

USING A MICROSCOPE TO MEASURE THE GLUE THAT HOLDS US TOGETHER

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We think that adhesion molecules hold cells together. This is the "glue" that holds us, and every other multicellular life form on this planet, together. Intriguingly, the atomic force microscope has recently been used to directly measure the binding force of one of these adhesion molecules. We now have direct evidence that adhesion molecules have the physical properties required to hold cells together. This essential new information shows that these molecules can perform the function that have been assigned to them. You'll be delighted to know that this molecule is more than strong enough to do its job!

As pointed out previously in this column,² the atomic force microscope (AFM) can be used to measure attractive forces between molecules. The key to this technique is that the spring constant for the cantilever of the AFM can be known, allowing for the forces that deflect the cantilever to be quantitated. Ulrich Dammer, Octavian Popescu, Peter Wagner, Dario Anselmetti, Hans-Joachim Güntherodt, and Gradimir Misevic used this principle to measure the binding strength between cell adhesion proteoglycans from a marine invertebrate.³ This molecule from the sponge *Microciona prolifera* is well characterized and is known to mediate cell recognition and aggregation *in vivo*. After isolating the proteoglycan, they imaged the molecules with an AFM and saw a ring-shaped molecule with a diameter of 200 nm and about 20 "arms" extending from the ring, each 180 nm in length. They demonstrated that AFM gave more 3-dimensional structural information about the molecule than can be seen by electron microscopy.

To measure interaction forces between adhesion proteoglycan molecules, they covalently attached the proteoglycans to the sensor tip of an AFM and to a flat surface. They advanced the cantilever tip toward the substrate surface in a series of approach-and-retract cycles. Since the binding events remained stable during the course of a given experiment, it was considered that none or very few of the functional adhesion sites on the proteoglycan molecules were irreversibly damaged. An approach-and-retract cycle showed that there was no adhesion until the molecules touched, then an adhesion

force was detected until the tip had been retracted about 200 nm from the surface. From this type of data it was suggested that there are long-range interactions between proteoglycan molecules, interpreted as the lifting and extension of the arms of the molecules, followed by further stretching until the elastic force of the cantilever equaled the strength of the binding. A sudden change in the bending of the cantilever indicated the lever "jumps off." In buffered seawater with a physiologic concentration of calcium ions, the jump offs indicated an adhesive force of 40 ± 15 piconewtons. The effect of varying the calcium ion concentration was consistent with expectations from aggregation studies. The fact that this study was carried out in a physiologic solution that can be manipulated is an important advantage. Also, an antibody that is known to inhibit adhesion proteoglycan-promoted cell adhesion reduced the interactive force in a predictable fashion. These three lines of evidence showed that the interactions between the proteoglycan molecules in the AFM resemble cell-to-cell adhesion events observed *in vivo*.

Further experiments suggested that the 40 piconewton step corresponded to the unbinding of a single pair of adhesion proteoglycan arms, with the variation caused by different amounts of overlap. Larger binding forces represented multiples of paired binding arms. It was also noted that the covalent bonds formed by these molecules were several times stronger than the binding forces, so that the molecules were not ripped apart by interactions with their neighbors. These quantitative measurements of properties of single molecules is exciting indeed.

Dammer *et al.* calculated that the 400 piconewton cohesive force between two individual adhesion proteoglycan molecules involving 10 pairs of arms could theoretically hold the weight of approximately 1,600 cells in a physiologic solution. With at least a thousand adhesion proteoglycan molecules per cell, this sponge seems to be well glued together! ■

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2 Carmichael, S.W., *Microscopy isn't just for microscopists, anymore*. *Microscopy Today* 94-5:24, 1994.

3 Dammer, U., O. Popescu, P. Wagner, D. Anselmetti, H.-J. Güntherodt, and G.N. Misevic, *Binding strength between cell adhesion proteoglycans measured by atomic force microscopy*, *Science* 267:1173-1175, 1995.

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