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OXIDATIVE DEGRADATION OF β- AND γ-AMINO ACIDS BY CONTACT GLOW DISCHARGE ELECTROLYSIS

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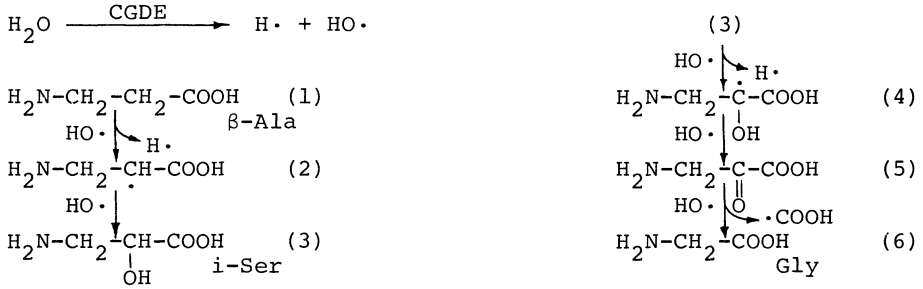
The degradation of β- and γ-amino acids in aqueous solutions by contact glow discharge electrolysis(CGDE) was studied. It was found that the reaction is actually a stepwise oxidative degradation by hydroxyl radical produced by CGDE.

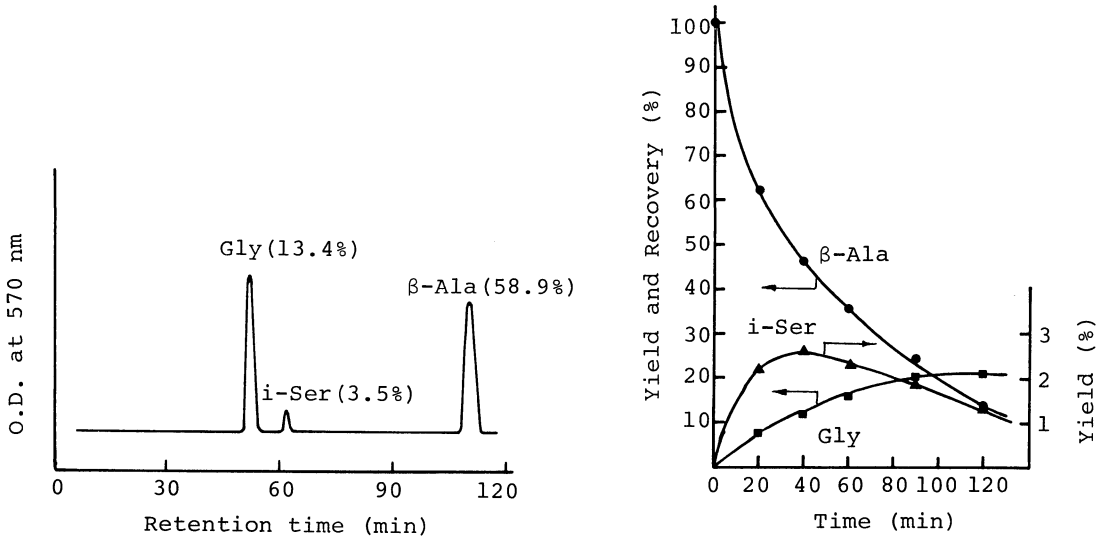
Contact glow discharge electrolysis(CGDE) is a type of chemical reaction carried out by means of an electric discharge between an aqueous solution containing a substrate and an electrode in contact with the solution.[1,2] Several studies on the application of CGDE to bioorganic compounds have been reported.[3,4,5,6,7,8] In the previous paper from this laboratory, the degradation of β-alanine(β-Ala), γ-amino-butyric acid(γ-NH_2-BA) and other β- and γ-amino acids were carried out.[9] From the results obtained, it was concluded that the α-methylene bound to the carboxyl group was apparently eliminated stepwise by CGDE.

In order to study the reaction pathway of the unusual degradation reaction by CGDE, the following speculations have been made to explain the stepwise degradation. In the aqueous solution, the primary product of CGDE would be hydroxyl radical, as in the irradiation of electron to the aqueous solutions.[10] The generated hydroxyl radical would oxidize the substrate as shown in the following scheme:



The α-hydrogen of the substrate(β-Ala)(1) was eliminated by a hydroxyl radical, and the resulting radical(2) reacted with hydroxyl radical to form isoserine(i-Ser)(3). The i-Ser was further oxidized by a hydroxyl radical to form aminopyruvic acid(5). The compound(5) could be easily oxidized radically to form the final product, glycine(Gly)(6). The degradation of β-aminobutyric acid(β-NH_2-BA) and γ-NH_2-BA by CGDE could also be explained in the same way as β-Ala, however, the reaction pathway would be more complex.

In order to confirm the hypothesis on the degradation of β- and γ-amino acids by CGDE described above, the time course of the degradation of β-Ala was studied



A, Amino acid analysis of the reaction mixture (40 min)

B, Time course of oxidation

Fig 1 Oxidation of β-Ala by CGDE

and the intermediates were carefully examined. The peak found after glycine in a diagram of amino acid analysis was confirmed as i-Ser by comparison with the authentic amino acid. The identification of i-Ser in the reaction mixture supported the hypothetical reaction pathway on the degradation of β-Ala by CGDE. From the diagram of the time course of β-Ala degradation, i-Ser could be the primary product of amino acid, and the amount of i-Ser decreased steadily depending on the reaction time. However, the amount of Gly, the final degradation product, increased steadily depending on the reaction time. From the proportion of i-Ser in the reaction mixture, the amino acid could be oxidized more easily than β-Ala by CGDE.

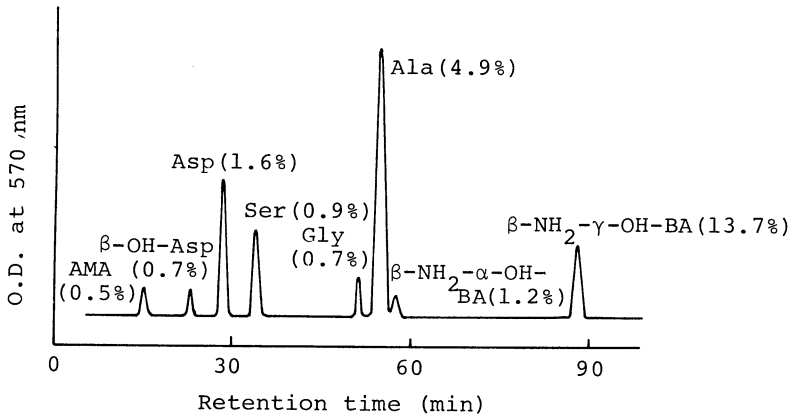


Fig 2 Oxidation of β-NH_2-BA by CGDE (40 min)

Fig 2 shows a diagram of the amino acid analysis of the degradation products of β-NH_2-BA by CGDE. Amino malonic acid(AMA), β-hydroxyaspartic acid(β-OH-Asp),

aspartic acid(Asp), serine(Ser), Gly, alanine(Ala), β-amino-α-hydroxybutyric acid (β-NH_2-α-OH-BA) and β-amino-γ-hydroxybutyric acid(β-NH_2-γ-OH-BA) were confirmed and the formation of these amino acids could be explained as shown in the following equations.

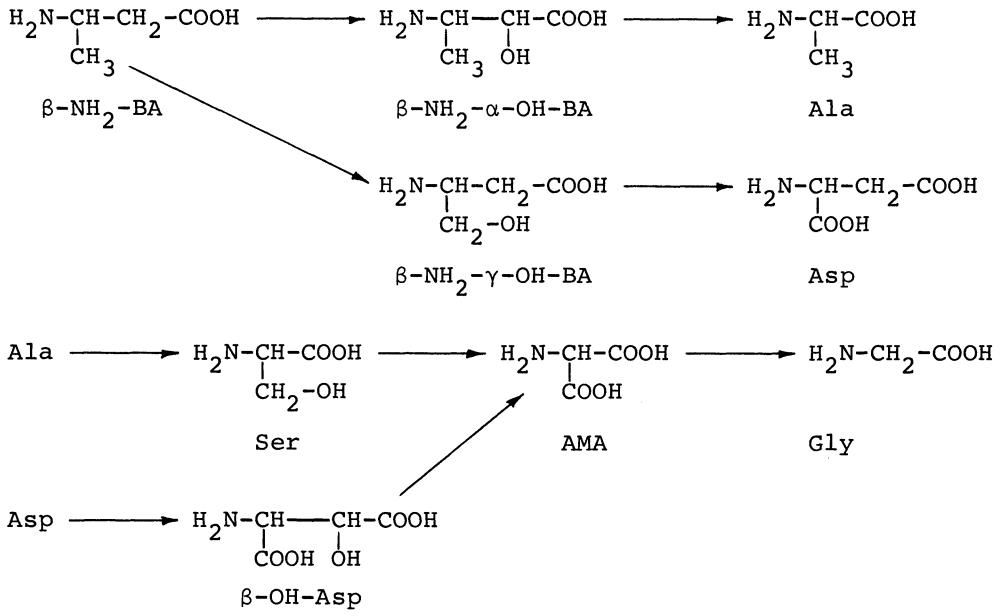


Fig 3 shows a diagram of amino acid analysis of the degradation products of γ-NH_2-BA by CGDE. The degradation products contain Gly, i-Ser, γ-amino-α-hydroxybutyric acid(γ-NH_2-α-OH-BA), γ-amino-β-hydroxybutyric acid(γ-NH_2-β-OH-BA) and β-Ala.

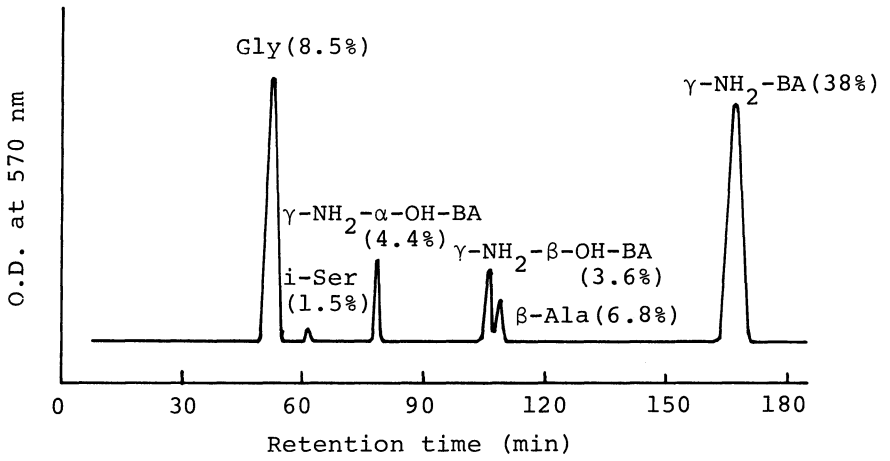
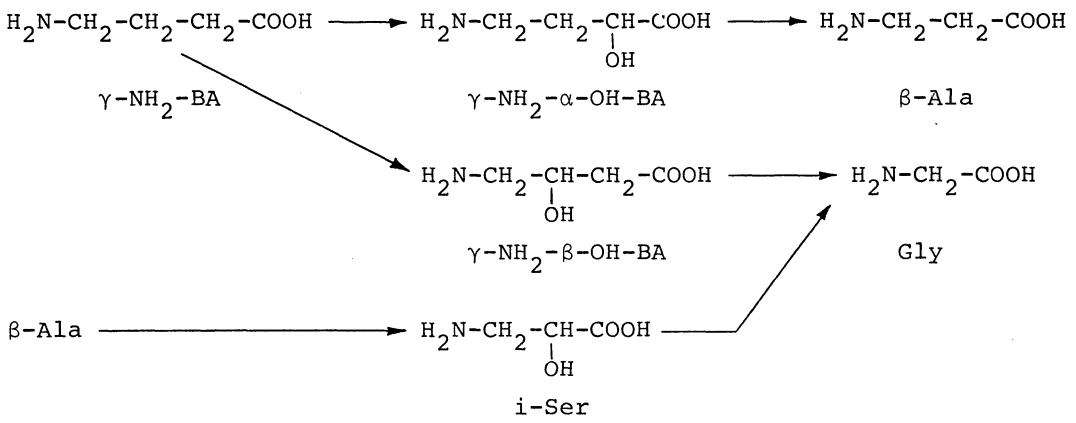


Fig 3 Oxidation of γ-NH_2-BA by CGDE (40 min)

The formation of these amino acids by degradation of γ-NH_2-BA could also be explained as shown in the next page.

The CGDE was applied to the aqueous solution(20 ml) containing 1.0 mmol of amino acids. The applied electric current was usually 50 mA at 600—1500 V. The reaction temperature was kept at 20 °C by cooling the reaction mixture in a methanol-dry ice bath. After the reaction was over, the solution was diluted appropriately for amino acid analysis(amino acid analyzer: Yanagimoro model LC-5S). The reaction mixture

was also treated with 2,4-dinitrofluorobenzene, and the resulting dinitrophenyl(DNP)-amino acids were separated by celite column chromatography,[11] followed by



identification using a thin layer chromatoplate. The major amino acid products were identified by comparing the R_f values with the authentic DNP-amino acids. Several authentic amino acids(i-Ser, β-NH_2-α-OH-BA, β-NH_2-γ-OH-BA, AMA, β-OH-Asp) were synthesized separately in order to compare the amino acids found in the reaction mixture.

The summarized results indicate that the apparently stepwise methylene group elimination of β- and γ-amino acids by CGDE is based on the oxidative degradation by the hydroxyl radical produced by CGDE.[12] The isolated intermediates show that the substrates were oxidized stepwise to form α-hydroxy acid, α-keto acid and then carboxylic acid by elimination of ·COOH radical. Such an oxidation is an interesting type of oxidation reaction without adding any oxidizing agent. The clean and powerful oxidation reaction by CGDE could be applied to various radical reactions in aqueous solutions in the synthesis and the reaction of bioorganic compounds.

References

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