Synthesis and Conformation of Poly(L-2-anthraquinonylalanine)

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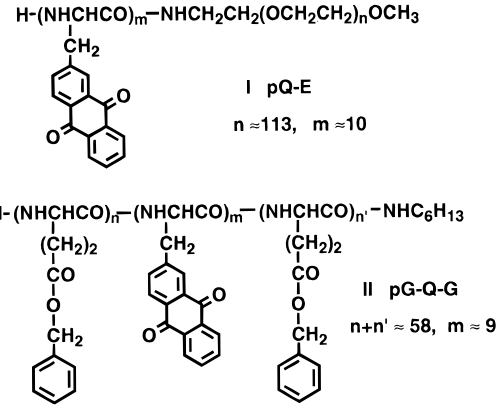
ABSTRACT: A novel nonnatural amino acid that carries a 2-anthraquinonyl group, L-2-anthraquinonylalanine, was first synthesized and converted to the corresponding *N*-carboxyanhydride (NCA). The NCA was polymerized to give poly(L-2-anthraquinonylalanine) in the form of a diblock copolymer with poly(ethylene glycol) that is linked to the C-terminal. A triblock copolymer was also prepared with poly(γ-benzyl L-glutamate)s that are attached to both N- and C-terminals. CD spectra of the diblock copolymer showed a helical conformation that is different from a right-handed α-helix. Conformational analysis and theoretical CD calculation suggested that the poly(anthraquinonylalanine) unit prefers a left-handed α-helix with the lowest energy side-chain orientation.

Anthraquinone has been known to work as an electron mediator between electrodes and redox molecules. Attempts have been reported to synthesize polymers carrying anthraquinonyl groups, which may be used for modifying electrodes to transport electrons to and from redox molecules in the surrounding media.[1,2] Polymerizations of vinyl monomers, such as vinylanthraquinone, with radical, cationic, and anionic initiators have not been very successful, probably due to the steric and electronic inhibitory effect of the anthraquinonyl group.[3] Gradwell et al.[4] reported radiation-induced polymerization of 2-vinylanthraquinone, but the degree of polymerization was low. Furthermore, since the solubility of poly(vinylanthraquinone) in common organic solvents is very limited, its electrochemical properties have been studied only in the solid state. Recently, a π-conjugated polymer of anthraquinonyl units has been reported by Etori et al.[5]

In this paper, we report the synthesis of a new nonnatural amino acid carrying the anthraquinonyl group, L-2-anthraquinonylalanine (anqAla) and polymerization of the corresponding *N*-carboxyanhydride (NCA). Due to the limited solubility of poly(anqAla), the polypeptide was prepared in the form of a diblock copolymer (pQE, **I**) with poly(ethylene glycol) (pEG) or in the form of a triblock copolypeptide (pGQG, **II**) with poly(γ-benzyl L-glutamate) [pGlu(OBzl)] units attached at both ends.

Since anthraquinonyl groups are densely packed in these polymers, unique electrochemical behavior that is different from that of monomeric anthraquinone is expected. In the accompanying paper, efficient electron migrations among the side-chain anthraquinonyl groups will be described.

Since the anthraquinonyl group is linked to the C^{α} atom with a single methylene group, the orientation of anthraquinonyl groups with respect to the polypeptide main chain is highly restricted, particularly when the main chain takes a helical conformation. Under these circumstances, the anthraquinonyl groups are densely packed with regular and helical arrangement. The regular and chiral side-chain orientation may be most conveniently studied by CD spectroscopy. In the latter section of this paper, the observed CD spectrum of the



polypeptide was theoretically analyzed on the basis of the exciton theory, together with the conformational energy calculations, to predict the most probable conformation and the side-chain orientation. Similar theoretical calculations have been reported for homopolypeptides of arylalanine-type amino acids with 1- or 2-naphthyl, 1-pyrenyl, and azobenzene side groups.[6]

Experimental Section

Outline of Synthesis. *N*-Acetyl-DL-anqAla was synthesized from 2-(chloromethyl)anthraquinone and diethyl acetamidomalonate. The racemic derivative was selectively deacylated with acylase to give L-anqAla. The optically active amino acid was converted to the NCA derivative, and the latter was polymerized with terminal aminated pEG to give the diblock copolymer **I**. Alternatively, Glu(OBzl) NCA, anqAla NCA, and Glu(OBzl) NCA were polymerized in this order to give a triblock copolypeptide **II**. In the following section, the synthesis is described in detail. All intermediates were checked for purity by TLC, HPLC, and ^{1}H NMR spectroscopy (200 MHz).

2-(Chloromethyl)anthraquinone. 2-Anthraquinonylmethanol (Aldrich, 1.00 g, 4.2 mmol) was dissolved in dioxane (15 mL) and phosphorus pentachloride (2.6 g, 12.6 mmol) was added, and the mixture was stirred for 4 h at room temperature. Methanol (3.4 mL) was added dropwise to the mixture and reacted for 1 h. The solvent was evaporated, and the remaining solid was recrystallized from THF. Yield: 1.01 g (94%). Mp: 171.5−172 °C.

Diethyl [(2-Anthraquinonylmethyl)acetamido]malonate. Sodium (0.05 g, 2.2 mmol) was added to dry ethanol (5 mL), and diethyl acetamidomalonate (0.51 g, 2.4 mmol) was dissolved. 2-(Chloromethyl)anthraquinone (0.50 g, 1.95 mmol) dissolved in dioxane (5 mL) was added to the mixture, and the mixture was refluxed for 8 h. The solvents were evapo-

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rated, and the remaining solid was redissolved in ethyl acetate. After removing insoluble inorganic components, the solution was concentrated and the remaining solid was recrystallized from dioxane/hexane. Yield: 0.81 g (95%). Mp: 219.5−220.5 °C.

***N*-Acetyl-DL-2-anthraquinonylalanine.** The above compound (1.15 g, 2.63 mmol) was dissolved in dioxane/ethanol (1/1, 15 mL), and 1N NaOH (10.5 mL) was added. The mixture was refluxed for 4 h and then acidified with 2 N HCl. After refluxing for 3 h, water was added to precipitate solid product. Yield: 0.75 g (84%). Mp: 248−249 °C. Anal. Calcd for $C_{19}H_{15}NO_5$: C, 67.64; H, 4.49; N, 4.15. Obsd: C, 67.21; H, 4.33; N, 4.20.

L-2-Anthraquinonylalanine. Acylase (from *Aspergillus* genus; Tokyo Kasei; 0.58 g) was dissolved in water (20 mL), and an insoluble part was filtered off. Cobalt chloride hexahydrate (0.017 g) was added to the solution. Acetyl-DL-anqAla (1.10 g, 3.26 mmol) was dissolved in 1 N NaOH (5 mL), and the pH was adjusted to 7.0 by gradually adding 1 N HCl. The above acylase solution (5 mL) was added to the latter mixture, and the mixture was gently stirred at 37 °C for 3 days. L-AnqAla was precipitated by adding a 3-fold amount of methanol, and the amino acid was collected, washed with methanol, and dried under vacuum. CD: $\Delta\epsilon_{266} = +58$ in trimethyl phosphate (TMP). Yield: 431 mg (45%). Mp 229−230 °C.

Racemization of Anthraquinonylalanine. Due to the strong electron-withdrawing property of the anthraquinonyl group, anqAla is expected to racemize easily. The racemization was actually followed by measuring CD intensity at 270 nm at various pH. When the pH was higher than 7.0, the CD peak totally disappeared after a few minutes at room temperature. This indicates that anqAla is extremely easy to racemize even under very mild alkaline conditions. Indeed, when the amino acid was reacted with di-*tert*-butyldicarbonate [$(Boc)_2O$] at pH = 8.5, the resulting Boc-amino acid was found to be totally racemized. This finding indicates that this amino acid cannot be used for conventional peptide synthesis in the optically pure form. We decided to convert it into the corresponding NCA derivative avoiding alkaline conditions and to polymerize the NCA to obtain homopolypeptides. As will be described later, the racemization is virtually suppressed after the amino acid was incorporated into polypeptides.

The optical purity of anqAla after the selective deacetylation with acylase at pH = 7.0 was checked by the diastereomer formation with (*S*)-(−)-1-(2,3-naphthalenedicarboxyimidyl)-propionyl fluoride (NIPF; Dojin, Japan). The amino acid (1 mg) was suspended in DMF, and 100 μL of the supernatant was mixed with the NIPF solution in acetonitrile (AN) (3.4 mM, 100 μL). After the solution had stood for 4 h at room temperature, the diastereomer mixture was analyzed by HPLC (ODS column). The elution was monitored at 328 nm. The different reactivities of NIPF against L- and D-amino acids were taken into consideration using racemic and pure L-phenylalanines as standards. Since a few percent of racemization was found to occur during the reaction of NIPF with L-phenylalanine, the optical purity was also corrected for this factor. The final optical purity of L-anqAla was found to be 92%.

L-2-Anthraquinonylalanine *N*-Carboxyanhydride. Trichloromethyl chloroformate (phosgene dimer, 0.15 mL) was dissolved in dry THF (2 mL) and stirred at 60 °C for 2 h. After cooling the mixture to 40 °C, the optically active amino acid (100 mg, 0.34 mmol) was added. The suspension was stirred for 7 h until the mixture became almost transparent. The solvent and remaining phosgene were removed under reduced pressure, and the remaining solid was recrystallized from DMF/diethyl ether. Yield: 59 mg (54%). An IR spectrum showed characteristic peaks of the NCA at 1780 and 1860 cm^{-1}.

Diblock Copolymer of L-2-Anthraquinonylalanine and Ethylene Glycol (pQE). AnqAla NCA (10 mg, 31 μmol) was dissolved in dry DMF (156 μL) and methoxypoly(ethylene glycol)aminoethyl ether (Shearwater Polymers, Inc., average degree of polymerization =113) (16 mg, 3 μmol) was added to initiate the polymerization. The characteristic IR peaks of NCA disappeared after 5 days, and the polymer solution was fractionated on a Sephadex LH60/TMP column. Since no low molecular weight component was found in gel chromatography, we assumed all anqAla units were incorporated into the diblock copolymer and estimated the average degree of polymerization of poly(anqAla) unit to be 10. Consequently, the total structure of the diblock copolymer is $(anqAla)_{10}-(EG)_{113}$. The latter structure is supported by the C/N ratio in the elemental analysis: calcd, 31.0; found, 33.9 ± 5.

Triblock Copolymer of (γ-Benzyl L-glutamate)-(L-2-Anthraquinonylalanine)−(γ-Benzyl L-glutamate) (pGQG). Glu(OBzl) NCA (25 mg, 93 μmol) was dissolved in dry DMF, and *n*-hexylamine (0.4 μL, 3 μmol) was added. The polymerization was followed by a decrease of the characteristic IR peaks of NCA. After completion of the first polymerization (5 h), anqAla NCA (10 mg, 31 μmol) was added. The second polymerization finished after 36 h. Then Glu(OBzl) NCA (25 mg, 93 μmol) was added to start the third polymerization. After 2.5 days, the mixture was poured into ether to precipitate the triblock copolymer. The polypeptide was fractionated on a LH60/TMP column to remove low molecular weight anqAla derivatives that were estimated to be about 10% of the total amount. The molar ratio of Glu(OBzl) unit to anqAla unit was calculated from ^{1}H NMR peaks (200 MHz in $CDCl_3$) to be 6.4 ± 0.6. Combining these data, the structure of triblock copolypeptide is expected to be $Glu(OBzl)_m-anqAla_n-Glu(OBzl)_{m'}$, with $n = 9$ and $m + m' = 58 \pm 5$.

Measurement. Absorption spectra were measured on a Jasco Ubest 560 spectrometer. CD spectra were recorded on a Jasco J500 or Jasco J720 instrument. Concentrations of polymers were determined from the absorbance of the anthraquinonyl group by using $\epsilon_{328} = 6000$ in TMP, DMF, and AN.

Calculations. Empirical energy calculations were performed on the PEPCON program,[7] that is an extended version of ECEPP[8] to include a variety of nonnatural amino acids. Only rotational angles were varied, and the bond lengths and bond angles were fixed in the calculation. Solvent molecules were not taken into consideration. Standard bond lengths and bond angles were assigned to the anthraquinionylalanine unit. Partial charges of the anthraquinone group were determined from CNDO/ON MO calculation[9] on *N*-acetylanthraquinonylalanine *N*-methylamide. Molecular models were drawn by a personal computer version of NAMOD.[10]

Theoretical CD calculation was made on the basis of the exciton theory.[11,12] The program (PEPCD) is essentially the same as that used for the CD calculation of poly(1- and 2-naphthylalanines), poly(1-pyrenylalanine), poly(L-9-anthrylalanine), and poly[L-*p*-(phenylazo)phenylalanine].[6] The electronic transitions of a single anthraquinonyl group were determined from the PPP−CI calculation with the Nishimoto−Mataga two-center repulsion integrals.[13] The PPP−CI program is our own version that includes calculation of monopole charges for ground state−excited state transitions and for excited state−excited state transitions. The excited states of poly(anqAla) ($n = 10$) were obtained through diagonalization of matrix elements that were evaluated as monopole−monopole interactions of the monomeric transitions. For each transition of poly(anqAla), the rotational strength was calculated, and for each rotational strength, a Gaussian curve was assigned to reproduce an experimental CD curve. The widths of the Gaussian curves were taken from the corresponding width of absorption peaks.

The conformational calculations and the CD calculations were carried out on MS-Fortran V5.1/MS-Windows 3.1, using an NEC PC9821Xn personal computer.

Results and Discussion

Polymerization of L-2-Anthraquinonylalanine NCA. Polymerization of anqAla NCA was carried out in DMF with terminal aminated poly(ethylene glycol) ($m = 113$) as the initiator to improve the solubility. The polymerization was carried out with NCA/amino group molar ratios of 10, 20, and 30. Due to the limited

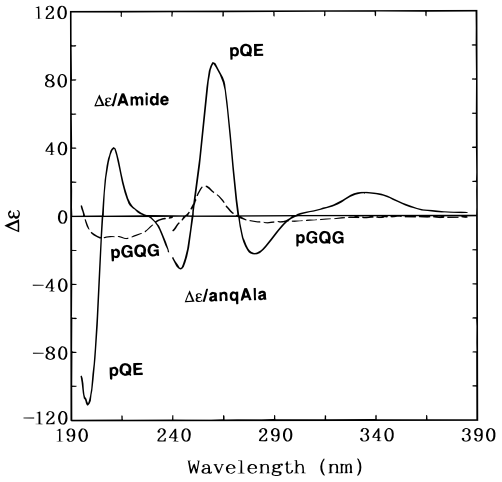

Figure 1. CD spectra of pQE (—) and pGQG (– –) in trimethyl phosphate. [anqAla] = 2.0×10^{-5} (pQE), 1.4×10^{-5} (pGQG) M.

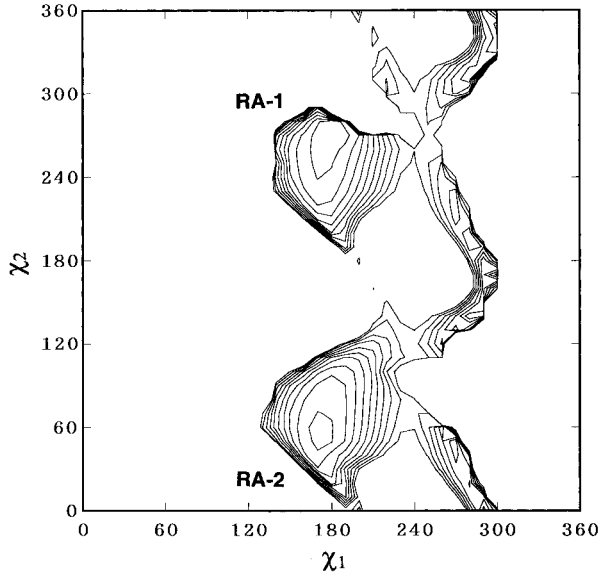

Figure 2. Energy contour map for the side-chain rotation of a poly(anqAla) unit in the right-handed α-helical main-chain conformation ($\phi = -62.5°$, $\psi = -42.3°$).

solubility of poly(anqAla), however, only the polypeptide with $n = 10$ was used in the following study.

Polymerization was also carried out with preformed pGlu(OBzl) in DMF. To improve the solubility further, Glu(OBzl) NCA was added again after completion of the polymerization of anqAla NCA. Molar ratios of Glu(OBzl)/anqAla/Glu(OBzl) were 30/10/30 and 40/20/40, respectively. The reaction mixture remained homogeneous even after the third polymerization. The mixture was poured into ether, and the polypeptides were fractionated with gel chromatography (Sephadex LH60/TMP). Fractions that appeared at the elution limit (MW > $\sim 5 \times 10^3$) were taken up. The triblock polypeptide of 40/20/40 contained no low molecular weight fractions, whereas that of 30/10/30 contained about 10% of the low molecular weight component that is assigned to unreacted anqAla NCA. Since the two triblock polypeptides showed very similar spectroscopic properties and the 40/20/40 polypeptide was less soluble than the 30/10/30 one, results of the 30/10/30 polypeptide will be described in the following section.

Suppression of Racemization of Anthraquinonylalanine Units in the Polypeptides. As described in the Experimental Section, anqAla is extremely easy to racemize under weak alkaline conditions. However, racemization during the NCA polymerization may not be significant, as judged from the more intense CD peak of the polypeptide pQE ($\Delta\epsilon_{260} = +90$ in TMP, Figure 1) than the original amino acid ($\Delta\epsilon_{266} = +58$ in TMP).

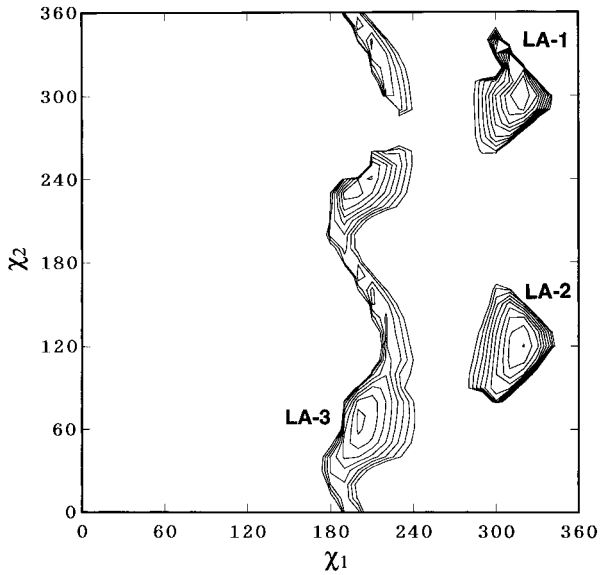

Figure 3. Energy contour map for the side-chain rotation of a poly(anqAla) unit in the left-handed α-helical main-chain conformation ($\phi = 62.5°$, $\psi = 42.3°$).

Racemization of anqAla units after they are incorporated into the polypeptide chain was checked by following the CD spectrum of an aqueous solution of pQE at pH = 7, 8, and 9. The $\Delta\epsilon$ value was unaffected for at least about 1 h under the alkaline conditions, indicating racemization is suppressed in the polypeptide.

No racemization was observed in the polypeptides also when the anthraquinonyl group was electrochemically reduced and reoxidized in AN. The CD profile changed reversibly during the redox processes, indicating no racemization is occurring. In contrast to the polypeptides, monomeric anqAla in TMP was totally racemized during the reversible redox processes.

Conformations of Poly(L-2-anthraquinonylalanine) in Solution. CD spectra of the diblock and triblock copolymers were measured in TMP (Figure 1). The CD spectrum of pQE shows intense positive and negative peaks that originate from exciton couplings of the asymmetrically arranged anthraquinonyl groups. The spectrum in the amide absorption region of pGQG shows a typical pattern of a right-handed α-helical conformation. The $\Delta\epsilon$ value at 222 nm was −13.0/amide bond, which is roughly the same as the value for a full right-handed α-helix (−11.5). The $\Delta\epsilon$/anthraquinone value of pGQG at the absorption region of anthraquinone (260 nm) is much smaller than that of pQE. If pQE exists in a right-handed α-helical conformation, the $\Delta\epsilon$/anthraquinone value must be the same for the two polymers. The CD profile in other regions is also different for the two polymers. Particularly, no strong contribution of anthraquinonyl groups is observed in the 190−240 nm region of pGQG. The smaller $\Delta\epsilon$ value and other differences in the CD profile of pGQG suggest that pQE does not take right-handed α-helical conformation.

Conformational energy calculations to predict the main-chain conformation and the side-chain orientation of poly(L-2-anthraquinonylalanine) ($n = 10$) were carried

Table 1. Results of Energy Minimization of Poly(L-2-anqAla), $n = 10$

	starting conformation					minimum-energy conformation V (kcal mol^{-1})					
structure	ϕ	ψ	ω	χ_1	χ_2	ϕ	ψ	ω	χ_1	χ_2	
RH-αI	−63	−42	180	180	280	−66	−42	180	182	279	−49.9
RH-αII	−63	−42	180	175	55	−65	−43	180	170	50	−39.3
LH-αI	63	42	180	320	310	53	53	180	314	312	−37.2
LH-αII	63	42	180	320	120	46	62	180	297	82	−36.3
LH-αIII	63	42	180	200	65	46	60	180	177	37	−22.5

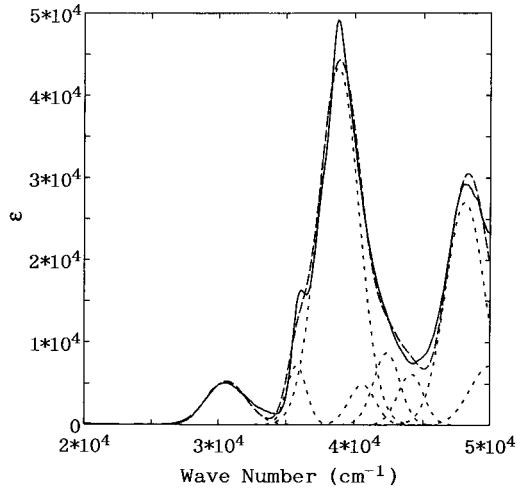

Figure 4. Absorption spectrum of anthraquinonyl methanol in trimethyl phosphate. The dashed lines are the result of least-squares resolution into Gaussian curves.

out. We assumed a helical symmetry for the polypeptide; i.e., rotational angles in a single amino acid unit (ϕ, ψ, χ_1, χ_2) were assumed to be the same for all 10 units. The starting main-chain conformations were a right-handed α-helix ($\phi = -62.5°$, $\psi = -42.3°$) and a left-handed α-helix ($\phi = 62.5°$, $\psi = 42.3°$), and the side-chain energy contour maps were drawn for the two main-chain conformations (Figures 2 and 3). Two side-chain orientations are found to be possible in the contour map for a right-handed α-helix, and three orientations are possible for a left-handed α-helix. Starting from the five possible conformations, energy minimizations were carried out by varying the four rotational angles (ϕ, ψ, χ_1, χ_2), assuming helix symmetry. The results of energy minimization are collected in Table 1. The calculation predicted that the right-handed α-helical conformation with type I side-chain orientation is the most stable one, but this may not be the case as indicated from CD spectroscopy. Among the rest of the four conformations, RH-αII, LH-αI, and LH-αII have about the same energy. The most likely conformation will be assigned from comparison of the experimental CD with the theoretical CD that is calculated for each possible conformation.

Theoretical CD Calculation. Theoretical CD of poly(anqAla) is calculated on the basis of the exciton theory. To begin with, we have to determine the energies and oscillator strengths of electronic transitions of an anthraquinone chromophore.[13,14] If we assume D_{2h} symmetry for the anthraquinone group, two B_{3U} and two B_{2U} transitions are predicted to appear at longer wavelengths than 200 nm. Also there are several B_{1G} transitions, but they are symmetry-forbidden. The absorption spectrum of 2-anthraquinonylmethanol is shown in Figure 4 with wavenumber as the abscissa. The dashed lines in Figure 4 show results of least-squares resolution of the absorption profile into Gaussian curves of different widths.

Table 2. Calculated and Observed Electronic Transitions of Anthraquinone

		calculated[a]		experimental[b]	
band	symmetry[c]	λ_{calc} (nm)	oscillator strength	λ_{obsd} (nm)	oscillator strength
I	B_{3U}	318	0.289	329	0.116
II	B_{2U}	275	0.608	277	0.08
III	B_{3U}	236	0.909	258	0.955
IV	B_{2U}	212	0.109	208	0.596
V	B_{1G}	325	0	forbidden transition	
VI	B_{1G}	212	0	forbidden transition	

[a] PPP−CI calculation with Nishimoto−Mataga two-center repulsion integrals. [b] Anthraquinonylmethanol in TMP. [c] Transitions of B_{3U} symmetry are polarized along the long axis, and those of B_{2U} are along the short axis.

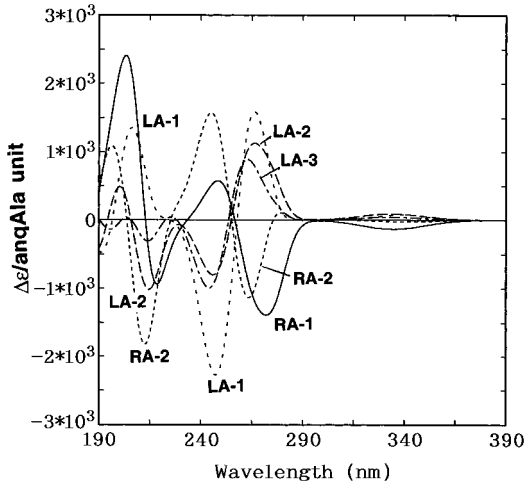

Figure 5. Theoretical CD spectra for the five possible conformations with the standard right- and left-handed α-helical main-chain conformations listed in Table 1.

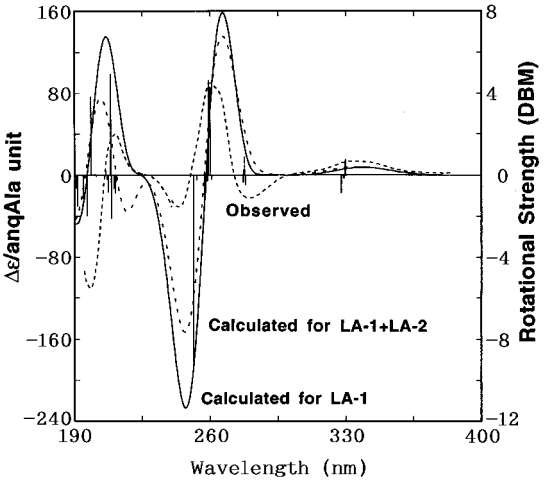

Figure 6. Comparison of the observed CD spectrum of pQE (- - -) with the theoretical spectrum for the LA-1 conformation (—). The rotational strength at each transition is also shown. The theoretical CD spectrum for the average of LA-1 and LA-2 is also shown (- - -).

Assignment of absorption peaks of anthraquinone has been determined from polarized absorption spectroscopy by Inoue et al.[15] They concluded that band I at 324 nm (3.04×10^4 cm^{-1} in Figure 4) and band III at 258 nm (3.88×10^4 cm^{-1}) are polarized along the long axis of anthraquinone and assigned to the B_{3U} transitions, whereas band II at 277 nm (3.61×10^4 cm^{-1}) and band IV at 208 nm (4.41×10^4 cm^{-1}) are polarized along the short axis and assigned to the B_{2U} transitions. These assignments are collected in Table 2.

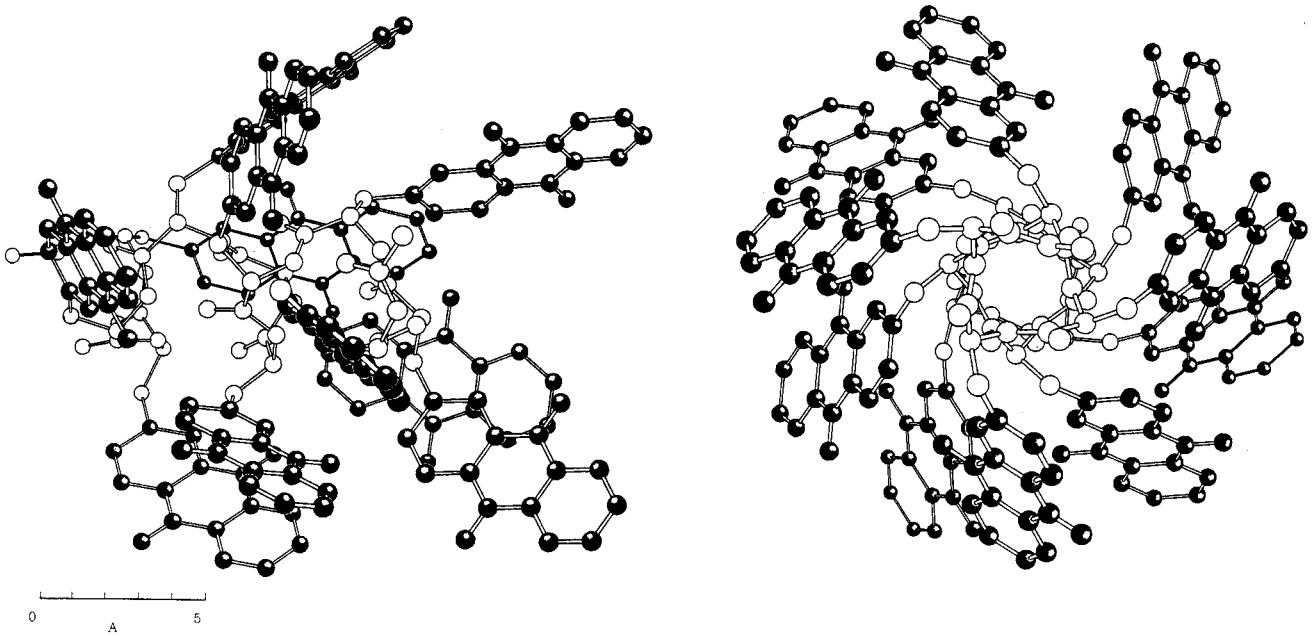

Figure 7. Most probable conformation (LA-1) of poly(anqAla)$_{10}$.

In the same table, observed oscillator strengths obtained from the least-squares resolution in Figure 4 are also shown. From the latter values, the transition dipole moments were calculated and used for the CD calculation. The oscillator strengths obtained from the PPP−CI calculation were, in some cases, significantly different from the observed values. Also, the experimental spectrum shows two small peaks between bands III and IV that cannot be assigned to any of the calculated transitions. Although these factors may render the present CD calculation somewhat ambiguous, the total CD profile that is determined essentially by the interactions between strong transition moments will not be much altered by these minor transitions.

According to Woody,[11,12] matrix elements between excited states of different anqAla monomers were calculated as the sum of monopole−monopole interactions, instead of the conventional dipole−dipole interactions. The monopole charges were obtained from the results of the PPP−CI molecular orbitals. The calculated monopole charges were normalized to reproduce observed transition moments in Figure 4. A monopole charge on the aromatic carbon or on the carbonyl oxygen was divided into two identical ones that are positioned 1.0806 Å above and below the aromatic plane.[12]

Thus, the transitions taken into consideration are $\pi\pi^*$ and $n\pi^*$ of each amide bond and bands I−IV of each anthraquinone chromophore. The transition energy, oscillator strength of the amide band, and the magnetic moment of the $n\pi^*$ transition were taken from Woody's paper.[12] A small $n\pi^*$ absorption of the anthraquinonyl group near 400 nm was neglected. Also, the forbidden B_{1G} transitions of anthraquinone were neglected, although they may have some influence on the CD profile, through $B_{1G}-B_{3U}$ and $B_{1G}-B_{2U}$ excited state−excited state interactions.

For the five possible conformations (right-handed α-helix with I and II side-chain orientations and left-handed α-helix with I−III side-chain orientations), theoretical CD spectra were calculated. Theoretical CD spectra for the standard right- and left-handed α-helices with the side-chain orientations listed in Table 1 are shown in Figure 5. Theoretical CDs of the minimum-energy conformations were not much different from the corresponding standard helical ones and are not shown.

Right-handed α-helical conformations with I or II side-chain orientations showed CD profiles that show opposite signs to the experimental CD of pQE. The opposite CD profile indicates that poly(anqAla) does not exist in a right-handed α-helical conformation. The conclusion is inconsistent with the observation of a very different experimental CD profile of pQE from that of pGQG. Possibly, the poly(anqAla) portion of pGQG cannot take the same conformation as pQE, because long pGlu(OBzl) chains on both sides are in the α-helical conformation.

Theoretical CD spectra for left-handed α-helices with three different side-chain orientations are also shown in Figure 5. Among the three, the conformation with the lowest-energy side-chain orientation (I) shows reasonable agreement with the experimental CD profile of pQE. The theoretical CD (solid line) and the experimental CD (small-dashed line) are compared in Figure 6, together with the rotational strengths of all the exciton transitions. The signs of all the coupled transitions are reproduced correctly. The positive and negative CD peaks around 200−250 nm are much stronger in the theoretical spectrum than in the experimental one. A possible reason for this discrepancy is that we have neglected several small transitions that are lying in this region but cannot be assigned to any transitions (see Figure 4). Of course, some disorders and fluctuations in the side-chain orientations may be another reason for the overestimation of the CD intensities.

Since conformational energies are about the same for the side-chain orientations I [$(\chi_1, \chi_2) = (320, 310)$] and II [(320, 120)] in the left-handed α-helix, it is likely that the anthraquinonyl side chains can take both orientations almost equally in a single polypeptide chain. In other words, the anthraquinonyl group may rotate around the $C^\beta-C^\gamma$ bond (χ_2). If each anthraquinonyl group may take the two orientations with equal probabilities, there are totally 2^{10} different orientations for a polypeptide of 10 anqAla units. Instead of calculating the 2^{10} CD spectra and taking their average, we took an average of two CD spectra for the left-handed

α-helical polypeptides with side-chain orientations I and II, respectively, for all the anthraquinonyl groups. The average spectrum is shown in Figure 6 by a large-dashed line. It seems that the average spectrum is somewhat more like the experimental one, indicating the possibility of side-chain rotations.

CD profiles were also calculated for other possible conformations, i.e., left- and right-handed 3_{10}-helices, and extended conformations with all possible side-chain orientations. However, no conformations gave reasonable CD profiles.

The left-handed α-helical conformation with the lowest-energy side-chain orientation is illustrated in Figure 7. The center-to-center distance between anthraquinonyl groups of the 1st and 2nd units is 9.8 Å, and that of the 1st and 4th units is 7.9 Å. The edge-to-edge distances are 6.4 and 4.1 Å for the 1−2 pair and the 1−4 pair, respectively. These distances are close enough to achieve fast electron hoppings that will be described in the accompanying paper.

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