Introduction

Binding of analyte (A) to the ligand (L) on the sensor chip can be described as:

\[
A_{\text{bulk}} \xrightarrow{k_m} A_{\text{surface}} + L \xrightarrow{k_a} AL
\]

The reaction is a two step event. First, the analyte is transferred out of the bulk solution towards the sensor chip surface. Second, the binding of the analyte to the ligand takes place. The first step is also known as mass transfer and is carried out by convection and diffusion (4). Both events have their own rate constants (\(k_m\) and \(k_a/k_d\)). The coefficient for mass transfer (\(k_m\)) is the same in both directions. With full or partial mass transfer, the diffusion from the bulk to the surface is slower than the rate of binding of the analyte to the ligand creating a shortage of analyte at the surface. The miniaturized flow cell in BIACORE reduces but cannot eliminate the potential contribution of mass transfer processes to the observed binding kinetics.

The mass transfer depends on the flow cell dimensions, the diffusion coefficient of the analyte (\(D\)) and the flow rate of the bulk solution (\(f\)). The binding rate depends on the association and dissociation constants of the analyte, local surface concentration of the analyte, density of binding sites and geometry (4). The diffusion coefficient (\(D\)) depends on the analyte molecular weight, asymmetry and solvent viscosity.

Although for kinetic measurements mass transfer in generally is avoided it can be exploited. Because under full and partial mass transfer limitation the binding rate is proportional to the analyte concentration, fast and simple concentration measurements can be done, even in crude mixtures (3, 6).

Mass transfer in practice

When the association rate constant (\(k_a\)) is greater than 1-10^6 M^-1s^-1 then the measured binding rate in some cases may reflect the transfer of analyte into the matrix rather than the reaction rate itself (2). In such cases, the binding rate often is constant during the initial phase of the interaction. After some time when most of the ligand sites are occupied the association shifts to a more kinetic association. Mass transfer limitations during the initial phase of binding are observed as a deviation from a straight line when dR/dt is plotted against R. Reaching equilibrium, kinetics take over and the dR/dt versus response plot will become more linear (4). Linear plots of dR/dt versus Response only indicate against significant mass-transfer when the analyte concentration is much higher than the dissociation constant \(K_D\). In addition, an increase in \(k_a\) with higher analyte concentrations is an indication for mass transfer limitation (4).
Controlling mass transfer

By varying the flow rate, a mass transfer limitation can be identified, since mass transfer is influenced by flow whereas the intrinsic reaction rate is flow independent. A higher flow rate will increase the association and dissociation rate constants until the mass transport limitation is slower than the kinetics.

<table>
<thead>
<tr>
<th>Ligand density 3000 RU</th>
<th></th>
<th>Ligand density 1260 RU</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fab concentration</strong></td>
<td><strong>kₐ (s⁻¹) x 10³</strong>: flow rate (µl/min)</td>
<td><strong>kₐ (s⁻¹) x 10³</strong>: flow rate (µl/min)</td>
</tr>
<tr>
<td>(nM)</td>
<td>5 20 50</td>
<td>5 20 50</td>
</tr>
<tr>
<td>8.25</td>
<td>1.43 1.87 2.18</td>
<td>26.6 2.73 2.92 3.24</td>
</tr>
<tr>
<td>16.5</td>
<td>1.57 1.95 2.23</td>
<td>53.2 2.86 2.98 3.23</td>
</tr>
<tr>
<td>33.0</td>
<td>1.77 2.07 2.31</td>
<td>83.1 2.98 3.02 3.23</td>
</tr>
<tr>
<td>66.0</td>
<td>1.93 2.16 2.38</td>
<td>113.0 3.04 3.04 3.24</td>
</tr>
<tr>
<td>132.0</td>
<td>2.01 2.21 2.42</td>
<td>219.0 3.18 3.07 3.28</td>
</tr>
</tbody>
</table>

Table: effect of analyte concentration on the dissociation rates constant of a Fab at different flow rates and ligand densities (5).

The balance between mass transfer and intrinsic reaction rate is influenced by the concentration of immobilized ligand. Reducing the ligand concentration reduces the binding flux and with the same diffusion flux staying constant the mass transfer limitation decreases (7). To be able to measure association rate constants as high as 1·10⁶ M⁻¹s⁻¹ the amount of immobilized ligand should be close or less than 2·10⁻¹⁴ mol. For a ligand of molecular weight of 10⁵ Da this corresponds to an immobilization level of approximately 2000 RU. Better even lower levels of immobilization (200-500 RU) help to keep intrinsic binding rates low so that mass transfer limitations are less marked (1).

Reducing the analyte concentration reduces the diffusion fluxes by the same magnitude resulting in a slower binding process. The kinetics will not change (7).

Mass transfer models

When analyzing the sensorgram it is tempting to add mass transfer to the equations. In some cases the fit will become better. However, always determine if there is any reason to do so.

Reaction equation

\[ \begin{align*}
A_{bulk} & \xrightarrow{k_i} A_{surface} + L & \xrightarrow{k_d} LA \\
\end{align*} \]

BiaEvaluation of BIACORE AB has two models, which takes mass transfer in account. In model 1: *Langmuir one to one interaction with mass transfer limitation*, the coefficient for mass transfer (kₚ) is calculated without the molecular size of the analyte. Because of this the mass transfer rate is in RU M⁻¹ s⁻¹, which is sometimes difficult to interpret.

In model 2: *1:1 binding with mass transfer – km*, the coefficient for mass transfer (kₚ) is calculated with the molecular size of the analyte and will give therefore the diffusion rate constant in m s⁻¹.
How To
Check for mass transfer?

References

1. BIACORE AB; Application note 301; Bia note; 1998.
6. Richalet, Secordel PM et all; Concentration measurement of unpurified proteins using biosensor technology under conditions of partial mass transport limitation; Analytical Biochemistry; 249 (2): 165-173; 1-7-1997.

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