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CONFORMATION OF SEQUENTIAL POLYPEPTIDES OF L-VALINE IN SOLUTION

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CD study of a number of sequential polypeptides of L-valine with L-alanine and glycine has been done in a mixed solvent system of hexafluoro 2-propanol (HFIP)-trifluoro ethanol (TFE) in order to elucidate the conformation of poly(L-valine) in solution. The results suggested that poly(L-valine) cannot take the α-helical conformation in HFIP-TFE. A new CD pattern was observed on the sequential polypeptide of L-valine with glycine.

A controversial problem remains on the conformations of poly(L-valine) in solution from the theoretical as well as the experimental aspects. Ooi et al[1] stated from their calculation of the conformational energy that α-helical conformation has the lowest energy for poly(L-valine). Experimentally the α-helix of the polypeptide of L-valine was observed on a block copolypeptide of $(DL\text{-Lys})_{18}(L\text{-Val})_{15}(DL\text{-Lys})_{16}$ in 98% methanol, but the copolypeptide existed in β-conformation in water.[2]

We wish to report in this communication an approach to elucidate the conformation of poly(L-valine) in solution from CD spectra of a number of the sequential polypeptides consisting of L-valine and other amino acids. Since poly(L-valine) itself is insoluble in almost all the solvents in which it will take the secondary structures, the sequential polypeptides as a modified polypeptide of L-valine were employed in order to improve the solubility in solvent. The use of these sequential polypeptides permits a clear explanation of the CD spectra compared with the random copolypeptides because the former have well defined sequences of amino acids.

The sequential polypeptides used in this study were prepared by a new method using N-carboxy α-amino acid anhydride (NCA) and an N-hydroxysuccinimide ester (OSu ester). The synthetic route of the sequential polypeptides by the new NCA-OSu method is illustrated by the synthesis of $(L\text{-Val-L-Val-L-Ala})_n$ shown in Scheme I.

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$$\text{H-L-Ala-OH} \xrightarrow[Na_2CO_3]{\text{L-Val NCA}} \text{H-L-Val-L-Ala-OH} \xrightarrow[NaHCO_3]{\text{Z-L-Val-OSu}} \text{Z-L-Val-L-Val-L-Ala-OH}$$

$$\xrightarrow{\text{HOSu, DCC}} \text{Z-L-Val-L-Val-L-Ala-OSu} \xrightarrow{\text{HBr}} \text{HBr·H-L-Val-L-Val-L-Ala-OSu}$$

$$\xrightarrow{N(Et)_3} (\text{L-Val-L-Val-L-Ala})_n$$

SCHEME I. Synthesis of $(\text{L-Val-L-Val-L-Ala})_n$ by the NCA-OSu method.

The reaction of L-alanine with L-valine NCA under the standard condition[3] of the NCA method for peptide synthesis gave L-valyl-L-alanine, which was reacted with Z-L-Val-OSu in a mixed solvent system of acetonitrile-water to yield Z-L-Val-L-Val-L-Ala-OH. The acylated tripeptide was converted to OSu ester without racemization. The monomer salt obtained by deacylation of the N-protected tripeptide OSu ester was polymerized quantitatively by addition of triethyl amine to $(\text{L-Val-L-Val-L-Ala})_n$. Thus obtained polypeptide contained no cyclic compounds after repeated washing with hot methanol. The other polypeptides were prepared in high yields by the same procedure as above. The paper described the detailed procedure of the synthesis will soon appear elsewhere.[4] The sequential polypeptides used in the CD study are listed in Table I with the reduced viscosity in dichloroacetic acid.

Table I. Sequential Polypeptides and Their Reduced Viscosities

Polypeptide	$\eta_{sp/c}^{a}$
$(\text{L-Val-L-Ala})_n$	0.12
$(\text{L-Ala-L-Val-L-Ala})_n$	0.23
$(\text{L-Ala-L-Val-L-Val-L-Ala})_n$	0.14
$(\text{L-Ala-L-Met-L-Ala})_n$	0.20
$(\text{L-Val-L-Val-L-Ala})_n$	0.16
$(\text{L-Val-L-Val-L-Val-L-Ala})_n$	0.06
$(\text{L-Val-Gly})_n$	0.17
$(\text{L-Val-L-Val-Gly})_n$	0.23
$(\text{L-Val-L-Val-L-Val-Gly})_n$	0.12

[a] Measured in dichloroacetic acid at 0.5 g/dl at 30°C.

The CD spectra of the sequential polypeptides were measured in a mixed solvent system of hexafluoro 2-propanol (HFIP)-trifluoro ethanol (TFE) at the concentration of 0.5 mg/ml. At this concentration, the association of the polypeptides may not present.[5] TFE is widely used as a good solvent which does not destroy α-helix of polypeptides, but it could not dissolve the sequential polypeptides of L-valine used in this study. Therefore we chose the mixed solvent system of HFIP-TFE. The solvent system containing HFIP has been successfully used as an α-helix supporting solvent for polypeptides[6] when an α-helix promoting solvent (eg. TFE) to be mixed with HFIP has a portion above 40% by volume. The solvent system used in this study may serve as such a mild solvent as supporting α-helix of the sequential polypeptides.

In Fig. 1 are shown the CD spectra of $(\text{L-Val-L-Ala})_n$, $(\text{L-Ala-L-Val-L-Ala})_n$, $(\text{L-Ala-L-Val-L-Val-L-Ala})_n$ and $(\text{L-Ala-L-Met-L-Ala})_n$. The last polypeptide $(\text{L-Ala-L-Met-L-Ala})_n$ consisting of amino acids classified[7] in α-helix forming group was used as a standard sample of the sequential polypeptide taking α-helix in the solvent. The CD curve of the polypeptide represented a positive peak at 192 nm (due to the π-π* transition) and two negative peaks at 207 nm (the π-π* transition) and 220 nm (the n-π* transition) characteristic of an α-helix. The polypeptides $(\text{L-Val-L-Ala})_n$, $(\text{L-Ala-L-Val-L-Ala})_n$ and $(\text{L-Ala-L-Val-L-Val-L-Ala})_n$ showed essentially identical CD spectra with that of $(\text{L-Ala-L-Met-L-Ala})_n$ having the positive peak at 190 nm and the negative peaks at 206 and 220 nm. This spectral pattern indicates obviously the presence of α-helical conformation in the sequential polypeptides of L-valine though coexistence of small amount of random coil component reflecting on the small magnitude of mean residue ellipticity and blue shift of the positive π-π* transition maximum around 190 nm. This result is consistent with far-infrared spectral study of this kind of polypeptides in solid state by Itoh et al.[8]

These results indicate capability of L-valine to form a stable α-helix in the copolypeptides having suitable sequences with α-helix forming amino acids[7] in accordance with the fact of the presence of L-valine in the α-helical parts of naturally occurring proteins such as myoglobin.[9] However in Fig. 2 the CD spectra of $(\text{L-Val-L-Val-L-Ala})_n$ and $(\text{L-Val-L-Val-L-Val-L-Ala})_n$ represent the typical β-conformation pattern having a positive peak at 195 nm and a negative peak at 215-217 nm. This shows that the polypeptides with successive sequences of L-valine fail to take α-helix in HFIP-TFE in spite of the incorporation of the amino acid that can form α-helix. From this result, we deduced that the preferable conformation of poly(L-valine) is not α-helix but β-structure in this solvent system.

Though the results obtained by the study of CD spectra of the sequential polypeptides of L-valine with L-alanine suggested that poly(L-valine) itself may not take α-helical conformation in HFIP-TFE, the fact that valyl residue can be incorporated in α-helix prompted us to study the conformations of the sequential polypeptides of L-valine with glycine as classified in non-α-helix forming amino acids. In Fig. 3 are shown the CD spectra of the sequential

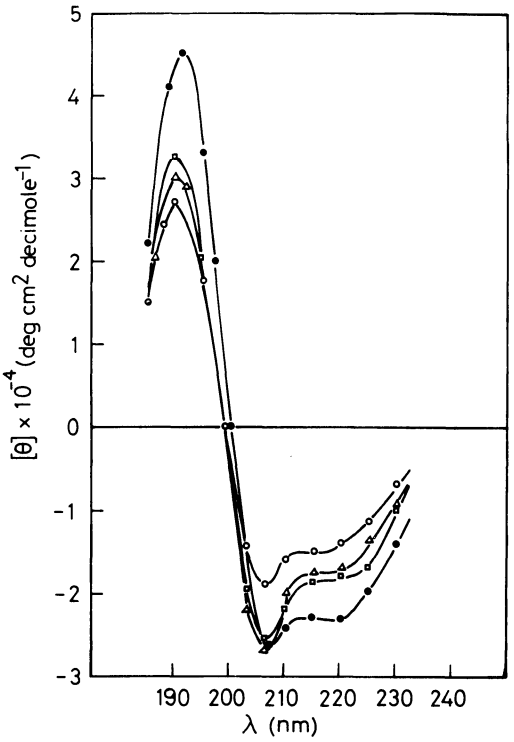

Fig. 1. The CD spectra of the sequential polypeptides of L-valine with L-alanine in HFIP-TFE (20:80 by volume) at room temperature.
○ : $(L\text{-}Val\text{-}L\text{-}Ala)_n$, △ : $(L\text{-}Ala\text{-}L\text{-}Val\text{-}L\text{-}Ala)_n$, □ : $(L\text{-}Ala\text{-}L\text{-}Val\text{-}L\text{-}Val\text{-}L\text{-}Ala)_n$, ● : $(L\text{-}Ala\text{-}L\text{-}Met\text{-}L\text{-}Ala)_n$

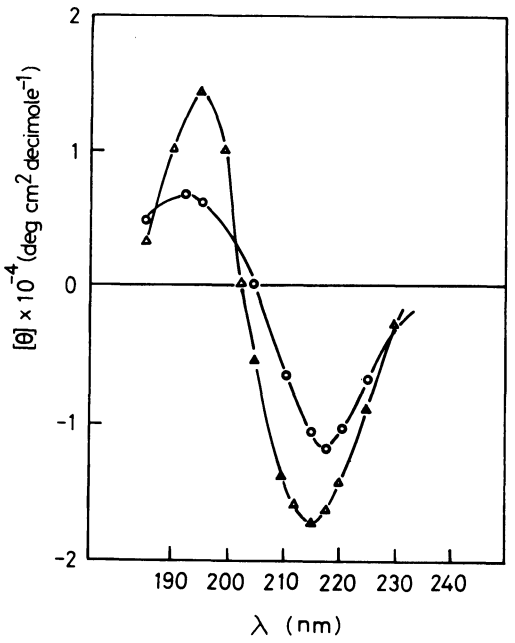

Fig. 2. The CD spectra of the sequentia polypeptides of L-valine with L-alanine in HFIP-TFE (30:70 by volume) at room temperature.
○ : $(L\text{-}Val\text{-}L\text{-}Val\text{-}L\text{-}Ala)_n$
△ : $(L\text{-}Val\text{-}L\text{-}Val\text{-}L\text{-}Val\text{-}L\text{-}Ala)_n$

polypeptides of L-valine with glycine in HFIP-TFE. The spectrum of $(L\text{-}Val\text{-}Gly)_n$ having a positive peak at 192 nm and negative peaks at 207 and 220 nm is similar to that of α-helix but the intensity of each band is relatively small. This α-helical conformation may be caused by reduced side chain interaction which is made by introduction of glycine in the polypeptide of L-valine.

Quite interesting spectrum was observed in case of the sequential polypeptide of $(L\text{-}Val\text{-}L\text{-}Val\text{-}Gly)_n$. The spectrum showing negative peaks at 189 nm and 220 nm and a positive peak at 203 nm is not assigned any spectral patterns characteristic of α-helix, β-structure and random coil. Then we propose tentatively that the polypeptide $(L\text{-}Val\text{-}L\text{-}Val\text{-}Gly)_n$ takes a new ordered structure in the solution. The CD spectrum of the sequential polypeptide is similar to that which is reverse to the CD pattern of poly(L-proline) II structure in sign of the ellipticity,[10] the polypeptide may exist in a similar extended state of the helical structure to the right-handed three-fold helix.

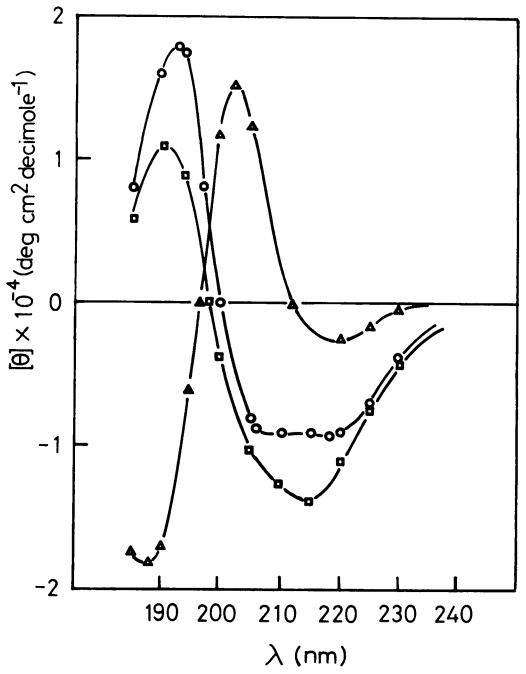

Fig. 3. The CD spectra of the sequential polypeptides of L-valine with glycine in HFIP-TFE (50:50 by volume) at room temperature.
○ : $(L\text{-Val-Gly})_n$, △ : $(L\text{-Val-L-Val-Gly})_n$,
□ : $(L\text{-Val-L-Val-L-Val-Gly})_n$

The polypeptide $(L\text{-Val-L-Val-L-Val-Gly})_n$ showed a CD pattern similar to that characteristic of β-conformation with a positive peak at 190 nm and a negative peak at 215 nm.

The sequential polypeptides $(L\text{-Val-Gly})_n$ and $(L\text{-Val-L-Val-L-Val-Gly})_n$ do not show the completely same CD spectra as those characteristic of α-helix and β-conformation, respectively, but quite similar to those. These results may suggest together with the new ordered structural CD pattern of $(L\text{-Val-L-Val-Gly})_n$ that the sequential polypeptides of L-valine with glycine take a deformed conformation of α-helix or β-structure and that the side chain-side chain interaction plays an important role on the conformation of the polypeptide. Further conformational investigation about a new ordered structure of $(L\text{-Val-L-Val-Gly})_n$ is now undertaken by nmr and ir techniques.

References

1) T. Ooi, A. Scott, G. Vanderkooi and H. A. Scheraga, J. Chem. Phys., 46, 4410 (1967)
2) R. F. Epand and H. A. Scheraga, Biopolymers, 6, 1551 (1968)
3) Y. Iwakura, K. Uno, M. Oya and R. Katakai, Biopolymers, 9, 1419 (1970)
4) R. Katakai, M. Oya, F. Toda, K. Uno and Y. Iwakura, Macromolecules, in press
5) M. Goodman, F. Naider and C. Toniolo, Biopolymers, 10, 1719 (1971)
6) J. R. Parrish, Jr., and E. R. Blout, ibid, 11, 1001 (1972)

7) E. R. Blout, "Polyaminoacids, Polypeptides and Proteins", Ed. by M.A. Stahmann, The University of Wisconsin Press, Madison, Wisconsin, (1962) pp 275

8) K. Itoh, H. Katabuchi and T. Shimanouchi, Nature New Biology, 239, 42 (1972)

9) J. C. Kendrew, H. C. Watson, B. E. Strandberg, R. E. Dickerson, D. C. Phillips and V. C. Shore, Nature, 190, 4 (1961)

10) M. L. Tiffany and S. Krimm, Biopolymers,11, 2309 (1972)

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