Helically Arranged Azobenzene Chromophores along a Polypeptide Chain. 2. Prediction of Conformations and Calculation of Theoretical Circular Dichroism

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ABSTRACT: Conformational energy calculations were carried out to predict main-chain conformations and side-chain orientations of sequential polypeptides with repeating units of Ala_m-azoAla (I; m = 1–3), Ala-azoAla-Aib-azoAla (II; m' = 1), and $Ala_{m'-1}$-azoAla-Aib) (II; m' = 2 and 3). The L-phenylazophenylalanine (azoAla) units in the sequential polypeptides were assumed to be in the trans state. Circular dichroism spectra were calculated for the predicted conformations on the basis of exciton theory. For both series of polypeptides theoretical CD spectra agreed with the experimental spectra for $m(m')$ = 1 and 2, in their profile and magnitude. The agreement indicates that the azobenzene groups are arranged regularly along the helix in the real polypeptides. In the case of $m(m')$ = 3, theory predicted opposite the CD profile with very small intensity compared with the experimental CD spectrum. The discrepancy was tentatively attributed to a partial unwinding of the helical conformation.

In the accompanying paper, six sequential polypeptides containing L-*p*-phenylazophenylalanine (azoAla) units were synthesized and a relation between the amino acid sequence and the circular dichroism spectrum (CD) was studied.[1] The CD spectra showed similar patterns of negative exciton couplet (positive peak at shorter wavelength and negative peak at longer wavelength) for the six polypeptides, although the spatial arrangements of azobenzene groups are quite different from each other. Furthermore, it was suggested that the main-chain conformations of the poly[$Lys(Z)_m$-azoAla] series are different from those of the Aib-containing polypeptides [Lys(Z) = N^{ϵ}-[(benzyloxy)carbonyl]-L-lysine, Aib = α-aminoisobutyric acid]. This paper describes theoretical approaches to the questions raised in the above experiment. The arrangement of the azobenzene side groups can be predicted from empirical energy calculations for the six sequential polypeptides. By using the spatial arrangement of azobenzene groups, theoretical CD can be calculated on the basis of exciton theory. The calculations will clarify (1) how the azobenzene groups are arranged spatially in each sequential polypeptide and how they fluctuate around the most stable orientation, (2) how the CD spectrum changes for different spatial arrangements of azobenzenes, and (3) how intense the CD spectrum could be when all irregularities and the fluctuation that are experimentally unavoidable were excluded.

Conformational energy calculations have been carried out successfully to predict side-chain orientations of polypeptides carrying arylalanine-type amino acids.[2-4] Since in the arylalanine-type amino acids, a bulky aromatic group is linked to the α-carbon by a single methylene chain, the reliability and accuracy of the calculation is high, due to the restricted rotational freedom of the side chain. This has been confirmed in the case of sequential polypeptides containing 1-naphthylalanine,[2] 1-pyrenylalanine,[3] and 9-anthrylalanine.[4] In this study, conformational energy calculations and theoretical CD calculations were carried out for the polypeptides containing another arylalanine-type amino acid, i.e., L-*p*-phenylazophenylalanine (azoAla).

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Procedures

Conformational Calculation. The conformational energy calculation was carried out by using the ECEPP potential energy parameters.[5] For simplicity, an alanine unit was replaced for a Lys(Z) unit. The ECEPP parameters for the Aib unit were taken from the literature.[6] Those of azoAla were determined in this study. The structural parameters for an azobenzene group were taken from crystallographic data of *trans*- and *cis*-azobenzenes.[7] The partial charges were calculated from CNDO/ON MO's of *N*-acetyl-L-phenylazophenylalanine *N*-methylamide in the trans and cis states.[8] The structural parameters and partial charges for *trans*-phenylazophenylalanine are listed in Figure 1. Those for *cis*-phenylazophenylalanine are available on request. The Simplex algorithm was employed for the energy minimization of conformations of sequential polypeptides. The calculations were carried out on an NEC PC9801 personal computer. The program has been coded by one of the authors (M.S.).[2-4]

Theoretical Circular Dichroism. Theoretical CD was computed on the basis of the exciton theory developed by Woody.[9] Since no strong CD was observed for polypeptides with *cis*-azobenzene groups, only CD spectra for the all-trans state were calculated. For the *trans*-azobenzene chromophore, only the lowest energy $\pi\pi^*$ transition at 325 nm was considered and the $n\pi^*$ transition that appears at 435 nm and other transitions at shorter wavelengths were neglected. The neglect of the $n\pi^*$ transition may be justified, because the coupling between the $\pi\pi^*$ transition and the $n\pi^*$ state should be small. The neglect of higher energy $\pi\pi^*$ transitions may also be justified by the experimental fact that no CD peak is observed in this region. The absence of the CD suggests that the higher energy transitions are mixtures of differently polarized transitions, as supported from a MO calculation.[10]

The transition moments and the monopole charges of *trans*-azobenzene were calculated from PPP–CI molecular orbitals.[10] The magnitude of the transition dipole moment for the lowest $\pi\pi^*$ transition (Bu) was 6.48 D, whereas the experimental transition dipole moment of *N*-acetyl-L-*p*-phenylazophenylalanine methyl ester in trimethyl phosphate was 6.92 D. Therefore, the monopole charges obtained from the PPP–CI calculation was multiplied by 6.48/6.92 to reproduce the experimental ab-

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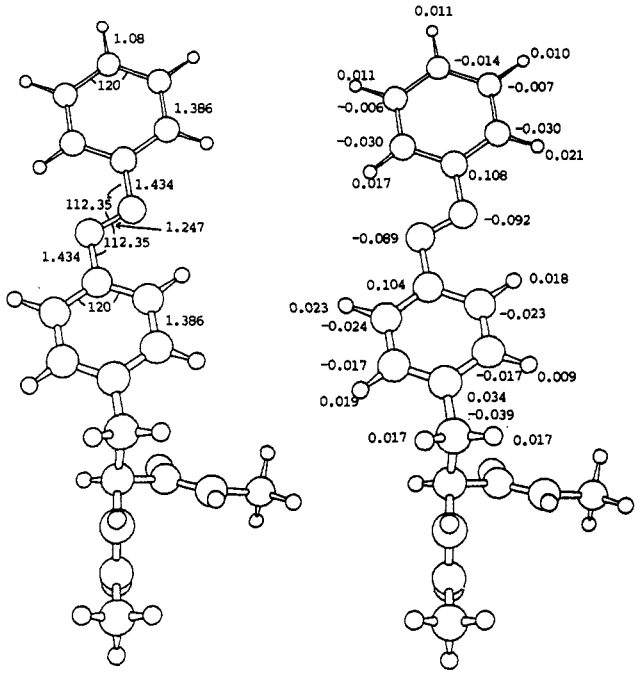

Figure 1. (Left) Partial charges (in electron charge unit) for atoms in the side chain of L-*p*-phenylazophenylalanine. The partial charges for atoms in the main chain are taken to be the same as those of phenylalanine.[5] (Right) Structural parameters of L-*p*-phenylazophenylalanine. Those in the main chain are taken to be the same as those of phenylalanine.[5]

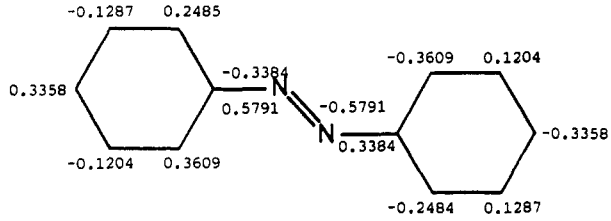

Figure 2. Monopole charges (in electron charge unit) for the lowest $\pi\pi^*$ transition (325 nm) of azobenzene. A pair of monopoles of the same charges are assumed to be located 1.0806 Å below and above the planar aromatic rings. The numbers indicate the charge on one of the pairs.

sorption spectrum. The polarization of the transition moment is tilted by 14° from the C–N bond of azobenzene. The peak wavelength of the experimental spectrum (325.5 nm) was used instead of the calculated peak position (315.5 nm), as the energy of the $\pi\pi^*$ transition. The monopole charges for the lowest $\pi\pi^*$ transition are listed in Figure 2.

Results and Discussion

Side-Chain Energy Contour Maps. Since the CD spectrum at the amide region showed a pattern of helix, side-chain energy contour maps for polypeptides Ala_m-azoAla (m = 1–3) were calculated assuming that the main chain is fixed to an α-helix ($\phi = -63°, \psi = -42°$)[11] or to a 3_{10}-helix ($\phi = -60°, \psi = -30°$).[6] Each side-chain rotational angle (χ_1 or χ_2) of an azoAla unit was assigned to be the same for all azoAla units in the polypeptides; i.e., a helical symmetry was assumed for the side-chain as well as for the main-chain conformation. The energy contour map for $(Ala_2\text{-azoAla(T)})_9$ is shown in Figure 3 [azoAla(T) = azoAla with *trans*-azobenzene group]. Only a single position around 180° is allowed for χ_1, but two positions are allowed with almost equal probabilities for χ_2. The two rotamers correspond to the orientations shown in Figure 4. In the following discussion the minimum at χ_2 = 80–90° will be called the A form and that at χ_2 = 260–279° will be called the B form.

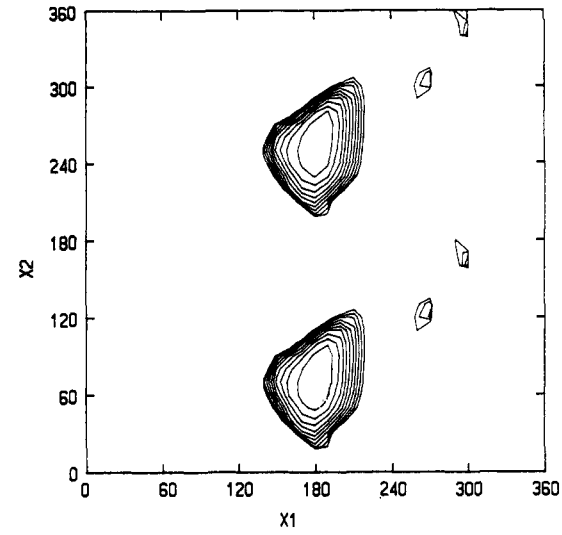

Figure 3. Side-chain energy contour map of poly(Ala_2-azoAla) in an α-helical main chain ($\phi = -63°, \psi = -42°, \omega = 180°$). The interval of contour lines is 0.5 kcal/mol.

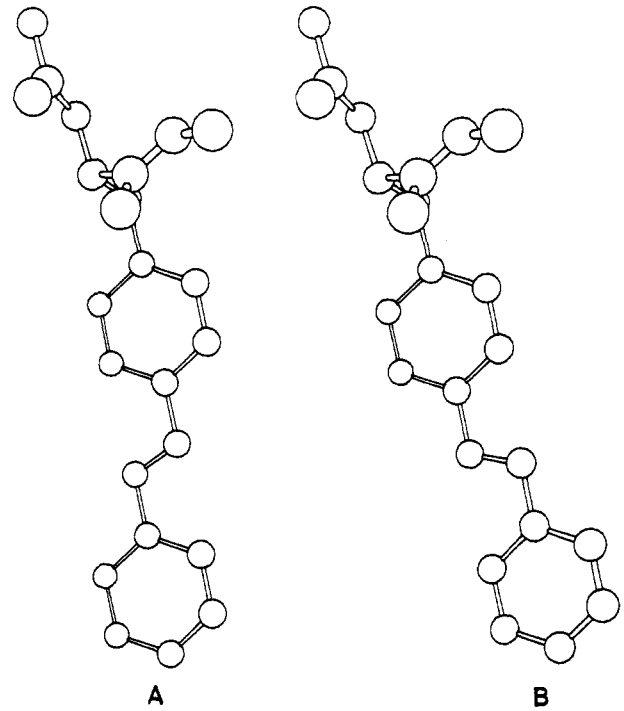

Figure 4. Two possible side-chain orientations. (Left) A form (χ_2 = 80°). (Right) B form (χ_2 = 260°).

The contour map is very similar to that of L-phenylalanine, indicating that the phenylazo group on the para position of phenylalanine does not influence the side-chain orientation. The contour maps for $(Ala_1\text{-azoAla(T)})_{15}$ and that for $(Ala_3\text{-azoAla(T)})_7$ were also calculated. But the maps were virtually the same as Figure 3. Therefore, interactions between the azobenzene side groups are weak in the sequential polypeptides. Side-chain energy contour maps were also calculated for 3_{10}-helical main chains. However, the contour maps were not much different from those of the α-helical main chain.

The side-chain orientation became highly restricted when Aib units were introduced at specific positions along a polypeptide chain. As examples, energy contour maps for the side-chain orientations of $azoAla_1$(T) and $azoAla_2$-(T) in α-helical poly[$azoAla_1$(T)-Ala-$azoAla_2$(T)-Aib] (n

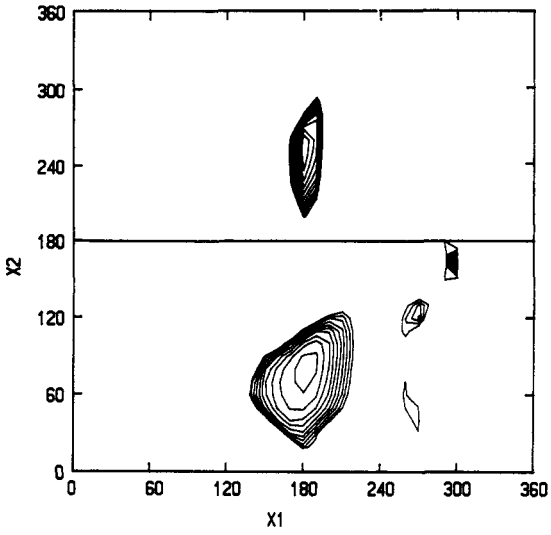

Figure 5. Side-chain energy contour map of azoAla$_1$(T) (bottom) and of azoAla$_2$(T) (top) in poly[azoAla$_1$(T)-Ala-azoAla$_2$(T)-Aib] in an α-helix. The interval of contour lines is 0.5 kcal/mol.

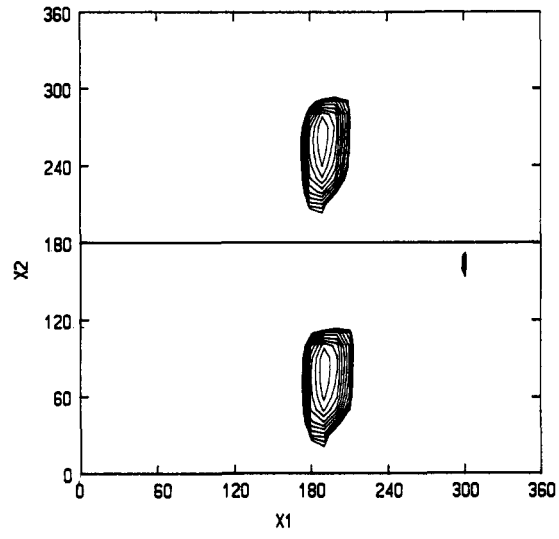

Figure 6. Side-chain energy contour map of azoAla$_1$(T) (bottom) and of azoAla$_2$(T) (top) in poly[azoAla$_1$(T)-Ala-azoAla$_2$(T)-Aib] in a 3_{10}-helix. The interval of contour lines is 0.5 kcal/mol.

= 7) are shown in Figure 5. Since, in each contour map, the lower half (χ_2 = 0–180°) is virtually the same as the upper half (χ_2 = 180–360°), only the lower half of the contour map of azoAla$_1$(T) and the upper half of that of azoAla$_2$(T) are shown together in Figure 5. The side-chain energy contour maps for the azoAla(T) unit in poly(Ala-azoAla(T)-Aib) and in poly(Ala-Ala-azoAla(T)-Aib) were also calculated. The map of the former polypeptide resembles that of azoAla$_1$(T) shown in the lower half of Figure 5, and that of the latter polypeptide resembles the upper half of Figure 5. These results indicate that the orientation of the azobenzene unit in the -Aib-*Xyz*-*Xyz*-azoAla- sequence is highly constrained. The orientational constraint is due to the steric overlaps between the α-methylene group of azoAla unit and the methyl groups of the Aib unit. This is a general rule applicable to any Aib-containing α-helical polypeptides.

Figure 6 shows the contour maps of azoAla$_1$(T) (lower) and azoAla$_2$(T) (upper) in 3_{10}-helical poly[azoAla$_1$(T)-Ala-azoAla$_2$(T)-Aib] (n = 7) (ϕ = –60°, ψ = –30°).[6] The constraint is partly relaxed in this case, because the β-methylene group of azoAla and the two methyl groups of the Aib unit become far separated in the 3_{10}-helix. However, the orientation of an azobenzene side group is still more constrained than that in polypeptides without Aib units.

Energy contour maps for *cis*-azobenzene groups were also calculated. Two enantiomers forms of *cis*-azobenzene (P and M) were considered. The orientation of a *cis*-azobenzene unit is more constrained than that of the trans unit when it is positioned in the same sequential polypeptide. The contour maps are available on request.

Energy Minimization. In the calculation of energy contour maps, the polypeptide main chain was fixed either to an α-helix or to a 3_{10}-helix and the stable orientations of the side chains were searched. To proceed further, main-chain rotational angles were also allowed to vary and the energy minimization was performed for the six polypeptides. The results are listed in Table I. The average main-chain angles of the polypeptides carrying no Aib units were converged to (ϕ = –67°, ψ = –39°). The rotational angles deviate from those of the α-helix (–63°, –42°),[11] but the deviation is common for the ECEPP parameters. For example, the energy minimization of poly(L-alanine) using ECEPP parameters gave (–68°, –38°) (ECEPP α-helix).

Energy minimization was also carried out for the three Aib-containing polypeptides. Average angles of the main chain were also converged to the ECEPP α-helix. Therefore, it is concluded that the azobenzene side groups do not affect the main-chain conformation significantly in the two series of polypeptides.

As typical examples for α-helical and 3_{10}-helical conformations, a ball-and-stick model of poly[Ala$_2$-azoAla(T)] in the minimum-energy conformation (Table I) is shown in Figure 7 and that of poly[Ala-azoAla(T)-Aib] in a 3_{10}-helix (–60°, –30°) is shown in Figure 8.[12] In the 3_{10}-helix, the azobenzene groups are arranged with a looser helical pitch than that of an α-helix.

Theoretical CD Calculations. CD spectra can be calculated on the basis of the spatial arrangements of azobenzene groups predicted from the above conformational energy calculations. The monopole charges required for the CD calculation are listed in Figure 2. The number of azobenzene groups taken into consideration was 12 for m = 1, 10 for m = 2, and 9 for m = 3 in the Ala$_m$-azoAla(T) series. It was 12 for azoAla(T)-Ala-azoAla(T)-Aib, 10 for Ala-azoAla(T)-Aib, and 10 for Ala$_2$-azoAla(T)-Aib. The theoretical CD spectrum is presented by $\Delta\epsilon$ values divided by the number of azobenzene groups in each polypeptide.

Theoretical CD spectra for the Ala$_m$-azoAla(T) series with *trans*-azobenzene groups in the A and B form are shown in Figures 9 and 10, respectively. The rotational angles are taken from Table I. To examine the effect of small changes in the main-chain conformation, CD spectra were also calculated for different sets of ϕ and ψ, i.e., for the Pauling–Corey α-helix (ϕ = –57°, ψ = –47°) and for the ECEPP α-helix (–68°, –38°). However, theoretical CD spectra were not sensitive to the small changes in the main-chain conformation.

CD calculations were also performed for the polypeptides containing Aib units. Theoretical CD spectra for the minimum-energy conformations listed in Table I are virtually the same as those shown in Figures 9 and 10. Since the Aib units often favor a 3_{10}-helix instead of an α-helix, CD spectra are also calculated for the standard 3_{10}-helical main chain (ϕ = –60°, ψ = –30°). In this case, the side-chain orientations were optimized assuming the 3_{10}-helical main chain. Theoretical CD spectra for the 3_{10}-helix with azobenzene groups in the B form are shown in Figure 11. The CD spectra in Figure 11 are similar to those in Figure 9, indicating that the effect of the main-chain conformation on theoretical CD spectra is small. In

Table I
Results of Main-Chain and Side-Chain Energy Minimization for Sequential Polypeptides Containing *trans*-L-Phenylazophenylalanine

repeating unit[b]	ϕ_{A1}	ψ_{A1}	ϕ_T	ψ_T	χ_1	χ_2	ϕ_{A2}	ψ_{A2}	ϕ_{A3}	ψ_{A3}
[A-T]	−66	−44	−68	−37	179	271				
$n = 15$			$\langle\phi\rangle = -67$, $\langle\psi\rangle = -40$							
[A_1-T-A_2]	−67	−42	−67	−37	177	258	−69	−39		
$n = 10$			$\langle\phi\rangle = -67$, $\langle\psi\rangle = -39$							
[A_1-T-A_2-A_3]	−66	−43	−67	−35	179	272	−68	−40	−68	−34
$n = 8$			$\langle\phi\rangle = -67$, $\langle\psi\rangle = -38$							

repeating unit[b]	ϕ_{A1}	ψ_{A1}	ϕ_T	ψ_T	χ_1	χ_2	ϕ_I	ψ_I	ϕ_{A2}	ψ_{A2}
[A-T-I]	−79	−40	−58	−36	184	257	−57	−38		
$n = 10$			$\langle\phi\rangle = -65$, $\langle\psi\rangle = -38$							
[A_1-T-I-A_2]	−57	−45	−70	−42	187	266	−57	−40	−74	−38
$n = 8$			$\langle\phi\rangle = -64$, $\langle\psi\rangle = -41$							

repeating unit[b]	ϕ_{T1}	ψ_{T1}	χ_{11}	χ_{21}	ϕ_A	ψ_A	ϕ_{T2}	ψ_{T2}	χ_{12}	χ_{22}	ϕ_I	ψ_I
[T_1-A-T_2-I]	−72	−36	180	274	−59	−46	−70	−44	181	257	−56	−44
$n = 8$				$\langle\phi\rangle = -64$, $\langle\psi\rangle = -42$								

[a] Starting conformation of the side chain is the B form. The rotational angles are written in degrees. [b] A = Ala, T = azoAla(T), I = Aib.

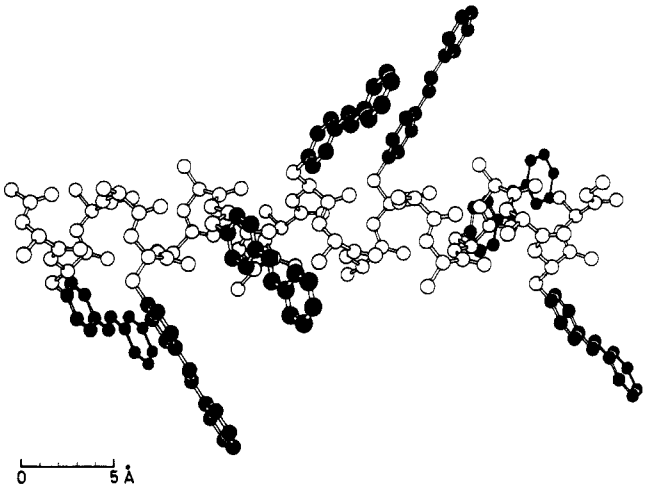

Figure 7. Ball-and-stick atomic model of the predicted conformation of poly[Ala_2-azoAla(T)] after energy minimization for main-chain and side-chain conformations (Table I).

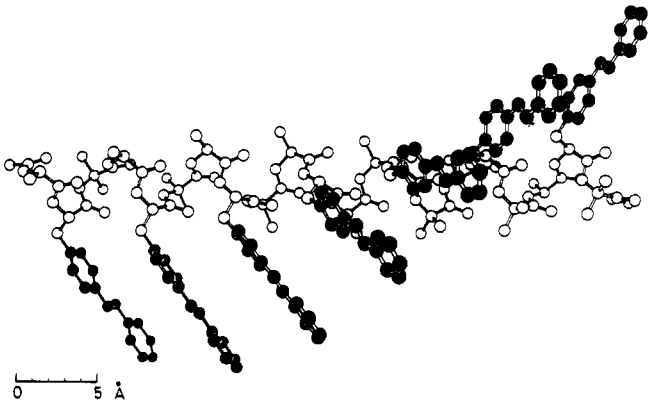

Figure 8. Ball-and-stick atomic model of the predicted conformation of poly[Ala-azoAla(T)-Aib] after energy minimization for only side-chain orientations. The main chain has been fixed to a standard 3_{10}-helix ($\phi = -60°$, $\psi = -30°$).

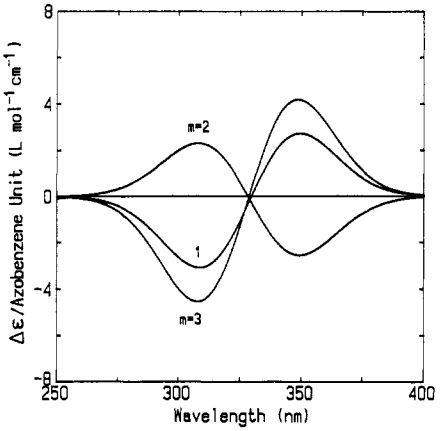

Figure 9. Theoretical CD spectra of poly[Ala_m-azoAla(T)] (m = 1–3) in the minimum-energy conformations. The orientation of the azobenzene group is the A form. The rotational angles of the minimum-energy conformation are almost the same as those listed in Table I, except for the χ_2 angle that is around 80–90°.

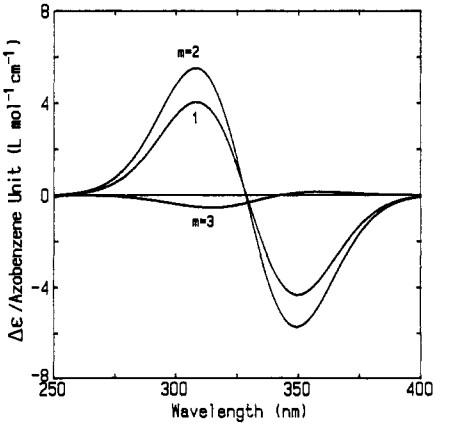

Figure 10. Theoretical CD spectra of poly[Ala_m-azoAla(T)] (m = 1–3) in the minimum-energy conformations listed in Table I. The orientation of the azobenzene groups is the B form.

both helices with their side chains in the B form, spectra for $m(m') = 1$ and 2 show a negative exciton couplet (negative peak at longer wavelength) and spectra for $m(m') = 3$ show a positive couplet. It is concluded from the calculation that different spatial arrangements of azobenzene groups along an α-helix and a 3_{10}-helix as shown in Figures 7 and 8 do not affect the CD spectrum significantly. This is explained in terms of the local nature of electronic interactions that determine the profile of the CD spectrum.

Theoretical CD spectra for $m(m') = 1$ and 2 agree with the experimental CD (Figures 3 and 4 in the accompanying paper) in their profile and magnitude when the B form is assumed. The agreement indicates that, in the real polypeptide, the azobenzene groups are arranged regularly along the α-helical main chain and the fluctuations around the most stable main-chain and side-chain conformation

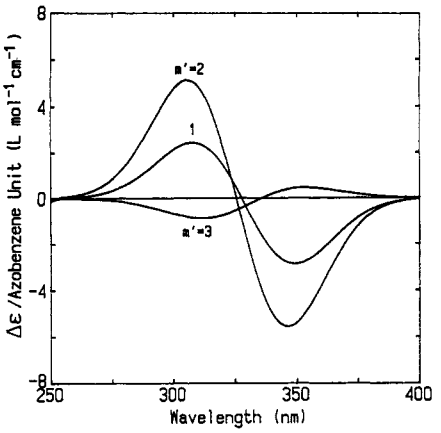

Figure 11. Theoretical CD spectra of poly[azoAla(T)-Ala-azoAla(T)-Aib] (indicated as $m' = 1$) and poly[$Ala_{m'-1}$-azoAla(T)-Aib] ($m' = 2$ and 3) in the standard 3_{10}-helix (-60°, -30°). Energy minimization was carried out only for side-chain conformations assuming the B form.

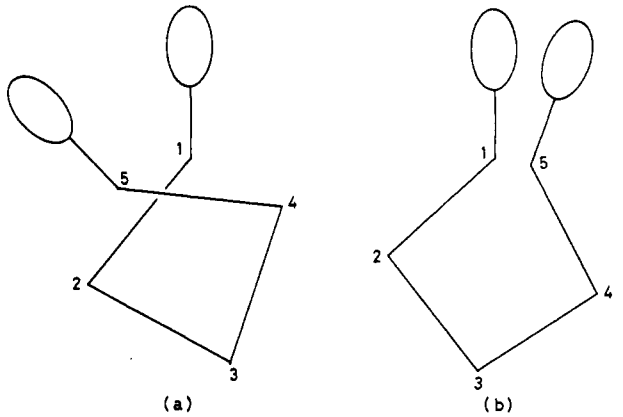

Figure 12. Top view of the helical conformation of poly[Ala_m-azoAla(T)]: (a) right-handed α-helix, (b) unwound structure that induces positive exciton couplet.

do not affect the CD spectrum significantly. Furthermore, a possible mixing of the A and B forms does not weaken the CD spectrum. Predominance of the A form is unacceptable, because the CD profiles for $m(m') = 1$ and 3 are opposite to experimental spectra and that for $m(m') = 2$ is very small for both the α-helical and 3_{10}-helical main chains.

On the other hand, theoretical CD for $m(m') = 3$ is very small and shows an opposite profile to the experimental spectrum, whichever side-chain orientation (A or B form) was assumed. The reason for the opposite CD profile for the $m(m') = 3$ cases was studied in detail. Small variations in the main-chain and the side-chain conformations did not change the profile markedly. Furthermore, any sequence of A and B forms in the side chains (i.e., ...ABABAB..., ...BABABA..., or random sequence) did not induce a negative exciton couplet that is experimentally observed. It was found that the arrangement of azobenzene groups in the $m(m') = 3$ polypeptide strongly favors positive exciton couplet, since the nearest azobenzene groups fixed on the α-helix or on the 3_{10}-helix are arranged in a right-handed screw configuration as illustrated in Figure 12a. The right-handed arrangement or a positive chirality parameter $\zeta = \mathbf{r}_{12}(\mathbf{m}_1 \times \mathbf{m}_2)$ is known to induce a positive exciton couplet.[13] Therefore, the negative exciton couplet observed experimentally is explained only when the main chain is largely unwound so that the relative arrangement of the nearest azobenzene groups assumes a left-handed screw configuration (Figure 12b). The unwound conformation may have very high energy and may not occur frequently. However, since a partial unwinding of a helix has been proposed to stabilize, the total energy of a helix,[14] the above speculation may have some theoretical background. Therefore, at present, the negative exciton couplet of the $m(m') = 3$ polypeptide can be explained in terms of very small CD in the most stable α-helical conformation and very strong negative CD induced at a partially unwound part of the helix. The effect of unwinding may be small in the $m(m') = 1$ and 2 cases, since in these cases the arrangement of azobenzene groups is determined by a local conformation of the helix.

Conclusions

Stable conformations for the six sequential polypeptides were proposed as in Table I and in Figures 7 and 8. It was shown that the side-chain orientation of an amino acid (Abc) is highly restricted in the sequence -Aib-*Xyz*-*Xyz*-Abc-, when the sequence is in an α-helix. The constraint is partially relaxed when the main chain is a 3_{10}-helix. Theoretical CD calculations were successful to predict the magnitude and profile of experimental CD spectra of $m(m') = 1$ and 2 polypeptides. The side-chain orientation of azoAla unit was found to favor the B form. The failure to predict the experimental CD of $m(m') = 3$ polypeptides was tentatively attributed to a partial unwinding of a helix that induces strong negative CD.

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References and Notes

(1) Sisido, M.; Ishikawa, Y.; Itoh, K.; Tazuke, S., previous paper in this issue.
(2) Sisido, M.; Imanishi, Y. *Macromolecules* **1986**, *19*, 2187.
(3) Sisido, M.; *Macromolecules* **1989**, *22*, 3280.
(4) Sisido, M. *Macromolecules* **1989**, 422, 4367.
(5) Momany, F. A.; McGuire, R. F.; Burgess, A. W.; Scheraga, H. A. *J. Phys. Chem.* **1975**, *79*, 2361.
(6) Paterson, Y.; Rumsey, S. M.; Benedetti, E.; Nemethy, G.; Scheraga, H. A. *J. Am. Chem. Soc.* **1981**, *103*, 2947.
(7) Brown, C. J. *Acta Crystallogr.* **1966**, *21*, 146.
(8) The CNDO/ON molecular orbital calculation was performed on the program kindly sent from Professor H. A. Scheraga of Cornell University.
(9) Woody, R. W. *Macromol. Rev.* **1977**, *12*, 181.
(10) Beveridge, D. L.; Jaffe, H. H. *J. Am. Chem. Soc.* **1966**, *88*, 1948.
(11) Blundell, T.; Barlow, D.; Borkakoti, N.; Thornton, J. *Nature* **1983**, *306*, 281.
(12) Beppu, Y. *Comput. Chem.* **1989**, *13*, 101.
(13) Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy—Exciton Coupling in Organic Stereochemistry*; University Science Books and Tokyo Kagaku Dojin: New York and Tokyo, 1982; Chapter 1.
(14) Saruyama, Y. *J. Chem. Phys.* **1985**, *83*, 413.