dynamic
BIOSENSORS

Application Note

Real-time measurements of DNA exonucleases

Key words: T5 exonuclease, Lambda exonuclease, DNA hydrolysis, molecular ruler

Background

DNA exonucleases are enzymes that catalyze the cleavage of mononucleotides from the 3' or the 5' end of a duplex DNA through hydrolysis. This mechanism contrasts with that of DNA endonucleases, which break phosphodiester bonds in the middle of the molecule. DNA exonucleases have various applications in molecular biology including the removal of overhangs and the generation of ssDNA from linear dsDNA.

Methods and Results

The enzymatic activities of T5 exonuclease and Lambda exonuclease (NEB), both highly efficient 5'→3' exonucleases, were investigated. A standard switchSENSE chip with 48 bp DNA tagged with a fluorophore at its 3'-end was used. The 5'-end was either free or modified with a protein or a small molecule. The activity was quantified in real-time by measuring the change in fluorescence that occurs when the dsDNA is degraded to ssDNA (molecular ruler assay, c.f. Scheme).

T5 exonuclease (1 µL) was diluted 50-fold in 1x NEBuffer 4 and different amounts of EDTA were added to control the concentration of free Mg^{2+} between 0 and 10 mM. The enzymatic activity was found to be the highest at 10 mM and steadily decreased with decreasing Mg^{2+} (Fig. 1).

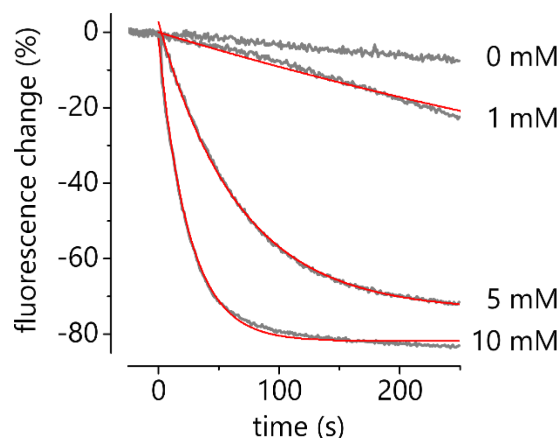

Figure 1 I Real-time measurement of T5 exonuclease (NEB) activity for different concentrations of Mg^{2+}-ions. Red lines are single exponential fits from which the nucleotide removal time is analyzed.

Mg^{2+}	T_{EXO}
1mM	768 ± 5 s
5mM	66.4 ± 0.4 s
10mM	24.8 ± 0.2 s

Next, Lambda exonuclease (1 µL) was diluted 50-fold in 1x Lambda exonuclease reaction buffer and the enzymatic activity was assessed at 25°C and 37°C. The preferred substrate of Lambda exonuclease is 5´-phosphorylated double stranded DNA. For the non-phosphorylated

duplex DNA used here, nuclease activity occurred at a reduced rate compared to that of T5 exonuclease, but the rate could be accelerated when raising the temperature from 25°C to 37°C (Fig. 2).

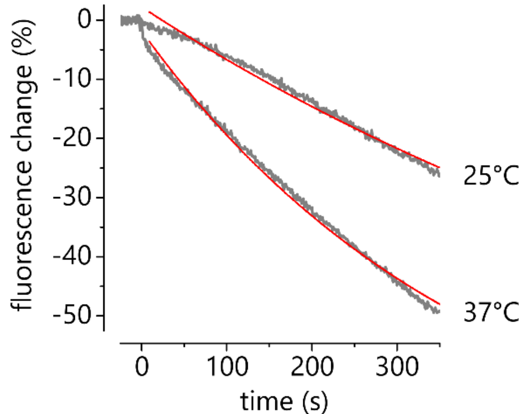

Figure 2 I Real-time measurement of Lambda exonuclease (NEB) activity at 25°C and 37°C (the temperature recommended by the supplier). Red lines are single exponential fits from which the nucleotide removal time is analyzed.

[T]	T_{EXO}
25°C	14.6 min
37°C	6.5 min

Finally, the effect of a 5'-modification of the DNA substrate on exonuclease activity was investigated. For this purpose, DNA substrates modified with human carbonic anhydrase 1 (hCA1), protein G (Pr G), the small molecule drug methotrexate (MTX) and an amino-group (NH2) were tested with T5 exonuclease (Fig. 3). Of interest, T5 exonuclease was able to degrade DNA molecules with the amino-group and also the small molecule MTX attached but could not cleave phosphodiester bonds when the 5'-end was blocked with a protein (hCA1 and Pr G).

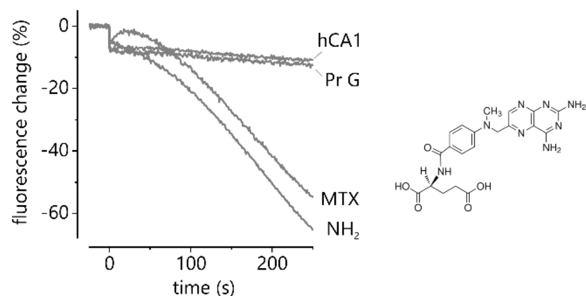

Figure 3 I Real-time measurement of T5 exonuclease (NEB) activity on different DNA substrates. The exonuclease is not active on DNA modified with human carbonic anhydrase 1 (hCA1) and protein G (Pr G), but displays activity on DNA with an amino terminus (NH2) and the small molecule methotrexate (MTX, shown on the right), an anticancer drug.

Conclusions

The activities of the 5' → 3' exonucleases T5 exonuclease and Lambda exonuclease could be analyzed using a switchSENSE molecular ruler assay. The assay can be applied for evaluating optimal working conditions (buffer, temperature). In addition, the tolerance of exonucleases for various substrates can be easily assessed (5'-phosphorylations, small-molecule modifications). By simply changing the orientation of the surface-immobilized DNA, the assay can be used likewise for 3' → 5' exonucleases.

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