

Analisis Inhibisi *Secretory Leukocyte Protease Inhibitor* (SLPI) rekombinan Asal Membran Amnion Manusia Terhadap Aktivitas Enzim *Porcine Pancreatic Elastase* (PPE)

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Shindy Purnamasari., 2011, Inhibitory analysis of recombinant *Secretory leukocyte protease inhibitor* (SLPI) from human amniotic membrane on *Porcine Pancreatic ELastase* (PPE) activity, Thesis is under guidance of Prof.Dr. Ni Nyoman Tri Puspaningsih, M.Si. and Dr. Elly Munadzirroh, M.Si, drg. Department of Chemistry, Faculty of Science and Technology, Airlangga University, Surabaya.

Abstract

Secretory leukocyte protease inhibitor (SLPI) is The 11.7kDa non-glycosylated protein is highly basic ($pI > 9.5$), stable in acidic conditions, and found in a variety of mucosal fluids, including saliva, respiratory secretions, and cervicovaginal fluids SLPI is a potent anti-microbial, anti-inflammatory protein that have wound healing activities. Previous study have expressed human SLPI as a polyhistidine-tagged protein (HisSLPI) using a recombinant *E.coli* expression system. The 11,7 kDa-tagged protein was then overexpressed in *E.coli* cells following a 4 hour induction of IPTG. The recombinant SLPI were purified to homogeneity from concentrated culture medium by one-step nickel-chelating affinity chromatography under non-denaturing conditions using FPLC, and analyzed by Coomassie-stained SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Purified recombinant SLPI was further characterized by kinetics studies. The recombinant SLPI was found to be fast-acting inhibitor of porcine pancreatic elastase, with KM values of 0,3359 mM, while V_{maks} 0,0151 $\mu\text{mol}/\text{min}$. The equilibrium dissociation constant K_i for the interaction of recombinant SLPI with its target enzymes was directly measured for *porcine pancreatic elastase* . K_i values were found to be in the 0,959 mM. Based on the kinetic parameters determined here, it may be concluded that recombinant SLPI may act as potent anti-inflammatory molecules and may be of therapeutic potential in the treatment of various inflammatory diseases.

Keywords: *SLPI, serine protease, IPTG induction, FPLC, enzyme kinetics.*