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Poster Abstract - F.01

DISULFIDE BONDS CONTRIBUTE TO THE RETENTION OF ZEOLIN, A NEW RECOMBINANT STORAGE PROTEIN, IN THE ENDOPLASMIC RETICULUM

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Disulfide bonds between cysteine residues are formed during protein folding and assembly in the endoplasmic reticulum (ER). Storage proteins of the 7S class usually do not have cysteine residues, whereas many prolamins are cysteine-rich. We have previously shown that a fusion between phaseolin (the common bean 7S protein) and the N-terminal half of γ-zein (a maize prolamin) is retained in the ER, forms protein bodies and is highly stable even in leaves of transgenic tobacco. The zein portion contains 6 out of the 15 cysteine residues of this prolamin. Analysis of the oligomeric state of zeolin in reducing or oxidizing conditions shows that these residues form inter-chain bonds and make the protein insoluble. We have investigated on the contribution of disulfide bonds to the destiny of zeolin and, more in general, on the effects that alterations of the redox conditions in the cell have on protein traffic along the plant secretory pathway. To this purpose, protoplasts prepared from leaves of wild-type or transgenic tobacco was treated with the reducing agent 2-mercaptoethanol and the destiny of secretory proteins determined by pulse-chase experiments. The results indicate that the reducing agent enhances interactions of passenger proteins with the ER chaperone BiP and inhibits protein traffic along the secretory pathway, suggesting that the folding of a number of secretory proteins is negatively affected. In spite of this general effect, 2-mercaptoethanol slightly stimulates the exit of zeolin from the ER, indicating that inter-chain disulfide bonds are an important determinant for zeolin high accumulation within this compartment, possibly because they cause the formation of oligomers that are too large to enter vesicular traffic.

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