Introduction of Side Chains into Polyglycine Dispersed on Solid Surface. I

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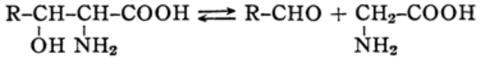
In 1953, Snell[1] reported a reversible catalytic cleavage of β-hydroxy amino acids in the presence of pyridoxal and certain metal ions in aqueous solutions of pH 3–12. Th. Wieland[2] observed recently, the conversion of glycyl-residue into seryl- or threonyl residue by Snell's method.



$$\underset{\displaystyle OH}{\text{R-CH}}\text{-}\underset{\displaystyle NH_2}{\text{CH}}\text{-COOH} \rightleftarrows \text{R-CHO} + \underset{\displaystyle NH_2}{\text{CH}_2}\text{-COOH}$$

In 1954, one of the authors (S. Akabori) postulated[3] a hypothesis that the "foreproteine" should have been produced by the following reactions in three steps.

1. Formation of amino-acetonitrile from formaldehyde, ammonia and hydrogen cyanide.

$$CH_2O + NH_3 + HCN \longrightarrow H_2N\text{-}CH_2\text{-}CN + H_2O$$

2. Polymerization of amino-acetonitrile on a solid surface, followed by the hydrolysis to polyglycine and ammonia.

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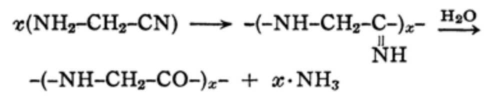
[1] E. Snell, D. Metzler and J. Longenecker, *J. Am. Chem. Soc.*, **75**, 2786 (1953).

[2] Th. Wieland and K. Dose, *Angew. Chem.*, **66**, 781 (1954).

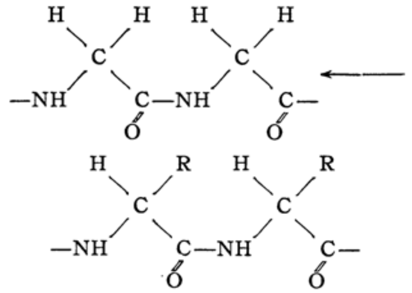
[3] S. Akabori, *Science* (Japan), Vol. **25**, 54 (1955).



$$x(NH_2\text{-}CH_2\text{-}CN) \longrightarrow \text{-(-NH-}CH_2\text{-}\underset{\overset{\|}{NH}}{C}\text{-)}_x\text{-} \xrightarrow{H_2O} \text{-(-NH-}CH_2\text{-CO-)}_x\text{-} + x\cdot NH_3$$

3. Introduction of side chains into polyglycine.

Akabori pointed out, further, that the asymmetric formation of amino acid residues could be explained, at least in one single peptide chain, by his hypothesis, assuming that the configuration of polyglycine absorbed on the solid surface is *cis*-form, as shown in the following scheme.



Experimental studies on the first and second reactions are now in progress in the author's laboratory. The present work deals with studies in regard to the reaction of the last step.

By the treatment of polyglycine spread on kaolinite with formaldehyde or acetaldehyde in the presence of a basic catalyst, glycyl residues are partly converted to seryl- or threonyl residues. By treating powdered polyglycine without solid bearer, with formaldehyde or acetaldehyde in the same way, practically no introduction of side chains was observed.

Experimental

Outline of Experiments.—Polyglycine was prepared, on one hand, through glycine N-carboxylic anhydride (G. C. A.) according to Leuchs[4] and on the other hand, by the polycondensation of glycine ester according to Kurtius[5]. Bulk preparations of polyglycine were obtained from G. C. A. by keeping its solution in dioxane, with a small quantity of pyridine, at room temperature for several days.

Materials.—Glycine N-Carboxylic Anhydride.[6]—Five grams of carbobenzyloxy glycine and 2.3 ml. of thionylchloride were dissolved in 5 ml. of acetic anhydride and heated to boiling for five minutes. On cooling the solution, fine crystals of G. C. A. were deposited. Yield 1.9 g.

Polyglycine in Bulk (without Bearer).—Two grams of G. C. A. were dissolved in 20 ml. of dry dioxane, 4 ml. of dry pyridine was added to it and the solution was kept in a sealed tube at room temperature. After four to five days, a mixture of ether and petroleumether was added to the reaction mixture, polyglycine precipitated in amorphous state was filtered, washed with ether and dried. The yield was 0.8 g. Polyglycine thus obtained was soluble in water with great difficulty, and gave positive biulet reaction.

Polyglycine Dispersed on Kaolinite.—a) From G. C. A.—Two grams of G. C. A. were dissolved, in 20 ml. of dry dioxane with 4 ml. of dry pyridine 15 g. of kaolinite were added to it and the whole mixture was placed in a sealed tube and allowed to stand at room temperature for eighteen hours under occasional shaking. The gelatinized reaction mixture was finally heated to 70°C for two hours. After cooling, the mixture was taken out from the tube and washed with ether, then with water. The dried polyglycine kaolinite weighed 16.5 g.

b) From Glycine Ester.—Glycine ethyl ester dissolved in ether was mixed with kaolinite, ether was distilled off and the remaining mixture was heated to 130°C for five hours. The product was washed with ether, then three times with hot water, and dried.

Several preparation of polyglycine kaolinite prepared in almost the same ways were analysed for their N contents. The results are shown in Table I.

TABLE I

Sample	Material Glycine ester	Kaolinite	Reaction Temp.	 Time	N Contents %
P. G. K. 1	5.9 g.	20.0 g.	130°C	5.0 hrs.	1.58
P. G. K. 2	4.9	15.0	130	5.0	1.26
P. G. K. 3	4.9	15.0	130	5.9	1.38
P. G. K. 4	4.9	15.0	130	5.9	1.34

Degree of Polymerization of Polyglycine Kaolinite.—One hundred and fifty mg. of polyglycine kaolinite (Sample P. G. K. 3) were suspended in 10 ml. of 4% aq. sodium bicarbonate solution mixed with 0.3 ml. of dinitrofluorobenzene dissolved in 10 ml. of ethyl alcohol and shaken for seven hours at room temperature. After the alcohol was distilled off, 10 ml. of 12 N hydrochloric acid were added and boiled for three hours. Dinitrophenyl-glycine formed was extracted with ether, purified by a silica gel column and determined by a photoelectric colorimeter. Terminal-N % 0.0098. Degree of polymerization; Total.N %/Term.-N % =140.

A determination of terminal. N% by van Slyke's method gave 0.008%, Total-N %/Term.-N % =172.

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4) H. Leuchs, *Ber.*, **39**, 857 (1906).

5) T. Kurtius, *Ber.*, **16**, 755 (1833).

6) A. Farthing, *J. Chem. Soc.*, **1950**, 3213.

Condensation of Formaldehyde or Acetaldehyde with Polyglycine Kaolinite.—Powdered polyglycine kaolinite was mixed with aq. formaldehyde or acetaldehyde and small quantity of various basic catalysts and kept at 60°C–100°C. After various reaction times, the solid matter was filtered, washed with alcohol and dried. An aliquot of the reaction products was boiled with 6 N hydrochloric acid for five hours. Kaolinite was filtered and filtrate was concentrated in vacuo. A small quantity of alcohol was added to the residue and evaporated again to removed hydrochloric acid. The remaining solution was diluted with water, neutralized to pH 7 and brought to 10 or 20 ml. A measured volume of the solution was pipetted out, dinitrophenylated by the usual method and the resulted DNP-amino acids were separated by a silica gel chromatography as shown in Fig. 1–2. The yellow bands corresponding to DNP-glycine and DNP-serine or DNP-threonine were cut out, eluted by methyl alcohol and determined by a photoelectric colorimeter. The results of experiments under various conditions are listed in Table II.

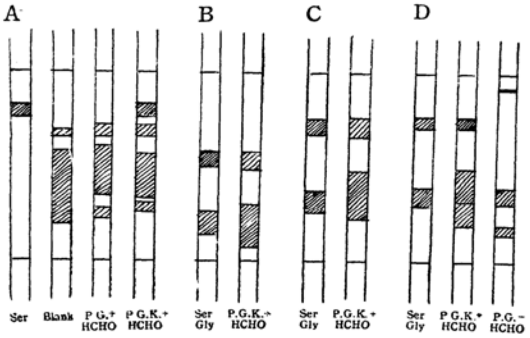

Fig. 1. D.N.P.-amino acids' column chromatograph of P.G.K.+HCHO.

Column.		Solvent
A.	Silica Gel-W.	AcOH−6.5 Phosph. Etp. Buff.
B.	Silica Gel-I.	20 ml. BuOH+80 ml. $CHCl_3$ −20% AcOH.
C.	Silica Gel-10	20 ml. BuOH+80 ml. $CHCl_3$ −20% AcOH.
D.	Celite	50 ml BuOH+100 ml. $CHCl_3$ −pH. 4.6 Acetate Buff.

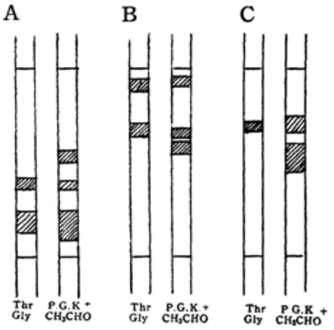

Fig. 2. D.N.P.-amino acids' column chromatograph of P.G.K.+CH_3CHO.

Column		Solvent
A.	Silica Gel-I.	20 ml. BuOH+80 ml. $CHCl_3$ −20% AcOH.
B.	Silica Gel-I.	5 ml. sec. BuOH+95 ml. $CHCl_3$ −2% AcOH.
C.	Silica Gel-X.	50 ml. BuOH+100 ml. $CHCl_3$ −pH. 4.6 Acet. Buff.

Discussion

From the results described above, it could be concluded that the methylene group of polyglycine adsorbed on kaolinite is quite susceptible as compared with polyglycine without bearer, to the condensation reaction with aldehyde giving rise to β-hydroxy amino acids residues. It is also clear from the results of end group determination of the polyglycine and molar ratio of serine/glycine in the reaction products, that the condensation occurred not only at amino terminal glycyl residues but also at methylene groups of non-terminal glycyl residues.

Summary

1) Polyglycine dispersed on the surface of kaolinite was prepared by the polycondensation of N-carboxylic anhydride or glycine ester on kaolinite.

2) It was concluded that a part of glycyl residues in polyglycine dispersed on kaolinite

TABLE II

Sample	Weight	Catalyst	Aldehyde	Solutions	Reaction Temp.	Reaction Time	Mol. ratio of Oxy amino acids/Gly.
PGK*	1.0 g.	K_2CO_3	HCHO	10 ml.	80°C	7 hrs.	0.031
PGK*	1.0	$NaHCO_3$	HCHO	10	80	7	0.030
PGK*	1.0	T. E. A.	HCHO	10	100	2.5	0.004
PGK3	1.0	K_2CO_3	HCHO	10	80	7	0.02–0.03
PGK*	0.5	K_2CO_3	CH_3CHO	10	60	50	0.015
PGK*	0.5	$NaHCO_3$	CH_3CHO	10	60	50	0.014
PG	25 mg.	T. E. A.	HCHO	3	100	3	—
PG	100 mg.	K_2CO_3	HCHO	10	80	7	+

PGK*, Polyglycine kaolinite which was prepared by N carboxylic anhydride method.

PG, Polyglycine without kaolinite. T. E. A., Triethylamine.

HCHO, 35% aq. solution. CH_3CHO, 20% aq. solution.

was easily converted to seryl or threonyl residues by the condensation with formaldehyde or acetaldehyde.

3) The condensation occurred not only at amino terminal glycyl residues but also at non-terminal glycyl residues.

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