ABSTRACT

Autoproteolysis accelerated by conformational strain - a novel biochemical mechanism.

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Natural fragmentation of polypeptide chains by autoproteolysis occurs in a number of protein families. It is a vital step in the maturation of several enzymes and in the formation of membrane-associated mucins that constitute a part of the protective mucus barrier lining epithelial cells. These reactions follow similar routes involving an initial N-O or N-S acyl shift starting with a nucleophilic attack by a hydroxyl or thiol group on a carbonyl carbon followed by resolution of the ester intermediate. Previous studies indicate that distortion of the scissile peptide bond may play a role in autoproteolysis. Our structural, biochemical and molecular dynamics studies of the autoproteolyzed SEA domains from human membrane-bound mucin MUC1 and human orphan receptor GPR116 confirmed this by revealing a novel biochemical mechanism where the folding free energy accelerates cleavage by imposing conformational strain in the precursor structure. This mechanism may well be general for autoproteolysis.

The structure of the cleaved MUC1 SEA domain was determined using NMR spectroscopy. It consists of four alpha-helices packed against the concave surface of a four-stranded anti-parallel beta-sheet. There are no disordered loops. The site of autoproteolysis is a conserved GSVVV sequence located at the ends of beta-sheets 2 and 3 where the resulting N- and C-terminal residues become integrated parts of these sheets after cleavage. The structure does not reveal any charge-relay system or oxyanion hole as would be expected if catalysis proceeded by way of transition state stabilization. The surface of the domain contains two hydrophobic patches that may serve as sites of interaction with other proteins, giving it a potential function in the regulation of the protective mucus layer.

Combined studies of autoproteolysis and adoption of native fold show that these mechanisms proceed with the same rate and that the autoproteolysis has a global effect on structure. Studies of the stability and cleavage kinetics were performed by destabilizing core mutations or addition of denaturing co-solvents. Analysis revealed that ~7 kcal mol^{-1} of conformational free energy is partitioned as strain in the precursor. The results corroborate a mechanism where the autoproteolysis is accelerated by the concerted action of a conserved serine residue and strain imposed on the precursor structure upon folding, that is, the catalytic mechanism is substrate destabilization.

The autoproteolysis of SEA is pH dependent. This is in line with a proposed mechanism with an initial N→O acyl shift, involving transient protonation of the amide nitrogen, and subsequent hydroxyl-mediated hydrolysis of the resulting ester. The mechanistic link between strain and cleavage kinetics is that strain induces a pyramidal conformation of the amide nitrogen which results in an increase of the pK_a and thereby an acceleration of the N→O acyl shift. Furthermore we propose a water hydronium as proton donor in this step. This explains the absence of conserved acid-base functionality within the SEA structure.

Keywords: SEA domain, autoproteolysis, MUC1, conformational strain, protein folding.

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