Fractal Analysis of Heparin-Protein Interaction Studies Occurring on Biosensor Surfaces

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Introduction

Heparin interacts with a wide variety of proteins and peptides (Lindahl, 1989). Zhang et al. (2002) indicate that it is a proteoglycan that is composed of highly sulfated linear polysaccharides of alternating uronic acid and glucosamine. In the human body heparin and its structurally-related heparan sulfate are the most acidic polysaccharides.

Zhang et al. (2002) indicate that heparin was previously immobilized on a sensor chip surface through an avidin-biotin bridge system. They emphasize that heparin binding proteins react specifically with avidin (Marks et al., 2001). Zhang et al. (2002) noted further that the streptavidin-biotin heparin chip was also unstable. They along with Van Delden et al. (1997) have since then analyzed heparin-protein interactions using immobilized albumin-heparin conjugates. Zhang et al. (2002) have recently analyzed heparin-protein interactions on a biochip using a preformed albumin-heparin conjugate.

The system by its very design is heterogeneous (for example, the receptors immobilized on the biosensor surface may exhibit some heterogeneity that is, surface roughness), and often other factors such as mass transport limitations (unless they are carefully eliminated or minimized) play a significant role and further complicate the design (especially its kinetic aspects) of the assay or the correct interpretation of the assay results. One possible way of monitoring for the presence of diffusional limitations and the heterogeneity that exists on the surface is by using fractals. A characteristic feature of fractals is self-similarity at different levels of scale. Fractals exhibit dilatational symmetry. Fractals are disordered systems, and the disorder is described by nonintegral dimensions (Pfeifer and Obert, 1989). Fractals have previously been used to analyze the binding and dissociation kinetics of a wide variety of analyte-receptor systems (Sadana, 2001). Fractals are particularly useful for this type of analysis because they help to characterize the heterogeneity that exists on the surface by a lumped parameter, the fractal dimension.

In this manuscript we provide an alternate analysis for the binding and dissociation of heparin binding proteins in solution to covalently immobilized heparin on a SPR biochip using a preformed albumin-heparin conjugate (Zhang et al., 2002). Binding and dissociation rate coefficients, as well as fractal dimension values for the binding and the dissociation phases are provided wherever applicable.

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:

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

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

The Dengue virus is a mosquito-transmitted virus that causes a febrile disease in humans (Chen et al., 1997). The authors further indicate that the Dengue virus envelope protein utilizes a sulfated form of heparin as a receptor. Figure 1a-d show respectively the binding and dissociation of 400 nM, 500 nM, 800 nM, and 1000 nM Dengue virus envelope protein in solution to heparin immobilized on a sensor chip surface (Zhang et al., 2002). Zhang et al. (2002) do indicate that there is tight binding between the Dengue virus envelope protein and the heparin. The binding and the dissociation phases shown in Figure 1a-d can be adequately modeled using a single-fractal analysis. The values of (a) the binding rate coefficient, k for a single-fractal analysis, and (b) the dissociation rate coefficient, k_d for a single-fractal analysis are given in Table 1a. The values of (a) the fractal dimension for the binding phase, D_f, for a single-fractal analysis, and (b) the fractal dimension, D_{fd} for the dissociation phase for a single-fractal analysis are given in Table 1b.

It is of interest to note that as the Dengue virus envelope protein concentration in solution increases by a factor of two from 400 to 800 nM (a) the binding rate coefficient, k increases by as factor of 4.28 from a value of 2.643 to 11.31, and (b) the fractal dimension in the binding phase, D_f increases by a factor of 1.15 from a value of 1.3926 to 1.5998. Changes in the binding rate coefficient appear to be in the same direction as changes in the Dengue virus envelope protein concentration and in the fractal dimension or the degree of heterogeneity on the sensor chip surface.

For the binding of 400 to 1000 nM Dengue virus envelope protein in solution to heparin immobilized on a sensor chip surface, Figure 2a shows the increase in the binding rate coefficient, k with an increase in the fractal dimension, D_f. For the data in Table 1a,b and in Figure 2a the binding rate coefficient, k is given by:

$$k = (0.0657 \pm 0.0066)D_f^{11.0457 \pm 0.8714} \qquad (3)$$

The binding rate coefficient for a single-fractal analysis, k is extremely sensitive to the degree of heterogeneity that exists on the surface as noted by the very high value (equal to 11.0457) of the order of dependence of k on D_f.

For the binding of 400 to1000 nM Dengue virus envelope protein in solution to heparin immobilized on a sensor chip surface, Figure 2b shows the increase in the binding rate coefficient, k with an increase in the Dengue virus envelope protein concentration in solution. For the data in Table 1a,b and in Figure 2b, the binding rate coefficient, k is given by:

$$k = (0.000176 \pm 0.000043)[\text{Dengue virus envelope protein}]^{1.6271 \pm 0.3034} \qquad (4)$$

The binding rate coefficient for a single-fractal analysis, k is quite sensitive to the Dengue virus envelope protein concentration in solution since it exhibits an order of dependence that lies between first and second (equal to 1.6271). The non-integer order of dependence exhibited reinforces the fractal nature of the system.

Conclusions

A fractal analysis is presented for the binding and dissociation of heparin binding proteins in solution to covalently immobilized heparin on a SPR biochip using a preformed albumin-heparin conjugate (Zhang et al., 2002). The binding and dissociation of Dengue virus envelope protein was analyzed. A single-fractal analysis was used to adequately model the binding and the dissociation kinetics. The regression analysis was performed using Corel Quattro Pro (1997).

References

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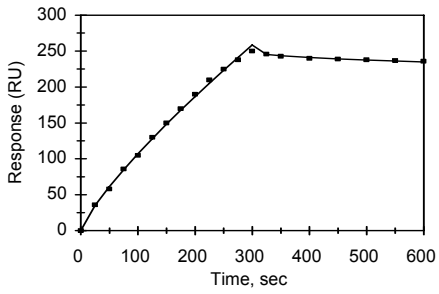
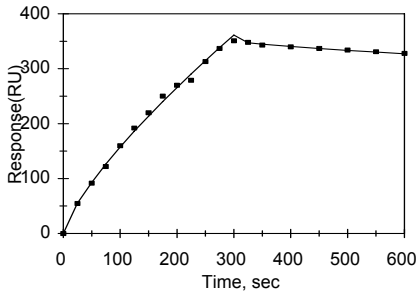
Figures



1a



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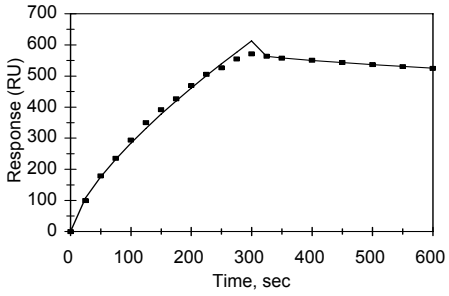

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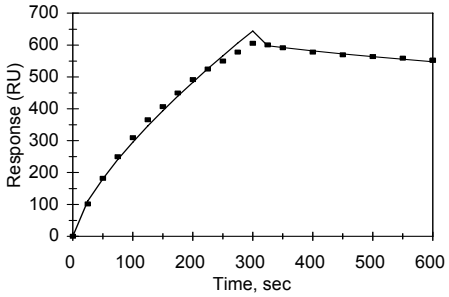
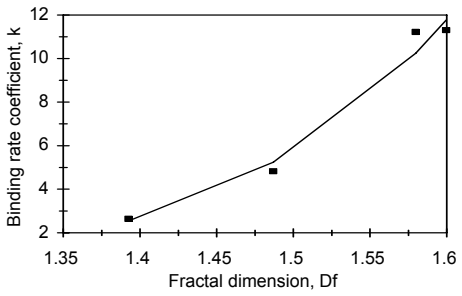
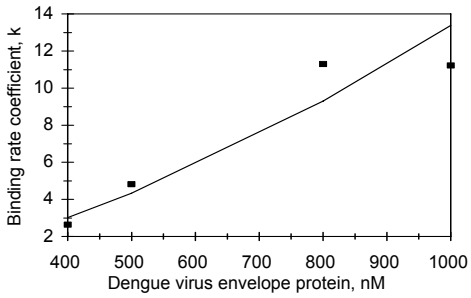


1d

Figure 1: Binding of different concentrations of Dengue virus envelope protein concentration in solution (in nM) to heparin immobilized on a sensor chip surface (Zhang et al., 2002): (a) 400 (b) 500 (c) 800 (d) 1000



2a



2b

Figure 2: (a) Increase in the binding rate coefficient, k with an increase in the fractal dimension, D_f
(b) Increase in the binding rate coefficient, k with an increase in the Dengue virus envelope protein concentration (in nM) in solution

Tables

TABLE 1a: Rate Coefficient Values for the Binding and the Dissociation Phase for Dengue Virus Envelope Protein and Heparin Interaction (Zhang et al., 2002)

Dengue virus envelope protein (analyte in nM) in solution/Heparin (receptor) on sensor chip surface	k	k_d
400	2.6425 ± 0.0707	9.844 ± 0.103
500	4.8282 ± 0.1373	0.3244 ± 0.053
800	11.307 ± 0.505	0.8876 ± 0.0065
1000	11.228 ± 0.539	0.5747 ± 0.0644

TABLE 1b: Fractal Dimension Values for the Binding and the Dissociation Phase for Dengue Virus Envelope Protein and Heparin Interaction (Zhang et al., 2002)

Dengue virus envelope protein (analyte in nM) in solution/Heparin (receptor) on sensor chip surface	D_f	D_{fd}
400	1.3926 ± 0.0210	2.0484 ± 0.0899
500	1.4870 ± 0.0222	1.4938 ± 0.1356
800	1.5998 ± 0.0348	1.6018 ± 0.0273
1000	1.5798 ± 0.0372	1.3808 ± 0.0956