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Introduction of Functional Groups to Polyalanine and Alanine by Contact Glow Discharge Electrolysis

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Summary

The alanine residue of poly-DL-alanine (DP=120) was converted to Asp, Thr, Ser, Glu and Gly residues by means of contact glow discharge electrolysis (CGDE) in a formic and an acetic acid solution. The total conversion of alanine residues to other amino acid residues was almost 20%.

Introduction

In order to clarify the mechanism for the formation of bioorganic compounds on the primordial earth, many investigators have performed experiments on the formation of amino acids from various gases, by the use of electric discharge which could be regarded as a simulation of lightning in the atmosphere on the primitive Earth (MILLER 1953, 1955 and 1957; HEYNS 1957). Contact glow discharge electrolysis (CGDE) is a type of chemical reaction carried out by means of an electric discharge between an aqueous solution containing a substrate and an electrode in contact with the solution (HICKLING 1971). Several applications of CGDE to bioorganic compounds were carried out (HARADA and IWASAKI 1974 and 1975; HARADA and SUZUKI 1977; HARADA and TERASAWA 1980; HARADA et al. 1978 and 1981; KOKUFUTA et al. 1980; SUZUKI et al. 1978; TERASAWA and HARADA 1980). In this paper, derivatization of alanine residues of poly-DL-alanine and also of DL-alanine by CGDE in aqueous solu-

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tions of formic and acetic acids are carried out.

Experimental

Methods

Poly-DL-alanine (18.5 mg) was dissolved in 10 ml of aqueous formic acid (14%) or acetic acid (14%), and the solution was applied to CGDE. The reaction temperature was kept ar 10 - 20 °C by cooling the reaction mixture in a methanol - dry ice bath. The applied electric current was 30 mA at 450 - 600 V, supplied with a power source (Toyo Solid State Power Supply Model 1515) for 0 - 6 hr.

A part of the reaction mixture was dialyzed against distilled water (300 ml x 10 times, 3 days) to eliminate the newly formed low molecular weight polyamino acids. The dialysis membrane used was Seamles Cellulose Tubing Model 18/32 of Visking Company. And the remaining part of the reaction mixture was hydrolyzed and the resulting amino acids were analyzed by a Yanagimoto Model LC-5S Amino Acid Analyzer. Infra-red spectra were measured with a Hitachi 260-50 Infrared Spectrophotometer. The degree of polymerization was determined by titration of the polymer in dimethylformamide (DMF) with N/50 - $HClO_4$/ acetic acid, using 0.2% thymol blue methanol solution as an indicator. A part of the hydrolyzed amino acids were converted to dinitrophenyl (DNP) amino acids by treatment with 2,4-dinitrofluorobenzene. The resulting DNP-amino acids were separated by a celite column which was treated with pH 4 phosphate - citrate buffer. The DNP-amino acids were eluted with chloroform/ether (9/1). The DNP-amino acids were further identified by thin layer chromatography (silica gel, deveroped with n-butanol saturated with water) by comparing the R_f values with those of authentic DNP-amino acids.

DL-Alanine (22.2 mg) was dissoluved in 10 ml of aqueous formic acid (14%) and the solution was applied

to CGDE in the same conditions as those of poly-DL-alanine, except dialysis and hydrolysis.

Materials

Poly-DL-alanine DL-Alanine N-carboxy anhydride (NCA) was prepared from DL-alanine and phosgene by the usual procedure. DL-Alanine (2.5 g, 0.028 mol) was reacted with phosgene in dioxane. After evapolation of the solvent, the residue was dissolved in acetone (10 ml) and treated with a column of active carbon mixed with silver oxide and molecular sieve for removal of halogen. The oily DL-alanine NCA [IR (NaCl, cm^{-1}) ; 1855 (s), 1810 (vs), 1750 (s)] was polymerized in 20 ml of DMF with N-triethylamine (0.28 ml) in dioxane as an initiator at an NCA/initiator ratio of 100/1 for 10 days at room temperature. After the polymerization was over, the reaction mixture was dissolved in 30 ml of water and was dialyzed against water (300 ml x 12 times) for 3 days and then lyophilized ; yield 0.4 g (20% from alanine). IR (KBr, cm^{-1}) ; 1640 (s, Amide I), 1540 (m, Amide II). Degree of polymerization was 120.

Results and Discussion

1) Introduction and convertsion of functional groups to polyalanine.

After the reaction was over, the reaction mixture was dialyzed against distilled water (300 ml x 10 times) for 3 days, and liophilized. The yield and the IR characteristic absorption bands are listed in Table I and Fig 1. The derivatized polyamino acids showed a new absorption band in the range of

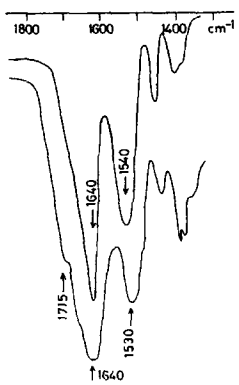


Fig 1. IR spectra of polyalanine and polyamino acid treated by CGDE in 14%-HCOOH for 1 hr, 30 mA, 500 V, 10 - 20 °C.

Table I Reaction yield and IR characteristic absorption bands of polyamino acids by CGDE

Sample	(1)	(2)	(3)
Yield(%)	20	41	39
Amide A	3470-3250	3520-3270	3550-3250
COOH	1715	1715	1710
Amide I	1640	1640	1640
Amide II	1530	1530	1530

Conditions : (1) 14%-HCOOH, (2) 24%-HCOOH, (3) 14%-CH_3COOH, current ; 30 mA, voltage ; 500 V, temperature ; 10 - 20 °C, reaction time ; 1 hr. Polyamino acids were dialyzed against H_2O for 3 days (300 ml x 10 times). IR spectra were measured in KBr disks (cm^{-1}).

1710 - 1715 cm^{-1} which is due to the carboxyl group.

2) Amino acid composition of the polyamino acids.

The polyamino acids shown in Table I were hydrolyzed with 6N - hydrochloric acid for 24 hr at 110 °C in a sealed tube under reduced pressure. After evaporation of hydrochloric acid, the residue was diluted appropriately and analyzed by an amino acid analyzer. The results are summarized in Table II. The amino acid composition of the polyamino acids obtained by CGDE in a formic acid solution was aspartic acid (Asp, 8 - 10%), serine (Ser, 3 - 4%), glutamic acid (Glu, 1 - 2%) and a small amount of threonine (Tre). On the other hand, the polyamino acids obtained by CGDE in an acetic acid solution, contained Glu (4%), Asp (6%) and Ser (5%).

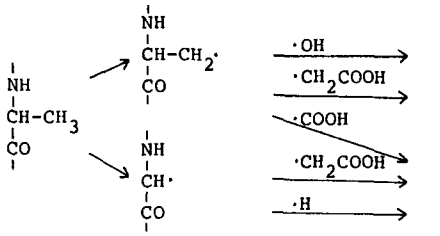
Consequentry, when polyalanine was subjected to CGDE in an organic acid solution, introduction of functional groups, such as CH_2COOH, COOH, OH, $CH(OH)CH_3$, to the side chain of alanine and glycine (Gly) residues took place. The methyl group of the alanine residue could also be removed by CGDE to form a glycine residue. On the other hand, the products of CGDE of alanine under the same conditions are only Asp (8.5%), Ser (2.1%) and Gly (0.7%). In this case, the CGDE reaction did not produce Glu and Thr. The formation of the new major amino acid residues by CGDE could be explained as shown in Scheme 1.

3) The time courses of the newly formed amino acid resi-

Table II Amino acid compositions of polyamino acids by CGDE (%)

Sample	(1)	(2)	(3)	(4)
Asp	8.2	9.5	5.7	8.5
Thr	0.6	0.3	0.8	-
Ser	4.0	3.3	0.8	2.1
Glu	2.1	1.3	4.3	-
Gly	6.9	5.3	5.5	0.7
Ala	77.3	79.6	78.4	88.6

Conditions : See to the footnotes of Table I.
(4) Alanine in 14%-HCOOH.



Scheme 1

dues produced by CGDE.

Fig 2a and Fig 2b show the time courses of amino acids from the reaction mixture of polyalanine and alanine in a formic acid solution by CGDE. The conversion of amino acids reaches their maximum values after 1/2 - 1 hr and then decreases under prolonged reaction time. The decomposition reaction took place simultaneously with the derivatization reaction because of the high energy reactions. Fig 2a and Fig 2b indicate that the derivatization of alanine and polyalanine took place in a similar way, however, the yields of the derivatized amino acid residues by CGDE is higher than that of alanine, and the degradation of amino acid residues of polyalanine took place faster than that of alanine.

Thus it was demonstrated that poly-DL-alanine could be derivatized in formic acid or acetic acid aqueous solution by using CGDE. The new derivatization method is interesting as a polymer chemistry and the result is also interesting from a chemical evolutionary standpoint, because the CGDE could be regarded as a model of a lightning striking on the aqueous surface of the primitive Earth.

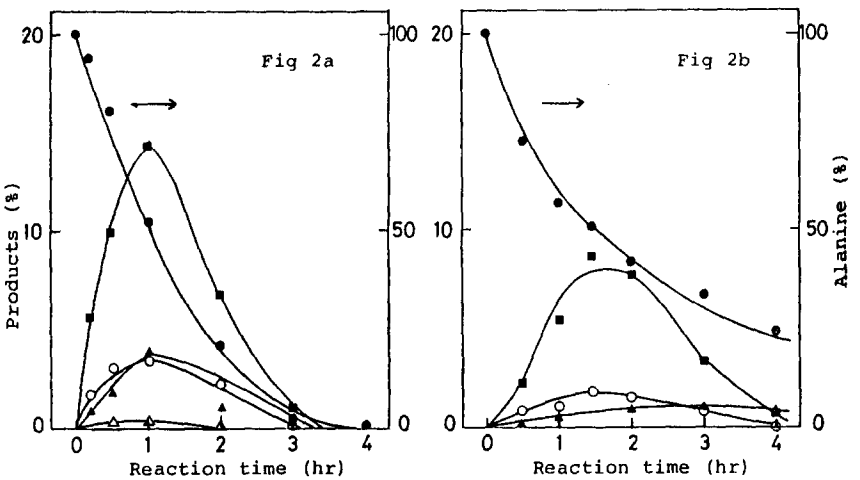


Fig 2a, Fig 2b. The time courses of the products of polyalanine and alanine by CGDE. 2a : Polyalanine in 14%-HCOOH, 2b : Alanine in 14%-HCOOH, 30 mA, 500 V, 10 - 20 °C. ●; Ala, ■; Asp, ○; Ser, ▲; Gly, △; Glu.

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