Studies on Dehydroalanine Derivatives. III. Formation of Amino Acids from Poly-dehydroalanine

By Shumpei SAKAKIBARA

(Received July 13, 1960)

In the previous papers of this series[1,2], the synthesis of *N*-carboxy-dehydroalanine anhydride and of poly-dehydroalanine was described. However, the synthesis of analytically pure poly-dehydroalanine was unsuccessful, and it was found that the polymer contained only about 10% dehydroalanyl residues in the molecule[2]. This suggested that the dehydroalanyl groups in the polypeptide chain are very reactive. The present investigation is on the reactivity of dehydroalanyl residues in polypeptide chains. Poly-dehydroalanine was used as test material, and the reagents used for the reaction were potassium carbonate, potassium cyanide, benzyl mercaptane and acetonitrile. The amino acids derived from the poly-dehydroalanine were examined after hydrolysis of the reaction products.

Experimental

Meterial.—Poly-dehydroalanine was prepared from *N*-carboxy-dehydroalanine anhydride[1]. It was polymerized in pyridine as described in the previous paper[2]. The freshly prepared polymer was used in these experiments.

Reaction Conditions and Detection of Amino Acids Formed.—The reaction mixtures shown in Table I were hydrolyzed for about 15 hr. at 105°C after addition of concentrated hydrochloric acid (an equal volume to that of the reaction mixture), and then concentrated to dryness. These concentrates were analyzed by Dowex 50 column chromatography using the conditions of Moore and Stein[3] (Fig. 1) and/or by two dimensional paper chromatrgraphy after dinitrophenylation under the improved conditions of Levy[4] (Fig. 2). Amino acids formed were analyzed quantitatively by Levy's method (Table II). The spot corresponding to DNP-aspartic or glutamic acid in Fig. 2 was rechromatographed on paper by descending method using 2.5 M phosphate buffer as solvent, as shown Fig. 4. Since the spot corresponding to DNP-aspartic acid from reaction mixture 3 contained a little DNP-glutamic acid, these two compounds were separated on a buffered celite column using 0.2 M phosphate-citric acid as buffer and chloroform-ether (90 : 10) as developer[5] (Fig. 3). Then, the bands corresponding to DNP-aspartic acid (A) and DNP-glutamic acid (B) were reconfirmed and repurified by paper

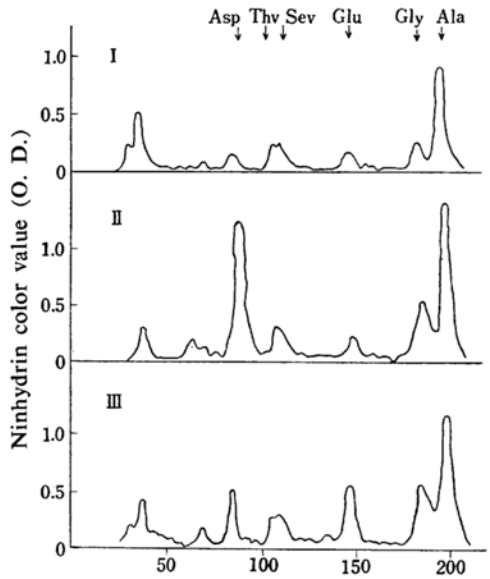


Fig. 1. Chromatographic analysis of ninhydrin-positive materials in the HCl-hydrolyzates (per 10 mg. of poly-dehydroalanine): I. Reaction mixture 1; II. Reaction mixture 3; III. Reaction mixture 5 (cf. Table I). Column: Dowex 50-X8, 100×0.9 cm.
Arrows indicate the position of the peaks of standard amino acids.

1) S. Sakakibara, This Bulletin, **32**, 13 (1959).
2) S. Sakakibara, ibid., **33**, 814 (1960).
3) S. Moore and W. H. Stein, *J. Biol. Chem.*, **192**, 663 (1951).
4) A. L. Levy, *Nature*, **174**, 126 (1954); G. Koch and W. Weidel, *Z. physiol. Chem.*, **303**, 213 (1956).
5) A. Courts, *Biochem. J.*, **58**, 70 (1954).

TABLE I. REACTIONS OF POLY-DEHYDROALANINE WITH VARIOUS REAGENTS

Reaction No.	Poly-dehydroalanine* apparent wt. (calcd. wt.) mg.	Reagent g.	Water ml.	Reaction conditions
1	5 (4.10)	K_2CO_3 0.04	2	A**
2	10 (8.20)	K_2CO_3 0.08	4	B†
3	65 (53.4)	KCN 0.4	20	A**
4	15 (12.3)	K_2CO_3 0.12 Benzyl mercaptane 0.1	6	C††
5	10 (8.20)	K_2CO_3 0.08 CH_3CN 1	4	B†

* Soluble poly-dehydroalanine contained 17.9% of volatile components. Since completely dry polymer was partially insoluble[2], the actual weight was calculated from the apparent weight.
** Kept at room temp. for about 15 hr. and then heated at 80°C for 1 hr. in a sealed tube.
† Irradiated with an UV-lamp (Mazda 15 W sterilizing lamp at the irradiation distance of 10 cm.) for 24 hr. at room temp. in a petridish (dia. 5 cm.) covered with cellophane (0.05 mm. thick).
†† Shaken at room temp. for about 15 hr. in a sealed tube.

TABLE II. QUANTITATIVE ESTIMATION OF DNP-AMINO ACIDS ANALYZED BY LEVY'S CHROMATOGRAPHICAL METHOD (FIG. 2) : μM/10 mg. OF DRIED POLY-DEHYDROALANINE

Spot No. in Fig. 2		1	2	3	4	5	6	7	8	9	10	11		12	13
Corresponding amino acid			Ala	Gly	Thr	Ser						Asp	Glu		*S*-Bz-CyS*
No. of reaction mixt.**	1	2.24	1.81	0.18	0.05	0.13	0.05	0.07	0.10	0.07	0.14	0.29	0.42	0.25	—
	2	2.78	1.74	0.78	0.18	0.20	0.06	0.06	0.23	0.09	0.17	0.50	0.90	0.19	—
	3	0.88	2.15	0.24	0.03	0.11	—	0.03	0.17	0.07	0.16	2.11	0.44	0.27	—
	4	1.01	1.27	0.19	0.05	0.25	—	0.12	0.16	0.12	0.13	0.14	0.19	0.09	0.96
	5	2.47	1.89	1.93	0.15	0.29	0.06	0.04	0.23	0.16	0.13	0.74	1.09	0.27	—

* Bz=Benzyl
** Confer Table I

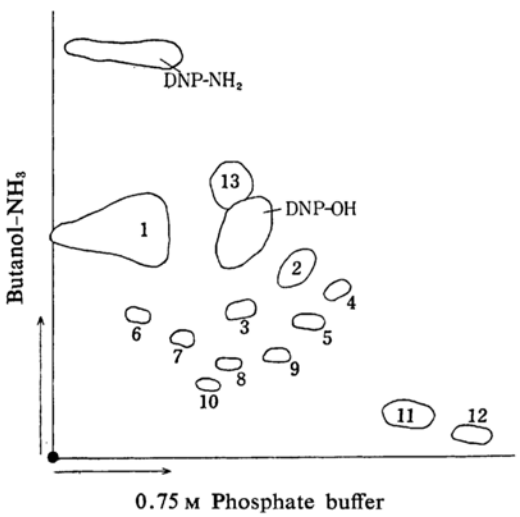


Fig. 2. Standard map of two-dimensional paper chromatogram of DNP-amino acids derived from every reaction mixture.
Details are listed on Table II.

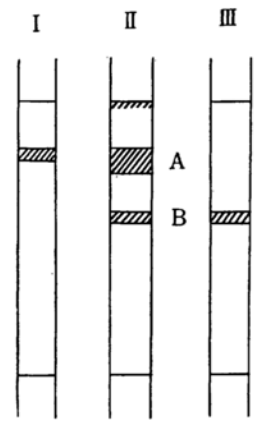


Fig. 3. Chromatographic separation of spot 11 in Fig. 2, reaction mixture 3; I. Standard DNP-aspartic acid; II. Spot 11 in Fig. 2, reaction mixture 3; III. Standard DNP-glutamic acid.
Column: Buffered celite; pH 4.2, 0.2 M phosphate-citric acid buffer.
Solvent: Chloroform-ether (90 : 10).

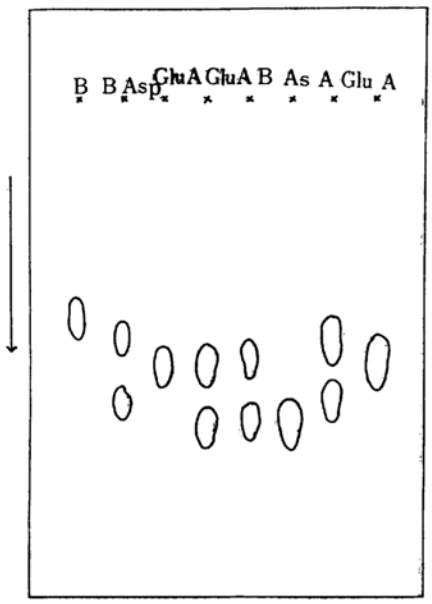


Fig. 4. Paper chromatogram for identification of bands A and B in Fig. 3 with authentic samples.
Solvent: 2.5 M phosphate buffer.
Descending method.

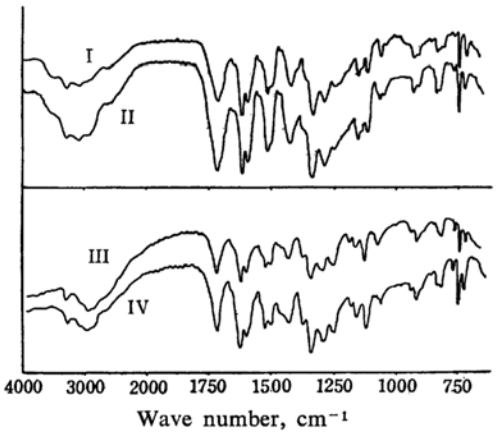


Fig. 5. Infrared absorption spectra of DNP-aspartic acid and DNP-alanine; I. Standard DNP-DL-aspartic acid; II. DNP-aspartic acid derived from the reaction mixture 3; III. Standard DNP-DL-alanine; IV. DNP-alanine derived from the reaction mixture 3.
Recorded on a Shimadzu IR spectrophotometer.
Liquid layer on KRS-5 plate.

chromatography (Fig. 4). The resulting DNP-aspartic acid was reconfirmed by comparison of its infrared absorption spectrum with that of an authentic sample (Fig. 5, I and II). The DNP-alanine formed in reaction mixture 3 was, like DNP-aspartic acid, identified by its infrared absorption spectrum (Fig. 5, III and IV).

In reaction mixture 4, *S*-benzyl cysteine was qualitatively detected by one-dimensional paper

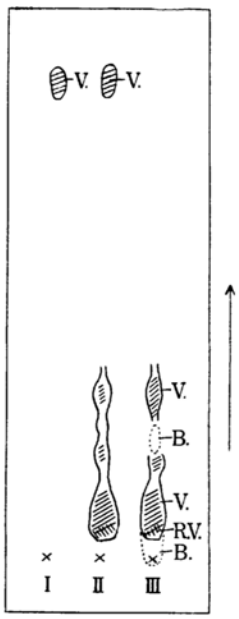
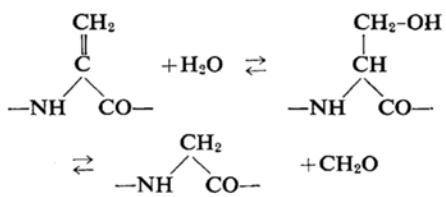


Fig. 6. One dimensional paper chromatogram of reaction mixtures 1 and 4. Identification of *S*-benzyl-cysteine formed in reaction mixture 4. I. Standard *S*-benzyl-cysteine; II. Reaction mixture 4; III. Reaction mixture 1.
V.···violet B.···brown R. V.···reddish violet

chromatography (butanol : acetic acid : water, 4:1:1) (Fig. 6).

Results and Discussion

Before hydrolysis, reaction mixtures 1 and 3 were clear light-brown solutions, 2 and 5 were clear light-yellow solutions, and 4 was a stable white emulsion. The hydrochloric acid hydrolyzates of these reaction mixtures contained small amounts of huminic material as mentioned in the previous paper[2]. As can be seen in the chromatograms of each reaction mixture (Figs. 1 and 2), several unknown bands and spots were observed besides bands and spots corresponding to natural-type amino acids such as alanine, glycine, serine, threonine, glutamic acid and aspartic acid. Formation of glycine and serine in each case could be explained by the following scheme.

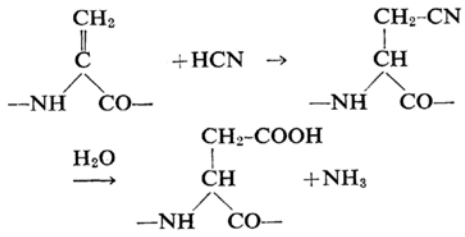


However, formation of amino acids such as alanine, glutamic acid, aspartic acid and threonine could not be explained. Perhaps, compounds such as carbon dioxide, formaldehyde,

ammonia and hydrochloric acid may react in various complex ways with the dehydroalanyl groups. It was very interesting to find spot 1 in the chromatogram shown in Fig. 2. This might correspond to di- or poly-DNP-derivative of some amino acid. Since the structure has not yet been confirmed, some kinds of basic amino acids or polyamino polycarboxylic acids may be derived from dehydroalanine.

Ultraviolet-radiation of dehydroalanyl groups resulted in the liberation of greater amounts of glycine.

As shown in Figs. 1, 2, 3, 4 and 5, and in Table II, formation of considerable amounts of aspartic acid in reaction mixture 3 was definitely confirmed, and this was explained by the following reactions:



In reaction mixture 5, the liberation of greater amounts of glycine, aspartic acid and glutamic acid was observed. However, the reason of their formation is as yet unknown.

S-Benzyl cysteine was clearly detected in reaction mixture 4 (Figs. 2 and 6). It was expected that cystine would have been found in some reaction mixtures containing sodium sulfide and thiosulfate, but this could not be confirmed. Similary, attempts to detect formation of tryptophane and tyrosine residues in the polymer using indol and phenol reagents, were unsuccessful because of the difficulty of detecting small amounts of these amino acids. Nevertheless, it was interesting that many kinds of amino acids were liberated from polydehydroalanine.

A few years ago, Akabori[6,7] proposed a hypothesis on the mechanism of formation of primordial protein. He suggested that initially polyglycine was formed by the polymerization of amino-acetonitrile[8] on the surface of clay. Then, many kinds of side chains were introduced into polyglycine through the reactions of this material with simple substances such as aldehydes or olefines. Subsequently, he confirmed experimentally the formation of serine, threonine[9] and leucine[7] from polyglycine, dispersed on kaolinite, on treatment with formaldehyde, acetoaldehyde and butene-2, respectively. If it is assumed that dehydroalanyl residues are formed from polyglycine during the dehydration reaction with formaldehyde, the formation of residues such as aspartic acid, glutamic acid, alanine and threonine, from polyglycine would be rationally explained. This suggestion is supported by the fact, as shown in the following communication, that *N*-phthaloyl-dehydroalanine can be derived from serine and phthalic anhydride. Furthermore, if experimental confirmation is possible, the formation of amino acids such as cystine, tryptophane and tyrosine could also be explained in a similar way.

Summary

Reaction of poly-dehydroalanine with various reagents such as potassium carbonate, potassium cyanide, benzyl mercaptane and acetonitrile were studied. In every reaction mixture, alanine, glycine, serine, threonine, glutamic acid, aspartic acid and several unknown amino acids were detected. Ultraviolet-radiation resulted in the liberation of greater amounts of glycine. It was observed that considerable amounts of aspartic acid, *S*-benzyl-cysteine and glutamic acid were formed in reaction mixtures of potassium cyanide, benzyl mercaptane and acetonitrile, respectively.

The author wishes to express his thanks to Professor Shiro Akabori and Professor Shunsuke Murahashi for their valuable advice and encouragement.

Institute for Protein Research
Osaka University
Kita-ku, Osaka

6) S. Akabori, *Science (Kagaku)*, **25**, 54 (1955).
7) S. Akabori, Proc. International Symposium on "The Origin of Life on the Earth", Pergamon Press, London (1959), p. 189.
8) H. Hanafusa and S. Akabori, This Bulletin, **32**, 626 (1959).
9) S. Akabori, K. Okawa and M. Sato, ibid., **29**, 608 (1956).