ENZYMIC DEHYDRATION OF L-GLUTAMIC ACID

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It is well known that glutamic acid (GA) is readily converted to its anhydride form, pyroglutamic acid (PGA), by heating in weak acid solution (Wilson and Cannan, 1937). The occurrence of the similar conversion in living organisms has been supposed from sometime before. For instance, Ratner (1944) demonstrated that a dog excreted D-PGA in urine after administration of D-GA, and Simonart and Yu Chow (1953) showed the formation of L-PGA at the expense of L-GA in Asp. oryzae. There are also reports that L-PGA is detected in plant tissues (Ellfolk and Synge, 1955; Fodidi, 1935; Freed and Hibbert, 1955; Rice and Pederson, 1954), but the mechanism of its formation is still unknown.

The present communication presents the occurrence of a new enzyme, called L-glutamic acid dehydrase, in a strain of Pseudomonas, which catalyzes the formation of L-PGA from L-GA.

The strain was isolated from soil, and from the taxonomical study, was identified as a variant of Pseudomonas cruciviae, and named "Pseudomonas cruciviae var. ovalis". This organism can utilize peptone, L-GA, L-PGA, and the members of TCA cycle (except acetate). Especially C_4-dicarboxylic acids are good carbon sources for their growth. On the other hand, such saccharic materials, as glucose, ribose, sucrose etc, are not utilizable. To carry out the experiments, the cells were grown in the following medium: 5 % succinic acid, 0.5 % ammonium sulfate, 0.001 % manganous sulfate, 0.001 % ferrous sulfate, 0.1 % potassium phosphate (monobasic), pH 7.0.

The mode of PGA formation from D-, DL-, or L-GA is shown in fig 1. As is evident from fig 1., the cultured broth has the outstanding PGA formation from L-GA, and the action is optically specific only to the L-forms. The identification of the product as the L-PGA was made comparing with the authentic sample by paper chromatography, melting point and optical rotation. And the product gave L-GA by acid hydrolysis.

This reaction is optimum at pH 8.0 and at the temperature 50°C. The reaction was reversible, and at the equilibrium the reaction mixture contained about 97 % of L-PGA and 3 % of L-GA in molar base. When the cells were boiled or treated with trichloroacetic acid, the activity of L-PGA formation was lost. From these ob-

servations, it is obvious that the reaction is wholly enzymatic, and the enzyme which catalyzes the reaction may well be referred to as L-glutamic acid dehydrase.

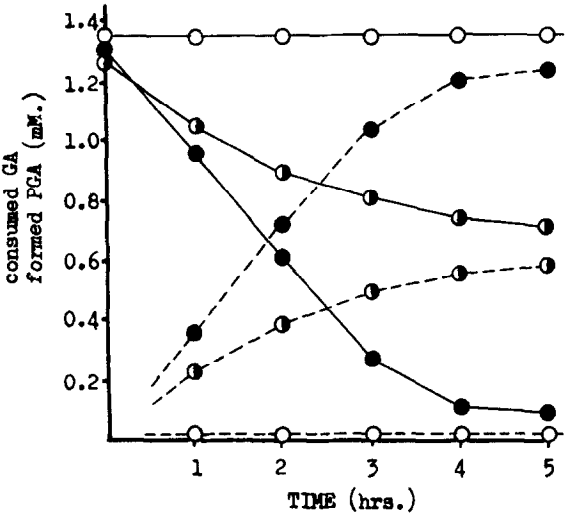

Fig 1. The Mode of PGA formation from GA by the cells of Pseudomonas cruciviae var. ovalis.

Experimental Conditions: As the cell suspension, 1 ml of cultured broth for 24 hrs. is used; GA (D-, DL-, or L-), 1.2-1.35 mM; phosphate, 300 M; total volume, 4 ml; pH 7.8; temperature 28°C

(-○- , D-GA; -◑- , DL-GA; -●- , L-GA; ——— , consumed GA; - - - - - , formed PGA)

The L-glutamic acid dehydrase was prepared from the cells in partially purified form as follows: (i) 8 gr portions of the lyophilized cells are treated with 10 kc. ultrasonic at 0-3°C in 80 ml M/15 phosphate buffer for 30 min. (ii) After centrifugation at 8000 g for 10 min., the supernatant is allowed to stand in 70°C water bath for 30 min. (iii) After centrifugation its supernatant (85 ml) is treated with 45 ml saturated ammonium sulfate solution at 0°C (to give 0.28 saturation). After 20 hrs., the precipitate is removed and discarded. Its supernatant (127 ml) is now brought to 0.62 saturation by addition of ammonium sulfate powder. The resulting precipitate is removed by centrifuge, and lyophilized. By this procedure, a purification of about 15-12 fold per unit amount of protein was achieved. The resulting precipitate showed single peak on the electrophoretic pattern in M/10 phosphate buffer at pH 8.0. This enzyme has no action on D-GA, N-acetyl-L-GA., L-γ-amino butyric acid, L-glutamine, L-GA-γ-ethylester, DL-aspartic acid, α-amino adipic acid, L-ornithine, L-Lysine, creatine, glycylglycine, diamino pimelic acid.

Recently, an enzyme, γ-glutamyl lactamase, forming L-PGA from L-γ-glutamyl peptide was reported by Connell and Hanes (1956). Our enzyme is quite distinct from theirs, since the γ-glutamyl lactamase has no action on L-GA. In the study of aerobic decomposition of L-PGA, using a strain of Pseudomonas, Maruyama and Nomura (1956) concluded that the first stage of the oxidation of L-PGA was not hydrolysis to L-GA. They suggested that the interconversion between GA and PGA did not take place

in their bacterium. However, as the L-glutamic acid dehydrase has a reversible reaction, L-PGA decomposition may arise via L-GA in some microbes.

The detailed account will be published elsewhere.

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