BIOTRANS
LA ROCHELLE 2023

N°458 / PC
TOPIC(s) : Enzyme production, immobilization / Enzyme discovery and engineering

Enhancing ferredoxin NADP+ reductase stability by heterologous expression in Pichia pastoris

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PURPOSE OF THE ABSTRACT

In nature, ferredoxin NADP+ reductase (FNR) is the main flavoenzyme which regenerates NADPH by receiving the electrons dispensed from photosystem I and catalyzing the two-electron reduction of NADP+ to NADPH. This catalytic capacity has been harnessed outside the cell in the so called ?electrochemical leaf?.1 Under these conditions, FNR catalyzes the reversible interconversion of NADPH into NADP+ when it is confined into an electrode surface. Moreover, this bioelectrocatalytic concept has been exploited as a cofactor regenerating tool when assembled with cofactor-dependent multi-enzyme systems.2 Among the reported FNRs, the one from the cyanobacterium Anabaena sp. (AsFNR) has been well recombinantly expressed in E. coli with relatively high protein yields, thus allowing been widely studied and kinetically characterized.3 Despite these advantages, AsFNR displays very poor stability (it is completely inactivated after spending one night stored at 4 ºC). This drawback hampers its application as a robust cofactor-regenerating system in large scale biotransformations. Several strategies have been focused on increasing the stability of this enzyme mainly by immobilization and rational mutations.4

Nowadays, the yeast expression system P. pastoris is one of the most popular and standard tool for the production of recombinant proteins in molecular biology. Overall the benefits of this strategy include appropriate folding and secretion of recombinant proteins to the external environment of the yeast cell.5 Moreover, N-glycosylation is one of the most common forms of protein post-translational modification in this yeast expression system; which is closely related with the increase in thermal stability of the resulting enzymes.6, 7 Based on these previous findings, herein we report for the first time the heterologous expression of AsFNR in P. pastoris aiming at increasing the operational stability of this enzyme through the natural N-glycosylation process. We have cloned a his-tagged AsFNR construct extracted from a pET28a vector and inserted it into a pGAPZ?A vector. After the successful clone construction, we optimized the heterologous expression of rAsFNR in P. pastoris in defined and enriched mediums. Once rAsFNR is secreted to the culture medium, we have purified it by IMAC chromatography and kinetically characterized. The obtained pure rAsFNR displays higher melting temperature and higher operational stability than the one expressed in E. coli expression system.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

stability | ferredoxin NADP+ reductase | glycosylations | P. pastoris

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