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Synthesis and Conformation of Sequential Polypeptides of L-Alanine and β-Aminobutyric Acid[1]

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ABSTRACT: Sequential polypeptides with the repeating units L-alanyl-(*S*)-β-aminobutyric acid, L-alanyl-(*R*)-β-aminobutyric acid, and L-alanyl-(*R,S*)-β-aminobutyric acid have been synthesized by polycondensation of the *N*-hydroxysuccinimide ester hydrochloride salts of the corresponding dipeptides. Circular dichroism and infrared spectroscopy studies of films of the polypeptides and circular dichroism study of their solutions in hexafluoro-2-propanol and hexafluoropropane-2,2-diol show the tendency of the polypeptides to adopt the β conformation in the solid state. In pure hexafluoro-2-propanol or hexafluoroacetone, the three polymers adopt what we interpret as random coil conformations. In mixtures of hexafluoro-2-propanol–water or hexafluoropropane-2,2-diol–water, the polypeptide containing the *S* isomer shows a definite tendency to form β structure. This tendency is not established for the *R* and the *R,S* isomers.

The conformation of poly(L-alanine), in solution or as a film, has been thoroughly investigated over the past fifteen years.[3–5] In both cases, the polypeptide exhibits a highly helical structure, as shown by circular dichroism (CD), infrared spectroscopy (ir), and x-ray crystallography.

The poly(β-amide) counterpart of poly(L-alanine), poly((*S*)-β-aminobutyric) acid, shows an antiparallel conformation in the solid state, as demonstrated by Schmidt[6] in 1970. In a recent paper, Chen et al.[7] have carried out CD, ir, and ultraviolet (uv) studies of this optically active polymer in hexafluoro-2-propanol (HFIP), hexafluoropropane-2,2-diol (HFPD), and methanesulfonic acid solutions. The addition of water to solutions of the polymer in HFIP or HFPD enhanced the β association of the chains before precipitation took place.

In recent years, L-alanine has been incorporated into many sequential polypeptides[8,9] and polydepsipeptides.[10] Its combination with amino acids such as Gly[11,13,14] or Glu[12] leads under defined conditions to β structured polypeptides.

In this paper, we present the synthesis and conformational analysis of sequential copolypeptides of L-alanine and of the optical isomers of β-aminobutyric acid, in order to investigate the influence of the configuration of the β-amino acid upon the conformation of the polypeptide, in the solid state and in solution.

Experimental Section

Reagents and Solvents. The amino acid derivative *t*-Boc-L-alanine was obtained from Bachem Fine Chemicals, Inc. Its purity was established by TLC. The compounds, (*S*)(−)-4-methylazetidinone, (*R*)(+)-4-methylazetidinone, poly((*S*)-aminobutyric acid), and poly((*R*)-aminobutyric acid), were generous gifts from Drs. J. Brandrup and E. Schmidt, Farbwerke Hoechst, Inc., Frankfurt, Germany. β-Aminobutyric acid was purchased from Aldrich Chemical Co., Inc. Dicyclohexylcarbodiimide (J. T. Baker) was purified by dissolving the commercial product in diethyl ether and removing the insoluble material by filtration and evaporation of the solvent in vacuo.

N-Hydroxysuccinimide, purchased from Aldrich, was used without further purification. The solution of 4 *N* hydrogen chloride in dioxane was obtained from the Pierce Chemical Co. *N,N*-Dimethylformamide (Mallinckrodt) was purified as described previously,[15] bp 40° (9 mm). Water and peroxides were removed from dioxane (Mallinckrodt) as described.[15] After distillation over sodium, it was stored under nitrogen in brown bottles over calcium hydride at 4°C. Triethylamine (Eastman) was refluxed over sodium for 2 hr and then fractionally distilled at atmospheric pressure (bp 88°C).

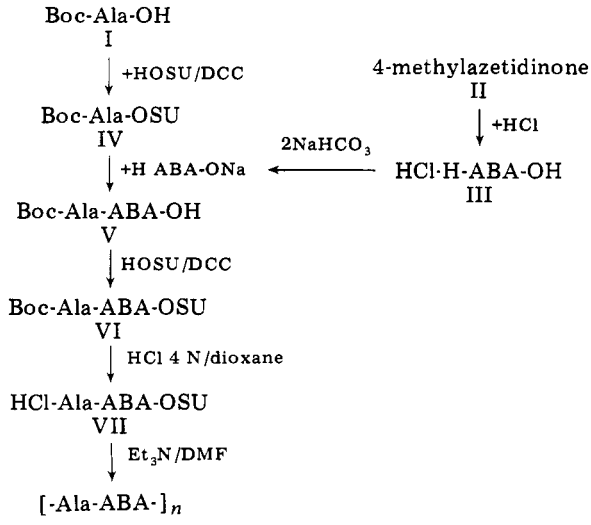
Trifluoroacetic acid (Eastman) was fractionally distilled from phosphorous pentoxide at atmospheric pressure. The fraction boiling at 71–72° was collected and stored over calcium chloride. The saturated solution of HBr in acetic acid (48%) was obtained from British Drug House. Other solvents and reagents mentioned in the experimental section were of "Analytical Reagent" quality and were used without further purification.

Analysis and Characterization of Intermediate Products. Melting points were determined on a Uni-Melt Capillary melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer Model 141 Polarimeter at 589 nm. Infrared spectra of the intermediate compounds were recorded as KBr pellets, using a Perkin-Elmer Model 180 spectrophotometer.

Proton magnetic resonance spectra were measured with a Varian T-60 spectrometer using tetramethylsilane as internal standard. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

Thin layer chromatography was carried out on precoated silica gel plates (Kodak 246F). The following solvent systems were used: 1-butanol–acetic acid–water (4:1:5, v/v) (BAW); acetone–acetic acid (98:2, v/v) (AA); chloroform–acetic acid (95:5, v/v) (CA); chloroform–methanol (1:1, v/v) (CM); chloroform–methanol–acetic acid (85:10:5, v/v) (CMA); chloroform–acetone (35:1, v/v) (CAC). Peptide spots were located on the plates by spraying with a 0.1% ninhydrin solution in acetone (free amine) and heating or treatment with hydrochloric acid vapor for 15 min (*tert*-butyloxycarbonylamine), ninhydrin spraying, and heating.

Scheme I



Synthesis. The synthesis of the three sequential polypeptides is shown schematically in Scheme I. We have deliberately avoided specifying the configuration of the β-aminobutyric acid (ABA), since the procedure is essentially the same for all three polymers. Later, "R" will be used to designate the intermediate containing an (*R*)-β-aminobutyric acid residue (example, IIIR), "S" an (*S*) residue and "R,S" a racemic residue.

***tert*-Butyloxycarbonyl-L-alanine *N*-Succinimidyl Ester (IV).** This compound was prepared according to Anderson et al.[16] using the solvent mixture ethyl acetate–dioxane (60:40 v/v). The reaction was carried out at 0° for 2 hr and then continued at 4° for 36 hr. Yield 75% (after two crystallizations from isopropyl alcohol); mp 161°C; $[\alpha]^{25}$D −52° (*c* 2, dioxane); R_F^{AA} 0.84.

(*S*)-β-Aminobutyric Acid Hydrochloride (IIIS). This was prepared from IIS as previously described:[7] yield 95%; mp 132–133°; $[\alpha]^{25}$D +21.30° (*c* 0.7, H_2O).

(*R*)-β-Aminobutyric Acid Hydrochloride (IIIR). Prepared as described for IIIS. $[\alpha]^{25}$D −21.30° (*c* 0.7, H_2O).

***tert*-Butyloxycarbonyl-L-alanyl-β-(*S*)-aminobutyric Acid (VS).** (*S*)-β-Aminobutyric acid hydrochloride (IIIS) (0.98 g, 7 mmol) and sodium bicarbonate (1.76 g, 21 mmol) were dissolved in water (30 ml) at room temperature and the solution cooled to 4°. A solution of IV (2 g, 7 mmol) in dioxane (20 ml) was then added and the reaction was allowed to proceed for 48 hr at 4°. The solvent was removed under reduced pressure, the residual oil dissolved in brine, cooled down to 0° and the pH adjusted to 3.0 with 1 *N* HCl without precipitation of the product. The saline solution was extracted four times with ethyl acetate at 0°C. These extracts were combined and washed three times with 0.05 *N* HCl saturated with NaCl at 0°C and three times with brine. The ethyl acetate solution was dried over $MgSO_4$, filtered, and evaporated in vacuo to an oil which was dissolved in ether and evaporated. After repeating this operation, a white solid was obtained which was recrystallized from 2-propanol–petroleum ether (bp 30–60°). The resulting white powder was homogeneous by TLC: R_F^{BAW} 0.68, R_F^{CMA} 0.90, R_F^{AA} 0.80; yield 1.36 g (70%); mp 78°; $[\alpha]^{25}$D −33.36° (*c* 2, $CHCl_3$); τ($CDCl_3$) 2.93 (1 H, doublet, peptide NH), 4.33 (1 H, doublet, urethane NH), 5.70 (2 H, multiplet, alanine α-CH, aminobutyric α-CH), 7.43 (2 H, doublet, aminobutyric CH_2), 8.53 (15 H, multiplet, Boc methyls, alanine and aminobutyric CH_3's). Anal. Calcd for $C_{12}H_{22}N_2O_5$: C, 52.55; N, 10.22; H, 8.03. Found: C, 52.77; N, 10.09; H, 7.95.

***tert*-Butyloxycarbonyl-L-alanyl-β-(*R*)-aminobutyric Acid (VR).** Prepared as described for VS, in the same yield and with identical R_F's, NMR, and melting point: $[\alpha]^{25}$D +1.8° (*c* 2, $CHCl_3$). Anal. Calcd for $C_{12}H_{22}N_2O_5$: C, 52.55; N, 10.22; H, 8.03. Found: C, 52.41; N, 10.12; H, 8.10.

***tert*-Butyloxycarbonyl-L-alanyl-β-(*R,S*)-aminobutyric Acid (VRS).** A similar procedure to that described for VR and VS was used. However, the β-amino acid residue was introduced as the free amine. Consequently, 2 instead of 3 mol of sodium bicarbonate were added per mole of amino acid: $[\alpha]^{25}$D −16.02° (*c* 2, $CHCl_3$); mp 54–58°C. Anal. Calcd for $C_{12}H_{22}N_2O_5$: C, 52.55; N, 10.22; H, 8.03. Found: C, 52.41; N, 10.18; H, 8.13. The product was obtained in the same yield and had identical R_F's and NMR spectrum.

***tert*-Butyloxycarbonyl-L-alanyl-β-(*S*)-aminobutyric Acid *N*-Succinimidyl Ester (VIS).** Compound VS (1.35 g, 5 mmol) was dissolved in dry ethyl acetate (25 ml), mixed with *N*-hydroxysuccinimide (0.58 g, 5 mmol) in dry dioxane (10 ml), and the mixture was cooled to 0°C. A solution of DCC (1.143 g, 5.5 mmol) in 10 ml of ethyl acetate was added to the cold solution and the reaction was allowed to proceed at 0° for 2 hr and then at 4° for 48 hr. Two drops of glacial acetic acid were then added and after 2 hr the precipitate of dicyclohexylurea (DCHU) was filtered off and the solution evaporated in vacuo. The syrupy residue was dissolved in 25 ml of ethyl acetate and stored overnight at 4°C. The DCHU which precipitated was filtered off and the ethyl acetate solution was washed, at 0°C, with 0.5 *M* sodium bicarbonate (3 × 25 ml) and three times with brine. The organic layer was dried over $MgSO_4$, filtered, and evaporated in vacuo. The oily residue was redissolved and evaporated twice with ether and the resultant white powder recrystallized from ethyl acetate–ether. Yield 1.5 g (80%); mp 128°C; $[\alpha]^{25}$D −71.0 (*c* 1, $CHCl_3$); R_F^{CMA} 0.88, R_F^{CAC} 0.64, R_F^{BAW} 0.77, R_F^{CM} 0.69, R_F^{AA} 0.80; τ ($CDCl_3$) 2.91 (1 H, doublet, peptide NH), 4.35 (1 H, doublet, urethane NH), 5.64 (2 H, multiplet, alanine α-CH, aminobutyric α-CH), 7.30 (6 H, multiplet, succinimide, aminobutyric CH_2), 8.60 (15 H, multiplet, Boc methyls, alanine and aminobutyric CH_3's). Anal. Calcd for $C_{16}H_{25}N_3O_7$: C, 51.75; N, 11.32; H, 6.74. Found: C, 51.49; N, 11.46; H, 6.95.

***tert*-Butyloxycarbonyl-L-alanyl-β-(*R*)-aminobutyric *N*-Succinimidyl Ester (VIR).** This was prepared as described for VIS in the same yield and with identical R_F's, NMR, and melting point: $[\alpha]^{25}$D +33.1° (*c* 2, $CHCl_3$). Anal. Calcd for $C_{16}H_{25}N_3O_7$: C, 51.75; N, 11.32; H, 6.74. Found: C, 51.31; N, 11.44; H, 6.68.

***tert*-Butyloxycarbonyl-L-alanyl-β-(*R,S*)-aminobutyric *N*-Succinimidyl Ester (VIRS).** Procedure, yield, R_F's, and NMR were as described for VIR and VIS: mp 104°C, $[\alpha]^{25}$D −18.10° (*c* 2, $CHCl_3$). Anal. Calcd for $C_{16}H_{25}N_3O_7$: C, 51.75; N, 11.32; H, 6.74. Found: C, 51.55; N, 11.35; H, 6.40.

L-Alanyl-β-(*S*)-aminobutyric *N*-Succinimidyl Ester Hydrochloride (VIIS). Compound VIS (1.4 g, 3.8 mmol) was dissolved in 10 ml of a 2 *N* solution of HCl in dioxane and after 1 hr at room temperature the solution was evaporated with exclusion of moisture. Trituration of the resultant oil with anhydrous ether gave a white solid, which was recovered by centrifugation. After decanting the supernatant the product was washed three times with ethyl acetate–ether, centrifuged, decanted, and dried in vacuo over P_2O_5: yield 1.15 g (quantitative); mp 76°; $[\alpha]^{25}$D −10.6 (*c* 1, 2-propanol); R_F^{BAW} 0.49, R_F^{AA} 0.10.

L-Alanyl-β-(*R*)-aminobutyric *N*-Succinimidyl Ester Hydrochloride (VIIR). The procedure, yield, mp, and R_F's were as described for VIIS $[\alpha]^{25}$D +11.1° (*c* 2, DMF).

L-Alanyl-β-(*R,S*)-aminobutyric *N*-Succinimidyl Ester Hydrochloride (VIIRS). This was prepared as described for VIIS and VIIR in the same yield and with identical R_F's: mp 67°C; $[\alpha]^{25}$D +11.0 (*c* 2, DMF).

Poly[L-alanyl-β-(*S*)-aminobutyric acid] (ASA). Compound VIIS (1.10 g, 3.7 mmol) was dissolved in 2.5 ml of DMF, the solution cooled to 0°, and triethylamine (0.4 g, 3.7 mmol) added. The reaction mixture solidified immediately and additional DMF (3 ml) was added. The thick slurry was stirred at 4° for 2 days, diluted further with DMF (2 ml), then stirred at room temperature for 2 days, additional DMF (2 ml) being added each day. The mixture was evaporated to dryness in vacuo and the solid residue was suspended in water. The suspension was dialyzed for 5 days in Spectrapor-3 dialysis bags (Spectrum Medical Industries, Inc., 3500 MW cut-off) against water. The resulting suspension was lyophilized, to yield 250 mg (43%) of fluffy white material: γ (KBr pellet) 3280, 1630, 1545 cm^{-1}.

Poly[L-alanyl-(*R*)-β-aminobutyric acid] (ARA). Using compound VIIR as a starting material, the procedure described above for ASA gave 285 mg of product (48%) with identical infrared spectrum (KBr pellet).

Poly[L-alanyl-(*R,S*)-β-aminobutyric acid] (ARSA). The product, prepared from VIIRS by the above procedure, had an ir spectrum identical with those from ARA and ASA: yield 175 mg (45%).

Molecular Weight Determination. The molecular weight of the three polymers was determined by amine end-group titration[17] in hexafluoro-2-propanol, using crystal violet (0.1% in glacial acetic acid) as an indicator. The polypeptide solution (3–5 mg in 1 ml of HFIP) was titrated with a standardized 0.100 *N* solution of $HClO_4$ in glacial acetic acid; each titration was repeated three times with different concentrations of polymer in HFIP and the average molecular weight was calculated. The titrations were carried out using

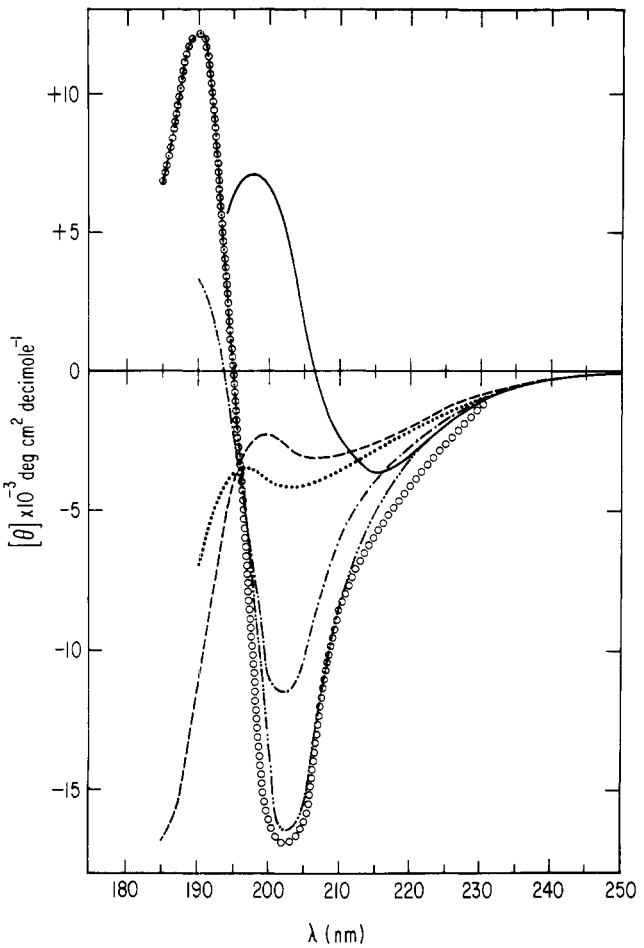


Figure 1. CD spectra of poly(L-alanyl-(*S*)-β-aminobutyric acid) in HFIP–water: (—) poly((*S*)-β-aminobutyric acid) in HFIP; (- - -) poly(L-alanyl-(*S*)-β-aminobutyric acid) in HFIP, 0% H_2O; (···) poly(L-alanyl-(*S*)-β-aminobutyric acid) in HFIP, 10% H_2O; (-·-) poly(L-alanyl-(*S*)-β-aminobutyric acid) in HFIP, 15% H_2O; (-···-) poly(L-alanyl-(*S*)-β-aminobutyric acid) in HFIP, 20% H_2O; (o) poly(L-alanyl-(*S*)-β-aminobutyric acid) in HFIP, 50% H_2O.

a radiometer-type SBUIa syringe buret equipped with a 0.5-ml syringe. Each division of the buret corresponded to 0.2 μl.

Circular Dichroism Measurements. Circular dichroism spectra were recorded at ambient temperature with a Cary 61 spectropolarimeter using cells with path lengths of 0.01, 0.1, and 1.0 cm. During the measurements the instrument was purged with nitrogen at a rate of 40 ft^3/min. The mean residue ellipticity $[\theta]_\lambda$, expressed as deg cm^2 $dmol^{-1}$, was calculated using the relationship

$$[\theta] = \theta_\lambda \mathrm{MRW}/10lc$$

where θ_λ is the observed ellipticity in degrees at wavelength λ, l is the cell path length in centimeters, MRW is the average molecular weight per residue (78 g/mol), and c is the sample concentration (g/ml). Concentrated stock solutions were prepared by weighing the appropriate amount of polypeptide in a volumetric flask and dissolving it in HFIP or HFPD. Solutions with the desired concentration were prepared by dilution of these stock solutions.

Polarized Infrared Spectra. Polarized infrared spectra of films of the polypeptides were recorded with a Perkin-Elmer Model 180 grating spectrophotometer, in the absorbance mode, with nitrogen purging (40 ft^3/min). Oriented films of the polypeptides in a matrix of polyethylene oxide (Poly-OX) were prepared according to the method of Ingwall et al.[18] by dissolving polypeptides in HFIP and mixing with a solution of poly-OX in TFE. The film was supported at a defined angle in a special Teflon holder.

Results

Number average molecular weights of poly(L-alanyl-(*R*)-β-aminobutyric acid), poly(L-alanyl-(*S*)-β-aminobutyric acid), and poly(L-alanyl-(*R,S*)-β-aminobutyric acid)

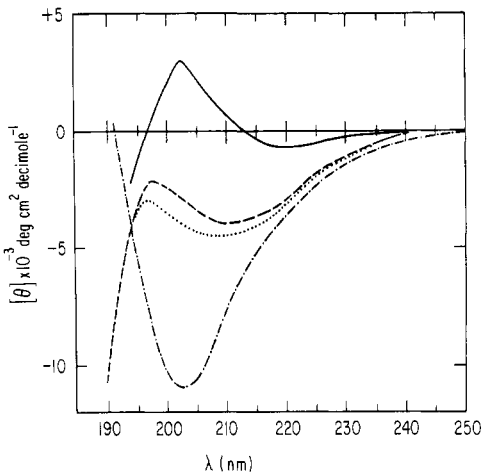


Figure 2. CD spectra of poly(L-alanyl-(*S*)-β-aminobutyric acid) in HFPD–water: (—) poly((*S*)-β-aminobutyric acid) in HFA; (- - -) poly(L-alanyl-(*S*)-β-aminobutyric acid) in HFA, 0% H_2O; (···) poly(L-alanyl-(*S*)-β-aminobutyric acid) in HFA, 20% H_2O; (-·-) poly(L-alanyl-(*S*)-β-aminobutyric acid) in HFA, 50% H_2O.

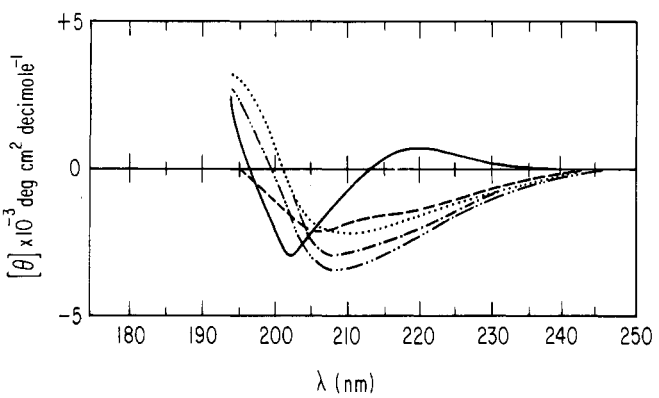


Figure 3. CD spectra of poly(L-alanyl-(*R*)-β-aminobutyric acid) in HFIP–water: (—) poly((*R*)-β-aminobutyric acid) in HFIP; (- - -) poly(L-alanyl-(*R*)-β-aminobutyric acid) in HFIP, 0% H_2O; (···) poly(L-alanyl-(*R*)-β-aminobutyric acid) in HFIP, 10% H_2O; (-·-) poly(L-alanyl-(*R*)-β-aminobutyric acid in HFIP, 20% H_2O; (o) poly(L-alanyl-(*R*)-β-aminobutyric acid in HFIP, 50% H_2O.

were respectively 10 500 ($\overline{DP}$ = 135), 7100 ($\overline{DP}$ = 89), and 7200 ($\overline{DP}$ = 90) as measured by amine end group titration.

The CD spectra of the three polypeptides in hexafluoro-2-propanol are shown in Figures 1, 3, and 5. Figures 2, 4, and 6 show the CD spectra of the polypeptides in hexafluoropropane-2,2-diol. These figures also show the effect of increasing water concentration on the CD. In each figure, the highest percentage of water represents the maximum amount that can be added to the solution without causing precipitation of the solute. These percentages of water depend on the original concentration of peptide in HFIP or HFPD. The CD spectrum of the corresponding poly-β-amino acid in pure solvent is shown in each figure for comparison. Figures 7, 8, and 9 show the CD spectrum of each polypeptide in the solid state. The films were cast directly onto the face of a quartz cell from a concentrated solution of each polymer in HFIP. The polarized infrared spectra of the stretched films of the polypeptides in poly-OX are shown in Figures 10, 11, and 12. We show the amide A, I, and II bands when the film is oriented both parallel and perpendicular to the polarized radiation. Fine crystalline powders of poly(L-alanyl-(*R*)-β-aminobutylic acid) and of poly(L-alanyl-(*S*)-β-aminobutyric acid) were obtained by grinding of the polypeptide films cast from hexafluoroace-

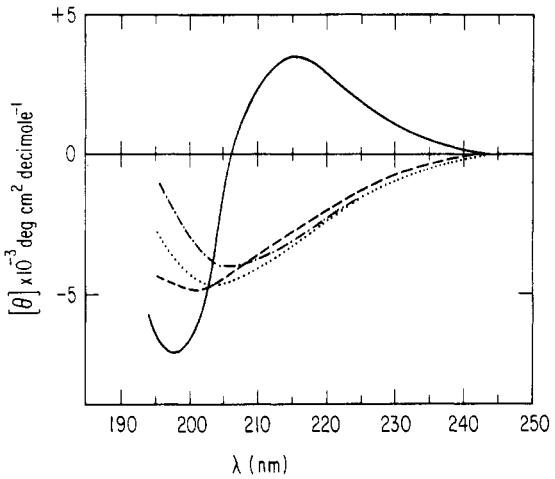


Figure 4. CD spectra of poly(L-alanyl-(*R*)-β-aminobutyric acid) in HFPD–water: (—) poly((*R*)-β-aminobutyric acid) in HFA; (- - -) poly(L-alanyl-(*R*)-β-aminobutyric acid) in HFA, 0% H_2O; (···) poly(L-alanyl-(*R*)-β-aminobutyric acid) in HFA, 10% H_2O; (-·-) poly(L-alanyl-(*R*)-β-aminobutyric acid) in HFA, 50% H_2O.

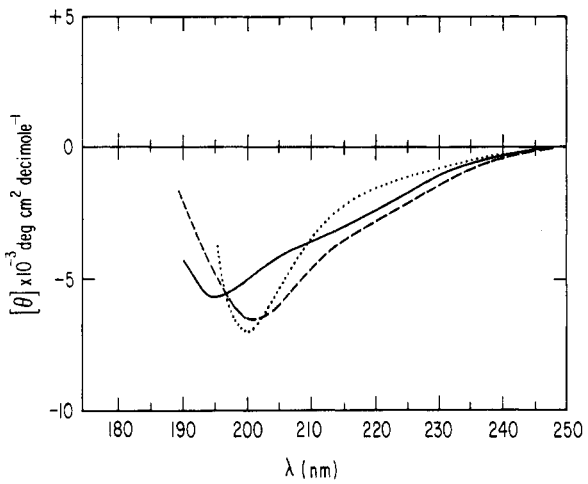


Figure 6. CD spectra of poly(L-alanyl-(*R,S*)-β-aminobutyric acid) in HFPD–water: (—) poly(L-alanyl-(*R,S*)-β-aminobutyric acid) in HFA, 0% H_2O; (- - -) poly(L-alanyl-(*R,S*)-β-aminobutyric acid) in HFA, 50% H_2O; (···) poly(L-alanyl-(*R,S*)-β-aminobutyric acid) in HFA, 80% H_2O.

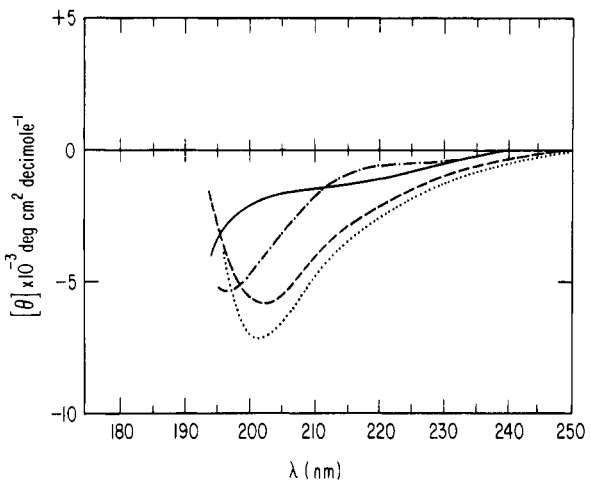


Figure 5. CD spectra of poly(L-alanyl-(*R,S*)-β-aminobutyric acid) in HFIP–water: (—) poly(L-alanyl-(*R,S*)-β-aminobutyric acid) in HFIP, 0% H_2O; (- - -) poly(L-alanyl-(*R,S*)-β-aminobutyric acid) in HFIP, 33% H_2O; (···) poly(L-alanyl-(*R,S*)-β-aminobutyric acid in HFIP, 50% H_2O; (-·-) poly(L-alanyl-(*R,S*)-β-aminobutyric acid) in HFIP, 90% H_2O.

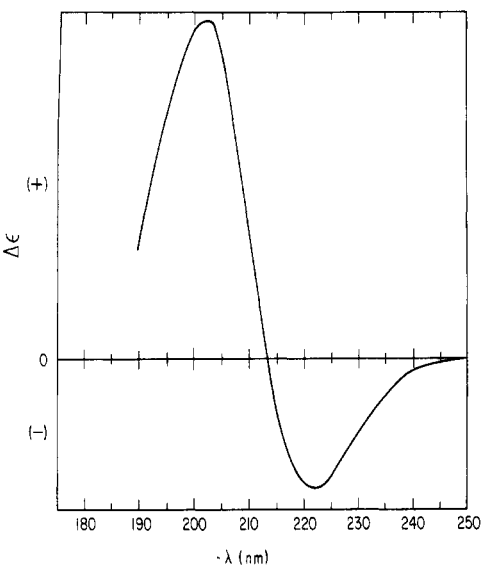


Figure 7. CD spectrum of poly(L-alanyl-(*S*)-β-aminobutyric acid) in the solid state.

tone sesquihydrate. Spacings derived from x-ray powder diagrams are listed in Table I.

Table I
Crystal Spacings from X-Ray Powder Diagram Spacing, Å

Poly(L-alanyl-(*R*)-β-aminobutyric acid)	Poly(L-alanyl-(*S*)-β-aminobutyric acid)
11.6	8.4
5.2	5.4
4.5	4.4
4.1	3.9
3.1	2.7
2.9	2.1

Discussion

The synthesis of intermediate compounds was accomplished in high yield and purity at each step merely by washing procedures or crystallization. The carboxyl terminus of the dipeptide was activated via the *N*-hydroxysuccinimide ester whose use for the synthesis of previously reported sequential polypeptides consisting of α-amino acids has led to high molecular weight material with minimum racemization.[21] On the other hand, no work has been reported, to our knowledge, on the racemization at the β carbon during coupling reactions of optically active β-amino acids via *N*-hydroxysuccinimide esters. It is, however, reasonable to suggest that racemization of β-amino acids through usual mechanisms such as oxazolone formation or proton abstraction is extremely unlikely because of the presence of the extra methylene group in the chain. We thus considered the polymerization of the dimer unit safe and did not carry out the usual racemization tests on the polymer.

It is noteworthy that cyclization by this procedure is normally a serious side reaction in the polymerization of dipeptide active esters. As a result the yield of polymer is generally low. In the case of the dipeptides discussed in this paper, the extra carbon atom in the β-aminoisobutyric acid residue would lead to a seven-membered ring. As a result the yield of polymer is high and cyclic dipeptide low.

The conformation of the three polypeptides in solution

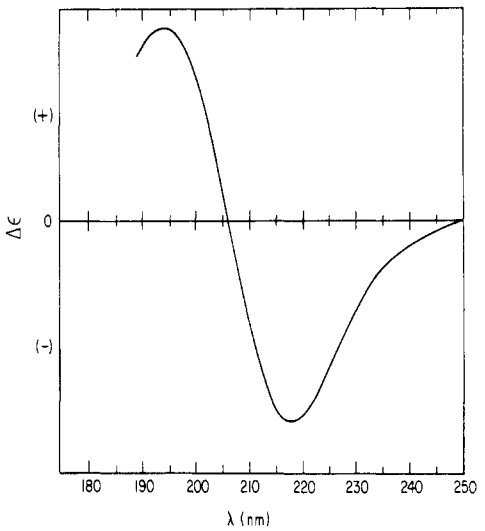


Figure 8. CD spectrum of poly(L-alanyl-(*R*)-β-aminobutyric acid) in the solid state.

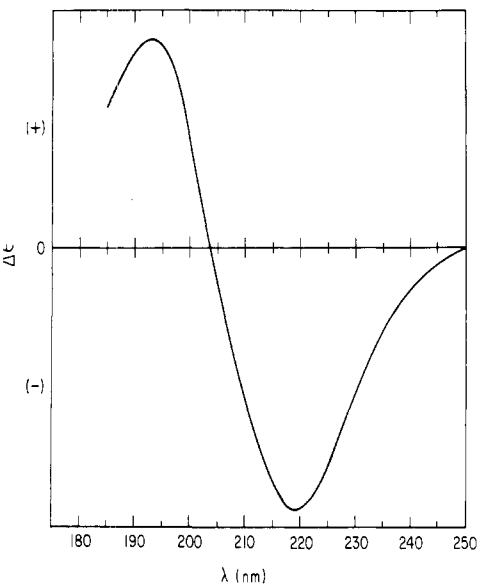


Figure 9. CD spectrum of poly(L-alanyl-(*R,S*)-β-aminobutyric acid) in the solid state.

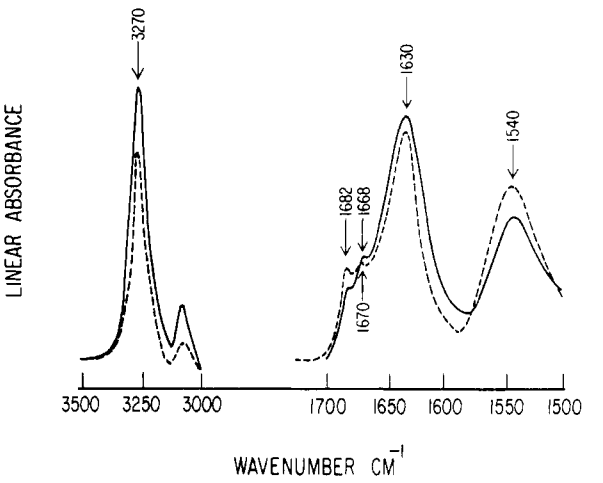


Figure 10. Polarized infrared spectrum of amide A, I, and II bands of an oriented film of poly(L-alanyl-(*S*)-β-aminobutyric acid) in a matrix of polyethylene oxide: (—) parallel orientation; (- - -) perpendicular orientation.

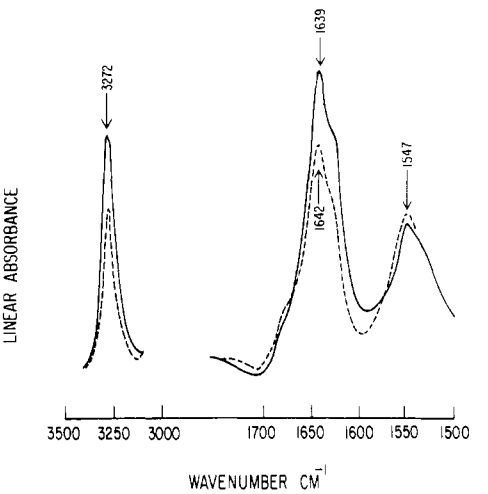


Figure 11. Polarized infrared spectrum of amide A, I, and II bands of an oriented film of poly(L-alanyl-(*R*)-β-aminobutyric acid) in a matrix of polyethylene oxide: (—) parallel orientation; (- - -) perpendicular orientation.

was first investigated by circular dichroism. In pure HFIP, they show weak ellipticity bands that could be ascribed to random coil conformation. The same is true of spectra in pure HFA.

The addition of water to HFIP or HFPD solutions of poly(L-alanyl-(*S*)-β-aminobutyric acid) causes significant changes in the spectra, as shown in Figure 1. At a polypeptide concentration of 0.2% (w/v) up to 50% water can be added before precipitation of the solute takes place. The precipitation of the polymer by addition of water is concentration dependent. More water is necessary to precipitate the solute at lower concentrations, without causing additional changes in the CD spectra, namely, a progressive random to β-type transition. Ellipticity of the trough at 202 nm and of the peak at 190 nm increases with increasing water concentration. The maximum ellipticity values we obtain are smaller than those reported by Chen et al.[7] for poly((*S*)-β-aminobutyric acid) under simiar conditions. Furthermore, the amount of water necessary to precipitate our polypeptide is greater than that necessary for the homopolypeptide.[7] We assume that molecular weights (200 000 compared with 7000) are partly responsible for these differences. Another reason may come from the difference in composition of the two polymers (namely, α and β amino acids on the one hand and only β-amino acids on the other hand). Such a change of composition can alter the hydration properties of the backbone of the polypeptides.

The addition of water to solutions of poly(L-alanyl-(*R*)-β-aminobutyric acid) and poly(L-alanyl-(*R,S*)-β-aminobutyric acid) in HFIP or HFA does not cause the sharp increase in the intensity of the n–π* and π–π* transitions that was observed for poly(L-alanyl-(*S*)-aminobutyric acid) (Figures 1 and 2). We assume that the *S* configuration of the β-amino acid unit is essential for the chains to adopt stable β conformations in water–organic solvent mixtures (Figures 3–6). As can be seen from Figures 5 and 6, poly(L-alanyl-(*R,S*)-β-aminobutyric acid) is considerably more soluble in aqueous solutions than its pure *R* or *S* counterparts. The same behavior is observed for many racemic polypeptides. We can conclude from Figures 3 and 4 that poly(L-alanyl-(*R*)-β-aminobutyric acid) remains highly disorganized even in the presence of water, although a

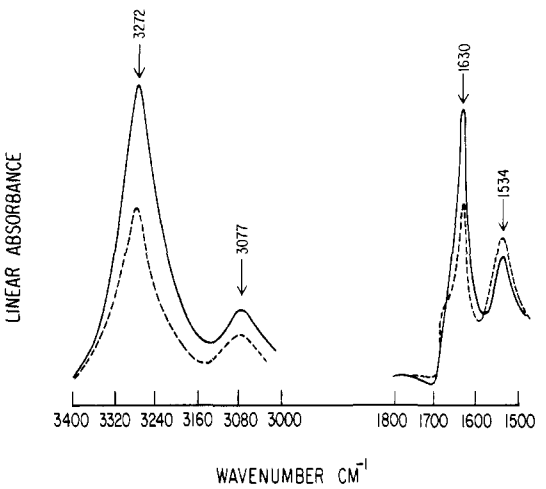


Figure 12. Polarized infrared spectrum of amide A, I, and II bands of an oriented film of poly(L-alanyl-(*R,S*)-β-aminobutyric acid) in a matrix of polyethylene oxide: (—) parallel orientation; (- - -) perpendicular orientation.

tendency toward β aggregation is suggested by the formation of a definite minimum between 200 and 210 nm.

Poly(L-alanyl-(*R,S*)-β-aminobutyric acid) has similar spectra in HFIP–H_3O and HFA–H_2O (Figures 5 and 6) although the minima around 200 nm are more intense than for poly(L-alanyl-(*R*)-β-aminobutyric acid). Its structure in water–organic solvent mixtures could be assumed to represent a compromise between organized β and random structures. As can be seen from Figure 7, the CD spectrum of poly(L-alanyl-(*S*)-β-aminobutyric acid) is similar in shape to known spectra of β-structured polypeptides such as poly(L-lysine).[19] However, the relative magnitude of the negative CD band of poly(L-alanyl-(*R*)-β-aminobutyric acid) (Figure 8) and of poly(L-alanyl-(*R,S,*)-β-aminobutyric acid) (Figure 9) is larger than expected for a β-like structure. It is likely that the CD spectra for the latter two polymers reflect a solid state comprised of both ordered and random conformations.

Polarized infrared spectra of oriented films of the polypeptides are consistent with CD evidence for their β structure. Films of poly-(L-alanine)[20] in the beta form show, like Figures 10, 11, and 12, bands around 1635, 1530, and 3280, corresponding to the amide I, II, and A transitions. However, the parallel dichroism of the amide I and A bands and perpendicular dichroism of the amide II bands are more suggestive of the cross-β form since poly(α-amino acids) in the cross-β form[18] display similar oriented ir spectra. Analysis of the amide I bands according to the procedure of Mizazawa and Blout[22] was performed using the recently derived vibrational parameters of Krimm and Abe.[23] The calculated frequencies of 1685 and 1635 cm^{-1} for the alanyl amide I band of the fully extended antiparallel β sheet are in substantial agreement with the experimental results obtained in oriented polyoxyethylene film for all three of the copolymers. However, the corresponding analysis for the β-amino amide was limited by the difficulty of choosing appropriate vibrational parameters.

A monomer repeat distance along the chain direction of approximately 8.5 Å is expected for the copolypeptides in the extended β conformation. As shown in Table I, a spacing of this magnitude was observed in the powder diagram of poly(L-alanyl-(*S*)-β-aminobutyric acid) but not in the powder diagram of poly(L-alanyl-(*R*)-β-aminobutyric acid). Further x-ray analysis of the polymers was inhibited by the great difficulty in obtaining oriented fibers.

This study of the copolypeptide of alanine with the *S* optical isomer of aminobutyric acid clearly shows a tendency to adopt the β conformation in both the solid state and in solution even though polyalanine forms very stable α-helices under normal conditions. Circular dichroism and infrared spectra of our polypeptides show absolutely no tendency toward α-helix formation as expected, since β-amino acid residues cannot possibly fit into the geometry of the α helix. The "dominant" residue, conformationally speaking, thus appears to be the β-amino acid. Its alternating presence in the chain also appears to be a predominant factor for the solvation of the polymer backbone, since it separates the amide groups responsible for hydrogen bonding and stabilization of a given structure by a greater distance than in poly(α-amino acids). At the same time, the extra methylene group of the β-amino acid increases the hydrophobicity of the backbone. This property of the backbone enhances the folding and aggregation of the chains in the presence of highly polar solvents. However, when two consecutive residues in the chain do not have the same configuration, as in the L-(*R*) and L-(*R,S*) polymers, aggregates do not form and the backbone is exposed to the solvent. Even the addition of water does not lead to formation of an ordered structure. We are continuing to investigate the conformations of these polymers.

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References and Notes

(1) Abbreviations used in this text are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature.
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