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Thermostable mutant variants of *Bacillus* sp. 406 α-amylase generated by site-directed mutagenesis

Research Article

Alexandr V. Kachan*, Anatoliy N. Evtushenkov

The Faculty of Biology,
Belarusian State University,
220030 Minsk, Republic of Belarus

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Abstract: Several mutations are known to increase the thermostability of α-amylase of *B. licheniformis* and other α-amylases. Site-directed mutagenesis was used to introduce similar mutations into the sequence of the α-amylase gene from mesophilic *Bacillus* sp. 406. The influence of the mutations on thermostability of the enzyme was studied. It was shown that the Gly211Val and Asn192Phe substitutions increased the half-inactivation temperature (T_m) of the enzyme from 51.94±0.45 to 55.51±0.59 and 58.84±0.68°C respectively, in comparison to the wild-type enzyme. The deletion of Arg178-Gly179 (dRG) resulted in an increase of T_m of the α-amylase to 71.7±1.73°C. The stabilising effect of mutations was additive. When combined they increase the T_m of the wild-type amylase by more than 26°C. Thermostability rates of the triple mutant are close to the values which are typical for industrial heat-stable α-amylases, and its ability to degrade starch at 75°C was considerably increased. The present research confirmed that the Gly211Val, Asn192Phe and dRG mutations could play a significant role in thermostabilization of both mesophilic and thermophilic α-amylases.

Keywords: *Thermal stability • α-amylase • Bacillus • Site-directed mutagenesis • Protein engineering*

Abbreviations:

AB406 - *Bacillus* sp. 406 α-amylase;
BAA - *Bacillus amyloliquefaciens* α-amylase;
BLA - *Bacillus licheniformis* α-amylase;
BStA - *Bacillus stearothermophilus* α-amylase;
DNS - 3,5-dinitrosalicylic acid;
dRG - the Arg178-Gly179 residues deletion;
SD - standard deviation.

1. Introduction

Nowadays, thermostable enzymes are used in various biotechnological processes [1]. Increased stability of enzymes to extreme temperatures allows the amount of enzyme in the reaction to be reduced. Increasing the reaction temperature improves the reaction yield and reduces the time taken for product accumulation.

α-amylases (EC 3.2.1.1) are used in industrial processing of starch-containing materials. The majority of these reactions are conducted at high temperatures, resulting in high demand for thermostable α-amylases.

Using rational engineering methods, several scientific groups have identified amino acid substitutions that lead to enhanced thermostability of α-amylases. Considerable success in this area has been reached in thermostabilisation of α-amylase from *Bacillus licheniformis* (BLA) [2]. Declerck and co-workers combined seven stabilising mutations in BLA which resulted in a 23°C increase in the half-inactivation temperature. Another example of thermostability engineering is the deletion of a short destabilising loop structure in the B domain of *B. amyloliquefaciens* α-amylase (BAA) [3]. Arg176-Gly177 residues, which form a projecting structure on the enzyme molecule surface, were deleted and led to an increase in BAA stability. Analogous deletion also stabilised the structures of α-amylases from *Bacillus* sp. KSM-K38

* *E-mail: av.kachan@mail.ru*