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A fractal analysis for the binding of riboflavin binding protein to riboflavin immobilized on a SPR biosensor

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Abstract

A fractal analysis is presented for the binding of riboflavin binding protein (RBP) in solution to riboflavin (Rf) derivative immobilized on a sensor chip. The influence of complexation of RBP with Rf in solution on its binding kinetics to immobilized Rf is also analyzed. A better understanding of the kinetics provides physical insights into these interactions and complements the original work of [I. Caelen, A. Kalman, L. Wahlstrom, Anal. Chem. 76 (2004) 137]. These authors [I. Caelen, A. Kalman, L. Wahlstrom, Anal. Chem. 76 (2004) 137] used a surface plasmon resonance (SPR) biosensor where no kinetic binding rate coefficients were provided. Numerical values of the binding rate coefficient are presented and linked to the degree of heterogeneity made quantitative by the fractal dimension, D_f, on the sensor chip surface. Both, single- and dual-fractal analysis are used to provide an adequate fit. The results presented here are consistent with the original work [I. Caelen, A. Kalman, L. Wahlstrom, Anal. Chem. 76 (2004) 137]. Predictive relations are presented for the binding rate coefficient, k, and for the fractal dimension, D_f, as a function of the RBP concentration in solution. The binding rate coefficient, k, is very sensitive to the degree of heterogeneity that exists on the surface (order of dependence equal to 6.583). In general, the changes in the degree of heterogeneity (fractal dimension, D_f) on the sensor chip surface and in the binding rate coefficient, k, are in the same direction. Both the fractal dimension, D_f, and the binding rate coefficient, k, exhibit rather low orders of dependence equal to 0.087 and 0.576, respectively, on the RBP concentration in solution. At the lowest RBP concentration (0.1 μg/mL) in solution, a dual-fractal analysis is required to describe the binding kinetics, whereas in the 0.2–10.0 μg/mL RBP concentration range in solution a single-fractal analysis is adequate. This indicates that there is a change in the binding mechanism at the lowest RBP concentration in solution (0.1 μg/mL).

Keywords: Fractals; Biosensor; Binding kinetics; Riboflavin; Riboflavin binding protein

1. Introduction

A promising approach in the understanding of biomolecular interactions is the development of biosensors. These biosensors are finding increasing application in the area of biotechnology, physics, chemistry, medicine, aviation, oceanography, environmental control, safe guarding of civilian infrastructures, protection of military and civilian personnel, and food product applications. Some of the not too distant future sensor applications include biomedical health monitoring, monitoring of terrestrial and aquatic environments, surveillance technology, and crisis management systems [1]. These future applications may or may not involve biological molecules as detectors; thus they are classified as sensors. In the not too distant past the mad cow incident in northwestern United States underscored the importance of protecting the food products fit for human consumption, and the need to identify the possible presence of pathogens in these products. Caelen et al. [2] have very recently developed a surface plasmon resonance (SPR) biosensor method for the detection of riboflavin (Rf) in milk-based products. These authors indicate that (a) riboflavin is an essential protein in human nutrition, and (b) the human body obtains a quarter of the average Rf intake from milk and milk-based products.

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Caelen et al. [2] used the Biacore Q system (Biacore AB, Uppsala, Sweden) and a carboxymethylated dextran sensor chip (CM5, Biacore AB), but did not provide a kinetic analysis and values of the binding rate coefficients and affinities. Lofas and Johnsson [3] indicate that the SPR technique permits a kinetic analysis, and the software that comes with it does provide values of the binding rate coefficients and the affinities (if the dissociation phase, if present, is also analyzed). The SPR software that provides the kinetic analysis does not incorporate the presence of diffusional limitations and the degree of heterogeneity present on the SPR chip surface. The SPR manufacturers (Biacore AB) claim that diffusional limitations may be minimized if the SPR biosensor is run properly. Also, for the analysis of low-molecular weight compounds, such as vitamins, as is analyzed by Caelen et al. [2] for riboflavin, an indirect assay is required [4–7].

In this manuscript, we re-analyze using fractal analysis the diffusion-limited binding data of riboflavin binding protein (RBP) in solution to riboflavin derivative immobilized on an SPR biosensor chip surface [2]. Fractal analysis has been used previously to analyze the diffusion-limited analyte–receptor reactions occurring on heterogeneous biosensor surfaces [8–10]. Caelen et al. [2] indicate that natural riboflavin does not bind to the chip surface since it does not contain the required chemical groups. Thus, it needs to be modified by introducing reactive ester groups at the *N*-3 position of the molecule [11]. Values of the binding rate coefficient and the fractal dimension, D_f, are provided. The fractal dimension, D_f, is a quantitative measure of the degree of heterogeneity on the surface. An increase in the value of the fractal dimension on the surface indicates an increase in the degree of heterogeneity on the sensor chip surface.

2. Theory

Havlin [12] has reviewed and analyzed the diffusion of reactants towards fractal surfaces. The details of the theory and the equations involved for the binding and the dissociation phases for analyte–receptor binding are available [8]. The details are not repeated here; except that just the equations are given to permit an easier reading. These equations have been applied to other analyte–receptor reactions occurring on biosensor surfaces [9,10]. Here we will attempt to apply these equations to the binding of riboflavin binding protein in solution to riboflavin derivative immobilized on an SPR biosensor chip surface [2]. For most applications, a single- or a dual-fractal analysis is often adequate to describe the binding kinetics.

2.1. Single-fractal analysis

2.1.1. Binding rate coefficient

Havlin [12] 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:

$$(\mathrm{Ab}\cdot\mathrm{Ag}) \approx \begin{cases} t(3 - D_{\mathrm{f,bind}})/2 = t^{p} & t < t_{\mathrm{c}} \\ t^{1/2} & t > t_{\mathrm{c}} \end{cases} \quad (1a)$$

here $D_{\mathrm{f,\,bind}}$ or D_{f} (used later on in the manuscript) is the fractal dimension of the surface during the binding step. The t_{c} is the cross-over value. Havlin [12] indicates that the cross-over value may be determined by ${r_{\mathrm{c}}}^2 \sim t_{\mathrm{c}}$. 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, $p = 1/2$ as it should be. Another way of looking at the $p = 1/2$ case (where $D_{\mathrm{f,\,bind}}$ is equal to two) is that the analyte in solution views the fractal object, in our case, the receptor-coated biosensor surface, from a 'large distance.' In essence, in the binding process, the diffusion of the analyte from the solution to the receptor surface creates a depletion layer of width $(Đt)^{1/2}$ where Đ is the diffusion constant. This gives rise to the fractal power law, (analyte–receptor) $\sim t^{(3-D_{\mathrm{f,\,bind}})/2}$. For the present analysis, t_{c} is arbitrarily chosen and we assume that the value of the t_{c} is not reached. One may consider the approach as an intermediate 'heuristic' approach that may be used in the future to develop an autonomous (and not time-dependent) model for diffusion-controlled kinetics.

2.2. Dual-fractal analysis

2.2.1. Binding rate coefficient

Sometimes, the binding curve exhibits complexities and two parameters (k, D_{f}) are not sufficient to adequately describe the binding kinetics. This is further corroborated by low values of r^2 factor (goodness-of-fit). In that case, one resorts to a dual-fractal analysis (four parameters; k_1, k_2, D_{f1}, and D_{f2}) to adequately describe the binding kinetics. The single-fractal analysis presented above is thus extended to include two fractal dimensions. At present, the time ($t = t_1$) at which the 'first' fractal dimension 'changes' to the 'second' fractal dimension is arbitrary and empirical. For the most part, it is dictated by the data analyzed and experience gained by handling a single-fractal analysis. A smoother curve is obtained in the 'transition' region, if care is taken to select the correct number of points for the two regions. In this case, the product (antibody–antigen; or analyte–receptor complex, Ab·Ag or analyte·receptor) is given by:

$$(\mathrm{Ab}\cdot\mathrm{Ag}) \approx \begin{cases} t^{(3-D_{\mathrm{f1,bind}})/2} = t^{p1} & t < t_1 \\ t^{(3-D_{\mathrm{f2,bind}})/2} = t^{p2} & t_1 < t < t_2 \\ t^{1/2} & t > t_c \end{cases} \quad (1b)$$

In some cases, a triple-fractal analysis with six parameters (k_1, k_2, k_3, D_{f1}, D_{f2}, and D_{f3}) may be required to adequately