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Membrane-membrane and membrane-substrate adhesion during dissection of gap junctions with the atomic force microscope

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ABSTRACT

The gap junction is a specialized region of the plasma membrane that consists of an array of cell-to-cell ion channels. These channels form where the membranes from two cells come together, and the gap junction is therefore composed of two lipid bilayers. The atomic force microscope (AFM) can be used to dissect the gap junction, removing one membrane and exposing the extracellular domains of the second. The force required to dissect the membrane, near 10^{-8} N vertical force for gap junctions adsorbed to mica, provides a measure of the strength of the interaction between the two membranes. Since a single membrane is left in contact with the mica, this interaction must be stronger than the membrane-membrane interaction. Non-junctional membrane attached to the gap junctions is easily removed with the AFM tip while the gap junction membrane remains attached to the mica, providing evidence that the interaction with the mica is mainly mediated by protein-mica interactions. Consistent with this hypothesis is the observation that material trapped under the membrane sometimes results in pieces of membrane above the material being pulled out during dissection. These results lay the foundation for examining the molecular details of the basis for membrane-membrane and membrane-substrate adhesion.

1. INTRODUCTION

The gap junction is an array of cell-to-cell channels thought to be important in a variety of biological functions such as growth control, synchronization of myocardial contractions, metabolic homeostasis, and development. These channels form where the membranes from two cells come together, and are found in most cell types. Gap junctions can be isolated with methods based on relative detergent resistance, as "plaques" of membrane about 1 μm diameter. This has allowed detailed study of gap junction structure by electron microscopy, X-ray diffraction and biochemical methods[1-5]. The basic unit of the channel is the connexon, a channel in one membrane thought to be composed of six identical or homologous protein subunits and 6-7 nm in diameter, that interacts with an identical structure in the apposing membrane to form a cell-to-cell channel. The connexons often have a nearly hexagonal packing with 8 to 10 nm center to center spacing. It has previously been shown that gap junctions can be imaged with the AFM, and that the plaque can be dissected exposing the extracellular surface of one membrane[6]. We here report recent results from an ongoing effort to explore the structure of this ion channel with the AFM.

2. MATERIALS AND METHODS

Rat liver gap junctions were isolated by a method similar to several previously described[7,8]. They were stored in water at -20° C before use. For imaging, a drop of solution containing gap junctions (c. 300 ng/ml) was adsorbed to freshly cleaved mica for 10 minutes and rinsed several times with phosphate buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, and 4.3 mM Na_2HPO_4 at pH 7.2). The mica was mounted in a fluid cell filled with PBS and imaging was performed on a NanoScope II or NanoScope III Scanning probe microscope, equipped with a D or J type scanner (Digital Instruments, Santa Barbara, California). For details on the operation and applications of the AFM see refs 9-11.

3. RESULTS AND DISCUSSION

3.1 Dissection of Gap Junctions

Like gap junction plaques adsorbed to glass, plaques on mica are stable when imaged at vertical tip forces of 10^{-9} N, and when then force is increased to 10^{-8} N the top membrane is dissected away (Fig. 1). The remaining membrane is 6-7 nm thick, close to half the thickness of the original membrane, and reveals the characteristic packing of channels. The force

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required to dissect the gap junction has many components and is not yet fully understood. Imaging force usually reported, as measured from the force versus distance curves[12], is only the vertical component and does not take into account any horizontal forces. The images in Fig.1 are taken in error signal mode (see Putman *et al.*, this volume) so the bright edge on the right of the membrane indicates significant cantilever deflection, and therefore increase in force, despite the movement of the z piezo to maintain constant applied force. This primarily horizontal interaction between the tip and the edge of the membrane is likely to be crucial to the dissection, but has not yet been measured quantitatively.

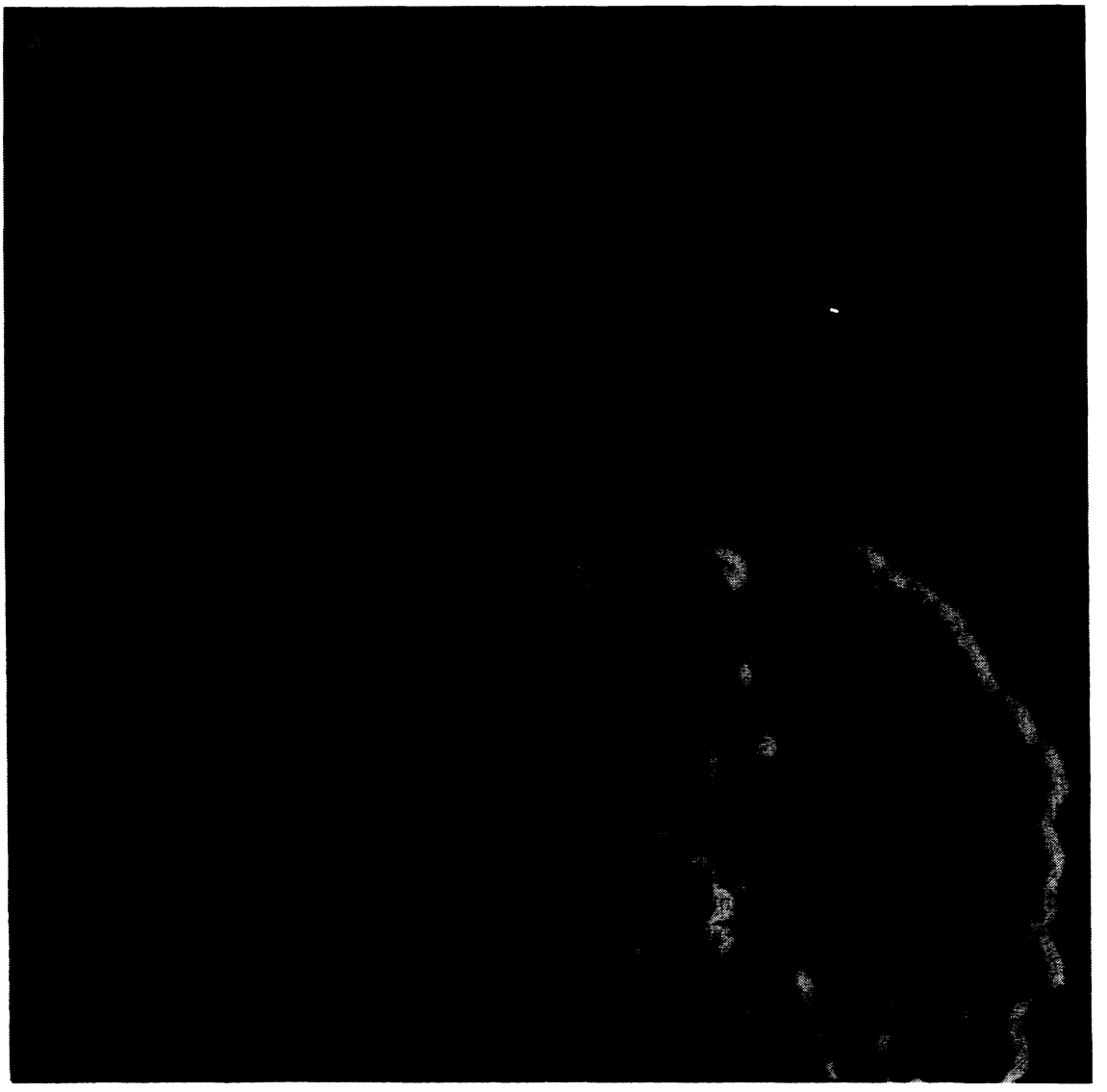

Fig. 1. Series of error signal images showing dissection of a gap junction plaque adsorbed to mica. (A) At low forces near 10^{-9} N the plaque is stable. Image is 1.4 μm on the side. (B and C) As the force is increased to near 10^{-8} N the plaque is top membrane is dissected away. Image is 1.1 μm on the side. (D) Finally a single membrane is left with the extracellular domains of the channels exposed in the characteristic hexagonal array. Image is 0.6 μm on the side.

3.2 Non-Junctional Membrane

Some isolated gap junctions adsorbed to mica have bare regions next to the array of channels. These regions appear, based on thickness and because they are contiguous with the gap junction membrane, to be naked lipid bilayers with no channels. The origin or the conditions under which these regions form is not understood, though similar regions have been seen by electron microscopy. In intact gap junction plaques this naked bilayer region is seen only on the bottom membrane as a single bilayer. It is possible that the corresponding top bilayer is very weakly adherent, as expected based on phospholipid head group repulsion, and therefore removed by the AFM tip on the first scan and never seen. Dissection of the gap junction first removes the top membrane, and at higher force removes the naked lipid bilayer leaving the bottom gap junction membrane (Fig. 2). These data suggest that the protein component of the gap junction is critical to the adhesion between the gap junction membrane and mica, and that phospholipid membranes deposited onto mica in PBS adhere weakly.

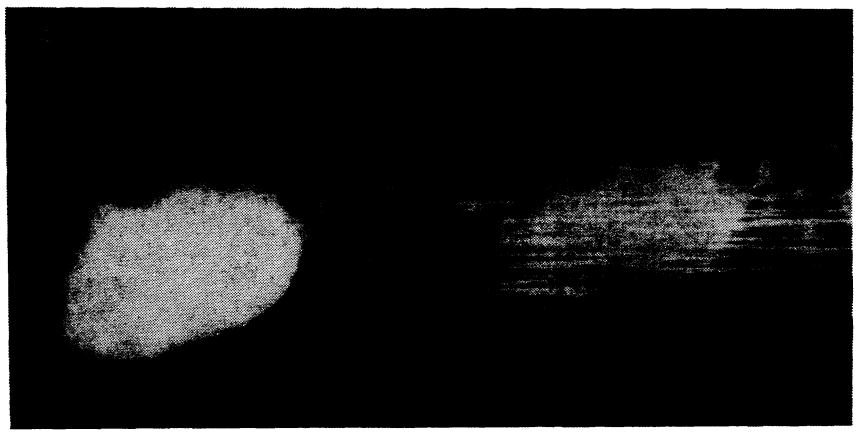

Fig. 2. Dissection of gap junction with non-junctional membrane. Images are 1.8 μm on the side. (A) The full thickness of the gap junction is 14-15 nm. A large region of smooth non-junctional membrane about 5-6 nm thick protrudes from the lower part of the gap junction membrane This region appears to be in contact the mica. (B) As the imaging force is increased the gap junction is dissected to the 6-7 nm single membrane, and the non-junctional membrane is completely removed.

3.3 Material Trapped Under the Membrane

Large scale undulations, roughly 50-100 nm wide and many nm tall, of the surface of gap junction membranes on glass have been previously described[6], and are often present in gap junctions adsorbed to mica. These undulatations do not result from material on the top surface of the gap junction, since dissected gap junctions with these undulations can also been seen. However, we have now observed many instances in which holes appear in the dissected membrane at positions corresponding to undulations in the whole gap junction (Fig. 3). This leads us to propose that these undulations form from material trapped between the gap junction and mica, which results in reduced adhesion between the two and pieces of the bottom membrane being pulled out when the top membrane is removed. An alternate explanation is that the undulations in the membrane provide a protrusion for the tip to push on, horizontally, making holes in the membrane. Since the edge of the whole bilayer provides a much larger protrusion, yet it is not removed at the forces used, that explanation seems unlikely. The nature of the material trapped under the membrane is not known. It is possible that the material is a functional molecule bound specifically to the gap junction membrane, however it is more likely a contaminant, glycogen for example is common in these preparations.

4. CONCLUSION

The work reported here defines some of the membrane-membrane and membrane-substrate adhesions we have observed in our study of gap junctions, and describes their relative strengths in terms of the vertical imaging force. The results

improve the basis for selecting substrates for imaging of biological membranes, and lay the foundation for detailed examination of the molecular basis of the interactions described here.

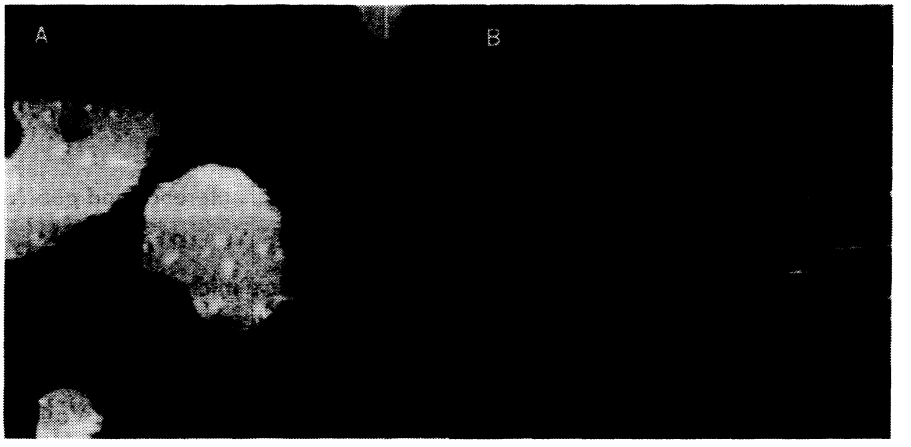

Fig. 3. Dissection of gap junctions with material trapped underneath results in holes in the remaining membrane where the trapped material was. Images are 3.2 μm on the side. (A) Gap junction plaques showing undulations in the surface (arrows) that are likely due to material trapped under the plaque. (B) Dissection of these plaques often results in holes in the membrane at positions material was trapped (arrows).

5. ACKNOWLEDGEMENTS

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