One step purification of PGA and immobilized reaction using IMAC

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The inexpensive large-scale production of pure PGA (Penicillin G Acylase) has been a commercial goal. PGA has been used as a model enzyme in the development of simple one-step purification method. In this study, the purification of poly-His tagged PGA protein secreted into the periplasmic space was carried out by using immobilized metal-ion affinity chromatography(IMAC). Effective secretion and high expression were examined as well as potential usefulness as a purification method. The PGA gene was obtained from *E. coli* ATCC 11105. Codons encoding histidines were fused at the C-terminus of the PGA gene by PCR. JM109harboring pPGA-HIS6 vector produced active his-tagged acylases in the presence of *lac* promoter during cultivation at 26 C3). The specific activity of the purified acylase by using one-step chromatography after osmotic shock was 38.5 U/mg and recovered with the yield of 70%. Both 23kDa(subunit) and 62kDa(subunit)1,2) were recovered by using IMAC with just C-terminus tagging of the subunit. The purification of the periplasmic fraction by osmotic shock was 2.6 fold and that of purified acylase was 19 compared to the crude extract. In addition, *in-situ* immobilization of the periplasmic poly-His tagged PGA was performed. Immobilized acylase was used for the production of 6-APA from penicillin G in batch reaction at 37C for 15min. The portion of protein bound to IMAC resin was higher than 95%. The production yield was about 80% and maintained during 10 successive operations.

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