Extended Abstract Journal of Molecular and Cellular Biochemistry 2021
Vol.5 No.3

Generation and characterization of some UnaG mutant

Numan Eczacioglu
Karamanoglu Mehmetbey University, Turkey.

Abstract

UnaG protein from Japan eel (Anguilla japonica) is a novel fluorescent protein with binding domain that acquires fluorescence when bound to unconjugated bilirubin (UC-BR). In this study, several point mutations (F17M, N57D, N57E, N57R, L41F, Y99F_Y134W, Y99M_Y134M, and W9F_W103F) were made on the UnaG nucleotide sequence via using a method for sequence and ligation independent cloning (SLIC). The aim of the mutations on UnaG is to figure out the change in fluorescence properties. The new mutagenic vector was transformed into the commercial competent cells (E. coli Mach1) by using heat shock at 42 °C for 2 minutes. Transformed cells were grown on and selected from the LB agar plate with ampicillin. (1:1000). The DNA sequencing results show that all these mutations have done correctly. The expression of the mutant proteins was made in the pTOLT expression system by inducing with IPTG. Cells were collected with high speed centrifugation. Before disrupting the cells, lysozyme enzyme was added to make break up the cells easier, some protease inhibitors (phenylmethylsulfonyl fluoride, benzamidine) were added for the protection from proteases of the protein and DNase and RNase were added on the cell pellet to avoid the DNA and RNA contaminations. Ultracentrifugation was applied on the cell lysate. Ni- NTA affinity chromatography system was used to get the pure mutant proteins from supernatant. SDS-PAGE and semi-dry Western blot were applied on the protein for the qualitative analyse. The pure protein bands were observed on the SDS-PAGE gel image. Additionally, the spectroscopic features of purified mutant proteins were measured after adding fresh UC-BR on fluorescence spectrophotometer. Excitation and emission spectra of the mutant proteins are similar; even so they have different fluorescence intensity at the same concentration. This study suggests that mutant UnaG proteins can be used to detect UC-BR level of cells/tissue.

This work is partly presented at International Conference on Biotechnology, Biomarkers & Systems Biology March 04-05, 2019 at Amsterdam, Netherlands