Analisis fungsi enzimatik protein katg rekombinan dengan mutasi baru pada mycobacterium tuberculosis yang resisten terhadap isoniazid = Analysis of enzymatic function of recombinant katg proteins harboring new mutations derived from izoniazid resistance mycobacterium tuberculosis

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Abstrak

ABSTRAK
Tuberkulosis (TB) masih menjadi masalah kesehatan utama di Indonesia. Salah satu penyebab tingginya kasus TB disebabkan adanya resistensi Mycobacterium tuberculosis (Mtb) terhadap obat anti tuberkulosis. Isoniazid (INH) yang merupakan salah satu obat lini pertama dalam pengobatan TB adalah pro-drug yang akan diubah menjadi bentuk aktifnya melalui aktivitas protein KatG Mtb. Mutasi pada gen katG yang mengkode protein KatG kemungkinan mempengaruhi aktivitas katalase dan peroksidase protein sehingga menyebabkan resistensi Mtb terhadap INH. Dalam studi ini, dilakukan kontruksi plasmid rekombinan protein KatG tipe liar dan protein KatG dengan mutasi baru pada residu N330D dan H400Y, serta mutasi yang umum dijumpai pada residu S315T dan S315N. Ekspresi protein KatG rekombinan dilakukan menggunakan host E. coli. Over ekspresi kelima protein rekombinan KatG terjadi setelah induksi IPTG. Purifikasi protein KatG rekombinan dilakukan berdasarkan prinsip kromatografi afinitas menggunakan Nikel sepharose. Setelah purifikasi diperoleh protein KatG yang murni. Aktivitas katalase dan peroksidase protein rekombinan KatG diukur pada berbagai konsentrasi substrat yang diperlukan dalam pengukuran efisiensi katalitik kedua aktivitas protein KatG. Hasilnya menunjukkan bahwa mutan protein KatG memiliki efisiensi katalitik yang lebih rendah dari protein KatG tipe liar. Penurunan efisiensi katalitik aktivitas katalase mutan N330D dan H400Y sebesar 31% dan 37% dan untuk aktifitas peroksidase sebesar 39% dan 3% dibandingkan KatG tipe liar. Struktur 3 dimensi protein KatG dari mutan tersebut dibuat menggunakan perangkat Modeller dan divisualisasikan menggunakan perangkat Pymol. Tidak terdapat adanya perubahan konformasi 3 dimensi protein KatG mutan dibandingkan dengan struktur 3 dimensi protein KatG tipe liar. Namun, letak residu N330D dan H400Y yang berada dekat daerah aktif ikatan INH pada protein KatG kemungkinan berpengaruh terhadap penurunan aktivitas enzimatik protein KatG.
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ABSTRACT
Tuberculosis (TB) is currently a major health problem in Indonesia. One of the many causes of the high incident of TB is due to the resistance of the Mycobacterium tuberculosis (Mtb) to anti-TB drugs. Isoniazid (INH), one of the first line anti-TB

drugs for TB treatment, is a pro-drug that is converted to its active form through the activity of Mtb KatG protein. Mutations in the katG gene encoding KatG may affect the catalytic efficiency of the catalase and peroxidase activities of the protein that eventually confers resistance to INH. In this study, recombinant plasmids containing katG gene that have new mutations on residue N330D dan H400Y, wild type, as well as mutant proteins with common mutations at residue S315T and S315N were constructed. Expression of recombinant KatG was performed using E. coli as an expression host. Over expression of recombinant KatG was facilitated by IPTG induction. Purification of recombinant KatG was performed using affinity chromatography employing Nickel sepharose. Pure recombinant proteins were obtained, and the catalase and peroxidase activities of the recombinant KatG protein were measured at various concentration of substrates. Result showed that mutant KatGs have a lower catalytic efficiency for both catalase and peroxidase activities than the wild type protein. Decreasing catalytic efficiency for catalase of mutants N330D and H400Y were 31% and 37% than that of wild type KatG, while catalytic efficiency for peroxidase of mutants N330D and H400Y were 39% and 3% lower than that of wild type. Three dimensional structures of mutant KatGs were generated using Modeller and visualized using PyMol softwares. The three dimensional structural of mutant KatG showed no conformational change compared with that of wild type KatG. However, the location of residues N330D and H400Y which are in the close proximity to the active site of KatG for INH binding is likely to have an effect on the decreased enzymatic activities of mutant KatG proteins.