Purification of the recombinant enhanced green fluorescent protein from Escherichia coli using alcohol + salt aqueous two-phase systems

ABSTRACT

The enhanced green fluorescent protein (EGFP) is widely used as a marker in life science. Currently, purifications of the EGFP mostly involve chromatographic methods, which are multistep, time-consuming and costly. In the present study, the recombinant EGFP expressed in Escherichia coli was purified using an economic aqueous two-phase system (ATPS). Short-chain aliphatic alcohol and organic salt were chosen as the phase-forming components owing to their recyclability and biodegradability, respectively. The partition behaviour of EGFP was evaluated under the varying conditions of ATPS, including types and concentrations of phase-forming components, feedstock concentration, and pH. In an optimal primary 2-g ATPS comprising 1-propanol and tripotassium citrate, a high recovery of EGFP (i.e., 92.1%) was attained in the salt-rich bottom phase. To facilitate the easy recovery of purified EGFP, a secondary ATPS was used to back extract the EGFP to a new top ethanol-rich top phase, and the ethanol was removed from the purified EGFP via evaporation. The 1-propanol and ethanol used in the primary and secondary ATPSs (i.e. 20-g system) were successfully recycled in three successive rounds of EGFP purification, yielding the average EGFP purification factor of 11.34 and 75.7% of EGFP yield. The purification of EGFP using the two stages of alcohol + salt ATPSs is efficient in terms of operation time, cost and process scalability.

Keyword: Enhanced green fluorescent protein; Aqueous two-phase systems; Alcohol; Salt; Back extraction