

Abstract

Glycosidases (EC 3.2.1.) *alias* glycoside hydrolases are enzymes that catalyze the cleavage of a glycosidic bond between two carbohydrates or between a carbohydrate and an aglycone. Under suitable conditions (especially reduction of water activity in the reaction mixture), these enzymes are also able to synthesize a glycosidic bond. By targeted mutagenesis of the catalytic centre of the enzymes, it is possible to suppress or completely abolish their hydrolytic activity. Enzyme synthesis using glycosidases makes it possible to prepare bioactive galactosides, for example galectin ligands.

The present work deals mainly with β -galactosidase from *Bacillus circulans*, its recombinant expression and mutagenesis. In the first part of the work, the commercially prepared plasmid of β -galactosidase from *B. circulans* isoform A that I designed was used for recombinant expression in *E. coli*. It was necessary to optimize the conditions of the enzyme production. As it is a large protein (189 kDa), the expression vector pCOLD II and cold production at 15 ° C were used. The enzyme is specific for the formation of the β -1,4 glycosidic bond and has been used to synthesize complex tri- and tetrasaccharide ligands that cannot be prepared with a crude commercial preparation containing undesirable enzyme activities. Furthermore, site-directed mutagenesis of the catalytic nucleophile at the active site of this enzyme was performed by PCR with mutant primers, creating a mutant at position E532G. Due to the resulting mutation, almost all hydrolytic activity of the enzyme was suppressed. In the third part of the work, a shorter variant of the enzyme was prepared by PCR with phosphorylated primers and subsequent ligation of only the desired part of the gene. All prepared enzymes were biochemically characterized and used in synthetic applications, which are documented in this thesis.

Keywords:

Bacillus circulans, *Escherichia coli*, β -galactosidase, galactosylation, glycosynthase, PCR, recombinant expression, targeted mutagenesis