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INFLUENCE OF THE DNA BINDING PROTEIN SSO7D FROM *S. SOLFATARICUS* ON THE ACTIVITY OF BOVINE DNA-EXOTRANSFERASE AND ITS TRUNCATED VARIANTS *

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Abstract

The Sso7d protein is widely used to develop fusion DNA polymerases with enhanced properties. In this study, we investigate the impact of the presence Sso7d in the reaction mix on the activity of terminal deoxynucleotidyl transferase (TdT) and its truncated variants. The aim of this work to assess the expediency of developing an improved fusion enzyme by leveraging Sso7d and TdT proteins.

Motivation and Aim

Although TdT was discovered in the 1960s, it is currently under specific focus of modern biology. TdT is a distinctive enzyme that can add nucleotides at the 3'-terminus of single-stranded DNA without the need for a template [1]. TdT is currently considered as the only way to realization of enzymatic *de novo* synthesis of DNA [2]. DNA-binding protein from *S. solfataricus* (Sso7d) has been successfully used before to developing improved tools for molecular biology through the genetic fusion of the genes encoding Sso7d and various enzymes such as DNA polymerases [3]. Sso7d has nonspecific binding activity to any dsDNA and RNA sequences and distort the structures of duplex DNA, which contributes to its stabilization and may negatively affect the activity of TdT. We anticipate gaining valuable insights into the impact of the presence Sso7d in the reaction mix on the activity of TdT and its truncated variants, which we can use for the development of new, improved fusion enzymes to realize enzymatic *de novo* synthesis of DNA.

Methods

The TdT and Sso7d genes optimized for *E. coli* expression were synthesized by chemical-enzymatic synthesis, amplified and subcloned into the pCWori vector (NdeI-HindIII sites). Truncated TdT at 110 and 148 N-terminus amino acids (110_TrTdT and 148_TrTdT) was obtained by PCR amplification of native TdT and ligated into the same vector. For protein expression we used *E. coli* strain BL21 (DE3) which cultivated in TB media at high aeration and 37 °C to A_{600} equal to 0.6 and protein synthesis was started by IPTG induction (1 mM) at 18 °C. After 24 h cultivation cells were separated from medium by centrifugation. The proteins were purified to homogeneity by immobilized metal affinity chromatography. The enzymatic activity of proteins was determined by the amount of reaction products (detected at a wavelength of 260 nm) fractionated on a C18 column (2.1 × 50 mm, 1.9 μm) by standard methods of ion-pair reverse-phase chromatography.

Results

A study reaction without Sso7d with substrates T_5, T_{15} T_{35} showed that the activity of the TdT, 110_TrTdT and 148_TrTdT increased with increasing oligonucleotide length. For the reaction with T_{15} ions of Co^{2+} has an activating effect, while Zn^{2+} cations reduce TdT and truncated variants activity. The presence of Sso7d in the reaction mix leads to a slight (up to 15 %) decrease in native TdT and 110_TrTdT activity for substrates T_5 and T_{15} and a more pronounced decrease for T_{35} (up to 30 %). Ions of Co^{2+} and Mn^{2+} were initially used as a replacement for Mg^{2+} in TdT reaction buffer to destabilize DNA duplexes, especially in the blunt-end extension reaction. It can therefore be assumed that the influence of Co^{2+} cations in the reaction mixture could be the reason for the less pronounced inhibitory effect of the Sso7d protein. In the absence of Me^{2+}, the inhibitory effect of Sso7d increases with oligonucleotide chain length, indicating that the mechanism of inhibition is not related to protein-protein interactions of Sso7d and TdT, but rather is mediated by the competitive interaction of Sso7d with DNA. It is curious that for 148_TrTdT, the presence of Sso7d in the standard reaction mixture, on the contrary, contributed to an increase in the amount of reaction products by 40 %.

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Conclusion

In all cases, the presence of Sso7d in the reaction mix leads to a reduction in TdT and 110_TrTdT activity. Only for 148_TrTdT we observed a positive effect from Sso7d, which can be used to construction of a fusion enzyme or to increase the amount of DNA obtained during synthesis.

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