Structural insights into the interaction of the conserved mammalian proteins GAPR-1 and Beclin 1, a key autophagy protein

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Mammalian Golgi-associated plant pathogenesis-related protein 1 (GAPR-1) is a negative autophagy regulator that binds Beclin 1, a key component of the autophagosome nucleation complex. Beclin 1 residues 267–284 have been shown to be required for binding GAPR-1. Here we report sequence analyses, structural modeling, mutagenesis combined with pull-down assays, X-ray crystal structure determination and small-angle X-ray scattering (SAXS) used to investigate the Beclin 1:GAPR-1 interaction. An equatorial surface groove on GAPR-1 was predicted to bind a peptide composed of Beclin-1 residues 267-284. Mutation of the five conserved residues within this groove, H54A/E86A/G102K/H103A/N138G, abrogates Beclin 1 binding as determined by pull-down assays. The 1.27 Å resolution X-ray crystal structure of this pentad mutant GAPR-1 revealed that the equatorial groove of the pentad mutant is shallower and more positively charged, and therefore may not efficiently bind Beclin 1 residues 267–284, compared to wild-type (WT) GAPR-1. Further, the self-dissociation constants of the pentad mutant and WT GAPR-1 was determined by isothermal titration calorimetry. Subsequently, the solution oligomeric state of pentad mutant and WT GAPR-1 were analyzed by SEC-SAXS and the presence of monomer and dimer species of the pentad mutant were determined by singular value decomposition analysis. Together these results indicate that not only the presence of the substituted residues in the predicted binding groove but also the altered monomer-dimer equilibrium of the pentad mutant GAPR-1 are responsible for disruption of the GAPR-1:Beclin 1 interaction.