Improved extracellular secretion of β-cyclodextrin glycosyltransferase from Escherichia coli by glycine supplementation without apparent cell lysis

ABSTRACT

The use of an effective inducer feeding strategy without causing cell lysis presents significant advantage to enhance the secretion of an enzyme to the culture medium of Escherichia coli. The cgt gene encoding β-cyclodextrin glycosyltransferase (β-CGTase) was cloned into pQE30xa as an N-terminal His-tagged protein and transformed into E. coli. The induction strategy was applied towards enhancing the extracellular secretion of the recombinant β-CGTase by increasing permeability of the outer membrane of E. coli. The supplementation of 1.2 mM glycine following 2 h of fermentation at 37°C enhanced the activity of β-CGTase to 38.295 U/mL, which was approximately 1.3-fold higher than the control (without induction). Further flow cytometry analysis was adopted as a rapid and highly reproducible approach to determine the effect of glycine supplementation on the viability of E. coli cells. The supplementation of glycine did not contribute to apparent cell lysis, with no adverse effects on cell viability, hence indicating the effectiveness of glycine in enhancing the extracellular secretion of β-CGTase. The recombinant β-CGTase was then purified through a combination of diafiltration and Ni-NTA affinity chromatography with 18.4-fold increase in purity. An effective glycine feeding strategy could enhance the extracellular secretion of β-CGTase without adverse effects on cell viability. This strategy could be applied potentially to enhance the secretion of a recombinant protein to the culture medium from E. coli cells without having cell lysis.

Keyword: Cell viability; Cyclodextrin glycosyltransferase; Extracellular secretion; Glycine; Inducer; Membrane permeability