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To my beloved parents, my family and my soulmate, my best friend; my husband and not forgetting to our newborn bub, Muhammad Rafiqi.

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ABSTRACT

Maltose binding protein (MBP) changes its conformational structure upon its ligand binding.This molecular recognition element that transduces a ligand-binding event into a physical one make MBP an ideal candidate for reagentless fluorescence sensing. MBP gene, (*malE)* was amplified from a pMaL-C4x plasmid vector and was fused to a Strep-Tag II pET-51b(+) vector. Strep-Tag II is a tag that will enable the MBP to be unidirectionally immobilized on solid supports. A cysteine mutant of the MBP was constructed by inverse PCR and the recombinant protein fusion was then purified by affinity purification using Strep-Tactin resin. To sense maltose binding, an environmentally sensitive fluorophore (IANBD amide) was covalently attached to the introduced thiol group. The tagged mutant MBP (D95C) was successfully generated and the protein was successfully purified with the expected molecular size of ~42 kDa observed on the SDS PAGE. The fluorescence measurements of the IANBD labeled of tagged mutant MBP (Strep-Tag II D95C) in the solution phase, showed an appreciable change in fluorescence intensity with dissociation constant, (K_d) of 7.6 ± 1.75 µM. Nonetheless, it could retain its ligand binding activity towards maltose. However, immobilization of Strep-Tag II D95C on solid surface suffered some limitation with the Strep-Tactin coated microwell plates because it did not give any dependable results to support the ligand binding activity of the site directed immobilized protein. Thus, this engineered mutant MBP (Strep-Tag II fused D95C) could be potentially developed for biosensor application with further improvement in protein immobilization method.

ABSTRAK

Protein pengikat maltosa (MBP) mengalami perubahan struktur konformasi semasa mengikat pada ligan. Molekul pengenalpastian yang menyebabkan transduksi pengikatan ligan kepada bentuk fizikal menjadikan MBP calon yang sesuai sebagai penderia pendaflour tanpa reagen. Gen MBP (*malE*) diamplifikasi daripada vektor plasmid pMaL-C4x dan kemudian digabungkan dengan Strep-Tag II yang terdapat pada vektor pET-51b(+). Strep-Tag II merupakan tag yang membolehkan MBP disekatgerak secara seragam kepada penyokong pepejal. Mutan MBP yang mempunyai satu residu sisteina telah dihasilkan melalui PCR berbalik dan gabungan protein rekombinan ini ditulen melalui penulenan afiniti yang menggunakan resin *Strep-Tactin*. Untuk mengesan pengikatan maltosa, flourofor yang sensitif pada persekitaran (IANBD amida) telah diikat secara kovalen kepada kumpulan tiol yang telah diperkenalkan pada protein itu. Mutan MBP yang bertag telah berjaya dijana dan melalui pemerhatian SDS-PAGE protein ini telah berjaya ditulen dengan saiz molekul ~42 kDa seperti yang dijangkakan. Ukuran pendaflour di dalam fasa larutan bagi mutan MBP yang bertag dan berlabel dengan IANBD menunjukkan perubahan ketara bagi keamatan pendaflour dengan pemalar penguraian K_d 7.6 ± 1.75 μM. Walau bagaimanapun, aktiviti pengikatan ligan terhadap maltosa boleh dikekalkan. Pemegunan protein Strep-Tag II D95C pada permukaan pepejal, berhadapan dengan beberapa kelemahan apabila piring mikrotelaga yang bersalut *Strep-Tactin* digunakan kerana ia tidak memberi keputusan yang dapat menyokong aktiviti pengikatan ligan oleh protein yang dipegunkan pada tapak khusus. Oleh itu, kejuruteraan mutan MBP gabungan Strep-Tag II-D95C berpotensi dibangunkan untuk aplikasi biopenderia dengan lebih penambahbaikan dalam kaedah pemegunan protein.