FRACTAL DIMENSION ANALYSIS OF FACTOR X ACTIVATION IN THE PRESENCE OF TISSUE FACTOR–FACTOR VIIa COMPLEX IN A CONTINUOUS FLOW REACTOR

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SUMMARY

The characteristics of a phospholipid surface are of major importance in the activation of factor X in the presence of tissue factor-factor VIIa (TF–VIIa) complex. A possible tool which provides a measure of the surface corrugation and roughness is the fractal dimension analysis. This paper uses the fractal characterization of a phospholipid surface to develop a model for analyzing surface based enzymatic reaction data. The modeling indicates that the fractal dimension (D) of a phospholipid surface is a function of the wall shear rate. The results also indicate that the fractal dimension of the phospholipid surface decreases from approximately 2.9 to 1.4 as the wall shear rate increases from 50 to 1600 sec⁻¹. At the same time the factor Xa production increases from 1.9 to 5.8 pmoles/(min·cm²).

The results of the fractal dimension analysis clearly indicate that the surface roughness of a phospholipid surface may have a significant effect on factor X activation.

INTRODUCTION

Kinetic studies in blood coagulation have in the past been generally restricted to batch and steady state type studies of individual reaction pathways. While such studies have told us a lot about the kinetics and reaction mechanism, there has always been a problem explaining the results of flow studies using classical reaction kinetics. One classical example of this type of problem is the effect of shear rate on the activation of factor X in the presence of tissue factor–factor VIIa (TF:VIIa) complex in a tubular flow reactor. Gemmell et al. (1988,1990) have reported that the activation of factor X is highly dependent on local flow conditions. In contrast with classical enzyme kinetic theory, there is a three-fold increase in maximum...
reaction velocity ($V_{\text{max}}$) as shear rate increased from 25 to 300 sec$^{-1}$.

The current kinetic and transport models provide an insight about the dependence of such system on the physical characteristics of the tube, the diffusion of substrate to the reaction surface and other hydrodynamic conditions. The basic assumption in these types of models is that the surface kinetics are assumed to be independent of reaction surface topography. In contrast, the kinetic activity of a reaction surface should strongly depend on its physical and chemical properties during the course of the surface reaction.

In general, it can be argued that under various conditions, surface can either activate or deactivate the kinetic efficiency of a given enzymatic reaction.

It is the objective of this paper to make an effort to explain the departure from classical enzyme kinetic theory using fractal dimension analysis.

**MATERIALS AND METHODS**

The instrumental setup for the automated continuous flow reactor system is as previously described by Contino et al. 1991. Borosilicate microcapillary tubes (0.27 mm inner diameter, length of 128 mm, 1.08 cm$^2$ inner surface area, and 7.3 µl volume) were cleaned by boiling in dilute detergent (0.2 % wt/wt Sparkleen, Fisher Scientific Co.) for 30 minutes and rinsed with several changes of distilled water. The tubes were then dried in an oven at 120°C and stored under dry conditions. The cleaned tubes were then filled with a suspension of 1 mM phospholipid vesicles containing 4.0 mM tissue factor in HEPES buffer (0.01 M HEPES, 0.14 M NaCl, 1 mg/ml bovine serum albumin, pH 7.5) and incubated for 20 minutes at room temperature. The phospholipid was expressed as its monomeric concentration. The phospholipid vesicles (100 to 150 nm in diameter) consisted of 30% (wt/wt) bovine brain phosphatidylserine and 70% (wt/wt) egg phosphatidylcholine (TF:PS/PC) (both lipids from Avanti Polar Lipids, Pelham, AL, USA). Thereafter, the tubes were flushed with HEPES buffer for 5 minutes with a volumetric flow rate of 116 µl/min, corresponding to a wall shear rate of 1000 sec$^{-1}$ at 37°C.

Perfusion of the capillary tubes with reaction mixture:
The coated tubes were then perfused with a reaction mixture containing factor VIIa (10 nM), factor X (800 nM), and Ca$^{2+}$ (5 mM) in HEPES buffer at 7.5 pH at a predetermined shear rate. The perfusate was collected at timed intervals into wells of ELISA plates containing 75 µl of 50 mM EDTA, 7.5 pH. The factor Xa concentration was measured after addition of 25 µl of 10 mM of