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Secretion Induces Cell pH Dynamics Impacting Assembly-Disassembly of the Fusion Protein Complex: A Combined Fluorescence and Atomic Force Microscopy Study

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Running Title: *Nanoscale pH Dynamics in Cellular Function*

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ABSTRACT

A wide range of cellular activities including protein folding and cell secretion, such as neurotransmission or insulin release, are all governed by intracellular pH homeostasis, underscoring the importance of pH on critical life processes. Nanoscale pH measurements of cells and biomolecules therefore hold great promise in understanding a plethora of cellular functions, in addition to disease detection and therapy. In the current study, a novel approach using cadmium telluride quantum dots (CdTeQDs) as pH sensors, combined with fluorescent imaging, spectrofluorimetry, atomic force microscopy (AFM), and Western blot analysis, enabled the study of intracellular pH dynamics at 1 milli-pH sensitivity and 80 nm pixel resolution, during insulin secretion. Additionally, the pH-dependent interaction between membrane fusion proteins, also called the soluble *N*-ethylmaleimide-sensitive factor activating protein receptor (SNARE), was determined. Glucose stimulation of CdTeQD-loaded insulin secreting Min-6 mouse insulinoma cell line demonstrated the initial (5-6 min) intracellular acidification reflected as a loss in QD fluorescence, followed by alkalization and a return to resting pH in 10 min. Analysis of the SNARE complex in insulin secreting Min-6 cells demonstrated an initial gain followed by loss of complexed SNAREs in 10 min. Stabilization of the SNARE complex at low intracellular pH is further supported by results from studies utilizing both native and AFM measurements of liposome-reconstituted recombinant neuronal SNAREs, providing a molecular understanding of the role of pH during cell secretion.

INTRODUCTION

Precision pH measurements especially of cells and biomolecules have greatly improved (1), although major challenges at the nanometer and milli-pH scale in cells remain. Nano-scale and single molecule intracellular measurements of pH, hold great promise in understanding an array of cellular functions at the molecular level and for applications from disease detection to therapy (1). Altered intracellular pH is causal to autophagy, mitophagy (2), and to a number of diseases; among them cancer and Alzheimer's (3,4). It is reported that while intracellular alkalization inhibits secretion from cells (5,6), acidification stimulates release (7,8). The role of intracellular acidification in cell secretion however is poorly understood, and is the subject of the current study. Secretion from cells involves the fusion of cargo containing membrane bound vesicles with the cell plasma membrane. Specialized membrane fusion proteins at the vesicle membrane called v-SNARE (9), and at the cell plasma membrane termed target or t-SNAREs interact and assemble in a t-/v-SNARE rosette or ring conformation to promote fusion between opposing lipid membranes (10,11). The t-/v-SNARE complex formed is very stable, requiring an ATPase called *N*-ethylmaleimide-sensitive factor (NSF) for its disassembly. The ATPase activity of NSF requires a basic pH optima (12,13). To determine the time-dependent shifts in intracellular pH following stimulation of cell se-