Computer Simulation of the Fibrinolytic Function of Lumbrokinase

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Lumrokinase is known to be very strong and novel fibronolytic enzyme could be extracted from the earthworm, Lumbricus rubellus [1]. Recently, a new technology was developed for improvement of the antithrombotic characteristics using Lumbrokinase immobilization onto the polymeric surface [2]. The fibronolytic function of the immobilized Lumbrokinase is investingating now. We tried to find some characteristics of the immobilized Lumbrokinase through computer simulation in this paper.

Materials and Methods

Enzyme kinetics

Immobilized lumbrokinase will degrade fibrin and fibrinogen directly. We can consider the fibrinolytic function of immobilized lumbrokinase from the adsorption characteristics of fibrinogen. The model of lumbrokinase function can be represented as follows, which was based on the Michaelis-Menten kinetics;

$$LK + F \underset{k_2}{\overset{k_1}{\rightleftarrows}} LKF \xrightarrow{k_3} LK + FDP$$

where LK represents the lumbrokinase, and F fibrinogen and/or fibrin, LKF the Lumbrokinase -Fibrinogen complex, and FDP the fibrinogen degraded products. k_1, k_2, and k_3 are rate constants. If the amount of the FDP is measured, it will follow the Michaelis- Menten curve. The surface density of immobilized lumbrokinase will also influence on its fibrinolytic activity.

Surface modelling and random sequential adsorption

The lumbrokinase immobilization can be modelled by modifiing the random lattice polymer surface[3]. The adsorption process was performed as follows;

1. Select a random position in N x N lattice in the lumbrokinase immobilized surface.
2. Try fibrinogen adsorption by considering only the number of the hydrophobic points in the fibrinogen box.
3. After a given interval is passed, the adsorbed fibrinogen is desorbed and the modified surface is recovered.
4. Repeat the above three steps until an equilibrium state reached.

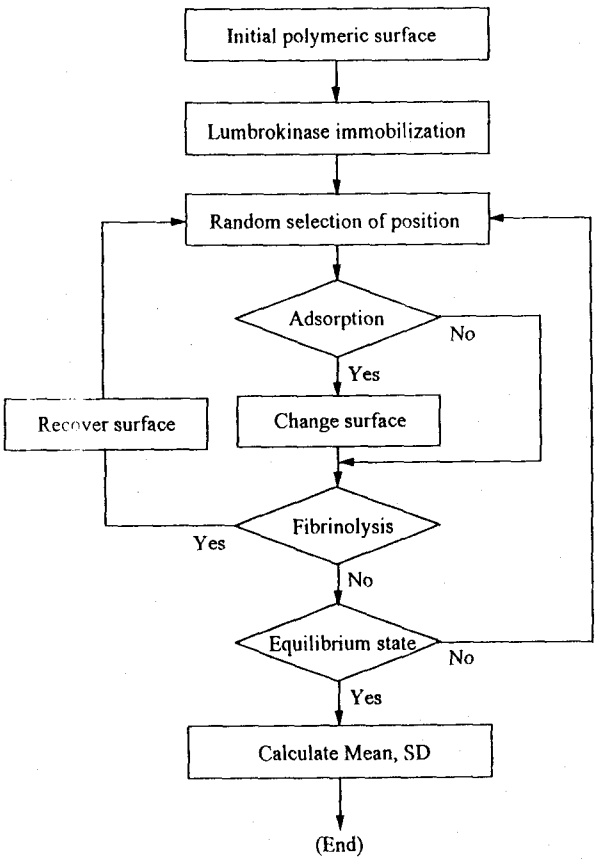

Figure 1 Flow chart of the fibrinogen adsorption on the lumbrokinase immobilized surface

The kinetic rate constant can be adjusted by the time interval of the fibrinogen degradation. Simulation was performed under the different number of the immobilized lumbrokinase and the several time intervals. According to the size and the molecular weight of the lumbrokinase, it was modelled as one point which was randomly positioned in the N x N polymeric lattice. Since there are some observations that lumbrokinase may promote fibrinogen adsorption, two kinds of models of the fibrinogen adsorption on the lumbrokinase-immobilized surface were developed. One is that initial adsorption of fibrinogen is promoted by the immobilized lumbrokinase. The other is that no interaction between fibrinogen and immobilized lumbrokinase is assumed. The schematic diagram of the simulation of the lumbrokinase-immobilized surface is shown in Figure 1. Equilibrium state was checked by the increase of adsorbed fibrinogen. Simulations were performed on the IBM-PC 486 with Borland C language.

Results and Discussion

The kinetic behavior of the immobilized lumbrokinase was simulated by adjustment of the equlibrium threshold. The kinetics are shown in Figure 2 in which the adsorption time is increased with the increase of the equilibrium threshold. The kinetics of the immobilized lumbrokinase was a little deviated from the Michales-Menten curve. The rate constant of the immobilized lumbrokinase was also changed by adjustment of the waiting time to desorption of fibrinogen. But the effect of the rate constant was not significant as shown in Figure 3, which implies that the equilibrium constant is much larger than the rate constant used in this simulation. Two interaction models of the immobilized lumbrokinase and fibrinogen demonstrated different results as shown in Figure 4. This insist is based on the surface concentration of the immobilized enzymes, but some experiments are needed to obtain the accurate rate constant of the lumbrokinase for more realistic simulations. This simulation techniques can be modified to other adsorption kinetics on the polymeric surface[4].

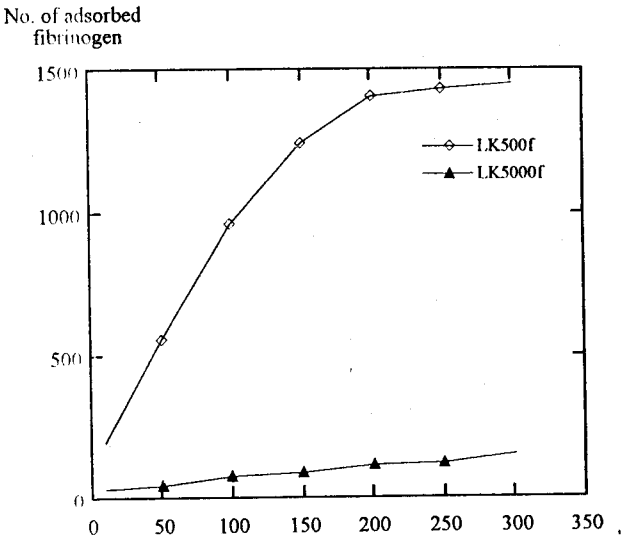

Figure 2 Fibrinogen adsorption kinetics on the lumbrokinase immobilized surface (Kinetic rate constant: 100)

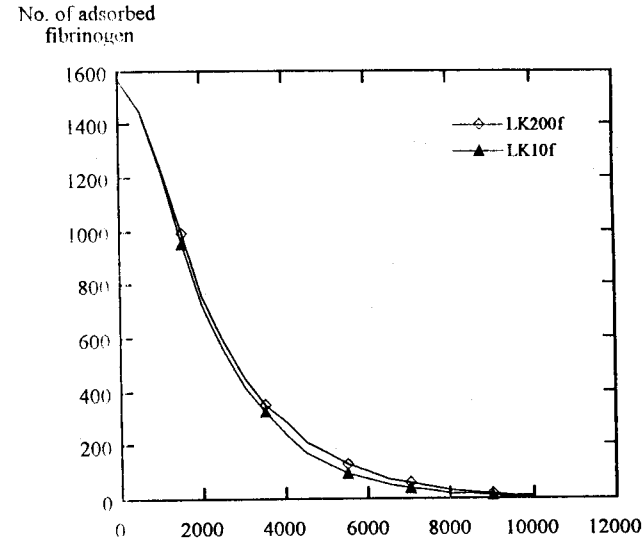

Figure 3 Fibrinogen adsorption on lumbrokinase immobilized surface (LK10f : rate constant 10) (Lk200f: rate constant 200)

References

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2. Gyu Ha Ryu, Ph.D. Thesis, Dept. of Biomedical Eng., Seou National University,1993
3. J. Kim, et al., 19th Annual Meeting of Society for Biomaterials, Birmingham, Alabama, 1993
4. J. Kim, et al., Autumn conference for KOSOMBE, Wonjoo, Korea, 1992

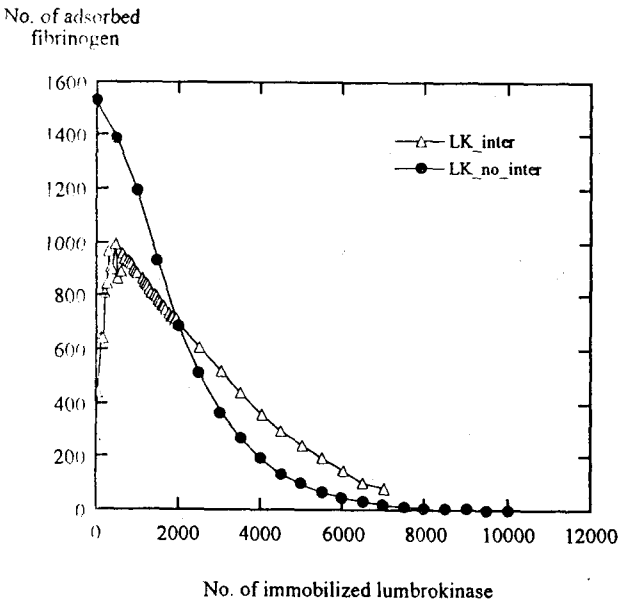

Figure 4 Fibrinogen adsorption on the co-immobilized surfaces; Comparison between interaction and non-interaction model for immobilized lumbrokinase