated by the generator matrix method based on the conformation energies computed for poly (L-alanine). The scattering function at intermediate angles varies systematically according to the variation of the helical content, demonstrating the sensitivity of intermediate-angle scattering to the conformational changes in the helix–coil transition. The comparison of the Guinier plots of the cross-section factors, calculated from the scattering functions at various helical contents, has indicated that the radius of gyration of the cross section of the helical sequence can be determined accurately from the plots even when the coil content is as much as 30%, and that the mass per unit length is determinable to within an error of 6% unless the coil content exceeds 10%.

(Keywords: scattering functions; poly(amino acid); helix–coil transition; radius of gyration of the cross section; intermediate-angle scattering; helical content)

INTRODUCTION

In previous papers[1,2], we have measured the small-angle X-ray scattering (SAXS) by poly(N^{ε}-carbobenzoxy-L-lysine) and poly(γ-methyl-L-glutamate) in several helicogenic solvents, and have observed that the conformations of the side chains were appreciably affected by the properties of the solvent. One of the most important parameters characterizing the side-chain conformations is the radius of gyration of the cross-section $\langle S_q^2 \rangle^{1/2}$ of the helix, because it is directly obtainable from analysis of measured scattering intensity without the aid of sophisticated scattering models. This analysis, which involves the Guinier plot of the cross-section factor, is based on the assumption that the molecule can be regarded as a rod over a certain range of the scattering vector. The accuracy of the determination of $\langle S_q^2 \rangle^{1/2}$ can, therefore, deteriorate due to various factors which give rise to deviations from this assumption. We have already pointed out that the length and its distribution of the helix have little effect on the cross-section factor as long as the helix is sufficiently long[3]. Here we present calculations of scattering functions of poly(amino acid) in the region of the helix–coil transition, and clarify the effects of the interruption of the helix and of the helical content on the scattering function and on the cross-section factor.

CALCULATION

If we consider a molecule comprising N residues divided into three sequences as shown in *Figure 1*, and let m be the number of residues constituting the helical sequence, and l and n be those numbers for the two different coil sequences. We have assumed that only one helical sequence of any size can exist in a molecule according to the Zimm–Bragg theory[4], in which m is interpreted as the number of residues involved in hydrogen bonding.

Since the free energies of the helical state and the coil state are considered to be independent of the position of the residue and of the length of the sequence, the partition function $Z(N)$ for the molecule is written as

$$Z(N) = 1 + \sigma \sum_{m=3}^{N} (N - m + 1) s^{m-2} \qquad (1)$$

where σ is the cooperativity parameter, and s is the

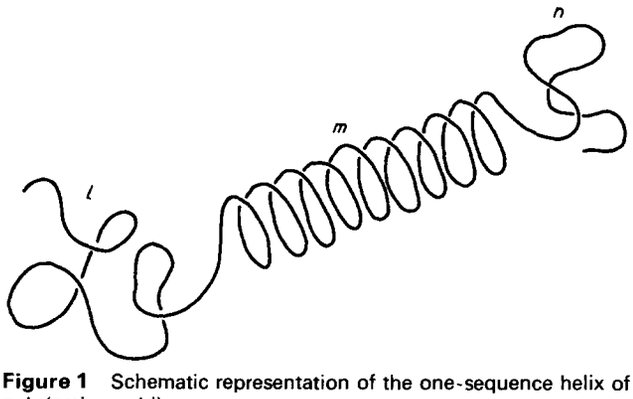

Figure 1 Schematic representation of the one-sequence helix of poly(amino acid)

0032-3861/84/091258-05$03.00

equilibrium constant. We have assumed that the residues near both ends of the molecule, which may be left unfolded in reality, can also be incorporated in the helical sequence. The helical content f_N, i.e. the average fraction of the helical residues, is given by

$$f_N=\sigma\sum_{m=3}^{N} m(N-m+1)s^{m-2}/NZ(N) \tag{2}$$

The probability $p(m;f_N)$ that the molecule contains a helical sequence consisting of m residues at fixed f_N is given by

$$p(m;f_N)=\sigma(N-m+1)s^{m-2}/Z(N) \tag{3}$$

Since $p(m;f_N)$ is independent of the position of the helical sequence, the scattering function $i(h)$ at f_N is expressed in the form:

$$i(h)=\sum_{m=3}^{N} i(h;m)p(m;f_N) \tag{4}$$

where $i(h;m)$ is the average scattering function for the molecules containing a helical sequence of m residues, and $h=(4\pi/\lambda)\sin\theta$ with wavelength λ and half of the scattering angle θ. This function $i(h;m)$ can be written as an average over all possible locations of the helical sequence with m fixed, an equal probability being assigned to all positions of the helical sequence.

$$i(h;m)=\sum_{l=0}^{N-m} i_{l,m,n}(h)/(N-m+1) \tag{5}$$

where $i_{l,m,n}(h)$ is the scattering function for the molecule consisting of two coil sequences of l and n residues and a helical sequence of m residues. Provided that the molecule is oriented at random, the scattering function $i_{l,m,n}(h)$ is generally expressed in the form:

$$i_{l,m,n}(h)=(N+1)^{-2}f(h)^2\sum_{i=1}^{N}\sum_{j=0}^{N}\left\langle\frac{\sin hr_{ij}}{hr_{ij}}\right\rangle \tag{6}$$

where r_{ij} is the distance between scattering units i and j, $f(h)$ is the scattering factor of the unit, and the angle brackets denote the statistical mechanical average over all elligible conformations of the molecule. We consider the centre of the scattering unit to be located at the junction of successive virtual bonds. Since the scattering function can be expressed as the sum of the products of the contribution from each sequence, $i_{l,m,n}(h)$ can be rewritten as

$$\begin{aligned}i_{l,m,n}(h)=(N+1)^{-2}f(h)^2\Bigg(&(N+1)+\sum_{i=0}^{l-1}\sum_{j=0}^{l-1}\left\langle\frac{\sin hr_{ij}}{hr_{ij}}\right\rangle\\&+\sum_{i=1}^{l+m-1}\sum_{j=1}^{l+m-1}\frac{\sin hr_{ij}}{hr_{ij}}\sum_{i=l+m}^{N}\sum_{j=l+m}^{N}\left\langle\frac{\sin hr_{ij}}{hr_{ij}}\right\rangle\\&+2\sum_{i=0}^{l-1}\sum_{j=1}^{l+m-1}\left\langle\frac{\sin hr_{ij}}{hr_{ij}}\right\rangle+2\sum_{i=l}^{l+m-1}\sum_{j=l+m}^{N}\left\langle\frac{\sin hr_{ij}}{hr_{ij}}\right\rangle\\&+2\sum_{i=0}^{l-1}\sum_{j=l+m}^{N}\left\langle\frac{\sin hr_{ij}}{hr_{ij}}\right\rangle\Bigg)\\&=(N+1)^{-2}f(h)^2\\&\quad\times[(N+1)+F_l(h)+F_m(h)+F_n(h)\\&\quad+2F_{lm}(h)+2F_{mn}(h)+2F_{ln}(h)]\end{aligned} \tag{7}$$

where the first three sums are over different units i and j. $F_l(h)$ and $F_n(h)$ represent interference within each coil sequence, and $F_m(h)$ interference within the helical sequence. Cross terms, $F_{lm}(h)$, $F_{mn}(h)$ and $F_{ln}(h)$ express the interference between the coil sequence of l units and the helical sequence, between the helical sequence and the coil sequence of n units, and between the two different coil sequences, respectively.

$F_m(h)$ representing the interference within the helical sequence of m units is given by

$$F_m(h)=2\sum_{t=1}^{m}(m+1-t)\frac{\sin hr_t}{hr_t} \tag{8}$$

where r_t is the distance between helical units separated by t units. The other five terms containing the contribution from the coil sequence may be expanded in a series in the even moments of r_{ij}^5, e.g.

$$\begin{aligned}F_{lm}(h)=&\sum_{i=1}^{l-1}\sum_{j=1}^{l+m-1}\exp(-v)\\&\times[1+g_{4;ij}(2v)^2+g_{6;ij}(2v)^3+g_{g;ij}(2v)^4+\cdots]\end{aligned} \tag{9}$$

with

$$\begin{aligned}v&=h^2\langle r_{ij}^2\rangle/6\\g_{4;ij}&=-2^{-3}[1-3\langle r_{ij}^4\rangle/5\langle r_{ij}^2\rangle^2]\\g_{6;ij}&=-2^{-4}[(1-3\langle r_{ij}^4\rangle/5\langle r_{ij}^2\rangle^2)\\&\quad-(1/3)(1-9\langle r_{ij}^6\rangle/35\langle r_{ij}^2\rangle^3)]\\g_{8;ij}&=-2^{-6}[(1-3\langle r_{ij}^4\rangle/5\langle r_{ij}^2\rangle^2)\\&\quad-(2/3)(1-9\langle r_{ij}^6\rangle/35\langle r_{ij}^2\rangle^3)\\&\quad+(1/6)(1-3\langle r_{ij}^8\rangle/35\langle r_{ij}^2\rangle^4)]\end{aligned}$$

where the sum in equation (9) extends over coil unit i and helical unit j. The other terms are given similarly.

Since rotations around virtual bonds in the coil sequence are independent of the states of neighbouring bonds[6], all moments $\langle r_{ij}^{2p}\rangle$ appearing in $F_l(h)$ and $F_n(h)$ can be replaced by $\langle r_t^{2p}\rangle$ without regard to the location of the sequence of $t=|i-j|$ units within the coil, which simplifies the evaluation of $F_l(h)$ and $F_n(h)$. The second and fourth moments were calculated according to the matrix generation method[7], while the higher moments up to the eighth were estimated by use of the 'equivalent freely jointed chain'[8]. The latter approximation may be rather inadequate for the evaluation of the higher moments in the cross terms especially when the helical sequence is relatively long. In that case, however, the contribution from the term $F_m(h)$, which can be obtained exactly, becomes dominant, keeping the error within a reasonable range.

The calculations were performed for poly(L-alanine), the conformation energies of which were available from Conrad and Flory[9,10]. The virtual bond length b was assigned the value 3.8 Å, and the value of σ equal to 10^{-4} [11]. For simplicity we have assumed that the scattering unit is a homogenious sphere of radius $R=1.9$ Å, i.e. a half of the virtual bond length. Although the choice of R is still somewhat arbitrary, the above value is reasonable for present purposes unless the side chain is very long and h is very large.

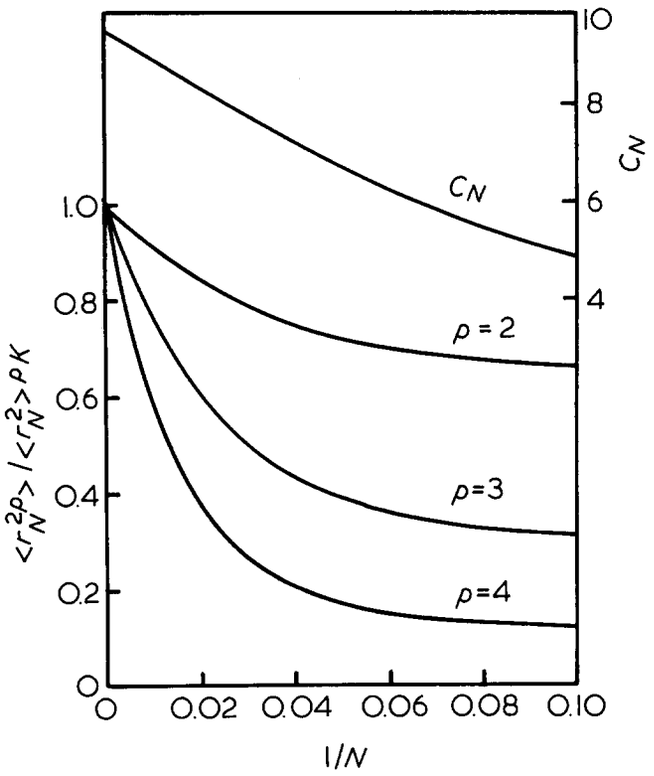

Figure 2 Characteristic ratio $C_N=\langle r_N^2\rangle/Nb^2$ and moment ratios $\langle r_N^{2p}\rangle/\langle r_N^2\rangle^p K$ for the coil conformation plotted against $1/N$. Moment ratios are normalized to unity at $N\to\infty$, K being the normalization factor

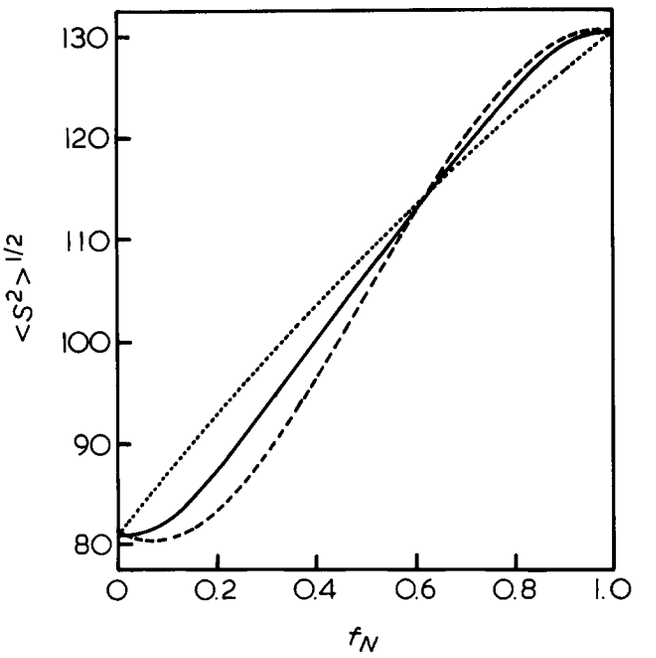

Figure 3 Radius of gyration $\langle S^2\rangle^{1/2}$ for $N=300$ vs. helical content f_N, calculated according to the Zimm–Bragg theory (———), all-or-none model (– – –) and model with fixed m (·····)

RESULTS AND DISCUSSION

In *Figure 2* the characteristic ratio $C_N=\langle r_N^2\rangle/Nb^2$ and the moment ratios $\langle r_N^{2p}\rangle/\langle r_N^2\rangle^p$ with $p=2, 3$ and 4 for the coil conformation are plotted against $1/N$. The moment ratios are normalized such that the intercept at $1/N=0$ has the value of unity, the normalization factors being 5/3, 35/9 and 35/3 for $p=2, 3$ and 4, respectively. The decrease in C_N and departures of the moment ratios from their limiting values with decreasing N,demonstrate the deviation from the Gaussian distribution of the distance between two residues. The limiting value of C_∞ is estimated to be 9.42, which is in good accord with the value 9.62 obtained by Conrad and Flory[9]. *Figure 3* shows the variation of the radius of gyration $\langle S^2\rangle^{1/2}$ for $N=300$ at various helical contents f_N. Results from the Zimm–Bragg theory are shown by the solid curve. The other curves are based on different assumptions; the dotted curve is calculated

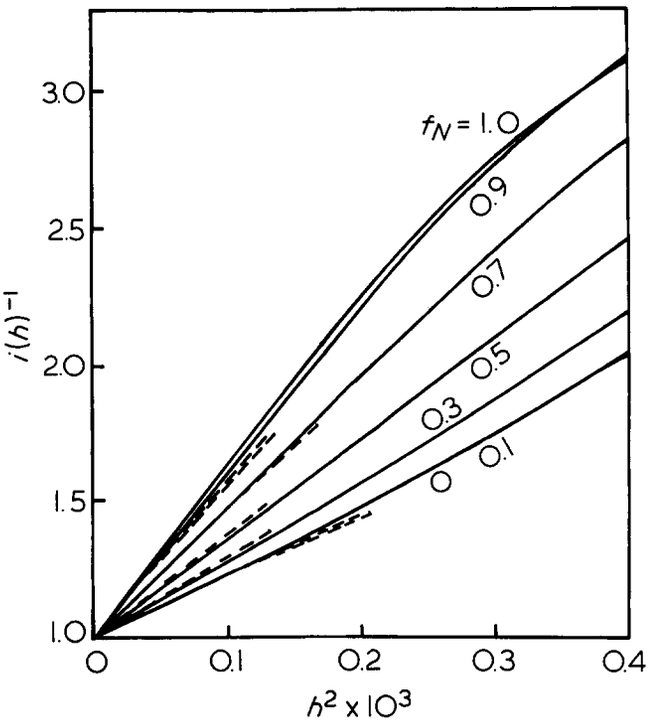

Figure 4 Reciprocal of $i(h)$ for $N=300$ at small angles vs. h^2 at various helical contents f_N, calculated according to the Zimm–Bragg theory. Dotted lines show the initial tangent to the curves

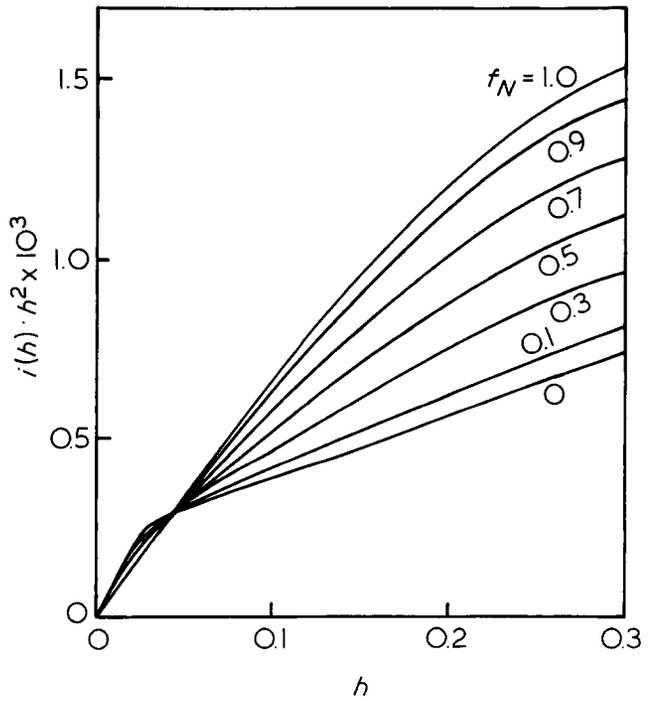

Figure 5 Kratky plots of scattering functions for $N=300$ calculated according to the Zimm–Bragg theory

assuming that the length of the helical sequence or m is fixed such that $m=f_N N$, and the dashed curve is obtained on the assumption that the system is a mixture of helical molecules and coil molecules (all-or-none model), the fraction of the former being f_N. The Zimm–Bragg theory and the all-or-none model yield an S-shaped curvature, while the model with fixed m leads to almost linear variation with f_N.

Figure 4 shows the scattering functions at small angles for $N=300$, as expressed by the Zimm plots, at various helical contents, calculated according to the Zimm–Bragg theory. The shape of the scattering functions in this angular range is insensitive to the helical content up to $f_N=0.1$, as expected from the variation of $\langle S^2\rangle^{1/2}$. The scattering functions at intermediate angles are shown in *Figure 5* in the form of Kratky plots, i.e., $i(h)h^2$ vs. h. Systematic variation with f_N is observed, demonstrating the possibility of determining the helical content by intermediate-angle scattering. Norisuye and Teramoto[12] calculated light-scattering functions for poly(amino acid) molecules based on a simplified model, in which the cross-section of a helical section was ignored by treating the helix as a succession of aligned scattering points.

Figure 6 shows the Guinier plots of the cross-section factor at various helical contents, i.e., plots of log $i(h)h$ vs. h^2, where $i(h)h$ is referred to as the cross-section factor. We can estimate the radius of gyration $\langle S_q^2\rangle^{1/2}$ of the cross-section and the mass per unit length M_q from the inclination and intercept of the tangent in the range of $1/L<h<1/\langle S_q^2\rangle^{1/2}$, where L is the length of the helical sequence. The dotted line shows the limiting form calculated for an infinitely long rod. The curve for the helix ($f_N=1.0$) has a long linear portion characteristic to

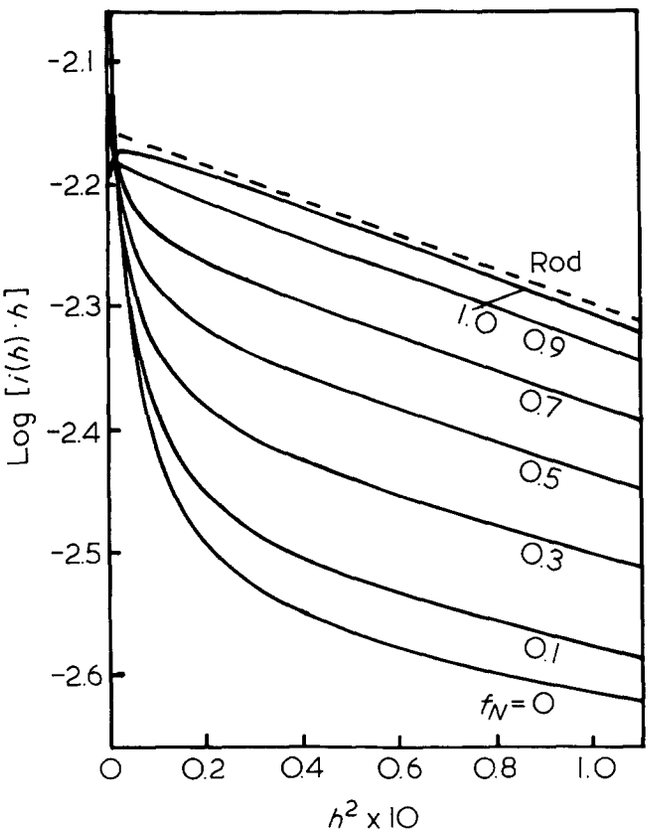

Figure 6 Guinier plots of the cross-section factor at various helical contents f_N according to the Zimm–Bragg theory. Dotted lines shows the limiting function for an infinitely long rod

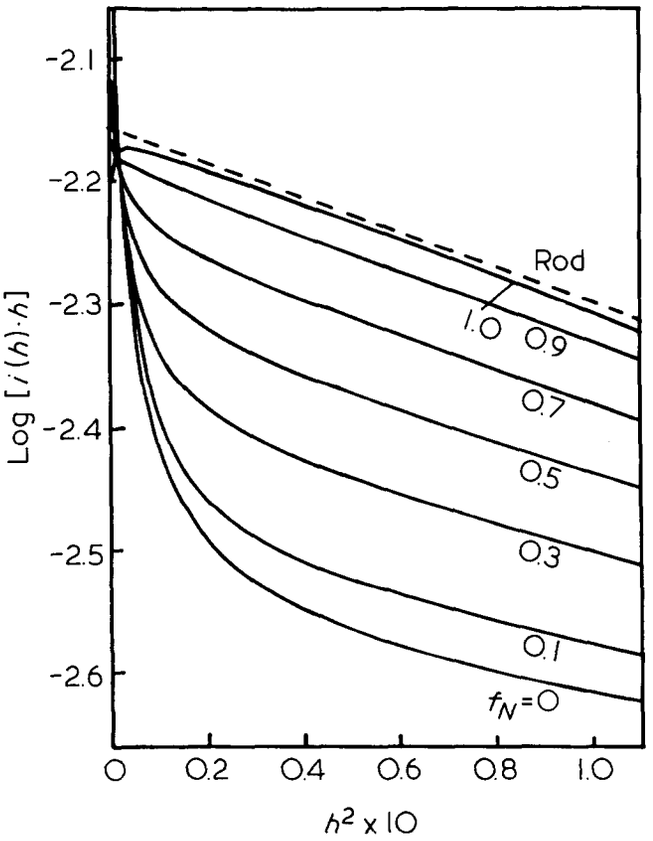

Figure 7 Guinier plots of the cross-section factor calculated with m fixed. Dotted line shows the limiting functions for an infinitely long rod

rodlike molecules, while, as the helical content decreases, the curves are shifted downward, exhibiting a steep slope in the low angle region, and the linear portion decreases. The values of $\langle S_q^2\rangle^{1/2}$ determined at $f_N=0.9$, 0.7 and 0.5 are almost identical to that of the helix, while the values of M_q are smaller by 6%, 18% and 30%, respectively, than that for the helix. When the helical content is below 0.3, we cannot any longer draw a reasonable tangent, indicating that the assumption on which this analysis is based is no longer appropriate. These observations lead to the conclusion that the presence of the coil sequences up to about 30% does not appreciably affect the determination of the radius of gyration of the cross-section of the helical sequence, and that the mass per unit length of the helical sequence can be obtained to within the error of ordinary measurements when the coil content is less than 10%.

Figure 7 shows Guinier plots of the cross-section factor calculated on the basis of the model with fixed m. Although these curves are similar to those in *Figure 6*, each curve is shifted slightly downward and differs in the low angle region. The curves based on the all-or-none model are almost identical with those calculated according to the Zimm–Bragg theory. Calculations were also made with $N=200$, and no significant difference was observed, demonstrating that the molecular weight has little effect on the shape of the scattering curve in the intermediate-angle region.

ACKNOWLEDGEMENT

The authors gratefully acknowledge Professor P. J. Flory of Stanford University and Dr J. C. Conrad of the

University of California, San Diego for providing the data on the conformation energy calculations of poly(L-alanine).

REFERENCES

1 Ishimuro, Y., Hamada, F. and Nakajima, A. *Macromolecules* 1978, **11**, 382
2 Ishimuro, Y., Hamada, F. and Nakajima, A. *J. Polym. Sci., Polym. Chem. Edn.* 1979, **17**, 1811
3 Ishimuro, Y., Hamada, F. and Nakajima, A. *Biopolymers* 1981, **20**, 2499
4 Zimm, B. H. and Bragg, J. K. *J. Chem. Phys.* 1959, **31**, 526
5 Nagai, K. *J. Chem. Phys.* 1963, **38**, 924
6 Flory, P. J. 'Statistical Mechanics of Chain Molecules', John Wiley & Sons, New York, 1969
7 Flory, P. J. *Macromolecules* 1974, **7**, 381
8 Yoon, D. Y. and Flory, P. J. *Polymer* 1975, **16**, 645
9 Conrad, J. C. and Flory, P. J. *Macromolecules* 1976, **9**, 41
10 Conrad, J. C. and Flory, P. J. private communication
11 Teramoto, A. and Fujita, H. J. *J. Macromol. Sci.* 1976, **C15**, 165
12 Norisuye, T. and Teramoto, A. *Polym. J.* 1970, **1**, 341