This work was supported by the Camille and Henry Dreyfus Foundation (Grant: TH-18-017), and by the NIGMS of the NIH (Grant: R15GM110606). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. The funders had no role in study design, data collection and analysis, or decision to publish.

104132, https://doi.org/10.1016/j.jbc.2023.104132

Abstract 2211

Minimal phosphorylation sites required for SIKE dimer to monomer transition

Michelle Bidwell Astaburuaga, *University of San Diego*

Danielle Etiel, Alyssa Pham, Erika Lopez Pena, Brianna Gonzalez Labastida, Ellis Bell, Jessica Bell

Structure dictates function and protein interactions mediate those functions. In the innate immune response to viral infection, Suppressor of IKK Epsilon (SIKE) is a protein of unknown structure and function, although several distinct protein interactions have been identified. Our long term goal is to understand how and why distinct SIKE interaction networks form and their impact on the innate immune response. We have defined SIKE as a primarily dimeric protein that undergoes a transition to a monomeric state when phosphorylated. We have also shown that SIKE phosphorylation modulates interactions with tubulin (enhances) and actinin (decreases). From our model of the SIKE dimer, five of SIKE's six phosphorylation sites per subunit are located at our predicted dimer interface suggesting that charge-charge repulsion of clustered phosphorylated serines mediates this quaternary state transition. Computational evaluation of these sites impact on dimer stability and their evolutionary conservation suggested that three of the five sites may be sufficient to induce this quaternary state transition. From these preliminary studies, we hypothesize that SIKE holds two distinct quaternary states that are regulated by phosphorylation at serines 187, 190, and 198. We have created a series of site-directed mutations representing individual and all combinations of these three serines mutated to glutamate. All bacterial expression of mutants was comparable to wild type levels. SEC of wild type SIKE shows multiple species, primarily dimer but also tetramer, which was confirmed by separating crosslinked WT-SIKE by SEC and assessing species present in peak fractions by SDS-PAGE followed by silver staining. Individual, double, and triple mutants were assessed in a similar manner. Results show how the introduction of negative charge redistributes the stable quaternary states and promotes the monomeric state.

This work was supported by NSF-REU grant # CHE-2050846 and USD PURE fellowship.

104133, https://doi.org/10.1016/j.jbc.2023.104133