

REPLACEMENT OF CYSTEINE RESIDUES OF A PROTEIN BODY-FORMING PROTEIN INCREASES SOLUBILITY AND CAUSES SECRETION

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Seed prolamins of a number of cereals accumulate in the endoplasmic reticulum (ER) as very large insoluble oligomers termed protein bodies (PB). The mechanisms of prolamins ER retention and PB formation are still poorly understood. We have previously shown that zeolin, a fusion between phaseolin (a vacuolar storage protein) and part of g-zein (a PB-forming protein), forms insoluble PBs in the ER of transgenic tobacco leaves, and that treatment of protoplasts with reducing agents increases zeolin solubility and leads to its partial secretion. We now show that reducing agents do not decrease the affinity of zeolin for the ER chaperone BiP, indicating that binding to BiP is not a major causing event for the ER retention of zeolin. Conversely, replacement of the six Cys residues of the g-zein domain with Ser residues by site-directed mutagenesis makes the mutated zeolin fully soluble even in the absence of reducing agents and causes quantitative secretion. These results point to disulfide bonds as a key structural feature for the formation of insoluble PBs and their ER retention.

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