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Structure and Function of Ecotin, a Potent Inhibitor of Pancreatic Serine Proteases

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Ecotin is a novel inhibitor of pancreatic serine proteases including trypsin, chymotrypsin and elastase. It is a dimer of 16 kDa subunits, and each of the subunit has a C-terminal flexible regions spanning 13 amino acids. Upon structural analysis by X-ray crystallographic studies, the C-terminal region has been reported to play a critical role in dimerization of the Ecotin subunits. It has also been shown that the dissociation constant is 390 nM. To evaluate the importance of the C-terminal region, site-directed mutagenesis was performed to eliminate the C-terminal region by introducing a stop codon at the codon of Trp130. The purified Ecotin mutant protein (Ecotin△13) was capable of inhibiting the pancreatic proteases 80-90% as well as the wild-type Ecotin. However, at 100-400 nM range, both the mutant and wild-type Ecotin behaved as dimeric molecules. Furthermore, both the proteins formed complexes with trypsin at a stoichiometric ratio of 1:1 (i.e., one dimeric Ecotin interact with two trypsin molecules. Nevertheless, Ecotin was more sensitive to inhibition by high concentration of acetonitril or urea than the wild-type, despite the fact that both the proteins remained as dimers under the same conditions. Thus, the importance of the C-terminal region in dimerization of Ecotin based upon the structural data should be re-evaluated.

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Purifications and Characterizations of a Yeast Ubiquitin Specific Protease Ubp6 from *E. coli*

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Ubiquitin (Ub) is a highly conserved 76-amino acid polypeptide present in all eukaryotes. It is generated from its linear fusion proteins and re-generated from Ub-protein conjugates. Ubiquitin specific proteases (Ubps) are thiol protease that cleave the α-peptide and/or ε-isopeptide bond after C-terminus of ubiquitin from either linear ubiquitin precursor proteins or the post translationally formed ubiquitin conjugates destined for proteolysis. To date, some 14 Ubp enzymes have been identified in DNA sequence databases by virtue of homology to conserved Cys and His residues.

The DNA encoding one of them, called Ubp6, was cloned into an *E. coli* expression vector, and Ubp6 was purified to apparent homogeneity using ammonium sulfate fractionation and DEAE-Sepharose and Q-Sepharose chromatographies. It consists of a single polypeptide with a size of 54 kDa. The purified protein can generate free ubiquitin from Ub-PEST, Ub-CEP80 and Ub-DHFR but not from diubiquitin. It has a Km value of 64 μM for Ub-PEST. The activity of Ubp6 was inhibited by sulfhydryl-blocking agents, iodoacetamide (IAA). And it is maximally active between pH 8.5 and 9.0. Ubp6 is the first Ub C-terminal hydrolase that can cleave off Ub from its large polypeptide adducts in yeast.