Conformational Studies on γ - Benzyl- *L*- Glutamate and *L*- Valine Containing Block Copolypeptides

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Abstract: Conformational studies on γ - benzyl- *L*- glutamate and *L*- valine containing block copolypeptides are reported using IR and CD spectra. The block copolypeptides contain valine block in the center and on both sides of the valine are γ - benzyl- *L*- glutamate blocks. The changes in conformation with increase in chain length of γ - benzyl- *L*- glutamate blocks are observed. When the chain length of γ - benzyl- *L*- glutamate block is 13, the block copolypeptide crystallized into beta conformation. With increase in chain length of γ - benzyl- *L*- glutamate block from 13 to 30 the conformation of the copolypeptide changes from beta sheet to alpha helix, but small amount of beta component also remains. With further increase in chain length of γ - benzyl- *L*- glutamate block from 30 to 45 the copolypeptide crystallizes exclusively into alpha helix conformation.

Keywords: γ - Benzyl- *L*- glutamate , *L*- Valine, block copolypeptides, IR and CD Spectra, α and β conformations.

Introduction

In the past, large number of polymers have been examined for biomedical applications and most popular polymeric materials are probably silicones, polyurethanes and hydrogels. More sophisticated polymeric materials such as block copolymers containing vinyl and polypeptide blocks have been designed¹⁻⁶. The polyvinyl blocks are selected for their good mechanical properties and polypeptides for their potential aptitude to improve the compatibility with blood and with different tissues. Additional advantages of the polypeptides are that their side chains can be modify at will. The hydrophobicity and the electrical state of the polypeptide can be changed.

Random copolymers are fibrous and often have good mechanically strength. Sequential copolymers of amino acid are also fibrous and have been studied as protein models⁷⁻¹⁰. Block copolymers have been of interest because they potentially represent the simplest model for globular protein^{11,12}. In practice, the rather low molecular weight

blocks available produced aggregated or micellar blocks^{12,13}. The AxBxXx copolymers fold like a globular protein with internal α -helical structure and external hydrophilic shell. The low molecular weight material forms aggregated micellar structure in aqueous solutions¹⁵⁻¹⁹. The peptide based biomaterials may have potential application in tissue engineering, drug delivery and biometric composite formation²⁰⁻²⁴. More recently poly amino acids and copoly- α -amino acids have been used for the preparation of biodegradable surgical dressing^{25,26}. The synthetic polypeptides^{1,6, 29} which have been used as materials at one time or other are derivatives of amino acids²⁹. Recently principles, applications and prospects of peptidic based material have been reviewed^{11,27}.

The solid state conformation and viscoelastic properties of high molecular weight triblock copolypeptides of γ -benzyl-*L*-Glutamate and *L*-valine or *L*-leucine have been studied^{7,14,28}. It has been shown that block copolymers self assemble into ordered nanostructure but random coil nature usually suppresses level of organization²⁰. The created self –assembly of block copolypeptides can be used as functional materials with nanoscopic dimensions. The so called supramolecular structures that can be obtained and their properties are essentially controlled at the molecular level by the right choice of the block copolymer^{4,5}. Among copolymer, it is possible to distinguish several types, namely block copolymer prepared by sequential homopolymerization (for example A-B-A- type triblock copolymers) and statistically random copolymers made by conventional copolymerization by adjusting the comonomer concentration using reactivity ratios^{29,30}. Many copolymers are of interest in which the molecules consist of long block capable of precipitating in glass like or crystalline domains separated by amorphous chain segments¹⁴.

In this manuscripts we describe synthesis and solid state conformational studies by circular dichroism (CD) and infrared spectroscopy (IR) of the $(\gamma\text{-bzl-}L\text{-Glu})_n\text{-(Val)}_{10}\text{-}(\gamma\text{-bzl-}L\text{-Glu})_n$ block copolypeptides. The intrinsic viscosity of block copolypeptides increases with increase in *n*. The γ - benzyl-*L*-glutamate is a strong α - former, where as *L*-valine is a strong β -former. The conformation of copolypeptides of γ -benzyl-glutamate and *L*-valine change from β to α - helix as *n* increases from 13 to 45.

Experimental

L-Valine and γ -benzyl-*L*-glutamate were from Sigma Chemicals, ethylenediamine (Sarabhai Chemicals) was triple distilled before use. Commercial grade solvent were purified and dried as per literature³¹. Benzyl alcohol and 4M phosgene solution in benzene were from Fluka. *N*-Carboxyanhydrides (NCA) of *L*- valine and γ - benzyl-*L*-glutamate were prepared by Goodman's method³².

Synthesis of block copolymers

L-Valine –*N*-carboxyanhydride (V) was polymerized using ethylenediamine as initiator with monomer (M) to initiator (I) ratio of 10 in benzene: dioxane (1:1v/v). The concentration of monomer was 21/mg/mL and polymerization was allowed to proceed for 3 days. The blocks of γ -benzyl-*L*-glutamate (G) were polymerized over *L*-valine blocks with varying degree of polymerization (M/I, 20,50 and 80) by adding appropriate amounts of γ -benzyl-*L*-glutamate and NCA. The polymerization was allowed to proceed for 7 days. The yields of block copolypeptides were about 75-80%. The block copolypeptides thus obtained were fractionated using ether as a non solvent. To fractionate the block copolypeptides, 800-900 mg of copolymer was dissolved in 25 mL of CHCl₃: TFA mixture and ether was added in three portions (40, 20 and 50 mL). The first (40 mL) and third (50 mL) fractions were discarded and only middle fraction was taken for further studies.

Amino acid analysis

Amino acid analysis of copolypeptides were done by following the relative integrated intensities of phenyl signals of γ -benzyl-L-glutamate (7.2 δ br) and gem dialkyl group signals of L-valine (0.9 δ br). For this purpose, a Perkin Elmer NMR Model, R-32 spectrometer was used. 30 mg of the copolymers were dissolved in 0.5 mL of trifluoroacetic acid or D-chloroform –trifluoroacetic acid mixture and their NMR spectra were recorded. To calculate the mole percent composition, the integration of the phenyl proton, signal was divided by 5 (as there are five protons in phenyl group) and integration of gem dialkyl group was divided by six. Then from the sum of integration the mole percent of each amino acid was calculated. The degree of polymerization “n” of γ -benzyl-L-glutamate was determined from amino acid composition assuming the degree of polymerization of (Val-Val) = 5.

Intrinsic viscosity $\{\eta\}$

A Ubbelohde viscometer was used to determine the intrinsic viscosity of polypeptides. Solution of block copolypeptides were prepared in chloroform: TFA (19:1 v/v). The solution were filtered through G-3 sintered glass crucible. The intrinsic viscosity was determined by extrapolation of plots of reduced viscosity against concentration. The intrinsic viscosity was expressed in dL/g.

Circular dichroism (CD) studies

A Jasco Spectro polarimeter Model J-500 DP was used to record CD spectra. The unit was appropriate standardized by using andosterone. The films for CD studies were casted from chloroform –trifluoroacetic acid (19:1) solution on a quartz plate, which were transparent to UV down to 185 nm. CD were recorded from 240 nm to down 185 nm wave length.

Infrared spectra

A Perkin Elmer (Model B-580) spectrometer was used. The IR of the copolypeptides were recorded in film form. The films were prepared by slow evaporation of dilute solution of copolypeptides in chloroform over clear surface of single crystal of sodium chloride. The films were dried in vacuum desiccators.

Results and Discussion

The amino acid composition, intrinsic viscosity $\{\eta\}$ data and conformation data of all the copolypeptides have been tabulated in Table 1. The intrinsic viscosity of γ - benzyl-L-glutamate and L-valine copolypeptides increases from 1.65 to 4.35 dL/g as the chain increases from 13 to 45. The increase in viscosity of block copolypeptides shows increase in molecular weight of the copolypeptides and confirms the increase in size of γ - benzyl - L-glutamates blocks. A conformational change from β to mixed α and β to α -helix was observed from increase in chain length from 13 to 45 of γ -benzyl-L-Glutamate.

Table 1. Conformational Studies on $(\gamma\text{-bzL-L-Glutamate})_n(\text{Val})_{10}(\gamma\text{-bzL-L-Glutamate})_n$

Copolypeptide	Mole Ratio		n	$\{\eta\}$ dLg ⁻¹	Conformation	
	G	V			CD	IR
GVVG	G	V				
	73	27	13	1.65	β	β
	86	14	30	2.65	$\alpha(\beta)$	$\alpha(\beta)$
	90	10	45	4.35	α	α

X= γ -bzL-L-glutamate, CD = Circular Dichroic spectra, IR = Infrared Spectra, $\{\eta\}$ = intrinsic viscosity, n = number of γ -bzL-L-glutamate units.

The IR spectra of the block copolypeptides are shown in Figure 1. The amide I band region has peaks characteristic of α -helix ($1647\text{--}1655\text{ cm}^{-1}$) for all the three block copolypeptides and also a band characteristic for parallel β - conformation ($1629\text{--}1640\text{ cm}^{-1}$) except for the system with $n=45$. IR spectrum of the block copolypeptide with $n=13$ shows a strong peak at 1629 cm^{-1} and a small peak at 1647 cm^{-1} suggesting that the system more or less has crystallized into β - conformation. The IR spectrum of the block copolypeptide with $n=30$ shows peaks of equal intensity at 1655 cm^{-1} and 1640 cm^{-1} suggesting the system crystallized into mixed α and β - conformation. The copolypeptide with $n=45$ shows peak at 1655 cm^{-1} indicating the presence of exclusive α – helical conformation³³.

Figure 1. IR Spectra of γ - benzyl-*L*- glutamate and *L*- valine copolypeptides, - ; $n=13$, - - ; $n=30$, - . - ; $n=45$.

Figure 2. CD Spectra of γ - benzyl-*L*- glutamate and *L*- valine copolypeptides, - ; $n=13$, - - ; $n=30$, - . - ; $n=45$.

The CD spectra of block copolypeptides are shown in Figure 2. The CD spectrum of block copolypeptide with $n=13$ shows a band at 216 nm indicating the presence of β - conformation. The CD spectrum of block copolypeptide with $n=30$ showed a strong band at 225 nm and a hump at 210 nm suggesting that the system has both α and β - conformation component present in it. The CD spectrum of block copolypeptide with $n=45$ shows band at 207 nm and 225 nm suggesting the presence of α conformation^{33,34}.

Both IR and CD spectra clearly show, block copolypeptide, with $n=13$, crystallizes into exclusive beta sheet structure. The block copolypeptide, with $n=30$, crystallizes into alpha helix conformation, but small amount of beta component is also present. With further increase in number of γ -benzyl- *L*-glutamate units to 45, the copolypeptide crystallizes into exclusive alpha helix structure. The results prove that γ -benzyl-*L*-glutamate influences the conformation of *L*- valine block. From these results it can be concluded that both beta sheet as well as alpha helical domain exist in γ - benzyl-*L*-glutamate and valine copolypeptides. Thus using this information, block copolypeptides of desired conformation may be prepared for various biomedical applications.

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