Stereotype Analysis of D- and L-Glutamic Acid in Poly-γ-Glutamic Acid by HPLC and Enzymatic Assay

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Abstract

γ-PGA is a multi-functional biopolymer and has been interested for its various applications. γ-PGA consists of D and/or L-glutamate and its ratio is different depend on its producing microorganism and culturing environments[1)]. Here we are reporting the two methods to determine the stereochemistry of D-and L-glutamate in γ-PGA. One is enzymatic and the other is HPLC method. The amount of D-glutamate in γ-PGA can be determined by assaying the amount of produced D-alanine followed by the reaction of D-amino acid aminotransferase in the presence of amino group acceptor. D-glutamate and D-alanine can be separated by TLC and D-alanine can be quantitatively analyzed by the elution of Ninhydrin spot. L-glutamate can be assayed by L-glutamate oxidas eand L-glutamate dehydrogenase. RP-HPLC and pre-column derivation with 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA) was the other method to assay D-and L-glutamate after hydrolysis of γ-PGA. Our method is more convenient, reproducible and precise than the previous method that is using CHIRALPAK column. Data obtained with HPLC exhibited good quantitative correlation with those from enzymatic analysis data of γ-PGA. Our methods would be useful and convenient for the quality control for the mass production of γ-PGA.

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Reference

1. Ashiuchi M, Shimanouchi K, Nakamura H, Kamei T, Soda K, Park C, Sung MH, Misono H., Enzymatic synthesis of high-molecular-mass poly-gamma-glutamate and regulation of its stereochemistry. (2004) *Appl Environ Microbiol.* 70(7), 4249-4255.