Abstract

YVC1 is yeast vacuolar channel that effluxes Ca2+ from the vacuole to the cytosol. Previous studies in this lab have shown that, there is an increase in the levels of cytoplasmic Ca2+ and YVC1 contributes to this increase. YVC1 contains 9 cysteine residues and since these cysteines are expected to play a role in its activation, we mutated 8 of the 9 cysteines to alanines. These Cys → Ala mutations were evaluated for protein expression, localization and function. C343A mutant was hyperactive. We also observed that C61A was defective in localization. Among the other mutants, we observed that C17A and C191A were nonfunctional and also these mutants were partially defective in glutathionylation. Functionality of C17A and C191A were also defective as detected by aequorin based assay for Ca2+. The results suggest a role for the two cysteine residues in YVC1 activation.