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Arsenic binds to redox sensor protein Keap1 directly through the thiol groups of critical cysteines in the linker region of Keap1

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Abstract

Arsenic is a well-documented environmental toxicant and human carcinogen. We have previously reported that arsenic potently induces cytoprotective genes by activating the Nrf2/Keap1/ARE system. Keap1, a Nrf2-binding partner, is rich in cysteine and may function as a redox sensor of antioxidant inducers. The mechanism by which arsenic and other toxic metals activate Nrf2 is currently unclear. Here we reported that arsenic directly binds with Keap1 through its thiol groups. Fluorescence-labeled arsenic was shown to bind to purified mouse Keap1 protein *in vitro* with a high affinity. A thiol reactive agent BAL or excess amount of arsenic competes with the fluorescent arsenic for Keap1 binding. An organoarsenical affinity matrix was developed and was shown to bind with *in vitro* translated Keap1, purified mouse Keap1, and endogenous Keap1 with a high affinity. Treatment of purified or endogenous Keap1 with arsenic, antioxidant tBHQ, or organoarsenic largely reduced the content of free thiol groups in Keap1. The strongest binding region of Keap1 with PAO matrix was mapped at the linker region of Keap1. Point mutations of cysteine to alanine at the linker region abolished or significantly reduced its binding to the arsenic affinity matrix. Together, these results revealed that arsenic binds with Keap1 directly and the thiol groups of cysteine residues in the linker region of Keap1 play a critical role in arsenic binding.

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