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Short communication

Distribution of olfactory marker protein on a tissue section of vomeronasal organ measured by AFM

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Abstract

Distribution of olfactory marker protein (OMP) on a tissue section of vomeronasal organ (VNO) was successfully measured by atomic force microscopy (AFM). Anti-OMP antibodies were covalently crosslinked with the tip of the AFM and were used as a probe to observe the distribution of OMP on a tissue section. First, force measurements were performed using a glass surface on which OMP was covalently immobilized to verify the success of tip modification. Clear differences of interaction forces were observed between a specific pair and the control experiments, indicating that the tip preparation succeeded. Next, distributions of OMP on the tissue section were observed by AFM and were compared with immunohistochemical observations. For large scale observation, a microbead was used as a probe in the AFM measurements. The results of the AFM measurements were well overlapped with that of immunohistochemistry, confirming the reliability of our method. A mapping of the AFM measurement with high resolution was also successfully obtained, which showed an advantage of the application of the AFM measurement in analysis of proteins on the tissue section.

Keywords: Atomic force microscopy; Olfactory marker protein; Vomeronasal organ; Interaction force; Microbead

1. Introduction

The noses of most vertebrates actually contain two sensory channels. The first is the familiar olfactory system, and the second channel is the vomeronasal complex [1]. The latter is a system that has its own separate organs, nerves, and connecting structures in the brain. The function of the vomeronasal system is the detection of pheromones, chemical messengers that carry information between individuals of the same species. Olfactory marker protein (OMP) is abundant 19-kDa cytoplasmic protein in olfactory neurons of vertebrate species. OMP plays an important functional role in olfactory chemoreceptor neurons, a hypothesis that has received strong support as a result of recent studies on mice rendered deficient in this protein. Specifically, mice rendered deficient in OMP by targeted gene deletion have a reduced ability to respond to odor stimuli [2,3].

In spite of these biological importances, quantitative analysis of OMP expression has not been performed at a single cell level. To realize this single cell level measurement, one of the promising approaches is to use atomic force microscopy (AFM). Since its invention in 1986 by Binnig et al. [4], AFM has been widely applied to the structural and functional study of biological systems [5–10]. As the tip of the AFM makes direct contact with the sample surface, the AFM has been used as a mechanical nanomanipulator. For example, when the tip of the AFM was penetrated into a cell, time-course expression of mRNAs were observed at the cell level [11,12]. In our previous study, we introduced a method of extending the measurement of cell adhesive interactions from a single molecular level to the cellular level using the AFM [13–15]. To study the distributions

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of receptors on a cell surface by AFM, a microbead was used as a probe to increase the area of contact with the cell surface. Distributions of receptors, such as integrins, were then observed for a living cell. Our previous method is a powerful tool to study expressions of proteins on a living cell surface, however, it is difficult to apply to proteins in a cell, such as OMP. In this study, a tissue section was used as a sample, and the same procedure as the measurement of cell adhesive interaction was applied. By using a tissue section, proteins which were expressed in a cell could be studied quantitatively with high spatial resolution. Here we report a result of the measurement of OMP expression on a tissue section.

2. Materials and methods

2.1. Preparation of AFM tip

For mapping of a small area, anti-OMP antibodies obtained from rabbits [16] were covalently crosslinked to an AFM tip (NP, Digital Instruments, Santa Barbara, CA, USA) as described below. The AFM tip was cleaned using UV-ozone cleaner and was reacted with 2% 3-aminopropyltriethoxysilane (Aldrich Chemical Co., Inc., Milwaukee, WL, USA) in toluene for 2 h. After washing with toluene and ethanol, 2 mM discuccinimidyl suberate (DSS, Pierce, Rockford, IL, USA) solution in 50% dimethyl sulfoxide and 50% ethanol was mounted onto the tip for 1 h. The tip was then washed with ethanol and pH 6.0 PBS, and was reacted with 0.5 mg/mL anti-OMP antibody for 15 min. After the reaction, 20 mM of glycine in pH 7.4 PBS was mounted on the tip and incubated for 30 min to block the remainder of the non-reacted parts. For the control experiment, a control antibody obtained from rabbit was immobilized to the tip using the same method.

For the mapping of a large area, a carboxylated polystyrene microbead (Polybead Carboxylate Microsphere, $r = 5$ μm nominally, Polyscience, Inc., Warrington, PA, USA) was attached to an AFM cantilever with epoxy resin using micro manipulators (MMN-1, MMO-202ND, and MN-153, Narisige Co., Tokyo, Japan) to increase the contact area [13]. Anti-OMP antibodies were covalently crosslinked to microbeads as described below. The carboxylated surface of the microbeads was reacted with 6 mM 1-ethyl-3 (3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, Pierce) and 15 mM *N*-hydroxysulfosuccinimide (Pierce) in pH 6.0 PBS for 15 min. After washing with pH 6.0 PBS, the beads were reacted with 0.5 mg/mL anti-OMP antibody in pH 7.4 PBS for 2 h and then washed with pH 7.4 PBS. After the reaction, 20 mM of glycine in pH 7.4 PBS was mounted on the beads and incubated for 30 min to block the remainder of the non-reacted parts.

2.2. Immobilization of OMP onto a glass surface

OMP [16] was immobilized onto a glass surface to verify the success of tip modification. An aminosilanized slide glass (MAS-coated glass, Matsunami Glass Ind., Ltd., Osaka, Japan) was activated with 5% glutaraldehyde (Sigma Chemical Co., St. Louis, MO, USA) for 30 min. After washing, 1 mg/mL OMP in pH 7.4 PBS was mounted on the activated glass for 1 h and washed with PBS.

2.3. Force measurement and immunohistochemistry

Force measurement experiments were carried out on an atomic force microscope (NVB-100, Olympus, Inc., Tokyo, Japan) at room temperature. Force measurements between OMP on a tissue section obtained from a goat [16] and anti-OMP antibodies were performed in PBS solution containing 0.1% BSA. A force mapping mode with a 1 μm Z scan size and at the scan speed of 1 μm/s Z scan rate was used for measurements. Maximum force for each assay was calculated, and maps of interactions between OMP on the tissue section and anti-OMP antibodies were obtained of several area sizes using the force value. For the mapping of a large area, a microbead was used as a probe, and the map was obtained in the same manner as that of the smaller one.

After the AFM analysis, the sections were incubated in a 10% blocking solution (BlockAce, Yukijirushi, Tokyo, Japan) consisting of 0.1% Triton X-100 in PBS (T-PBS) at room temperature for 30 min. Without washing, the cells were incubated with anti-OMP antibody diluted with T-PBS for 2–3 days at 4 °C. Following five rinses in T-PBS, the sections were incubated at 37 °C for 2 h with anti-rabbit Ig, fluorescein linked whole antibody (GE Hearthcare Bio-Sciences Corp., Piscataway, NJ, USA; 1: 25). After washing the sections five times with T-PBS, they were mounted using an antifade reagent (PermaFluor™ Aqueous Mounting Medium, ThermoShandon, PA, USA) and observed under a confocal laser microscope (LSM510, Carl Zeiss, Oberkochen, Germany). Each confocal image was taken at 0.5 μm depth intervals.

3. Results and discussion

To verify the success of tip modification, the interaction force between OMP and its antibody was measured using a glass surface on which OMP was covalently immobilized, and the result was compared with that of the control experiments. Table 1 shows average values of forces measured using AFM tips on which several kinds of modifications were carried out. In comparison with the control experiments, strong forces were measured in the case between OMP and its antibody. In the presence of 5 μg/mL anti-OMP antibody in the solution, interaction forces were greatly reduced (Table 1). These results indicate that the tip modification was successful, and the distribution of OMP

Table 1
Average values of interaction forces for several combinations of modifications

Tip modification	Sample	Average force [pN]
Anti-OMP antibody	OMP	75 ± 33
Anti-OMP antibody	OMP in the presence of 5 μg/mL anti-OMP antibody	34 ± 14
Control antibody	OMP	27 ± 16
Unmodified	OMP	25 ± 11