Quantitative detection of a DNA ligase reaction on a quartz-crystal microbalance

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ABSTRACT
We report here kinetic analyses of DNA ligation by using a DNA-immobilized quartz-crystal microbalance (QCM), which enables in situ real-time monitoring of both the binding of ligase and ligation reaction on DNA strands, as mass changes.

INTRODUCTION
DNA ligase is one of the responsible enzymes for replication and repair of DNA. It has been used as a tool for in vitro DNA manipulation and cloning techniques. The reaction mechanism of DNA ligation has been mainly studied by measuring the combination of RI-labeled substrates and gel electrophoresis. Despite several improvements, these techniques have still some difficulties such as the requirement isotope-labeling of probes and of special techniques. To know total reaction mechanism, it is more useful to monitor the whole reaction steps such as both binding of the enzyme and ligation processes on DNA strands on the same device. We have reported that total reactions of DNA polymerase (binding of enzyme to DNA strands, elongation of complementary nucleotides along a template, and release of the enzyme from the polymerized DNA) could be followed and obtained kinetic parameters of each process by using a highly sensitive 27 MHz QCM.

In this paper, we describe that the bottom DNA-immobilized 27 MHz QCM is a useful tool to detect directly and quantitatively each step of ligation reaction in aqueous solution (see Figure 1).

RESULTS AND DISCUSSION
A schematic illustration of experimental setup and chemical structures of oligonucleotides are shown in Figure 1. Oligonucleotides having various terminal ends were immobilized on the one side of Au electrode of the 27 MHz QCM through avidin-biotin linkages. The coverage of DNA strands on the QCM was maintained to be 17% of the electrode area to avoid the steric hindrance for enzyme bindings.

Figure 2 shows typical times courses of frequency decreases (mass increases) of the bottom DNA-immobilized
Figure 2. Binding behavior of ligase onto various strands (20 °C, pH 7.5, 5 mM Tris-HCl, [ATP] = 1 mM, 40 mM NaCl, 6.6 mM MgCl₂, and 10 mM DTT).

(a) \( \text{TCCP} \), (b) \( \text{TC} \), (c) \( \text{TTCC} \), and (d) \( \text{TTCCP} \)

Table 1. Kinetic parameters for ligase binding on the bottom DNA having various terminal ends

<table>
<thead>
<tr>
<th>Terminal End</th>
<th>( k_{on} ) / ( 10^4 ) M⁻¹ s⁻¹</th>
<th>( k_{off} ) / ( 10^{-3} ) s⁻¹</th>
<th>( K_a ) / ( 10^8 ) M⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{TCCP} )</td>
<td>90</td>
<td>20</td>
<td>0.5</td>
</tr>
<tr>
<td>( \text{TC} )</td>
<td>20</td>
<td>8</td>
<td>0.3</td>
</tr>
<tr>
<td>( \text{TTCC} )</td>
<td>80</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>( \text{TTCCP} )</td>
<td>80</td>
<td>1</td>
<td>8</td>
</tr>
</tbody>
</table>

\( ^{a}20^\circ \text{C}, \text{pH} 7.5, 5 \text{ mM Tris-HCl, [ATP]} = 1 \text{ mM}, 40 \text{ mM NaCl, 6.6 mM MgCl}_2, \text{and 10 mM DTT} \)

Table 3. Kinetic parameters for ligase binding on the bottom DNA having various terminal ends

In conclusion, the QCM is useful to detect kinetically and quantitatively both the enzyme binding and ligation processes on the same device.

REFERENCES