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Size reduction of Sesquiterpene cyclase BcBOT2 via site directed mutagenesis and further protein optimizations for future NMR studies

AUTHORS
Trang NGUYEN / LEIBNIZ UNIVERSITY HANOVER, SENATOR-BAUER-STRASSE 2, HANOVER

PURPOSE OF THE ABSTRACT
BcBOT2 is a sesquiterpene cyclase from the fungus Botrytis cinerea and has the potential to convert farnesyl diphosphate and its derivatives into a structurally more complex sesquiterpene such as presilphiperfolan-8ß-ol. Since only homology models are available so far, the aim is therefore to achieve a structural elucidation of this protein in solute form with the help of NMR spectroscopic methods. Due to its initial size of 47 kDa, the enzyme had to be reduced in size by removing parts of the N- and C-terminus via site-directed mutagenesis. For this purpose, a mutant with an unchanged enzymatic activity could be generated, which would come to a size of 37.6 kDa by the additional removal of the 6x-His tag. Since triple labelling (15N, 13C, 2H-labelling) is required in this case, optimization experiments regarding protein expression, solubility and stability were carried out before the actual enrichment.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

BIBLIOGRAPHY