Recombinant protein immobilisation and display by alginate

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ABSTRACT

Biopolymers are a diverse group of organic materials with important applications in a number of industries. Their ability to adsorb and encapsulate compounds has been widely utilised in both biotechnologies and pharmaceuticals. In the last decade, biopolymers have been given new and enhanced functionality, including the separation and purification of compounds. This field is of increasing relevance as advances in the bacterial cell culture process have improved productivity in the biomanufacturing industry, with the establishment of several bacterial host cell lines and optimised protein production systems. This increase in upstream productivity is leading to bottlenecks in downstream processing as current technology platforms reach their limits of throughput and scalability. While previous studies have generated functionalised protein biopolymers using polyhydroxyalkanoate (PHA) biopolyester beads, very few studies have examined the commercially significant biopolymer alginate. Alginate is an exopolysaccharide produced by algae and some bacteria, and is widely utilised in food, pharmaceutical, and biomedical industries because of its stabilising, haemostatic, biocompatible properties and its modifiable structure. In this study, a partially functional alginate-binding recombinant protein was produced, which contained an α-amylase domain from *Bacillus licheniformis* (BLA) translationally fused to the alginate-binding domain of *Pseudomonas aeruginosa* AlgX – an alginate acetyltransferase. An Ssp DnaB mini-intein was included between BLA and AlgX to facilitate recovery of BLA, following immobilisation and display on the surface of alginate. However, aberrant activity of the intein caused total cleavage of the recombinant protein between its BLA and AlgX domains before it could be recovered from the protein production system. Additionally, the absence of a key cysteine residue in the alginate-binding domain prevented the formation of a disulfide bond, which is an essential structural element for the folding and functionality of this region. While this study was unable to overcome intein hyperactivity, functional analysis of the BLA domain showed consistent and significant levels of α-amylase activity, leading to a positive outlook for the functionality of a full-length recombinant protein if proper intein activity can be restored and the necessary cysteine included. In this way, alginate could be specifically functionalised with a desired protein, and in turn, alginate beads could be used for the separation and enrichment of target proteins.

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LIST OF ABBREVIATIONS

A full list of abbreviations.

˚C	Degree Celsius
A	Absorbance
AGE	Agarose gel electrophoresis
Ap	Ampicillin
APS	Ammonium persulfate
BLA	*Bacillus licheniformis* α-amylase
bp	Base pairs
BSA	Bovine serum albumin
Δ	Delta (deleted)
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTPs	Deoxyribonucleotide triphosphates
dsDNA	Double-stranded deoxyribonucleic acid
DTT	Dithiothreitol
ETOH	Ethanol
EDTA	Ethylenediaminetetraacetic acid
g	Gram/gravity
GDP	Guanine diphosphate
His-tag	Polyhistidine-tag
HRP	Horse radish peroxidase
kbp	Kilo base pairs
kDa	Kilodaltons
λ	Lambda phage
LB	Luria-Bertani (broth)
MOPS	3-(N-morpholino)propanesulfonic acid
MW	Molecular weight
OD	Optical density

ORF	Open reading frame
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
REase	Restriction endonuclease
RNAase	Ribonuclease
Rpm	Rotations/revolutions per minute
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
TB	Terrific Broth
TBE	Tris-borate-EDTA buffer
TBST	Tris-buffered saline and Tween 20
Tet	Tetracycline
TEMED	Tetramethylethylenediamine
T_m	Primer melting temperature
Tris	Trishydroxymethylaminomethane
vol	Volume
v/v	Volume per volume
w/v	Weight per volume

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