INTISARI

Luciferase-like monooxygenase (LLM) merupakan salah satu flavoprotein enzim yang termasuk ke dalam golongan *luciferase-like family*. LLM diketahui memiliki peran sebagai biokatalisator dalam berbagai reaksi oksidasi seperti, epoksidasi, halogenasi, dan oksidasi *baeyer villiger*. Pada penelitian sebelumnya, ORF *llm1* yang berasal dari *Priestia megaterium* telah berhasil dikloningkan ke dalam vektor *pET28a*(+) serta diekspresikan dalam *Escherichia coli* BL21 (DE3). Pada penelitian ini dilakukan pengujian kelarutan, purifikasi, dan karakterisasi parsial rekombinan LLM1. Pada persiapan *pre-culture*, *Escherichia coli* BL21 (DE3) yang memiliki *pET-llm1* dikultivasi dalam medium *Luria Bertani* (LB) cair mengandung 50 µg/ml kanamisin pada suhu inkubasi sebesar 37^0C, agitasi sebesar 150 rpm, selama 18 jam. Sebanyak 2% *pre-culture* diambil dan diinokulasi kembali pada 50 mL medium LB cair berkanamisin. Kultur diinkubasi selama 1 jam hingga nilai absorbansi (OD_{600}) pertumbuhan kultur mencapai 0.6 - 0.8, kemudian IPTG berbagai perlakuan (0,1 mM; 0,5 mM; dan 1 mM) ditambahkan ke dalam medium. Kultur diinkubasi kembali hingga 3 jam, kemudian sel dipanen dan disonikasi dalam larutan 10 mM Tris-HCl pH 8.8. Hasil berupa LLM1 terlarut dipurifikasi, dialisis, dan dianalisis dengan SDS-PAGE. Karakterisasi biofisik-kemikal parsial dilakukan dengan menggunakan analisis spektrum UV-Vis spektrofotometri. Pengujian stabilitas LLM1 dilakukan dengan mereaksikan protein dengan denaturan *guanidine hydrochloride* berkonsentrasi 1 M hingga 3 M. Hasil pengujian kelarutan menunjukkan bahwa LLM1 terlarut dalam 10 mM Tris-HCl pH 8.8 pada induksi IPTG 0,1 ; 0,5 ; dan 1 mM berturut – turut 68, 60, dan 69 %. LLM1 dapat dipurifikasi secara parsial menggunakan metode penukar ion. Analisis spektrum absorbansi ($OD_{200-250}$) menunjukkan bahwa LLM1 memiliki nilai puncak absorbansi 206,5 nm pada kondisi terlipat dan 230 nm pada kondisi terdenaturasi. LLM1 juga terbukti memiliki ketahanan terhadap *guanidine hydrochloride* dengan konsentrasi maksimal 1,2 M.

Kata kunci : Purifikasi, Karakterisasi, Kelarutan, LLM1

ABSTRACT

Luciferase-like monooxygenase (LLM) is a flavoprotein enzyme that belongs to the luciferase-like family. LLM is known to have a role as a biocatalyst in various oxidation reactions such as epoxidation, halogenation, and baeyer villiger oxidation. In a previous study, ORF llm1 derived from Priestia megaterium has been successfully cloned into pET28a (+) vector and expressed in Escherichia coli BL21 (DE3). In this study, solubility assay, purification, and partial characterization of recombinant LLM1 were carried out. To prepare the pre-culture, Escherichia coli BL21 (DE3) with pET-llm1 was cultivated in liquid Luria Bertani (LB) medium containing 50 µg/ml kanamycin at an incubation temperature of 37^0C, agitation at 150 rpm, for 18 hours. A total of 2% of the pre-culture was taken and re-inoculated into 50 mL of liquid LB medium with kanamycin. The culture was incubated for 1 hour until the absorbance value (OD_{600}) of culture growth reached 0.6 - 0.8, then IPTG of various concentrations (0.1 mM, 0.5 mM, and 1 mM) was added to the medium. The culture was re-incubated for up to 3 hours, then cells were harvested and sonicated in 10 mM Tris-HCl pH 8.8 solution. The soluble product of LLM1 was purified, dialyzed, and analyzed by SDS-PAGE. Partial biophysical-chemical characterization was performed using spectrophotometric UV-Vis spectra analysis. A stability assay of LLM1 was carried out by interacting the protein with guanidine hydrochloride in concentration of 1 M to 3M. Solubility assay results showed that LLM1 dissolved in 10 mM Tris-HCl pH 8.8 at IPTG induction of 0.1, 0.5, and 1 mM was 68, 60, and 69 %, respectively. LLM1 was able to be partially purified using the ion exchange method. Absorbance spectrum analysis ($OD_{200-250}$) showed that LLM1 had a peak absorbance value of 206.5 nm in the folded condition and 230 nm in the denatured condition. LLM1 was also shown to have a tolerance to guanidine hydrochloride with a maximum concentration of 1.2 M.

Key words : Purification, Characterization, Solubility, LLM1