Physicochemical Properties of Amino Acid Polymer

Polyglycine I

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Polyglycine was synthesized with the improved method from glycine monomer which was a typical fundamental amino acid. However, this synthesis reaction belonged to a comparative difficult one for obtain a polymerized compound. Now, there are two typical structural forms in polyglycine. One is β-form which extended nearly zigzag chain and is specificity of polyglycine I, another one is a helical form which is specificity of polyglycine II.

At first, the order of synthesis reaction was decided as an approximate second order in the process of reaction. At second, the structural form of synthesized polyglycine was decided as a zigzag form in β-conformation. Some new physicochemical properties were determined predominately with infrared and farinfrared spectrophotometers in the spectral region of 4000 cm^{-1} to 50 cm^{-1}. Then, some new assignments to the absorption bands were carried out for the molecular vibration modes.

Experimental and results

Synthesis process :

N-carboxyanhydride[1] of glycine ; The higher ordered structure of polyglycine I, the fundamental polypeptide which was polymerized with glycine, was investigated from the various viewpoints. In the present investigation, the polyglycine had to be synthesized in pure chemical process at first and be in high purity. 8 g pure glycine, which was purified two times and was ground carefully, was suspended in 375 ml pure dioxane which was purified, dehydrated and distilled two times. And glycine was reacted with phosgene for 6 hrs at 60℃ under stirring condition by magnetic stirrer. In the case of glycine, this reaction was not easy in comparison with other α-amino acid, but the purity of reaction product with this method was higher than other method. At the end of reaction, dried nitrogen gas which was better than carbon dioxide passed into the solution to release exess phosgene. The solution was filtered with sintered glass filter 11G4 under dry condition. The filtrate was condensed protecting entrance of moisture. And purified dried ethyl ether was added into it and its solution was evaporated. This procedure was repeated several times. This addition of ethyl ether was made

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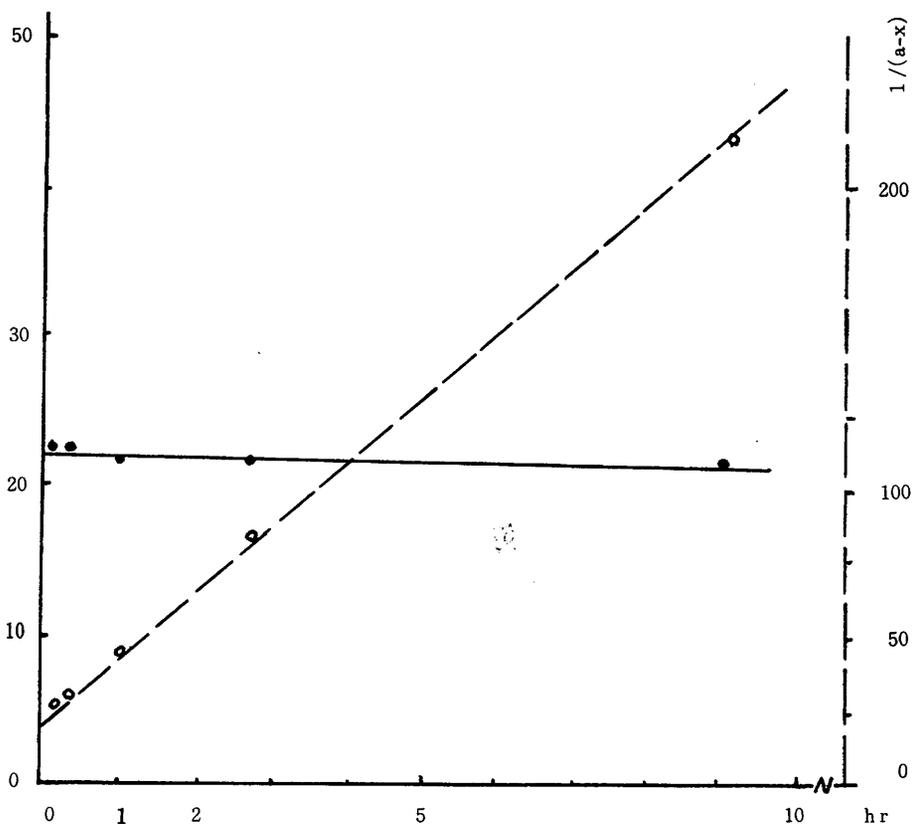
Fig. 1. The order and rate constant of the synthesis reaetion of polyglycine I.

to crystallize the reaction product. The crude crystal obtained was 9 g. And it was dissolved in purified dried ethyl acetate. And it was filtered and purified dried petroleum ether was added to recrystallize. This procedure was repeated three times carefully. The white crystal was dried on phosphoric oxide under reduced pressure. The yield was 7. 8 g.

The order of synthesis reaction ; The released gas in process of synthesis reaction was absorbed into two step-apparatuses of 50% potassium hydroxide under a complete protecting condition for moisture which invaded into the synthesis system. And its analysis line was treated very carefully. The step-

apparatuses which were original design were isolated completely except the phenomenon of reaction. The determination was carried out gravimetrically, because the reaction obeyed the law of mass action. Of course, spectro-photometrical determination was carried out in parallel. Generally, the phenomenon has to be determined with two and more methods at the same time. The order of synthesis reaction was decided as an approximate second order. Its obtained result was shown in Fig. 1.

Polyglycine I[2] ; 4. 4 g N-carboxyanhydride (Leuche anhydride) of glycine was dissolved in 180 ml purified dried dioxane. And 0. 02

mol triethylamine as an initiator was added into the dioxane solution under stirring condition by magnetic stirrer. The reaction was continued for 90 hrs at the instrument room which was regulated at 25℃ ±1℃. During reaction process, the amount of released carbon dioxide was determined at regular intervals to decide the order of reaction or the rate of reaction at the constant temperature room. Much purified dried ethyl ether was added into the dioxane solution to precipitate the reaction product, polymerized material. And the polymer was washed with 200 ml purified dried ethyl ether using centrifuger and it was dried under reduced pressure.

For purification of polyglycine I, dichloroacetic acid which was dried, distilled three times was better than several solvents. And trifluoroacetic acid was more suitable than other some solvents for preparation. As a matter of fact, there was one fundamental strict restriction that the molecular structure did not change with use of solvent. It was recognized that these two solvents did not induce any change in the molecular structure.

Calculation :

Internal co-ordinates ; Polyglycine I is classified to the factor group C_{2v} from the investigative results[3,4,5,6] of X-ray. And the normal vibrations which are characteristic amide vibrations and skeletal vibrations are grouped into nine A_1, nine B_1, four A_2, and four B_2. Then, the calculations were carried on using Wilson's GF matrix method.[7,8] And the internal co-ordinate vectors which belonged to the m-th unit of infinite molecular chain of the polyglycine I were represented as $R^{(m)}$, $R'^{(m)}$. The methylene groups were calculated as mass points. The internal co-ordinates used are as following,

$R_1^{(m)}$ $\Delta r_{NH} = \Delta r_{15}$

$R_2^{(m)}$ $\Delta \gamma_{NH} = (1/\sqrt{2})\Delta(\alpha_{513'} - \alpha_{215})$

$R_3^{(m)}$ $\Delta \beta_{NH} = (1/\sqrt{6})\Delta(2\alpha_{3'12} - \alpha_{513'} - \alpha_{215})$

$R_4^{(m)}$ $\Delta r_{NH} = \Delta r_{12}$

$R_5^{(m)}$ $\Delta \alpha_{NMC} = \Delta \alpha_{321}$

$R_6^{(m)}$ $\Delta r_{MC} = \Delta r_{23}$

$R_7^{(m)}$ $\Delta r_{CO} = \Delta r_{34}$

$R_8^{(m)}$ $\Delta \gamma_{CO} = (1/\sqrt{2})\Delta(\alpha_{432} - \alpha_{1'34})$

$R_9^{(m)}$ $\Delta \beta_{CO} = (1/\sqrt{6})\Delta(2\alpha_{231'} - \alpha_{1'34} - \alpha_{432})$

$R_{10}^{(m)}$ $\Delta r_{CN} = \Delta r_{31'}$

$R_1'^{(m)}$ $\Delta r'_{NH} = \Delta r_{1'5'}$

$R_2'^{(m)}$ $\Delta r'_{NH} = (1/\sqrt{2})\Delta(\alpha_{31'5} - \alpha_{5'1'2'})$

$R_3'^{(m)}$ $\Delta \beta'_{NH} = (1/\sqrt{6})\Delta(2\alpha_{2'1'3} - \alpha_{31'5'} - \alpha_{5'1'2'})$

$R_4'^{(m)}$ $\Delta r'_{NM} = \Delta r_{1'2'}$

$R_5'^{(m)}$ $\Delta \alpha_{NMC} = \Delta \alpha_{1'2'3'}$

$R_6'^{(m)}$ $\Delta r'_{MC} = \Delta r_{2'3'}$

$R_7'^{(m)}$ $\Delta r'_{CO} = \Delta r_{3'4'}$

$R_8'^{(m)}$ $\Delta \gamma'_{CO} = (1/\sqrt{2})\Delta(\alpha_{2'3'4'} - \alpha_{4'3'1})$

$R_9'^{(m)}$ $\Delta\beta'_{CO} = (1/\sqrt{6})\Delta(2\alpha_{13'2'} - \alpha_{2'3'4'} - \alpha_{4'3'1})$

$R_{10}'^{(m)}$ $\Delta r'_{CN} = \Delta r_{3'1}$

where r_{ij} represented the bond length between atom i and atom j, α_{ijk} represented the bond angle formed by atom i, atom j, and atom k.

The G matrix was constructed with these internal co-ordinate vectors. And it is expressed in the form shown in Fig. 2.

Potential energy ; The equation of Urey-Bradly[8] potential energy was as follows,

$$V = \sum_{m,i,j} K'_{ij} r_{ij}^{(m)}[\Delta r_{ij}^{(m)}] + (1/2)K_{ij}[\Delta r_{ij}^{(m)}]^2$$

$$+ \sum_{m,i,j,k} H'_{ijk} r_{ij}^{(m)} r_{jk}^{(m)}[\Delta\alpha_{ijk}^{(m)}] + (1/2)H_{ijk} r_{ij}^{(m)} r_{jk}^{(m)}[\Delta\alpha_{ijk}^{(m)}]^2$$

$$+ \sum_{m,i,j,k} F'_{ik} q_{ik}^{(m)}[\Delta q_{ik}^{(m)}] + (1/2)F_{ik}[\Delta q_{jk}^{(m)}]^2$$

where m represented the index for chain units, $r_{ij}^{(m)}$, $r_{jk}^{(m)}$ and $q_{ik}^{(m)}$ represented the equilibrium value of the interatomic distance. The summation was taken for all i and k combinations which corresponded to the index i and k in α_{ijk} for the term containing F'_{ik} and F_{ik}.

Reduction of secular equation ; Polyglycine I has a plane of symmetry as a two-fold screw axis. The vibrations which are active optically are classified into four species, A_1, B_1, A_2, and B_2. The A_1 and B_1 vibrations are symmetric to molecular plane, the A_2 and B_2 vibrations are antisymmetric to it. The G and F matrices for A_1 and B_1 were constructed by the symmetry co-ordinates.

$$S_i = (1/\sqrt{N})\sum_m (R_i^{(m)} + R'^{(m)}_i)$$ for A_1 species

$$S_i' = (1/\sqrt{N})\sum_m (R_i^{(m)} - R'^{(m)}_i)$$ for B_1 species

where N was the total number of chain units.

The secular equations[10] (10th order) for the A_1 and B_1 vibrations

$$|G_{A_1}F_{A_1} - \lambda E| = 0$$
$$|G_{B_1}F_{B_1} - \lambda E| = 0$$

were solved by an large electronic computrer.

The force constants and the molecular parameters used in this calculation are listed in Table I.

Then, polyglycine I is in antiparallel chain pleated sheet structure in the crystalline state. Each of the internal vibrations splitted

	...	$R'^{(m-1)}$	$R^{(m-1)}$	$R'^{(m)}$	$R^{(m)}$	$R'^{(m+1)}$	$R^{(m+1)}$	...
$R'^{(m-1)}$	$\tilde{B}$	A	B					
$R^{(m-1)}$		$\tilde{B}$	A	B				
$R'^{(m)}$			$\tilde{B}$	A	B			
$R^{(m)}$				$\tilde{B}$	A	B		
$R'^{(m+1)}$					$\tilde{B}$	A	B	
$R^{(m+1)}$						$\tilde{B}$	A	B

Fig. 2. Form of G, F matrix of polyglycine I.

Table I. Force constants (mdyn./Å) and molecular parameters

$K(N{-}H)$	5. 6	$H(H{-}N{-}C)$	0. 35	$F(H. N. C)$	0. 50
$K(N{-}C)$	5. 5	$H(C{-}N{-}M)$	0. 30	$F(C. N. M)$	0. 30
$K(N{-}M)$	3. 4	$H(H{-}N{-}M)$	0. 15	$F(H. N. M)$	0. 50
$K(M{-}C)$	3. 1	$H(N{-}M{-}C)$	0. 20	$F(O. C. M)$	0. 50
$K(C{=}O)$	8. 5	$H(O{=}C{-}M)$	0. 30	$F(M. C. N)$	0. 50
		$H(M{-}C{-}N)$	0. 30	$F(O. C. N)$	1. 50
		$H(O{=}C{-}N)$	0. 35	α_{HNC}	120°
		r_{NH}	1. 00 Å	α_{CNM}	120°
		r_{NH}	1. 47 Å	α_{MNH}	120°
		r_{MC}	1. 54 Å	α_{NMC}	120°
		r_{CO}	1. 21 Å	α_{OCM}	120°
		r_{CN}	1. 32 Å	α_{MCN}	120°
				α_{NCO}	120°

into two normal vibrations as follows[11,12,13,14,15],

Molecule	Crystal	Selection Rule in IR	Molecule	Crystal	Selection Rule in IR
A_1	$(0, 0)$	inactive	A_2	$(0, 0)$	inactive
	$(0, \pi)$	active		$(0, \pi)$	active
B_1	$(\pi, 0)$	active	B_2	$(\pi, 0)$	active
	(π, π)	active		(π, π)	active

Then, except for the amide I, II, and III vibrations, the frequency difference of each pair of splitted bands may be very small in the case of in-plane vibrations.

Amide vibrations ; Among the vibrations characteristic of polypeptides, four vibrations, amide I, II, III, IV, were treated as the in-plane vibrations.

Skeletal streching vibrations ; There are four skeletal streching vibrations, two of A_1 species and two of B_1 species.

Skeletal deformation vibrations ; There are four skeletal deformation vibrations, two of A_1 species and two of B_1 species.

The absorption bands of the KBr pellet and its-self pellet of glycine or polyglycine I in the spectral region of 4000 cm^{-1} to 50 cm^{-1} are listed in Table II and Table III respec-

tively.

Five spectrophotometers, Perkin-Elmer 121 (NaCl, KBr prisms), Perkin-Elmer 521 (grating), Hitachi EG-1 (grating) for infrared range, Perkin-Elmer 201 C (450 cm^{-1} to 290 cm^{-1}, 280 cm^{-1} to 185 cm^{-1}, 190 cm^{-1} to 120 cm^{-1}, 140 cm^{-1} to 110 cm^{-1}, 120 cm^{-1} to 95 cm^{-1}, 100 cm^{-1} to 65 cm^{-1}) and Hitachi FIS-1 for farinfrared range were used to determine many absorption bands.   For various calculations the operated large electronic computers were placed at Osaka University, The University of Tokyo, and Tohoku University.

The absorption bands of the film of poly-glycine I which was prepared with trifluo-roacetic acid were determined under dried and wetted conditions using the special cell.

The special cell was invented by the author was very convenient to other objects. The dried state was kept by concentrated sulphuric acid. The wetted state was kept by saturated sodium perchloride solution of water or heavy water.

The calculated frequencies and energy distribution for molecular vibration modes are listed in Table III.

Table II. Vibrational Frequencies of Glycine in the Spectral Region of
4000 cm^{-1} to 50 cm^{-1}. (cm^{-1})

KBr pellet (NaCl prism range in infrared range)	KBr pellet (KBr prism range in infrared range)	Its-self pellet (Farinfrared range)
3170 s	912 s	361
2890 s	894 s	283
2618 s	836 s	245 sh
2381 sh	694 s	192 ww
2128 m	666 sh	139
1595 s	604 s	110 ww
1520 s	503 s	84
1451 s		73
1420 s		
1340 s		
1115 m		
1033 m		
896 s		

Table III. Vibrational Frequencies and Assigments of Polyglycine I in the
Spectral Region of 4000 cm^{-1} to 50 cm^{-1}. (cm^{-1})

KBr pellet (NaCl prism range in infrared range)	Its self film (Grating, in infrared range)			νcalc.	Assignment Energy distribution
	H_2SO_4 (Dried)	H_2O (Wetted)	D_2O (Wetted)		
	3450 sh	3450 sh	3450 sh		
3333 s				3387 B_1, B_2	$\varDelta NH$
				3340 A_1, B_1	
	3290 s	3290 s	3290 s		
3095 s	3070 sh	3070 sh	3055 sh		
2950 s					
		*2480 s			
				1687 B_1	$\varDelta CO(75)$

				1682	$\Delta CO(75)$
				1652 A_1	
1645 s	1627 s	1625 s	1620 s	1644 B_2	$\Delta CO(72)$
				1647 B_1	
				1547 B_1	$\Phi NH(73)$, $\Delta NC(30)$
				1542	$\Phi NH(71)$, $\Delta NC(33)$
				1539 B_2	$\Phi NH(65)$, $\Delta NC(37)$
				1518 A_1	
1524 s	1518 s	1515 s	1520 s	1512 B_1	
			*1470 m		
1439 s	1430 s	1430 s	1430 s		
1381 s					
1344 m					
				1308 B_1	$\Delta NC(44)$, $\Delta CM(22)$
				1305 A_1	
				1299	$\Delta NC(41)$, $\Delta CM(20)$
				1295 B_1	
				1292 B_2	$\Delta NC(36)$, $\Delta CM(22)$
			1265 w		
	1235 sh	1235 m	*1240 m		
1222 s					
	1202 m				
	1200 m	1205 m	1205 m		
1166 sh					
1111 sh	1135 m	1135 m	1135 m		
				1101 A_1	
	1055 w	1055 w	1055 w	1076 B_1	$\Delta NM(70)$, $\Delta CM(23)$
1029 sh					
1018 s	1018 m	1018 m	1018 m		
1000 sh				1001 B_1	
	990 w	990 w	993 w	991 A_1, B_1	$\Delta CM(22)$
				979 B_2	$\Delta NM(50)$, $\Delta NC(12)$
				977	$\Delta NM(49)$, $\Delta NC(13)$
			*950 sh		
				920	$\Delta CM(50)$
				915 B_2	$\pi CM(47)$
				874 B_1	
815 sh	835 w	835 w	835 w		
	800 w	800 w	800 w		
				770 B_1	$\pi NH(94)$

				756 B_2	$\pi NH(91)$
				756	$\Phi lN\cdots H\cdots O(91)$
				741 B_1	
	713 s	713 s	713 s		
705 s					
666 sh					
(KBr prism range in infrared range)					
977 s					
761 sh					
				723	$\Delta CM(21)$, $\Phi CO(23)$
				721 B_1	$\pi CM(23)$, $\Phi CO(22)$
704 sh					
				655 B_2	$\pi CO(85)$
				654	$\pi CO(85)$
				645 B_1	$\pi CO(50)$
625 sh	630 w	630 w	630 m		
	605 s	603 s	603 s		
591 s	590 s	590 s	590 s	591 B_1	$\Phi CO(63)$
				589 A_1	
				541 B_1	
500 s					
Its self pellet (Farinfrared range)	Its self pellet (Farinfrared range)				
	488 ww				
360	363			352 B_2	$tNC(51)$
				351	$tNC(51)$
	282			278 B_2, B_1	$\angle MNC(35)$, $\Phi CO(35)$
				276	$\angle MNC(35)$, $\Phi CO(35)$
				262 B_1	$\angle MNC(52)$
223	219			223 A_1	
				213 B_1	$tNC(24)$, $\Phi lN\cdots H\cdots O(16)$
				176 A_1	
144				143	$\Delta H\cdots O(104)$
133	137 sh			138 B_1	$\angle CMN(32)$, $tCN(28)$
118	112 sh				
				109	$tCM(39)$, $\pi NH(27)$

87 sh

79 sh

69

61	B_1	$\Phi lN\cdots H\cdots O(51),$
		$t\bar{N}C(39)$
27	B_2	$\Phi lN\cdots H\cdots O(55),$
		$t\bar{N}C(19)$

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

νcalc. : calculated frequency (cm^{-1})

Summary

Polyglycine was synthesized from N-carboxyanhydride of glycine which was the second starting material. And the first starting material was glycine. The synthesis method was improved by the author partially. And the synthesized polyglycine was concluded by polyglycine I which was β-chain polypeptides in β-conformation, the all-trans zigzag conformation.

The order of polymerization reaction was an approximate second order.

These assignments to absorption bands were carried out in comparison with the calculated frequencies which were calculated by Wilsons GF matrix and Urey-Bradly potential energy equation fundamentally. And the energy distribution was shown at the same time.

The various vibrational frequencies observed in absorption spectra were attempted to assign as possible. Especially, the assigns of some new absorption bands were attempted to decide against some corresponding vibration modes.

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〔**内容抄録**〕 アミノ酸重合物の物理化学的性質　ポリグリシンI

堀津圭佑

　第2出発物質であるグリシンの N-カルボキシ無水物から ポリグリシンを合成した。第1出発物質はグリシンであった。この合成法は著者により部分的に改良され，また合成したポリグリシンは β-空間配位の β-鎖ポリペプチドで全トランスジクザク空間配位のポリグリシンI と決定された。合成反応の次数は大体2次であった。ウイルソン GF 行列式とユーレイーブラドレイのポテンシャルエナージー式を基本的に使用して，計算された振動数と比較して測定された吸収帯の帰属をした。同時にエナージー分布も示した。

　吸収スペクトルにおいて測定された各種の振動数は可能の限り帰属を試みた。特にいくつかの新しい吸収帯の帰属はいくつかのそれらに相当する振動様式に対して決定するために 試みられた。