

Calcium and pH Induced Closure of Gap Junction Hemichannels as Visualized by Atomic Force Microscopy

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Gap junctions help to mediate cell-cell communication. The term “gap junction” refers to a cellular structure containing multiple intercellular channels spanning the two plasma membranes and the narrow extracellular “gap” that separates them. Gap junction channels, dodecamers of the connexin protein, are dynamic macromolecular complexes capable of opening and closing the channel pore in response to a number of stimuli such as divalent cations, signaling molecules, phosphorylation, pH and modulators of specific isoforms. While there exists strong cell biological, biochemical and biophysical measurements for the effects of these modulators, not much is known at the structural level as to the conformational changes that occur when the pore closes in response to these stimuli. Previously, we had shown using atomic force microscopy (AFM) that force dissected connexin26 (Cx26) gap junction hemichannels could be reversibly opened and closed in response to Ca^{2+} in a reductionist imaging system containing only the gap junction plaques and an experimental buffer with or without the ligand [1]. We use preparations of gap junctions from over-expressing Cx26 HeLa cells. We image the extracellular surface at molecular resolution and obtain measurements of domain height above the membrane and pore shape and diameter. We are able to resolve individual subunits in these topographs. An advantage in using AFM is that we can identify structural rearrangements on the same gap junction channels or hemichannels non-destructively imaged both spatially and temporally because the samples are held in a buffered flow-system chamber, maintain their hydration and are non-destructively imaged. Thus, we can correlate physiological treatments with any molecular rearrangements. These studies complement the static structures that we obtain using cryo-electron microscopy [2].

The closure of connexin channels in cells by acidification has been well documented both in model tissue culture systems [3] and in playing a functional role in tissue [4], however, it is not known whether the acidification affects the connexin itself or other modulating proteins or compounds that in turn close the channel pore. An intracellular drop in pH has been advocated as one mechanism by which cells control their intercellular communication through gap junctions. Bevans and Harris [5] have shown that protonated aminosulfonate compounds directly inhibit connexin channel activity in an isoform specific manner. High-resolution AFM topographs observed that in HEPES buffer the pore of homomeric connexin26 channels was closed at $\text{pH} < 6.5$ and reversibly opened by increasing the pH to 7.6 (see FIG. 1A and B). Above pH 7.6 the connexon channels remained fully opened. This conformational change was completely reversible. This pH dependent channel closure could not be observed in maleate or phosphate buffered solutions in accordance with the results of Bevans and Harris [5]. Our results indicate that the binding of protonated aminosulfonates promote a pH induced conformational change at the extracellular surface associated with a gating event. While the pore diameter increased gradually with pH, the outer connexon diameter remained unchanged. This channel opening was concomitant with an $\sim 6.5^\circ$ rotation in connexon lobes at the extracellular surface. From these topographs, we deduce a mechanism for closure of the extracellular “loop” gate, being different from the Ca^{2+} induced closure observed previously (FIG. 1C).

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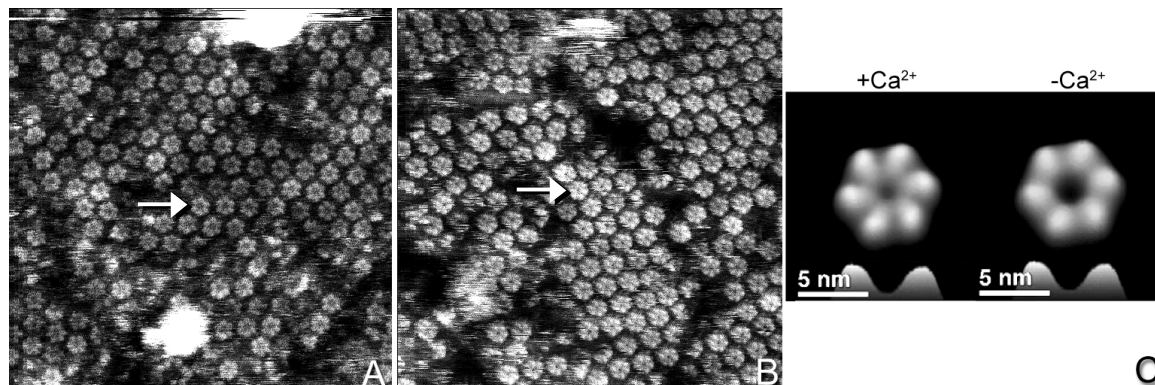


FIG 1 (A). Force dissected Cx26 gap junction at pH 8 and (B) pH 6.5. Note the difference in pore size as indicated by the arrow. (C) Averages of surface views of Cx26 hemichannels open and closed by Ca^{2+} (adapted from [1]).