Conformational Studies of ϵ -CBz- *L*-Lysine and *L*-Valine Block Copolypeptides

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Abstract: Conformational studies of ϵ -CBz-*L*-lysine and *L*-valine block copolypeptides using x-ray diffraction and CD spectra are described. The block copolypeptides contain valine block in the center and on both side of the valine are ϵ -CBz-*L*-lysine blocks. The conformation of the copolypeptides changes with increases in the chain length of ϵ -CBz-*L*-lysine blocks. When length of ϵ -CBz-*L*-lysine blocks is 9, the block copolypeptide has exclusive beta sheet structure. With the increase in chain length of ϵ -CBz-*L*-lysine blocks from 9 to 14, the block copolypeptide shows presence of both alpha helix and beta sheet components. With further increase in chain length of ϵ -CBz-*L*-lysine blocks, the beta sheet component disappears and block copolypeptides exhibits exclusive α -helix conformation.

Keywords: ϵ -CBz-*L*-lysine and *L*-valine, Copolypeptides, x-Ray diffraction and CD spectra, α and β conformation

Introduction

The synthetic polypeptides used as, synthetic silk fiber analogues, biocompatible materials and in surface coatings, microphones, piezoelectric membranes are derivatives of amino acids¹⁻⁸. Random copolymers are fibrous and often have good mechanical strength^{9,10}. Sequential copolymer of amino acid are also fibrous have been studied as protein models¹¹⁻¹³. Block copolymers have been of interest because they potentially represent the simplest model for globular protein¹⁴. In practice, the low molecular weight block copolymers produce aggregates or micellar blocks¹⁵. The AxBxXx copolymers fold like a globular protein with internal α -helical structure and external hydrophilic shell¹⁶⁻²⁰. It has been shown that block copolymers are able to self assemble into ordered nanostructure but random coil nature usually suppress any further level of organization²¹.

The peptide based biomaterials may have potential application in tissue engineering, drug delivery and biometric composite formation²¹⁻²⁵. More recently poly amino acids and co-poly-amino acids have been used for the preparation of biodegradable surgical dressing^{26,27}. Recently principles, applications and prospects of peptidic based biomaterial have been reviewed^{28,29}.

The solid state conformation and viscoelastic properties of high molecular weight triblock copolypeptides of γ -Bzl-*L*-glutamate and *L*-valine or *L*-leucine have been studied^{7,10,30}.

In this present paper, we describe synthesis and solid state conformational studies by circular dichroism (CD) and X-ray diffraction of the (ϵ -CBz-Lysine)_n-(Val-Val) - (ϵ -CBz-Lysine)_n. The intrinsic viscosity of block copolymers increases with increase in *n*. The ϵ -CBz-lysine is α -former, where as *L*-valine is β -former. The block copolypeptide of ϵ -CBz-lysine and *L*-valine the conformation change from β to α -helix.

Experimental

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

Synthesis of block co-polymers

L-Valine-*N*-carboxyanhydride was polymerized using ethylenediamine as initiator with monomer (M) to initiator (I) ratio of 10 in benzene:dioxane (1:1 v/v). The concentration of monomer was 21 mg/mL and polymerization was allowed to proceed for 3 days. The blocks of ϵ -CBz-lysine were polymerized over *L*-valine blocks with varying degree of polymerization (M/I, 20, 30, 50 and 80) by adding appropriate amounts of ϵ -CBz-lysine NCA. The polymerization was allowed to proceed for 7 days. The yields of block copolymers were about 75-80%. The block copolymers thus obtained were fractionated using, ether as a non solvent. To fractionate the block copolymers, 800-900 mg of copolymer was dissolved in 25 mL of (9:1 v/v) CHCl₃:TFA mixture and ether was added in three portions (40 mL, 20 mL 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 copolymer was done by following the relative integrated intensities of phenyl signals of ϵ -CBz-lysine (7.2 δ) and gem dialkyl group signals of *L*-valine (0.9 δ). 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 ϵ -CBz-lysine 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 (Figure not shown). 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 was recorded from 240 nm to down 185 nm wave lengths.

X- Ray diffraction studies

A Philips (PW -1010) x- ray diffractometer was used. Samples for x-ray diffractions were prepared by casting thick films from copolymer's chloroform: trifluoroacetic acid (19:1 v/v) solution, over glass slides. The films were oriented by stroking the solution by fine knife in one direction as evaporation occurred. The films were then kept in vacuum desiccator for two days. The following relationship was used to calculate d_{hkl} values.

$$d_{hkl} = \frac{n\lambda}{2 \sin \Theta}$$

CuK radiation filtered with Ni film was used to record the diffraction pattern from $2\Theta = 3^\circ$ to 30° , the Bragg angle. A chart speed of 2Θ per minute was used.

Results and Discussion

The amino acids compositions, intrinsic viscosities and conformation data of all the block copolypeptide are tabulated in Table 1. The intrinsic viscosity of ϵ -CBz- lysine and valine copolypeptides increase from 1.5 to 6.20 dL/g as the chain length of ϵ -CBz- lysine blocks increase from 9 to 57. The increase in viscosity of block copolypeptides showed increase in molecular weight of copolypeptides which confirms the increase in chain length of ϵ -CBz- lysine blocks. CD spectra of the copolypeptide are shown in Figure 1. The CD spectrum of block copolypeptide with $n=9$ showed a band at 214 nm which is indicative of β -sheet structure. The CD spectrum of block copolypeptide showed bands at 222 nm and a hump at 207 nm which shows the presence of both β -sheet and α -helical components. The CD spectrum of block copolypeptide with $n=26$ showed broad band in the range of 207 - 222 nm, this exhibit the presence of both the β -sheet and α -helical components in the peptide. The CD spectrum of block copolypeptide with $n=57$ showed two separate bands at 205 nm and 225 nm which shows the presence of exclusive α -helical conformation.

Table 1. Conformational Studies on $(X)_n(\text{Val-Val})_5(X)_n$.

Copolypeptide	Mole Ratio		n	$\{\eta\}$ dLg ⁻¹	Conformation		
	K	V			CD	X- Ray, d_{hkl}	
GVVG	64	36	9	1.50	β	β	13.6+ 8.8+ 4.8(s)
	73	27	14	2.10	β, α	α, β	12.9(s), 4.85(s)
	84	16	45	3.20	α	α (β)	12.9 (s), 4.8 (m)
	92	80	57	6.20	α	α	12.9 (s) , 4.8(f)

S=strong, m=medium, f=faint, Poly-L-valine, 4.8 Å°, Poly-CBZ-lysine 12.9Å°

The CD spectra indicate that a transition from exclusively β -sheet with $n=9$ system to a mixed β -sheet and helical form occurred with $n=14$ system. The ellipticities of the $[\pi-\pi^*]$ (205), $n-[\pi^*]$ (225 nm) bands of $n=26$ and $n=57$, copolypeptides were nearly equal, indicating that the β -sheet component if present at all is present as very minor component³³.

x- Ray diffraction profile of $(\epsilon\text{-CBz-lysine})_n-(\text{Val-Val})_5-(\epsilon\text{-CBz-lysine})_n$ films casted from chloroform: TFA (19 : 1 v/v) and are shown in Figure 2. The block copolypeptide with $n=9$ shows a sharp diffraction at 4.8° d_{hkl} and small diffraction at d_{hkl} 13.6 $^\circ$ Å and 8.8 $^\circ$ Å indicating the presence of β -sheet structure. The block copolypeptide with $n=14$ shows diffraction of almost equal intensity at d_{hkl} 12.9 $^\circ$ Å and 4.8 $^\circ$ Å indicating the presence of both β -sheet structure and α -helical structure. The block copolypeptide with $n=26$ shows

a sharp diffraction at d_{hkl} 12.9 °Å and a broad diffraction at d_{hkl} 4.8 °Å suggesting the presence of mainly α – helical structure in this system. The block copolypeptide with $n= 57$ diffracted strongly at d_{hkl} 12.9 °Å and very weakly at d_{hkl} 4.8 °Å indicating the presence of exclusive α - helical structure in this system. The X- ray diffraction profile shows a transition from exclusively beta form ($n=9$) to a mixed beta and left handed alpha form ($n=14, 26$). The system, $n=57$ have almost alpha helical form^{34,35}.

Figure 1. CD Spectra of ϵ -CBz-*L*-lysine and *L*-valine copolypeptides ,
-; $n=9$, - - ; $n= 14$, - . - ; $n= 26$, -.-, $n= 57$.

Figure 2. x-Ray diffraction pattern of spectra ϵ -CBz-*L*-lysine and *L*-valine copolypeptides , -; $n=9$, - - ; $n= 14$, - . - ; $n= 26$, -.-, $n= 57$.

Thus both CD and x- ray diffraction studies show that block copolypeptide having the chain length of ϵ - CBz- lysine 9 crystallizes into exclusive beta sheet structure. When chain length of ϵ - CBz- lysine increases to 14, the block copolypeptide has two domains and crystallizes into mixed alpha and beta conformation. When the chain length of the ϵ -CBz-lysine blocks is increased to 45, the CD spectrum indicates that copolypeptide crystallizes into exclusive alpha helix and x- ray diffraction profile shows that the copolypeptide acquires alpha helix conformation but small amount of beta sheet component is also present. With further increase in chain length of ϵ -CBz-lysine blocks to 57, the copolypeptide crystallizes exclusively into alpha helix.

Therefore using the above observations, block copolypeptides which either crystallize into exclusive beta sheet structure, mixed beta and alpha structure or exclusive alpha helix structure may be prepared for biomedical and other applications.

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