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ENZYMATIC ACTIVITY OF THE FUSION PROTEIN OF TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE WITH DNA-BINDING PROTEIN SSO7D FROM *S. SOLFATARICUS*[*]

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Abstract

Gene fusion techniques have demonstrated promising results in developing more active, processive DNA polymerases. In the present work, we isolated and investigated the activity of a fusion enzyme combining terminal deoxynucleotidyl transferase (TdT) with the DNA-binding protein from *Sulfolobus solfataricus* (Sso7d). The fusion proteins exhibit enhanced 3′-5′ exonuclease activity, which substantially impairs their nucleotide incorporation efficiency.

Motivation and aim

The TdT is a unique polymerase that incorporates nucleotides at the 3′-terminus of single-stranded DNA primers in a template-independent manner. This biological function has propelled the development of numerous biomedical and bioengineering applications [1]. The extensive utilization of TdT is constrained by its low expression levels in *E. coli*, low optimal operating temperature, and difficulty in controlling its enzymatic activity [2]. To address these limitations, we designed and isolated a fusion enzyme combining TdT and the Sso7d. Sso7d exhibits nonspecific binding activity toward dsDNA and RNA sequences, distorts the structure of duplex DNA, and has previously been successfully employed as a fusion domain for developing improved molecular biology tools [3]. We expect that the obtained fusion enzymes could serve as prospective candidates for novel, specific applications.

Methods

The fusion genes were obtained by overlap extension PCR and cloned into the pCWori (TdT_Sso7d) and pET20b (Sso7d_TdT) vectors. All proteins were expressed in *E. coli* BL21 (DE3) cells, which were cultivated in TB media and purified by immobilized metal affinity chromatography. Enzymatic activity was quantified by measuring the reaction products, which were separated using a C18 column under standard ion-pair reverse-phase chromatography conditions and detected at 260 nm.

Results

The Sso7d on C-terminus of the TdT showed a complete loss of catalytic activity in the addition of nucleotides and more pronounced 3′-5′ exonuclease activity in the deletion of nucleotides from the coding end. In contrast, the Sso7d_TdT demonstrated less nucleotide addition activity (0.14 U/μM for Sso7d_L3_TdT and 0.18 U/μM for Sso7d_L2_TdT) than native TdT (0.27 U/μM) and exhibited a pronounced deletion of nucleotides from the coding end.

The presence of Co^{2+} and Mn^{2+} ions in the reaction mixture with Sso7d_L2_TdT leads to fewer nucleotide deletions, increased processivity, and enhanced nucleotide-addition activity, correlating with stronger binding to the substrate and nucleotide in the enzyme's active site due to these metal ions [4]. Incorporation of Ca^{2+} into the assay buffer produced a near-complete loss of TdT's catalytic activities. Zn^{2+} ions decrease nucleotide-addition activity and, to a greater extent, nucleotide-deletion activity. Moreover, Zn^{2+} binding to nucleobases shifts the enzyme from a processive to a distributive mode by disrupting dNTP interactions within the active site [4].

The nucleotide incorporation and processivity of Sso7d_L2_TdT increased with the extension of the oligonucleotide sequence, similar to TdT, but only up to the T_{25} substrate, for which the activity was significantly reduced. Further substrate elongation led to a significant decrease in activity, which could be related to enhanced binding with the Sso7d domain. The use of a substrate containing secondary structure significantly reduces the activity and processivity of the native TdT, restricting it to a single product. This effect is even more pronounced with Sso7d_L2_TdT, which exhibits increased affinity for dsDNA.

Conclusion

The significant loss of nucleotide addition activity and enhanced nucleotide deletion activity render the obtained fusion proteins unsuitable for *de novo* DNA synthesis or translational applications. The increased nucleotide deletion

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activity, combined with the addition activity, which can be controlled by the presence of metal ions, may be advantageous for specific applications such as aptamers engineering, mutagenesis studies, or gene editing.

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