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原子間力顕微鏡によるフォンビルブランド因子と血小板膜糖タンパク Ibαのせん断依存的な粘着力の測定

Measurement of Shear Dependent Adhesive Force between von Willebrand Factor and Glycoprotein Ibα by Atomic Force Microscopy

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1. Introduction

Von Willebrand Factor (vWF), a large multimeric plasma glycoprotein, plays an important role in regulation of hemostasis and thrombosis formation especially under high-physiological shear rate such as arterial flow and pathological shear rate. Under these conditions at first vWF binds to exposed subendothelial structures and enables blood platelet arrest through the interaction between its A1 domain and the platelet glycoprotein Ibα (GPIbα) receptor [1]. The mechanism of vWF under high shear rate is still unclear. The previous studies found that vWF change their steric structure under high shear rate and this conformation change is probably critical factor of their shear rate dependent role [2]. Other study suggested the possibility of two types of conformation changes of vWF. One is large length scale changes, which may occur at high shear rates more than 2,300 s^{-1}. Another one is smaller changes of domain level features at physiological shear rate less than 3,000 s^{-1} [3]. In spite of the previous studies which tried to find the shear dependent vWF role by AFM imaging[2], flow chamber with fluorescence microscope [4] or optical tweezers [5], the shear dependent conformational change and adhesive force change are not clear.

In this study, we tried to detect the binding force between vWF and GPIbα at the molecular level, as well as its shear rate dependence by AFM force-displacement curve.

2. Materials and Methods

We coated recombinant GPIbα (rGPIbα) to a cantilever and 10 μg/ml vWF to a cover glass. The adhesive force between vWF and GPIbα could be measured with the force-displacement curve (Fig. 1) and the function (1) with the cantilever spring constant k and the deflection of cantilever δ.

$$F = k\delta \quad (1)$$

We compared the adhesive force between vWF and GPIbα for different shear rate, no flow (0 s^{-1}), low-physiological shear rate (300 s^{-1}), high-physiological shear rate (800 s^{-1}) and pathological shear rate (1,500 s^{-1}). We also evaluated the dependence of adhesive force on the duration after flow loading.

3. Results

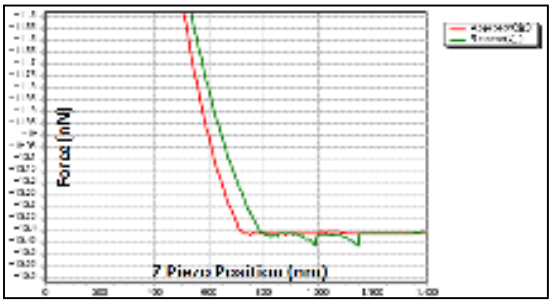

Fig. 1 Force curve of adhesive force between vWF-GPIbα

We measured the binding force between vWF and rGPIbα where the concentration of vWF was low (10 μg/ml) and Fig. 1 shows a example of force-displacement curve of its adhesive force. There were significant differences of the adhesive force between “without flow/with low-physiological shear rate” and “with high-physiological shear rate/with pathological shear rate”. The changes of binding force continued for at least 60 min.

4. Discussion and Conclusion

In this study, we detected the binding force between vWF and rGPIbα at the molecular level by AFM force-displacement curve measurement. As a conclusion, under high shear rate (high-physiological shear rate and pathological shear rate), vWF acquire the ability to bind to GPIbα with high binding force. Based on the two facts 1)the correlation of peaks distance and vWF molecure structure and 2)long time scale reversibility or even irreversibility of this binding force change, this change is a result of the rearrangeement of domain level features of vWF.

This shear induced domain level change of vWF and its adhesive force are critical factors of thrombus formation of platelet and our result can lead to reveal the mechanisms of many vWF related diseases.

5. Reference

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