Fractal Analysis of Binding and Dissociation Kinetics of Thrombin on Biosensor Surfaces

Atul M. Doke* **and Ajit Sadana**
Chemical Engineering Department
University of Mississippi
PO Box 1848
University, MS-38677

Introduction

The prevention of clot formation due to thrombin is an important area of investigation. Deinum et al. (2002) have recently presented the binding and dissociation kinetics of three potent thrombin inhibitors, Inogatran, Malagatran, and CH-248 in solution to human α-thrombin immobilized on a surface plasmon resonance (SPR) biosensor.

The BIACORE biosensor based on the surface plasmon resonance (SPR) principle is being used to identify thrombin inhibitors. It is important to obtain kinetic and dissociation rate coefficients for the thrombin inhibitor interactions in order to obtain better physical insights into these reactions. An analysis of the binding and dissociation interaction, wherein human-thrombin is immobilized on the sensor chip of a surface plasmon resonance biosensor and the corresponding analyte is in solution, is an initial step in this direction.

The SPR biosensor protocol analyzes the binding (and dissociation where ever applicable) kinetic curves using classical saturation models involving analyte-receptor binding generally under diffusion-free conditions and assuming that the receptors are homogeneously distributed over the sensor surface. Computer programs and software that come with the equipment provide values of the binding (and the dissociation) rate coefficients (Biacore, 2002). Though a careful analysis and experimental protocol may eliminate or minimize the influence of diffusional limitations; realistically speaking, it is more appropriate to include a heterogeneous distribution of receptors on the sensor surface. It is for this reason that the computer programs and the software indicated above, and provided by the manufacturers (Biacore, 2002) are not used in the present analysis. Instead, an alternate analysis is used that incorporates theoretically in the kinetic model the heterogeneous distribution of receptors on the sensing surface. This is a more realistic approach to the real-life situation. This would become more significant if the degree of heterogeneity of the receptors on the surface affects the binding and the dissociation rate coefficients to a large degree.

One possible way of accounting for the presence of heterogeneity that exists on the surface is by using fractals. A characteristic feature of fractals is the self-similarity at different levels of scale. Fractals are particularly useful for this type of analysis because they help characterize the heterogeneity that exists on the surface by a lumped parameter, the fractal dimension.

In this manuscript we use fractals to analyze the binding and dissociation of the thrombin inhibitor CH-248 in solution to human α-thrombin immobilized on a SPR biosensor surface (Deinum et al., 2002). The data has been analyzed before by the software that comes along with the SPR biosensor (Deinum et al., 2002). But, in the analysis to be presented in this manuscript we include (and as indicated above) the involvement of heterogeneity on the receptor surface. This aspect has been neglected in the previous study (Deinum et al., 2002). Binding and dissociation rate coefficients, affinity values, as well as fractal dimension values for the binding and dissociation phases are provided for the thrombin-thrombin inhibitor system.

Theory

Single-fractal analysis

Binding rate coefficient

Havlin (1989) indicates that the diffusion of a particle (analyte [Ag]) from a homogeneous solution to a solid surface (e.g. receptor [Ab]-coated surface) on which it reacts to form a product (analyte-receptor complex; (Ab.Ag)) is given by:

$$\mathbf{(Ab.Ag)} \approx \begin{cases} \mathbf{t}^{(3-D_{f,bind})/2} = \mathbf{t}^{p} & \mathbf{t < t_c} \\ \mathbf{t}^{1/2} & \mathbf{t > t_c} \end{cases} \qquad \mathbf{(1)}$$

Here $D_{f,bind}$ is the fractal dimension of the surface during the binding step. t_c is the cross-over value. Above the characteristic length, r_c, the self-similarity of the surface is lost and the surface may be considered homogeneous. Above time, t_c, the surface may be considered homogeneous, since the self-similarity property disappears, and 'regular' diffusion is now present. For a homogeneous surface where D_f is equal to 2, and when only diffusional limitations are present, $p = ½$ as it should be.

Dissociation Rate Coefficient **:**The diffusion of the dissociated particle (receptor [Ab] or analyte [Ag]) from the solid surface (e.g., analyte [Ag]-receptor [Ab]) complex coated surface) into solution may be given, as a first approximation by:

$$\mathbf{(Ab.Ag)} \approx \mathbf{-t^{(3-D_{f,diss})/2} = t^{p}} \qquad \mathbf{(t>t_{diss})} \qquad \mathbf{(2)}$$

Here $D_{f,diss}$ is the fractal dimension of the surface for the dissociation step. This corresponds to the highest concentration of the analyte-receptor complex on the surface. Henceforth, its concentration only decreases. The dissociation kinetics may be analyzed in a manner 'similar' to the binding kinetics.

Results

A fractal analysis will be applied to the data obtained for thrombin-thrombin inhibitor complex binding and dissociation taken from the literature (Deinum et al., 2002). The fractal analysis is only one possible approach to analyzing the diffusion-limited binding kinetics assumed to be present in the systems analyzed.

Deinum et al. (2002) have analyzed the binding of different low-molecular mass,

active-site-directed thrombin inhibitors (299-575 Da) to human α-thrombin using a BIACORE surface plasmon resonance (SPR) biosensor.

Figure 1a-d shows the binding and dissociation of 24 nM CH-248 in solution at 6°C, 15°C, 25°C, and 30°C to human-thrombin immobilized on a biosensor chip surface. A single-fractal analysis is adequate to describe the binding and the dissociation kinetics in first three cases while at 30°C a dual-fractal analysis is required to adequately describe the binding kinetics. The values of (a) the binding rate coefficient, k, and the dissociation rate coefficient, k_d are given in Table 1a, and (b) the values of the fractal dimensions for the binding phase, D_f and for the dissociation phase, D_{fd} are given in Table 1b.

It is of interest to compare the binding and the dissociation rate coefficient values for 24 nM CH-248 at 6°C and at 15°C. In both cases, a single-fractal analysis is adequate to describe the binding as well as the dissociation kinetics. An increase in the temperature from 6°C to 15°C, leads to (a) an increase in the binding rate coefficient, k value by a factor of 1.96 from 0.1136 to 0.2222, and (b) to a decrease in the dissociation rate coefficient, k_d value by 19.1% from 0.0324 to 0.0262.

For the 24 nM CH-248 concentration in solution, the affinity, K value is 8.48. It is of interest to note that for the 24 nM CH-248 concentration in solution, an increase in the temperature from 6 to 15°C, leads to an increase in the affinity, K value by a factor of 2.42 from 3.506 to 8.48.

Table 2 shows the affinity values obtained for CH-248 at 6, 15, 25, and 30 °C for its binding in solution to human α-thrombin immobilized on a sensor chip surface. The highest affinity value (equal to 8.48) is at 15°C, and the lowest value (equal to 0.13) is at 25°C.

Conclusions

A fractal analysis of the binding and dissociation of potent thrombin inhibitors in solution to human α-thrombin immobilized on a SPR sensor chip surface provides a quantitative indication of the state of disorder or the degree of heterogeneity on the biosensor surface. The analysis of both the binding as well as the dissociation steps provide a more complete picture of the reaction occurring on the surface besides providing a value of the affinity, K. This is the ratio of the rate coefficient for binding, k, and for dissociation, k_d, steps. This is important, as indicated by Deinum et al. (2002), that analyte inhibitors (for example, for thrombin) selected as drug candidates are often chosen on the basis of K_i (= 1/K) values. In our case, the lowest K_i value for 24 nM CH-248 occurs at 15 °C, and is equal to 0.118.

The fractal dimension value provides a quantitative measure of the degree of heterogeneity that exists on the sensor chip surface for the thrombin inhibitor-thrombin interactions. The degree of heterogeneity for the binding and dissociation phases is, in general, different. Both types of examples are presented wherein either a single- or a dual-fractal analysis is required to describe the binding kinetics. The dissociation kinetics

is adequately described by a single-fractal analysis. The dual-fractal analysis is used only when the single-fractal analysis did not provide an adequate fit (sum of least squares less than 0.98). This was done by regression provided by Quattro Pro 8.0 (Corel Quattro Pro, 1997).

References

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Deinum, J., Gustafsson, L., Gyzander, E., Kullman-Magnusson, M., Edstrom, A., & Karlson, R. (2002). *Analytical Biochemistry*, *300*, 152-162.

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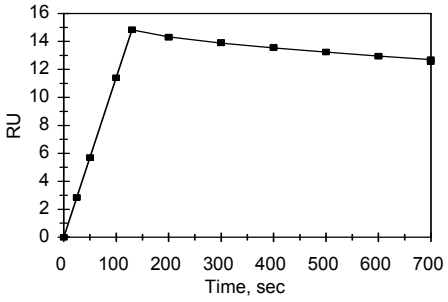
Figures



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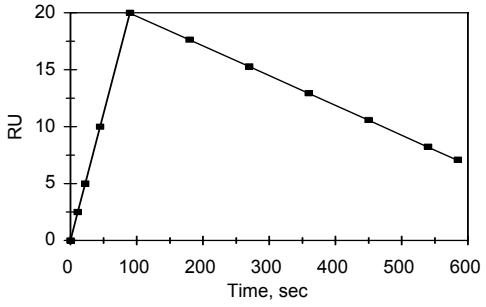

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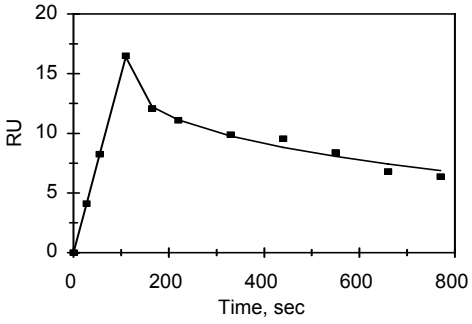

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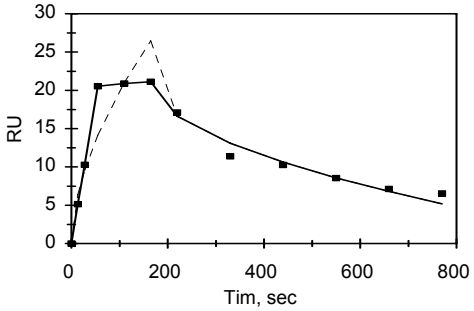

1d

Figure 1: Binding of a potent thrombin inhibitor, CH-248, H-(R)-Cha-Pro-Arg [$CH_2OCH_2CF_3$] (520 Da) in solution at different concentrations and at different temperatures to human α-thrombin immobilized on a sensor chip surface: (a) 24 nM, 6 °C (b) 24 nM, 15 °C (c) 24 nM, 25 °C (d) 24 nM, 30 °C

Tables

Table 1a: Rate coefficients for the binding and the dissociation phase for CH-248 in solution to thrombin immobilized on a sensor chip surface (Deinum et al., 2002)

Analyte in solution/Receptor on surface	Temperature	k	k1	k2	kd	kd1	kd2
24 nM CH-248/ immobilized thrombin	6°C	0.1136 ±0.0006	na	na	0.0324 ±0.0019	na	na
24 nM CH-248/ immobilized thrombin	15°C	0.2222 ±0.0	na	na	0.0262 ±0.00002	na	na
24 nM CH-248/ immobilized thrombin	25°C	0.1494 ±0.00007	na	na	1.1499 ±0.0734	na	na
24 nM CH-248/ immobilized thrombin	30°C	0.1137 ±0.00002	na	na	0.2031 ±0.0397	0.08669 ±0.0223	0.8289 ±0.020

Table 1b: Fractal dimensions for the binding and the dissociation phase for CH-248 in solution to thrombin immobilized on a sensor chip surface (Deinum et al., 2002)

Analyte in solution/Receptor on surface	Temperature	D_f	D_{f1}	D_{f2}	D_{fd}	D_{fd1}	D_{fd2}
24 nM CH-248/ immobilized thrombin	6°C	1.0 ±0.0008	na	na	1.6766 ±0.0682	na	na
24 nM CH-248/ immobilized thrombin	15°C	1.0 ±2.2E-15	na	na	1.0012 ±0.0011	na	na
24 nM CH-248/ immobilized thrombin	25°C	2.0 ±0.001	na	na	2.3456 ±0.0556	na	na
24 nM CH-248/ immobilized thrombin	30°C	1.0042 ±0.00036	na	na	1.6574 ±0.1812	0.08669 ±0.0223	0.8289 ±0.020

Table 2: Affinity values for a potent thrombin inhibitor, 24 nM CH-248 (analyte) in solution to human α-thrombin (receptor) immobilized on a BIACORE sensor chip surface

Temperature °C	Affinity
5	3.51
15	8.48
25	0.13
30	0.559