An altered DNA ligase enzyme influences wild type DNA ligase to increase ligation reaction of DNA on RNA template

DNA can break or nick during DNA replication. But to repair this damage, DNA ligase, an enzyme that can seal DNA breaks, is in the living system. In molecular biology, DNA ligase is extensively used in recombination of genes. This means that DNA is broken in one place and then joined to a different DNA molecule. The function of DNA ligase is dependent on the cofactor ATP.

The most commonly used DNA ligase is that from phage named T4, which is commercially available, efficient and stable. T4 DNA ligase is an Mg^{2+}- and ATP-dependent enzyme that seals DNA nick in three steps: activation of the enzyme with the help of ATP, formation of an intermediate of enzyme-DNA complex and finally catalysis of the nick sealing reaction. T4 DNA ligase is very efficient for ligation of DNA on a DNA template (a model or pattern). It also ligates DNA on RNA template but the reaction is inefficient. In case of RNA-templated DNA ligation, DNA ligase can do the first two steps of the reaction very efficiently but the third step reaction becomes very slow. As a result, a large fraction of intermediate products (enzyme-DNA complexes) are formed and accumulated.

But why do we need to ligate of DNA efficiently on an RNA template? In general, genes are expressed to mRNA first and then converted to protein. The use of a DNA probe to ligate with targeted mRNA directly to quantify gene expression is not possible due to slow ligation rate of DNA with RNA. That why, it's also not possible to detect mRNA in a single cell or in tissue. In conventional present techniques, RNA needs to be converted DNA to detect targeted mRNA in situ and to quantify specific gene expression.

In this study an altered T4 DNA ligase was used that influenced ligation of DNA on an RNA template when mixed with T4 wild type (wt) DNA ligase. Altered T4 DNA ligase cannot perform the ligation reaction alone. It cannot do the first two steps of the reaction but can do the third step of the reaction very efficiently. Here I found that in the presence of lower concentration of ATP (10 μM) and Mg^{2+} (10mM), a combination of a high concentration of T4 wt DNA ligase (0.5 U/ μl) and an altered T4 DNA ligase (0.05 U/μl) could ligate DNA on an RNA template very efficiently. I have also done this experiment in the presence of only wt T4 DNA ligase and in combination of wt T4 and altered T4 DNA ligase. I found that the ligation reaction occurred very rapidly within one hour when using a combination of enzymes but slowed down after two hours. I predicted that other enzymes might be mixed with the purified altered T4 enzyme that inhibited the ligation reaction at later times.

If the altered T4 DNA ligase is purified further, this novel DNA ligase may be used for efficient ligation of DNA on an RNA template, which will be a useful mechanism for sensitive and accurate detection and distinction of RNA sequence variants and other broad applications.

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