J. Am. Chem. Soc. 1983, 105, 4077–4082

One-Dimensional Aromatic Crystals in Solution. 2. Synthesis, Conformation, and Spectroscopic Properties of Poly(L-2-naphthylalanine)

Masahiko Sisido,* Syun Egusa, and Yukio Imanishi

Contribution from the Research Center for Medical Polymers and Biomaterials, and the Department of Polymer Chemistry, Kyoto University, Kyoto 606, Japan.

Received December 3, 1982

Abstract: A novel aromatic poly(α-amino acid), poly(L-2-naphthylalanine), was synthesized, and its circular dichroism (CD) and fluorescence spectra were measured in solution. The CD spectrum in trimethyl phosphate showed a strong exciton splitting around the ${}^1B_b$ absorption band, indicating the presence of a polymeric band structure as a result of the electronic interactions among the helically arranged naphthyl groups. The fluorescence spectrum was dominated by a monomer fluorescence accompanied by a small excimer fluorescence. These spectroscopic properties suggest that poly(L-2-naphthylalanine), as well as the 1-naphthyl isomer, works as a molecular conductor which may transport electrons across biological membranes. Empirical potential energy calculations were carried out for side-chain conformations (χ_1, χ_2), when the main-chain conformations were fixed to the standard right- and left-handed α, δ, and 3_{10} helices. Starting from the minima in the side-chain conformational maps, the total energy was minimized, varying the four dihedral angles (ϕ, ψ, χ_1, χ_2) simultaneously, and eight minimum-energy conformations were found. For the latter conformations, theoretical CD calculations were performed taking two amide transitions ($n\pi^*$ and $\pi\pi^*$) and three naphthyl vibronic transitions (${}^1L_b$, ${}^1L_a$, and ${}^1B_b$) into account. Judging from the energy and the CD calculations, the most likely conformation of poly(L-2-naphthylalanine) is a left-handed α-helix-like conformation. The theoretical CD of poly(L-1-naphthylalanine) was also recalculated taking the vibronic peaks into account.

A rodlike aromatic polypeptide, in which aromatic groups are arranged regularly along the helix axis, is promising as a molecular conductor which may carry electrons across biological membranes. In a previous paper,[1] the synthesis, conformation, and spectroscopic properties of poly(L-1-naphthylalanine) [poly(L-1-NapAla)] were described. It was demonstrated that the arrangement of the aromatic groups was helical, and the electronic interactions between the chromophores resulted in the formation of the exciton state which was evidenced by an intense splitting of the ${}^1B_b$ circular dichroic band. The fluorescence spectrum showed little excimer emission, indicating the absence of energy traps along the polymer chain.

As an extension of the study, the synthesis and conformation of another aromatic poly(α-amino acid), poly(L-2-naphthylalanine) [poly(L-2-NapAla)], were undertaken and are described in this paper. As in the previous paper,[1] empirical conformational calculations[2] were performed and several possible helical conformations were selected. For these conformations, theoretical CD calculations according to the exciton theory[3] were carried out and the resulting CD curves were compared with the experimental one.

The theoretical CD calculation in this study differs from the previous one in that each vibronic transition in the ${}^1L_b$, ${}^1L_a$, and ${}^1B_b$ bands was treated as an independent transition. The directions and the monopole distributions for the vibronic transitions which belong to the same band were assumed to be the same. The oscillator strengths for each vibronic peak are evaluated from the experimental spectrum of 2-methylnaphthalene in trimethyl phosphate. A revised CD calculation was also carried out for poly(L-1-NapAla).

Experimental Section

Materials. Polymer Samples. Racemic 2-naphthylalanine was synthesized according to Berger et al.,[4] mp 209–211 °C dec. Anal. Calcd for $C_{13}H_{13}NO_2$: C, 72.54; H, 6.09; N, 6.51. Found: C, 72.42; H, 6.32; N, 6.54. The optical resolution was carried out by the selective deacylation of the N-acetylated amino acid with acylase.[5] The optical purity was tested by ${}^1H$ NMR spectroscopy in the presence of a chiral shift reagent, tris[3-(trifluoromethylhydroxymethylene)-*d*-camphorato]europium(III), in deuteriochloroform. Racemic 2-naphthylalanine methyl ester showed two OCH_3 peaks in the presence of 0.4 mol of the shift reagent, whereas the methyl ester of the resolved amino acid showed only one peak under the same conditions. The amino acid was converted to the *N*-carboxy anhydride (NCA) derivative with trichloromethyl chloroformate (phosgene dimer). The phosgene dimer (0.5 mL) in dry tetrahydrofuran (50 mL) was warmed at 60 °C for 1 h; then the amino acid (100 mg) was suspended in the solution. The mixture was stirred at 50 °C for 3 h until a clear solution was attained. After concentrating the solution in vacuo, the NCA was precipitated with hexane. The solid obtained was recrystallized repeatedly from ethyl acetate/hexane, yield 30 mg, mp 135–136 °C. The formation of NCA was confirmed by a pair of IR peaks characteristic for the NCA ring (1850, 1780 cm^{-1}). The block copolymer of γ-benzyl DL-glutamate and L-2-NapAla was prepared following Gratzer and Doty.[6] The NCA was added to a dichloroethane solution of a preformed poly(γ-benzyl DL-glutamate) (degree of polym-

Table I. Oscillator Strengths and Peak Positions of the Vibronic Peaks of 2-Methylnaphthalene in Trimethyl Phosphate[a]

electronic transition	peak position (cm^{-1})	oscillator strength
${}^1L_b$	31 270	0
	32 670	0
	34 070	0
	35 470	0
	36 870	0
${}^1L_a$	34 727	0.030 3
	36 127	0.045 5
	37 527	0.041 2
	38 927	0.028 6
	40 327	0.018 7
${}^1B_b$	44 583	0.993
	45 884	0.597
	47 183	0.324

[a] Refractive index = 1.40.

---

[1] Sisido, M.; Egusa, S.; Imanishi, Y. *J. Am. Chem. Soc.* **1983**, *105*, 1041.

[2] Momany, F. A.; McGuire, R. F.; Burgess, A. W.; Scheraga, H. A. *J. Phys. Chem.* **1975**, *79*, 2361.

[3] Woody, R. W. *J. Polym. Sci., Macromol. Rev.* **1977**, 12, 181.

[4] Berger, A.; Smolarsky, M.; Kurn, N.; Bosshard, H. R. *J. Org. Chem.* **1973**, *38*, 457.

[5] Fu, S-C. J.; Birnbaum, S. M.; Greenstein, J. P. *J. Am. Chem. Soc.* **1954**, *76*, 6054.

[6] Gratzer, W. B.; Doty, P. *J. Am. Chem. Soc.* **1963**, *85*, 1193.

0002-7863/83/1505-4077$01.50/0

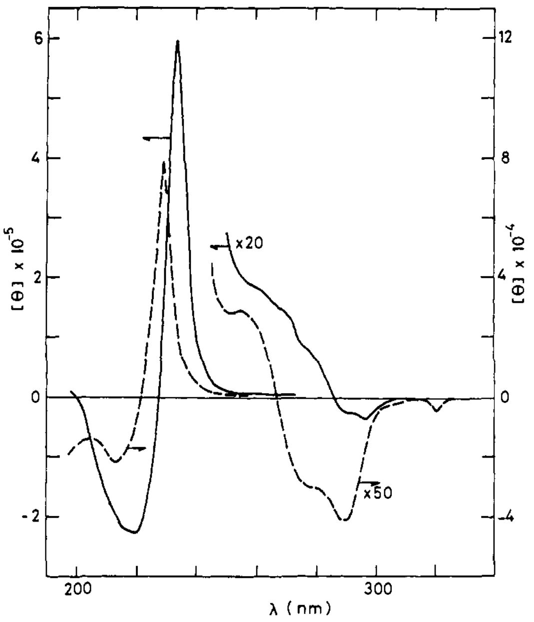


Figure 1. Circular dichroism of poly(L-2-NapAla) (—) and *N*-acetyl-L-2-naphthylalanine (---) in trimethyl phosphate containing 1 vol % of dichloroethane: [Nap] = 2.0×10^{-4} M, cell length = 1 cm (250–350 nm) and 1 mm (190–250 nm), room temperature.

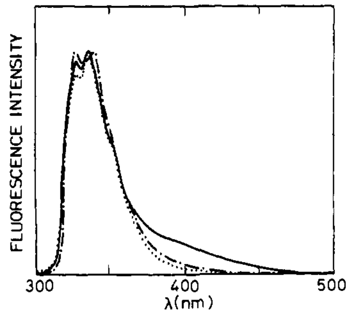


Figure 2. Fluorescence spectra of poly(L-2-NapAla) (—), poly(L-1-NapAla) (-·-), and *N*-acetyl-L-2-naphthylalanine (---) in trimethyl phosphate containing 0.1 vol % of dichloroethane: [Nap] = 1.0×10^{-5} M, excitation wavelength = 285 nm, room temperature.

erization = 120) ([prepolymer] = 2.5×10^{-3} M, [NCA] = 0.1 M). The characteristic IR peak of the NCA disappeared after 24 h.

The number-average degree of polymerization of the 2-naphthylalanine block should be equal to the stoichiometric ratio of the NCA to the terminal amino group of the preformed polymer;[7] it was 40 in the present case. An approximate molecular weight of the block copolymer was estimated by a gel chromatography (TOYOPEAL HW-50 in dimethylformamide). The molecular weight was around 3×10^4 which is consistent with the theoretical value of 3.42×10^4. Although a homopolymer of 2-NapAla was insoluble in any solvents, its block copolymer with poly(γ-benzyl DL-glutamate) dissolved in dichloroethane, dimethylformamide, trimethyl phosphate, and other polar solvents.

Since the NCA polymerization using the preformed poly(γ-benzyl DL-glutamate) as an initiator was found to proceed more smoothly than the previous case using poly(ethylene glycol)–poly(sarcosine) copolymer,[1] a block copolymer of poly(L-1-NapAla) with poly(γ-benzyl DL-glutamate) was also prepared, and its CD and fluorescence spectra were reexamined.

Other Reagents. A low molecular weight model compound, *N*-acetyl L-2-naphthylalanine ethyl ester, was prepared by the acetylation of the amino acid ethyl ester, mp 149.5–150.5 °C (lit.[4] 133–136 °C for the D isomer). Anal. Calcd for $C_{17}H_{19}NO_3$: C, 71.56; H, 6.71; N, 4.91. Found: C, 71.38; H, 6.70; N, 4.84. 1- and 2-methylnaphthalene (analytical grade) were used without further purification. Trimethyl phosphate, dimethylformamide, and other solvents for spectroscopic measurements were distilled before use.

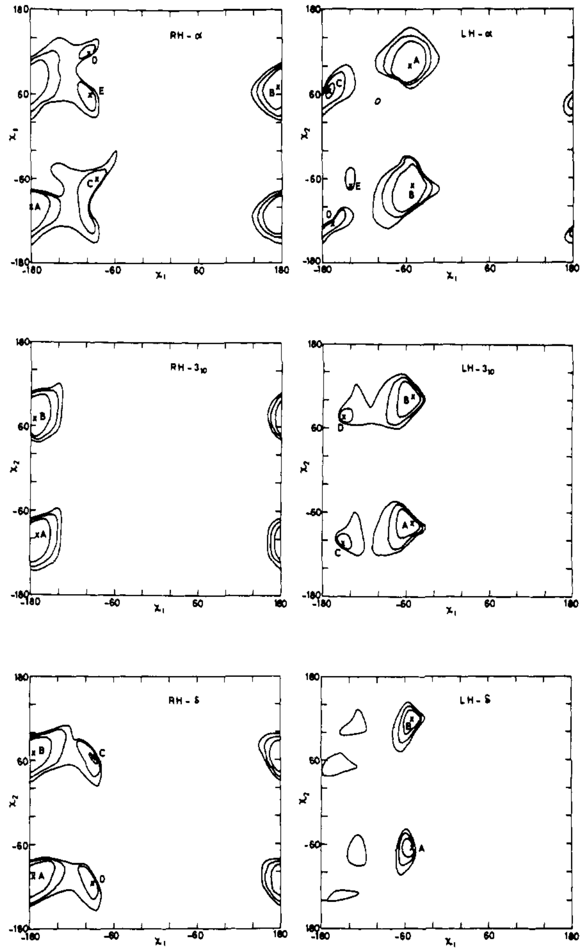


Figure 3. Energy contour maps for side-chain conformations of helical poly(L-2-NapAla). Main-chain conformations are fixed to right- and left-handed α, 3_{10}, and δ helices. The contour lines represent the energies higher by 2.5, 5.0, and 7.5 kcal mol^{-1} than the minimum indicated by "A" in each map.

Measurements. The following spectrometers were used: Hitachi EPS-3T (UV and visible absorption), Hitachi MPF-4 (fluorescence), and JASCO J-20 (CD). Absorption and CD measurements were carried out under aerated conditions. Fluorescence spectra were measured after bubbling N_2 gas for 20 min.

Since the block copolymer became insoluble when it was isolated as a powder, the polymerization mixture was diluted to appropriate concentrations for the spectroscopic measurements. Although the phenyl groups of the poly(γ-benzyl glutamate) block hampered the UV measurement of the naphthyl group, CD and fluorescence spectra were not affected.

Empirical Conformational Energy Calculation

A conformational energy calculation was caried out for a helical heptamer of poly(L-2-NapAla). All nonbonded pairs in the heptamer were taken into consideration. The geometry and the empirical potential energy functions[2] were the same as those used in the previous paper,[1] except for the change of the bond position from 1-naphthyl to 2-naphthyl. The conformations of local energy minima were searched by setting the main-chain conformations (ϕ, ψ) to one of several standard helical conformations, i.e., right- and left-handed α, 3_{10}, and δ helices. The energy contour maps were obtained for the conformational space of the side chain (χ_1, χ_2). Starting from the local minimum-energy conformations found in the side-chain energy maps, total-energy minimization was carried out varying the four dihedral angles (ϕ, ψ, χ_1, χ_2) simultaneously.

Theoretical CD Calculations

For the stable conformations obtained in the procedure of energy minimization, theoretical CD curves were computed according to the method reported by Woody.[3] The polymer was assumed to be a pentadecamer (n = 15) of 2-NapAla in a regular helical conformation. The electronic parameters for the amide and the naphthyl chromophores were

---

[7] Bamford, C. H.; Elliott, A; Hanby, W. E. "Synthetic Polypeptides"; Academic Press: New York, 1956; Chapter III.

Table II. Stable Side-Chain Conformations of Poly(L-2-NapAla)

main-chain conformation[a]	side-chain confor-mation	χ_1, deg	χ_2, deg	E,[b] kcal mol^{-1}
RH-α ($\phi = -50°$, $\psi = 56°$, $t = 99°$, $h = 1.47$ Å)	A	−180	−100	4.7
	B	175	70	5.0
	C	−85	−60	5.0
	D	−95	115	7.8
LH-α ($\phi = 49°$, $\psi = 57°$, $t = -99°$, $h = 1.47$ Å)	A	−55	100	2.3
	B	−50	−70	2.4
	C	−170	65	4.5
	D	−165	−125	5.6
RH-3_{10} ($\phi = -60°$, $\psi = -30°$, $t = 111°$, $h = 1.75$ Å)	A	−170	−95	5.5
	B	−175	70	5.5
LH-3_{10} ($\phi = 60°$, $\psi = 30°$, $t = -111°$, $h = 1.75$ Å)	A	−50	−75	3.0
	B	−50	105	3.1
	C	−150	−105	6.4
	D	−150	75	6.6
RH-δ ($\phi = -75°$, $\psi = -4°$, $t = 121°$, $h = 2.00$ Å)	A	−175	−105	8.0
	B	−175	70	8.2
	C	−90	65	10.2
	D	−90	−115	11.1
LH-δ ($\phi = 75°$, $\psi = 4°$, $t = -121$, $h = 2.00$ Å)	A	−50	−65	10.3
	B	−50	120	10.4

[a] Values in the parentheses are the main-chain rotational angles (ϕ, ψ), the rotational angle per residue about the helix axis, t, and the translation per residue along the helix axis, h. [b] (Empirical potential energy for the heptamer)/7.

Table III. Results of Total Energy Minimization for Poly(L-2-NapAla)

starting confor-mation[a]	converged confor-mation	ϕ	ψ	χ_1	χ_2	E, kcal mol^{-1}
		degrees				
RH-α-A RH-3_{10}-A RH-δ-A	RH-ME-A	−67	−40	−178	−94	2.4
RH-α-B RH-3_{10}-B RH-δ-B	RH-ME-B	−67	−40	−180	75	2.7
RH-α-C RH-δ-D	RH-ME-C	−59	−46	−84	−58	6.3
RH-α-D RH-δ-C	RH-ME-D	−65	−39	−88	122	6.6
LH-α-A LH-3_{10}-B LH-δ-B	LH-ME-A	54	50	52	104	1.8
LH-α-B LH-3_{10}-A LH-δ-A	LH-ME-B	55	49	−51	−71	1.7
LH-α-C LH-3_{10}-D	LH-ME-C	51	53	−169	65	4.0
LH-α-D LH-3_{10}-C	LH-ME-D	52	51	−169	−119	3.8

[a] Starting conformations for the total energy minimization were written in an abbreviated form; i.e., RH-α-A indicates right-handed α helix with side-chain conformation of the A form. For other notations, see Table II.

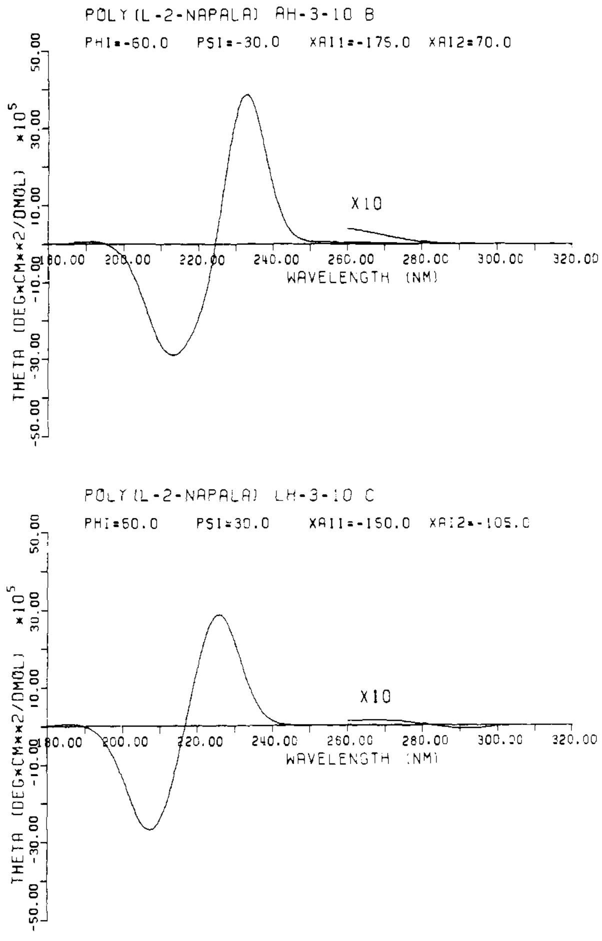


Figure 4. Theoretical CD curves for the standard helical conformations listed in Table II. Only CD curves which are compatible with the experimental spectrum are shown.

the same as those used in the previous paper.[1] However, in the present calculation two modifications were made. (1) The transition energies and the transition moments for the 2-naphthyl group were taken from the absorption spectrum of 2-methylnaphthalene in trimethyl phosphate, instead of in *N*-acetyl-L-1-naphthylalanine ethyl ester as in the previous case. (2) Each vibronic peak in the ^1L_b, ^1L_a, and ^1B_b bands of the naphthyl group was treated as a single transition. The direction of the transition moment and the mode of the monopole-charge distribution of each vibronic transition were taken to be the same within each band, which have been listed in Tables II and III in the previous paper.[1] The magnitude of the vibronic transition moments and the absolute values of the monopole charges were calculated from the partial oscillator strengths of each vibronic peak, which were obtained by the least-squares curve resolution of the experimental absorption spectrum of 2-methylnaphthalene in trimethyl phosphate. In the curve resolution, each vibronic peak was assumed to have a Gaussian shape with a half-width of 1000 cm^{-1} for the ^1L_a band and 800 cm^{-1} for the ^1B_b band. The highest peaks in the experimentally observed ^1L_a and ^1B_b bands were assigned to the 0–1 and the 0–0 vibronic peaks, respectively.[8] The rest of the vibronic peaks were positioned with the same intervals of 1400 cm^{-1} for the ^1L_a band and 1300 cm^{-1} for the ^1B_b band, respectively.[9]

The experimental peak at 31 270 cm^{-1} was assigned to the 0–0 vibronic peak of the ^1L_b band.[8] The spacing for this band was assumed to be the same as that of the ^1L_a band. Five vibronic peaks were considered for the ^1L_b and ^1L_a bands and three for the ^1B_b band. The oscillator strengths and the peak positions of the vibronic peaks considered are listed in Table I.

For the calculation of the Hamiltonian matrix elements between local excited states within the same naphthyl group, dipole moments and monopole charges for the transitions between excited states, i.e., ^1L_b–^1L_a and ^1L_b–^1B_b, are necessary. Those quantities have been calculated from the Hückel–CI molecular orbitals[10] in the previous paper. However, neither experimental nor theoretical information is available for the oscillator strengths of the vibronic transitions between excited states. Therefore, two different calculations were tried. In the first calculation only vibronic transitions between excited states with the same vibrational quantum number were considered. This is physically equivalent to assuming the same equilibrium nuclear configuration for the two excited states. In the second calculation, the effective nuclear displacements of the two excited states with respect to the ground-state configuration were estimated from the band shapes of the experimental spectrum of 2-methylnaphthalene (0.077 Å for ^1L_a and 0.045 Å for ^1B_b), and their difference was used as a displacement for the ^1L_a–^1B_b transition. Then

---

(8) Birks, J. B. "Photophysics of Aromatic Molecules"; Wiley-Interscience: London, 1970, p 70.

(9) Miller, K.; Murrell, J. N. *Theor. Chim. Acta* **1965**, *3*, 231.

(10) Pariser, R. *J. Chem. Phys.* **1956**, *24*, 250.

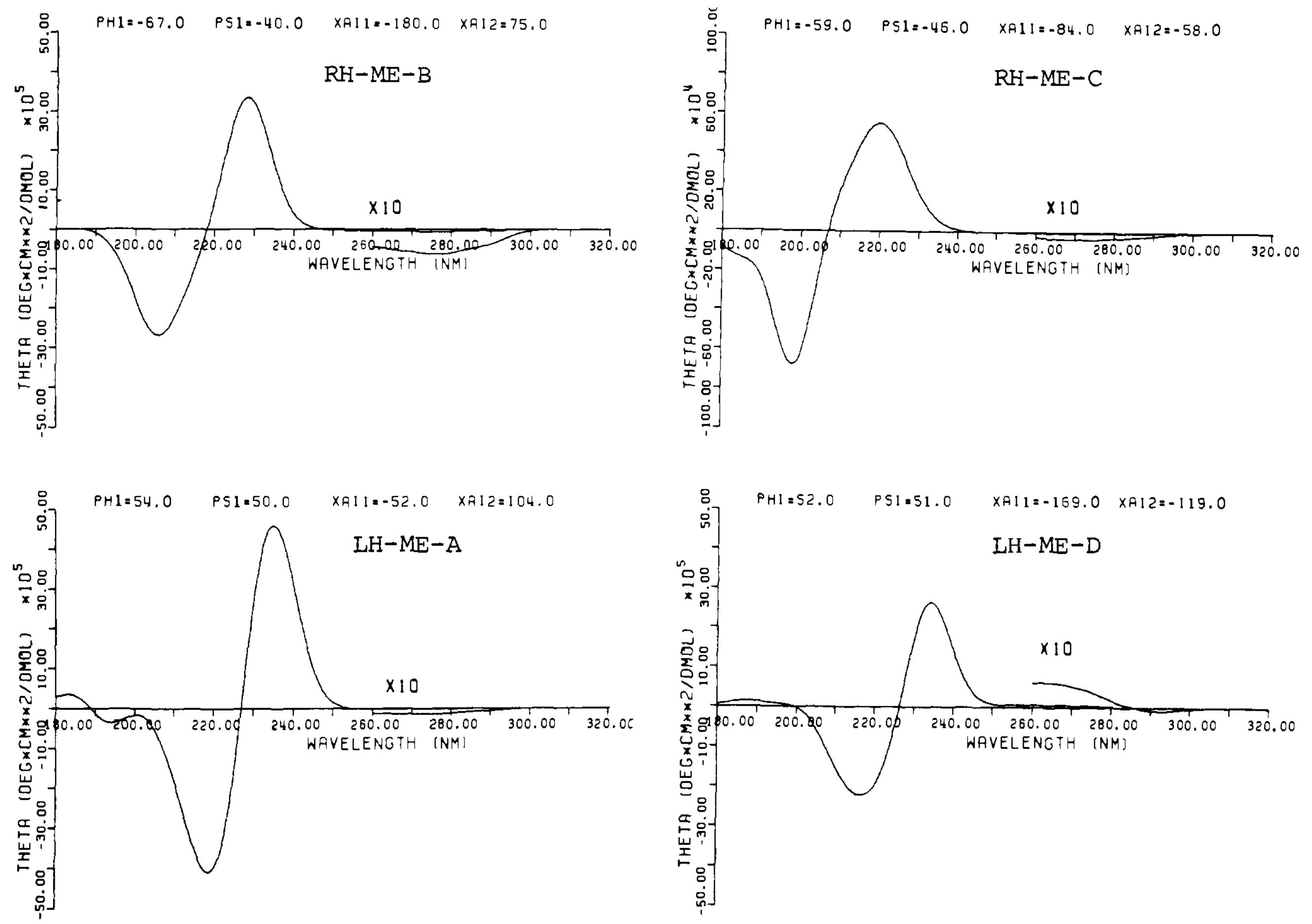


Figure 5. Theoretical CD curves for the minimum-energy conformations listed in Table III. Only CD curves which are compatible with the experimental spectrum are shown.

the vibronic band shape for the ^1L_a–^1B_b transition was calculated from the overlap integral between the Hermite polynomials having the displaced origins. The vibronic band shape of the ^1L_b–^1B_b transition was assumed to be the same as that for the ^1L_a–^1B_b transition. This assumption was rationalized by the fact that the fluorescence band shape from the ^1L_b excited state almost coincides with the mirror image of the ^1L_a absorption band. The band shape of the experimentally observed ^1L_b band provides no information to determine the nuclear configuration of the ^1L_b excited state, since its intensity is determined by coupling with the vibrational transitions.[11]

Despite the above elaboration, the two kinds of calculations gave the same theoretical CD curves. This is expected since the two transitions between excited states are electronically forbidden, having a quadrupole (^1L_b–^1L_a) or an octapole (^1L_b–^1B_b) moment, and electronic interactions with other states are very small. Pure vibrational transitions within each absorption band were neglected.

Since 15 transitions (one for amide $\pi\pi^*$, one for amide $n\pi^*$, three for naphthyl ^1B_b, five for naphthyl ^1L_a, and five for naphthyl ^1L_b) were considered for each naphthylalanine unit, there are 225 local excited states for the pentadecamer of poly(L-2-NapAla). The Hamiltonian matrix of the dimension of 225 × 225 was diagonalized and the energies and the wave functions of the polymer were obtained. The theoretical CD curve was calculated from these data in the usual manner.[1,3]

Results and Discussion

CD Spectra of Poly(L-2-NapAla) and Its Model Compound. Figure 1 shows CD spectra of poly(L-2-NapAla) and its model compound, *N*-acetyl-L-2-naphthylalanine ethyl ester, in trimethyl phosphate. The polymer spectrum shows an intense exciton splitting at the ^1B_b band. The molar ellipticity per naphthyl group reaches 6.0×10^5 deg cm^2 dmol^{-1} at 234 nm and -2.3×10^5 at 219 nm. The large exciton splitting is undoubtedly a result of a regular helical arrangement of naphthyl groups along the polypeptide chain and electronic interactions among the chromophores. The CD intensity at the ^1L_a band is much smaller than that of ^1B_b band but is larger than the intensity of the low molecular weight model compound. Virtually the same CD pattern was observed in dichloroethane, unless a strong organic acid, such as trifluoroacetic acid, was added.

Very interestingly, the CD spectrum of poly(L-2-NapAla) resembles that of poly(L-1-NapAla).[1] Since the CD pattern depends on the geometrical arrangement of naphthyl groups along the polypeptide chain, the resemblance indicates a similar helical arrangement of the chromophores. However, the conformational energy calculation and the theoretical CD prediction, which will be described in the next section, suggested different conformations for the two polymers and, therefore, the resemblance may be accidental.

Fluorescence Spectra. Figure 2 compares fluorescence spectra of poly(L-2-NapAla) and its model compound. The spectrum of poly(L-1-NapAla), which was newly prepared with a preformed poly(γ-benzyl DL-glutamate) and has a higher molecular weight than the previous one, is also shown in the figure. No excimer emission is seen for poly(L-1-NapAla), and little excimer emission is observed for poly(L-2-NapAla). Since the excitation energy migrates along the polymer chain and is efficiently trapped into excimer sites, the number of the excimer forming sites should be much less than that estimated from the excimer/monomer intensity ratio in the fluorescence spectrum.

The fact that the excimer site is nearly or completely absent may be preferable for these aromatic poly(α-amino acid)s to be utilized as a molecular conductor which can carry excitation energies or electrons through the helical chain. Furthermore, the rodlike shape of the helical chain is convenient as the molecular

---

[11] Mataga, N.; Kubota, T. "Molecular Interactions and Electronic Spectra"; Marcel Dekker: New York, 1970; Chapter 3.

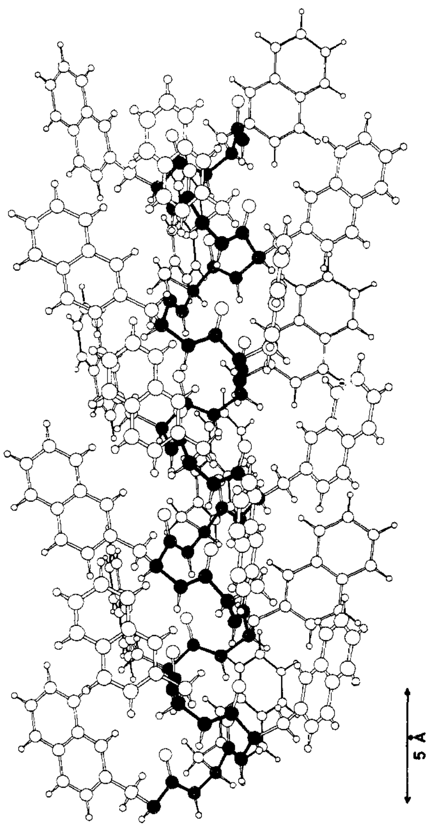


Figure 6. Molecular display of poly(L-2-NapAla) in LH-ME-D conformation. Solid circles represent atoms involved in the peptide chain.

conductor to be incorporated vertically into a membrane plane and to transport electrons across the membrane.

Empirical Conformational Energy Calculation. Empirical energies were computed for the side-chain conformations (χ_1, χ_2) of poly(L-2-NapAla), the main-chain conformation being fixed to right- and left-handed α helices $(\phi, \psi) = (\mp 50°, \mp 56°)$, 3_{10} helices $(\mp 60°, \mp 30°)$, and δ helices $(\mp 75°, \mp 4°)$. The β structure was not considered because of the absence of excimer emission. Side-chain energy contour maps for the six main-chain conformations are shown in Figure 3. Local minima in each map are labeled as A, B, C, . . . in increasing order of the energy. Table II lists the positions, the helix parameters,[12] and the energies of the local minima found in the contour maps.

Starting from the local minima, total-energy minimization was performed, varying the four dihedral angles simultaneously. The results are listed in Table III. It was found that three right-handed main-chain conformations which have similar side-chain conformations converged into the same right-handed conformation, and three left-handed conformations also converged into the same left-handed conformation. Furthermore, the converged main-chain conformations with different side-chain conformations are almost indistinguishable ($\phi = -67° \sim -59°$, $\psi = -46° \sim -39°$ for the right-handed helix and $\phi = 51° \sim 55°$, $\psi = 49° \sim 53°$ for the left-handed helix), regardless of the side-chain conformations. The converged main-chain conformations are not much different from the standard right- and left-handed α helices. Therefore, the conformations corresponding to the minimum total energy will be referred to right- and left-handed minimum-energy α helices hereafter.

Theoretical CD Spectra. Theoretical CD curves were computed for all local minima listed in Table II and total energy minima in Table III. Figures 4 and 5 collect the CD curves which are compatible with the experimental curve shown in Figure 1; that is, the computed curves which show the same splitting pattern at the ^1B_b band as the experimental curves are listed in the figures. The theoretical CD for left-handed minimum-energy α-helix D form (LH-ME-D) reproduces the experimental CD most satisfactorily. Those for LH-ME-A, RH-Me-B, RH-3_{10}-B, and LH-3_{10}-C, however, cannot be discarded. The last two conformations were converged into RH-ME-A and LH-ME-D conformations, respectively, upon total-energy minimization. Therefore, three independent conformations were found to be acceptable on the basis of the potential energy calculation and the CD prediction. Among the three, LH-ME-D conformation seems most likely, since its theoretical CD correctly reproduces the CD pattern at the ^1L_a band. Although the potential energy of the LH-ME-D conformation (3.8 kcal mol^{-1} per naphthylalanine unit) is somewhat higher than that of the conformation of the total minimum energy (LH-ME-B, 1.7 kcal mol^{-1}), it may be within a permissible range.

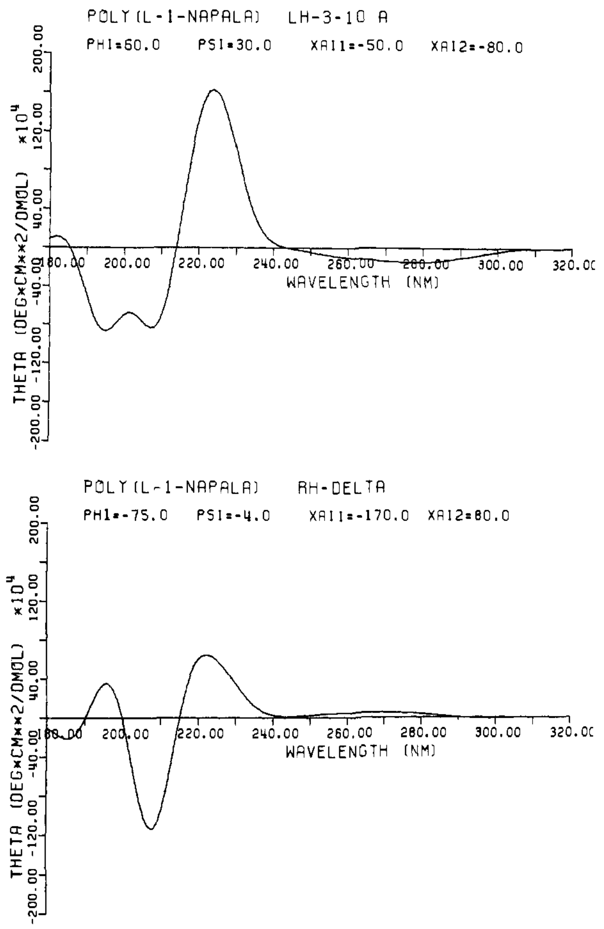


Figure 7. Theoretical CD curves for the standard helical conformations of poly(L-1-NapAla) which are listed in Table IV in ref 1. Vibronic peaks are explicitly taken into consideration.

Figure 6 illustrates the atomic model of poly(L-2-NapAla) in the LH-ME-D conformation. The NAMOD molecular display program was used.[13] The center-to-center distance between the nearest naphthyl groups, which are attached to the next turn of the helix, is 6.0 Å, whereas that between the adjacent naphthyl groups along the polymer sequence is 9.3 Å. These interchromophore distances are too far to form excimers and consistent with the fluorescence spectra, but they are short enough for a dipole-dipole interaction to form a excitonic state which has been observed as a CD splitting at the ^1B_b absorption band.

In the previous paper,[1] two candidates were proposed for probable conformations of poly(L-1-NapAla) in solution. Since in the previous CD calculation vibronic peaks were not taken into consideration, the calculation was repeated here. The oscillator strengths and the positions of the vibronic peaks were taken from the least-squares peak resolution of the absorption spectrum of 1-methylnaphthalene in trimethyl phosphate. The theoretical CD

---

(12) Sugeta, H.; Miyazawa, T. *Biopolymers* **1967**, *5*, 673.

(13) Beppu, Y. Quantum Chemistry Program Exchange, No. 370, Indiana University, Indiana, 1979.

computed for the left-handed 3_{10}-helix A form and the right-handed δ helix of poly(L-1-NapAla) are shown in Figure 7. As compared with the previous results (Figure 6 in the previous paper), the present CD curves show a broader ^1L_a band, which makes the theoretical curves more realistic. However, the pattern of the CD curves, especially at the ^1B_b band, is not so affected as to alter the previous conclusion.

Registry No. (±)-2-Naphthylalanine, 14108-60-2; 2-naphthylalanine NCA derivative, 85421-92-7; poly(γ-benzyl DL-glutamate), 25087-28-9; *N*-acetyl L-2-naphthylalanine ethyl ester, 37440-00-9; poly(L-2-naphthylalanine), 85421-91-6.

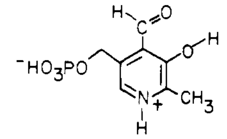
Transimination Kinetics of Pyridoxal 5′-Phosphate Schiff Bases

Shu-Huan Weng and Daniel L. Leussing*

Contribution from the Department of Chemistry, The Ohio State University, Columbus, Ohio 43210. *Received June 21, 1982. Revised Manuscript Received February 9, 1983*

Abstract: A direct transimination pathway for the exchange of ethylamine in $H_iPLPEta$ Schiff bases by alaninate or aspartate has been established. This rate is roughly twice as fast as that for exchange proceeding along an indirect pathway involving the reaction of aminoacidate with PLP freed by the hydrolysis of $H_iPLPEta$. The rate expression for direct exchange can be written in terms of the simple second-order reactions of aminoacidate with the various protonated forms of the Eta Schiff base. No effects by excess solution components or added buffers consistent with a requirement for catalysis of proton transfer between the intermediate gem diamines were observed, in contrast to results reported for imine exchange between MCB^+ and NH_2OH (ref 16). It is concluded that proton transfers in the PLP imines are internally catalyzed by the PLP phosphate side group or the phenoxide ring substituent. This latter site may also serve as a catalyst in the enzymic reaction.

Pyridoxal 5′-phosphate (PLP), shown as the triprotonated molecule in **1**, is one of the active forms of the vitamin B_6 group

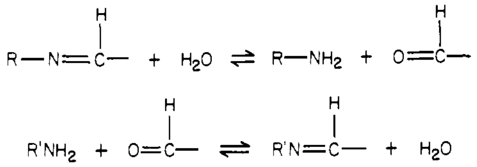


1 H_3PLP

of compounds which are essential enzymatic cofactors in a number of biological reactions.[1,2] An important class of reactions are those involving the aminotransferases where amino acids and α-keto acids are reversibly interconverted. The reactions proceed through a shuttle mechanism in which a PLP Schiff base of the amino acid tautomerizes from an aldimine to a ketimine. Hydrolysis releases the keto derivative of the amino acid and leaves pyridoxamine 5′-phosphate bound to the enzyme. This latter substance is available to convert a reactant keto acid to amino acid in the reverse sequence.[1-7]

Initially PLP is covalently bonded to the enzyme through a Schiff base linkage to a terminal amine group of a protein lysine.[8a-c] In aspartate aminotransferase, one of the most studied of these enzymes, the binding site has been identified as Lys-258.[8c,d,9] The first step in the reaction must therefore be the replacement of the lysine amine group by that of the amino acid. An indirect exchange pathway involves the hydrolysis of the PLP–lysine Schiff base followed by condensation of the PLP with an aminoacidate ion (intermediate carbinolamines[10-12] have been omitted for simplicity) (Scheme I).

Scheme I



$$R-N{=}\overset{H}{C}- + H_2O \rightleftharpoons R-NH_2 + O{=}\overset{H}{C}-$$

$$R'NH_2 + O{=}\overset{H}{C}- \rightleftharpoons R'N{=}\overset{H}{C}- + H_2O$$

Another possible pathway involves the direct displacement of one amine by another in a process termed transimination.[13-15] In a study of the displacement of the pyrrolidine residue by NH_2OH from N-P-methoxybenzylidinepyrrolidinium cation (MCB^+) Hogg, Jencks, and Jencks[16] established the reaction mechanism shown in Scheme II.

A proton situated on the imine nitrogen atom is necessary for attack by the entering amine. To drive the reaction to completion a proton transfer from the entering group of the intermediate gem diamine to the leaving group is required, and as a consequence

---

(1) (a) A. E. Braunstein, *Enzymes, 2nd Ed.*, **2** (1960). (b) A. E. Braunstein, *Enzymes, 3rd Ed.*, **9**, 379 (1973).

(2) D. E. Metzler, M. Ikawa, and E. E. Snell, *J. Am. Chem. Soc.*, **76**, 648 (1954).

(3) E. E. Snell, *J. Biol. Chem.*, **154**, 313 (1944).

(4) D. E. Metzler and E. E. Snell, *J. Am. Chem. Soc.*, **74**, 979 (1952).

(5) V. I. Ivanov and M. Ya. Karpiesky, *Adv. Enzmol. Relat. Areas Mol. Biol.*, **32**, 21 (1969).

(6) D. E. Metzler, *Adv. Enzymol. Relat. Areas Mol. Biol.*, **50**, 1 (1979).

(7) A. E. Martell, *Adv. Enzymol. Relat. Areas Mol. Biol.*, **53**, 163 (1982).

(8) (a) W. Terry Jenkins and Irwin W. Sizer, *J. Am. Chem. Soc.*, **79**, 2655 (1957). (b) R. C. Hughes, W. T. Jenkins, and E. H. Fischer, *Proc. Natl. Acad. Sci. U.S.A.*, **48**, 1615 (1962). (c) Y. A. Ovchinnikov, A. E. Braunstein, C. A. Egorov, O. L. Polyanovsky, N. A. Aldanova, M. Yu Feigina, V. M. Lipkin, N. G. Abdulaev, E. V. Grishin, A. P. Kiselev, N. N. Modyanov, and V. V. Nosikov, *Dokl. Acad. Nauk SSSR*, **207**, 728 (1972). (d) S. V. Shlyapnikov, S. V. Myasnikov, A. N. Severin, E. S. Myagkova, M. A. Torchinsky, M. Yu, and A. E. Braunstein, *FEBS Lett.*, **106**, 385 (1979).

(9) Geoffrey C. Ford, Gregor Eichle, and Johan U. Jansonius, *Proc. Natl. Acad. Sci. U.S.A.*, **77**, 2559 (1980).

(10) Byeong H. Jo, Vasu Nair, and Leodis Davis, *J. Am. Chem. Soc.*, **99**, 4467 (1977).

(11) In PLP Schiff base formation the rate of formation/dissociation of carbinolamine is fast followed by slower dehydration/hydration (ref 12). Therefore, the rate expressions for these reactions are described by the simple equations shown in Table II.

(12) Steve Hershey and Daniel L. Leussing, *J. Am. Chem. Soc.*, **99**, 1992 (1977).

(13) K. Koehler, W. Sandstrom, and E. H. Cordes, *J. Am. Chem. Soc.*, **86**, 2413 (1964). D. Mackay, *Biochim. Biophys. Acta*, **73**, 445 (1963).

(14) William P. Jencks, "Catalysis in Chemistry and Enzymology", McGraw-Hill, New York, 1969.

(15) Peter S. Tobias and Roland G. Kallen, *J. Am. Chem. Soc.*, **97**, 6530 (1975).

0002-7863/83/1505-4082$01.50/0