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Physicochemical Properties of Amino Acid Polymer
Molecular Structures of Polyglycines

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Introduction

There are two typical types in molecular structure of polyglycine which is synthesized from glycine. The one is a socalled polyglycine I, zigzag form in β-conformation. The another is a socalled polyglycine II, helical form in α-helical conformation.

Infrared spectra and X-ray diffraction patterns of two molecular configurations of polyglycines are very significant for investigation of molecular structure. Then, these physicochemical properties were determined to decide these molecular structures with infrared and farinfrared spectrophotometers at low, room, and high temperatures mainly and X-ray diffractometer at room temperature complementally. The obtained infrared spectra and X-ray diffraction patters of polyglycines revealed that the presence of β-conformation for polyglycine I and α-helical conformation for polyglycine II.

Experimental and Results

Various infaraed and farinfrared absorption bands of polyglycines, polyglycine I and polyglycine II, were published on the previous papers.[1,2] One more, those experimenteal results were recognized partially with new synthesized polyglycine I and polyglycine II. And, the solubility to alkali metal salt solution, the specificity of physicochemical properties depended upon temperature change, the specific action of reagents, and X-ray diffraction of powder material were determined with infrared spectrophotometer and X-ray diffractometer respectively.

Thus, infrared and farinfrared spectra and X-ray powder photographs were useful to reveal the presence of β-conformation for polyglycine I and α-helical conformation for polyglycine II.

N-carboxyanhydride of glycine[1,3] : 6 g pure glycine was suspended in 285 ml pure dioxane. And glycine was reacted with phosgene for 6 hrs at 60°C under stirring condition. At the end of reaction, dried nitrogen gas passed into the solution to release excess phosgene. The solution was filtered with 11 G 4 under dry condition attentively. Pure dried ethyl ether was added to the condensed solution with protect from entrance of moisture. The addition of ethyl ether and evaporation procedure was repeated several times. This procedure was useful to crystallize the reaction product. The crude crystal obtained was 6.8 g. And it was dissolved in purfied dried ethyl acetate. And it was filtered and purified petroleum ether was added to recrystallize. The white crystal was dried on phosphoric oxide under reduced pressure. The yield was 5.8 g. This detailed procedure was published on the prevoious paper.[1,2]

Polyglycine I : 4 g N-carboxyanhydride (Leuche anhydride) of glycine was dissolved in 160 ml purfied dried dioxane. And 0.02 mol triethylamine as an initiator was added into the dioxane solution under stirring condition. The reaction was continued for 9

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hrs at 25±1°C. Much purified dried ethyl ether was added into the dioxane solution to precipitate the reaction product. And the polymer produced was washed with 200 ml purified dried ethyl ether by centrifuger and it was dried under reduced pressure.

Polyglycine II : 1 g polyglycine I was dispersed in 100 ml saturated lithium bromide aqueous solution. After the dispersed solution was complete homogeneous state, it was centrifuged with high gravity to separate the undispersed material. The precipitate was not observed. Then, the supernatant fraction was poured into excess water as much as possible without the polymer precipitating out. The precipitate was then washed and centrifuged several times to remove all lithium bromide. The white precipitate, crude polyglycine I, was obtained. Then, this procedure was repeated again to purify the material. The material was washed with pure dried acetone and pure dried ethyl ether. And it was dried under reduced pressure. The yield was 0.95 g. Of course, the material was dried directly under reduced pressure was possible. The former was more smaller powder than the latter. During purfication procedure, the infrared spectra of the materials were determined to detect the contamination and the molecular change. It was found that the best way to obtain polyglycine II without any trace of polyglycine I. The structure is unlikely to be a hydrated form and is quite stable. It is dispersible only in the solvents which disperse polyglycine I. In this respect it differs from other synthetic polypeptides which have remarkedly different solubilities depending on whether are in the α or β . By adjusting the conditions of precipitation it is possible to control the particle size. Then the solubility is very important. The detailed preparation procedure was published on the previous paper.[2)]

Conversion : Polyglycine I is converted into polyglycine II with alkali metal salt solution like the above description. Also, polyglycine I is converted into polyglycine II with alkali earth salt (e.g. calcium chloride) organic solvent (e.g. formic acid). In the case of calcium chloride formic acid, it is effective on

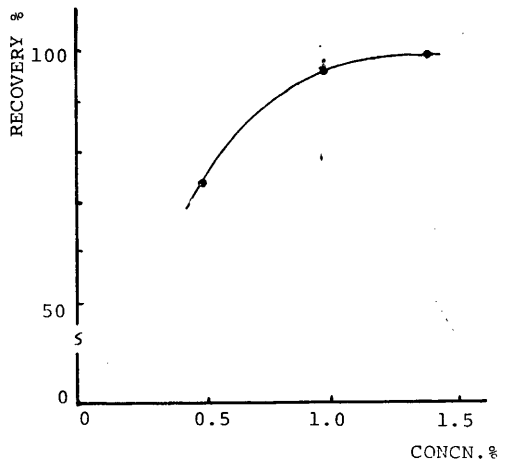

Fig. 1. The Solubility Curve of Polyglycine to Saturated Aqueous Lithium Bromide Solution

such a conversion. However, in this procedure of remove from the salt, the extraction with ethyl alcohol was not easy. And the trace of salt gives the contamination in specimen. So the lithium bromide method is better and safer than the calcium chloride method.

Solubility : The solubility of polyglycine to alkali metal solution was not detail on the previous paper. Then, the part of the experiment of solubility is described. The solubility of polyglycine to saturated lithdium bromide aqueous solution is expressed by the recovery percentage. The recovery percentage against 0.5%, 1.0%, and 1.4% solution that the polyglycine was contained 0.5% (weight/weight), 1.0%, and 1.4 % in each saturated lithium bromide aqueous solution respectively was 73.7%, 96.8%, and 99.9%. The obtained result is shown on Fig. 1.

So, this examination was carried out by 1.0% solution. The small loss is also significant to avoid the contamination of impure material. The object of examination is on an acquirement of pure material for the physical investigation.

The procedure was followed with the above described method on "preparation of polyglycine II." Now the author proposes an one expression that the polyglycine is dispersed in the lithium bromide solution. Because, it is formed polypeptide chain, polymer, and

its solubility is not large. And the molecular weight, numbor average molecular weight, weight averagemolecular weght has to be determined with osmometer, light scattering apparatus, ultracentrifuger, or others. Then, these tries may be carried on at the next experimental step.

Temperature change : The experimental temperature was controlled at -22 ± 2°C, 25 ± 2°C, and 70 ± 2°C respectively. Of course, other higher or lower temperature was considered. But the experimental conditions, cell structure, window material (KSR5), temperature control unit, restricted this determination to the described temperatures. However the improvement in the cell is trying by the author. On the other hand, many absorption bands were determined in the spectral region of 4000 cm^{-1} to 400 cm^{-1}. The typical difference depending upon temperature change in spectral absorption bands was not observed between three temperatures. The farinfrared absorption bands were determined at room temperature (25 ± 2°C).

Infrared and farinfrared spectrophotometers : Various spectral determinations were carried out with Perkin-Elmer 521, Hitachi EG-1, Hitachi FIS-1, and partially Perkin-Elmer 201 C.

Polarized radiation : Polarized radiation was obtained with inserting a silver bromide wire grid polarizer made by Hitachi in the stead of other polarizer, e. g. selenium transmission polarizer.

Calculation : The various calculations followed with the described method on the previous[1~21] papers. The description on this paper is abbreviated. It is too long.

X-ray diffractions : X-ray diffractions were determin ed with Geiger-Flex X-ray Diffractometer made by Rigaku Denki, 2001. The condition was Cu-Kα_1, 35 KV, 15 mA at room temperature regulated.

Then the obtained representative vibrational frequencies of film and themselves pellets of polyglycine I and polyglycine II, and the assignments are listed in Table 1. Other various obtained data may be publish on other paper.

The existence of two modifications of polyglycine has not been found and investigated. As one reason, the separation or purification of polyglycine from natural material, silk, etc., has not been so easy. But the synthesis of polyglycine has been more difficult than other polypeptides, and co-polymers. On the other hand, polyglycine is a fundamental important amino acid polymer, and is an interesting material biologically. Recently, some publications pointed at the existence.

The specific physicochemical properties, infrared and farinfrared spectra, vibrational frequencies, assignments of absorption bands, energy distribution for molecular vibration modes of synthesized polyglycine I and polyglycine II were published by the author. However, the syntheses of polyglycine I and polyglycine II were carried out again, because the amount of synthesis is limited by various conditions at the author's laboratory.

The existence of a folded form of some polypeptide chain is supported by experimental evidence obtained from an examination of infrared absorption spectra and X-ray diagram of synthetic or natural material. The results obtained from infrared and farinfrared spectra are shown on this paper, but the results from X-ray diffraction may be reported on the other paper. For materials which have been examined in a well-crystallized, oriental form (poly-γ-benzyl-L-glutamate, poly-γ-methyl-L-glutamate) the X-ray evidence is strongly suggestive of a helical fold of the kind proposed by Pauling et al.[22] The subject has been reviewed by Crick.[23] The α-helix of Pauling et al. was arrived at from consideration of conditions for minimum molecular structural energy in a folded polypeptide chain. These considerations exclude many folds which are sterically possible and suggest that the α-helix is the most stable conformation of fold with internal hydrogen bonds. Then, the physical properties of various synthetic polypeptides were investigated with the exception of polyglycine. As one reason, the difficulity of chemical synthesis of pure polygylcine made this exception. In the case of polyglycine, the indications that two conformations of chain occoured according to the method of preparation, namely, the

Table 1. Vibrational Frequencies and Assignments of Film and Themselves Pellets of Polyglycine I and Polyglycine II in the Spectral Region of 4000 cm^{-1} to 50 cm^{-1}. (cm^{-1})

Polyglycine I		Polyglycine II	
Frequency, cm^{-1}	Assignment	Frequency, cm^{-1}	Assignment
3451 sh			
3292 s	NH str.		
		3272 s	NH str.
		3084 m	Amide II×2
3072 sh	Amide II×2		
		2931 m	CH_2 antisym. str.
1681 sh, 1628 s	Amide I (C=O str.)		
		1640 s	Amide I (C=O str.)
		1552 s	Amide II (CN str. +NH in-plane def.)
1519 s	Amide II (CN str.+NH in-plane def.)		
1431 s	CH_2 bend.		
		1416 s	CH_2 bend.
		1376 m	CH_2 wag.
		1284 s, 1281 s	Amide III (CN str.+NH in-plane def.)
		1252 s	CH_2 twist.
1234 sh	Amide III (CN str.+NH in-plane def.)		
1201 m	CH_2 twist.		
1199 m		1181 sh	
		1136 s	
1134 m			
1056 w			
		1028 s	Skel. str.
1017 m	Skel. str.		
991 w			
		902 s	CH_2 rock.
		836 m	
833 w			
802 w			
		801 w	
		752 sh	Amide V (NH out-of-plane def.)
714 s	Amide V (NH out-of-plane def.)		
631 w	Amide IV (C=O in-plane def.)		
606 s	Skel def.		
591 s	Amide VI (C=O out-of-plane def.)	493 ww	
489 ww			
		369	Amide VII (CO-NH torsion)
364			
		285	
283			
218			
		215 ww	
		146 ww	
136 sh			
112 sh			
		104 ww	
88 sh		(88)	

	Molec, Struc Polyglycine
	(80)
78 sh	
	(74)
68	
	(67)

Intensity : s=strong, m=medium, w=weak, ww=very weak, sh=shoulder

extended β-chain type, and the α-helix type were recognized from the experimental results published on the previous paper.[1,2] This reasoning agreed with the results obtained.

The question of the stability of these folds is considered, as a result of which it appears that the stability of these folds may not be greatly inferior to that of the α-helix. According to the usual considerations, various analyses are proposed. The infrared spectra of α-helix polypeptides are characterized by a carbonyl streching mode in the region 1652—1657 cm^{-1} for optically active polypeptides, and a slightly higher range for *meso* forms. The vibrational frequency of this band changes on going from the solid form to solution in an inert solvent, and there is no reason for doubting that the folded form is preserved in those conditions. Then, absorption band may be shift slightly by the condition of preparation. And x-ray diffraction method is very useful for analysis of molecular structure of polymer. The synthetic α-helix polypeptides pack at least approximately hexagonally, and it is possible to get an idea of the $10\bar{1}0$ speacing in particular case from the density or from considerations based on van der Waals' radii, etc. With this information, the presence of an α-helix may be recognized by the occurence of a strong ring of the appropriate diameter on the powder photograph.

It will be seen from the foregoing that two criteria may be applied to recognize α-helices in unoriented polypeptides, one based on the frequency of the C=O mode, the other on the presence of a dominant ring in the X-ray diffraction. If orientation can be produced then the characteristic infrared dichroism and X-ray fibre pattern may also be sought.

The problem of the existence of a regular type of fold in crystalline or globular proteins has received much attention, and after the establishment of evidence of the presence of α helices in synthetic polypeptides, it was natural to look for evidence of the same fold in proteins. The postulates from which Pauling deduced the α-helix contain nothing to suggest that this will not be the stable form in proteins, and indeed he claimed to have found evidence that such a helix occured in hemoglobin. However, the parallel rod-like arrangement seen in Patterson diagrams of some crystalline proteins, should be much more marked if parallel α helices were present, though the data for hemoglobin are not incompatiable with α helices if the molecule are not parallel, and Carlisle et al.,[24] in a detailed examination of ribonuclease, have concluded that there is insufficient evidence for the presence of α helices in this protein. The low infrared dichroism in this protein gives so cause for belief that they are made up of α helices.

Since in crystalline proteins the range of side-chain size is great, and in addition active side chains are present, it is perhaps likely that α helices, if present, would be so distorted as to be difficult to recognize. For this reason, a protein such as silk, which consists largely of the residues of the small amino acids, glycine and alanine, has advantages. The molecular weight is large and there are apparently no cross-links, so the properties may be expected to resemble the synthetic polypeptides, about whose conformation much is already known. The very low content of ionizable side-groups in silk ensures that infrared absorption bands from ionized COO^- groups will not be present to any appreciable extent ; such bands may, in certain cases, appear and may be confused with bands from the peptide groups.

Polyglycine I is a typical β conformation spectrum, with the NH streching mode at 3292 cm^{-1}, the C=O mode at 1628 cm^{-1}, and the complex band which arises partly from NH deformation at 1519 cm^{-1}. If a

rather strong band appears at ca. 1690 cm^{-1} in polyglycines, it was attributed to small peptides of low molecular weight. In this experiment, its absorption band was not appeared completely. So, the synthesized polyglycine I in this experiment now reported was free from molecules of low molecular weight. There was a band of considerably reduced intensity at 1681 cm^{-1} which is really attributable to polyglycine. It shows parallel dichroism, and in this respect resembles a very weak band which appears at 1695 cm^{-1} in the spectrum of β-poly-L-alanine, but not in α-poly-L-alanine. A band at about the same vibrational frequency appears in other β-polypeptides, and in silk. There is a band at 1431 cm^{-1} which may be a CH_2 bending mode, the band at 1017 cm^{-1} is useful for diagnostic purposes. And this band is influenced by preparation method of specimen.

Polyglycine II shows a typical α-helical conformation spectrum. The NH streching mode is at 3272 cm^{-1} and the band which usually accompanies NH streching modes, at 3084 cm^{-1} is stronger than in other synthetic polypeptides. Polyglycine II is interesting in having a C=O band at 1640 cm^{-1}, considerably higher than normal β frequencies but lower than the range of frequencies found in α polypeptides. The NH deformation mode is at 1552 cm^{-1}. The band at 1681 cm^{-1} of polyglycine I was not observed in the spectrum of polyglycine II, and this phenomenon affords proof that this band does not arise from end groups or impurities. There is a band at 1416 cm^{-1} which may be a CH_2 bending mode. The band at 1028 cm^{-1} is useful for diagnostic purposes, and shows that whereas polyglycine precipitated from solution consists wholly of form II. As the preparation or casting of specimen, and the selection and usage of reagent decide the form of polymer, the method of examination has to be cautioned. The effect that this band, 1028 cm^{-1}, is associated with a diglycyl group is considered as one reason. So, the consideration to these problems claimes the investigations in similar or homologous materials.

Turning to the physicochemical properties of β silk, biologically more interesting material, the great similarity of *Bombyx mori* and of *Anaphe moloneyi* fibroin is thought as one of subject of investigation. The great similarity of the spectra in the region of overtone and combination bands is rised to a valuable, interest, and reasonable experimental viewpoint. According to Lucas,[25] *Anaphe moloneyi* silk contains approximately 39 glycine+50 alanine out of a total of 100 residures, whereas *Bombyx mori* silk contains 41 glycine+28 alanine, with a corresepondingly higher proportion of bulkly side chains than is found in *Anaphe moloneyi* silk.

Table 2. Vibrational Frequencies of Absorption Bands, Optical Specificities, and Assignments. (cm^{-1})

Polyglycine II (β)	*Anaphe moloneyi*	*Bombyx mori*	Dichroic Character	Assignment (Atomic group)
	1452	1453	∥	CH_3
	1446	1447	⊥	CH_3
1234	1231	1235	∥	Amide
	1168	1166	∥	CH_3
1017	1001	999	∥	
	975	978	∥	
		928	⊥	

The observed vibrational frequencies of absorption bands in spectra, optical specificities and assignments of polyglycine are listed on Table 2 in comparison with the above described two kinds of silk.

The following conclusion is thought from data of Table 2 at the first step.

The similarity between these two spectra shows how dominant is the effect of the glycine and alanine residues, an effect which has been pointed out by Astubury et al.[26] on the basis of frequency measurements on an unspecified silk. The correspondence is greatly enhanced by the similarity of the dichroic character of the bands. Thus, the determination of dichroism is very important to analyze the information of the molecular structure may be easy to understand. The peptide bonds in polyglycine I have the usual dichroism found in β structures is considered. Then, especially the dichroism of the amide was paied attention to the band at 1681 cm^{-1} in polyglycine I. The strong parallel band at 1231—1235 cm^{-1} is almost

certainly such a band, amide group, and may contain contributions both from glycine and from alanine residues. Also, the parallel band at ca. 1266 cm^{-1} is not observed in polyglycine. A number of the stroger bands may be correlated with the glycine and the alanine residues of silk. The assignment of three of these bands to methyl group frequencies is reasonably certain. The splitting of the 1450 cm^{-1} asymmetrical CH_3 deformation mode is ascribed to steric effects. It may perhaps be significant that the vibrational frequency difference for this band between polyglycine I (1017 cm^{-1}) and polyglycine II (1028 cm^{-1}) is 11 cm^{-1} and the corresponding figure for *Anaphe moloneyi* silk (β form and freeze-dried form) is 12 cm^{-1}. The β form or the freeze-dried form was produced by different preparation. The infrared spectrum of freeze-dried *Anaphe moloneyi* silk suggests the absence of a β conformation. And the environment of all the C=O groups is substantially the same. Also, attempt to synthesize poly-α-amino-isobutyric acid in a conformation form corresponding to polyglycine II is carring on under the improved different experimental condition, the polymer is thought in a form giving the characteristic α-helix spectrum and X-ray powder diagram. This suggests that the absence of an asymmetric carbon atom is not the primary cause of polyglycine not folding in the α-helix conformation.

On the other hand, a perfectly regular fold appears to be excluded by single, broad ring shown by X-ray diffraction diagram. This would appear to leave two probabilities.

(1) The hydrogen bonds are random, comprising both inter- and intrachain, but nevertheless the immediate environment of the C=O groups does not vary greatly throughout the material.

(2) The hydrogen bonds may all be formed within one chain in a fold which allows considerable molecular flexibility by rotation around the α carbon single bonds.

Thus, infrared investigation companies with X-ray diffraction is greatly significant to determine the detail molecular structure of polymer.

Then, the consequence or molecular structure for the two silks requires the synthesis of polyalanine. At the next chance, the author may try to synthesize polyalanine.

Summary

Polyglycine is classified into two conformations. The one is a β-form which is a extended zigzag chain, and is called polyglycine I. The another is a helical form, and is called polyglycine II.

The physicochemical properties, molecular structures, of new prepared polyglycines were determined with infrared and farinfrared spectrophotometers in the spectral region of 4000 cm^{-1} to 50 cm^{-1} mainly at low, room, and high temperatures under regulatory condition, and were determined with X-ray diffractometer complementally.

The evidence which these new synthesized polyglycine I and polyglycine II were β-conformation and α-helical conformation respectively was recognized.

Absorption bands of polyglycines were tried to assign the corresponding molecular vibration modes in parallel with the calculations of vibration energy and energy distribution.

The reagents, dichloroacetic acid and trifluoroacetic acid, had to be cautionary in using, casting procedure especially. Alkali metal salt, lithium bromide, was useful for the conversion between β-conformation and α-helical conformation.

The dichroism, and the solubility of polyglycine, and the specific effect of reagent on molecular structure were investigated parallely.

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アミノ酸重合物の物理化学的性質
ポリグリシンの分子構造

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ポリグリシンは2つの典型的立体配位（構造）に区分される．1つは伸長したジグザグ鎖のβ形で，ポリグリシンⅠとよばれ，他の1つはラセン形で，ポリグリシンⅡとよばれる．新につくられたポリグリシンの物理化学的性質，分子構造を赤外および遠赤外分光光度計で制御された低，室，高温において 4000 cm^{-1} から 50 cm^{-1} の分光領域について，主として測定し，またX線回折計で補助的に測定もした．これらの新しく合成されたポリグリシンⅠとポリグリシンⅡはそれぞれβ立体配位とαラセン立体配位であるという証を確認した。

ポリグリシンの吸収帯につき，分子振動様式の帰属を試みた。ジクロロ酢酸，トリクロロ酢酸は使用上，塑造過程上特に注意されねばならなかった．アルカリ金属塩，臭化リチウムはβ立体配位とαラセン立体配位間の転移に対して有効であった．ポリグリシンの二色性や溶解度，試薬による分子構造への特性を振動エネルギーとエネルギー分布の計算と平行してこの実験中に研究した．