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Handbook of Proteolytic Enzymes 2nd Edn
ISBN 0-12-079610-4

616. UmuD and UmuD′ proteins

Databanks

MEROPS name: UmuD protein
MEROPS classification: clan SF, family S24, peptidase S24.003
Species distribution: More than one superkingdom
Sequence known from: *Escherichia coli*, IncJ plasmid R391, *Morganella morganii*, plasmid pKM101, plasmid Rts1, *Salmonella enterica*, *Salmonella typhimurium*, *Serratia marcescens*, *Shigella flexneri*, *Vibrio cholerae*
Tertiary structure: Available

Name and History

The *Escherichia coli umuC* locus was identified in the late 1970s in genetic screens for strains that were **U**V-non**mu**table (Kato & Shinoura, 1977). In their manuscript, Kato and Shinoura reported the identification of three discrete loci that they called *umuA*, *umuB* and *umuC*. Mapping studies indicated that *umuA* and *umuB* mutants were likely to reside in *lexA* and *recA* respectively, while *umuC* appeared to be a novel locus involved in damage-induced mutagenesis (Kato & Shinoura, 1977). Shortly thereafter, in an independent study, Steinborn similarly isolated mutants of *E. coli* that were also nonmutable after exposure to UV light, which he called *uvm* (for **UV**-**m**utagenesis) (Steinborn, 1978). The use of the *uvm* name was, however, dropped in the early 1980s when it was realized that the locus is allelic with *umuC*.

Cloning and sequencing of the *umu* locus revealed that instead of encoding a single gene, it in fact consists of a two-gene operon that is regulated by the transcriptional repressor LexA (Bagg *et al*., 1981; Shinagawa *et al*., 1983; Elledge & Walker, 1983; Kitagawa *et al*., 1985; Perry *et al*., 1985). The larger of the two genes was called *umuC*, while the smaller gene was called *umuD*. The *umuD* gene is located upstream of *umuC* with the TGA stop codon of *umuD* and the ATG start codon of *umuC* overlapping by one base pair (Kitagawa *et al*., 1985; Perry *et al*., 1985). Perry *et al*. also noticed that the smaller *umuD* gene encodes a protein with similarity to the C-terminal domain of LexA, including conserved cleavage site and active-site residues, suggesting that UmuD may undergo post-translational processing (Perry *et al*., 1985). Indeed, in 1988, Shinagawa *et al*. and Burckhardt *et al*. demonstrated that the 15 kDa UmuD protein undergoes both RecA-mediated cleavage and autocatalysis at high pH to generate peptides of ~3 kDa and ~12 kDa (Burckhardt *et al*.,

1988; Shinagawa *et al*., 1988). At the same time, Nohmi *et al*. (1988) reported that unlike LexA, which is inactivated for its repressor functions upon proteolysis, the larger of the two UmuD cleavage products, called UmuD′, is active and actually required for damage-induced mutagenesis.

In the years since their initial discovery, several orthologs of *umuDC* have been identified, cloned and characterized. Many share the same general operon organization with a *umuD*-like gene located immediately upstream of a *umuC*-like gene. Various names have been given to these orthologs including *mucAB* (**m**utagenesis, **U**V and **c**hemical) (Perry & Walker, 1982; Perry *et al*., 1985); *samAB* (***Sa****lmonella* **m**utagenesis) (Nohmi *et al*., 1991); *impAB* (**I**-group **m**utagenesis and **p**rotection) (Glazebrook *et al*., 1986; Lodwick *et al*., 1990), *rumAB* (**R**-plasmid ***umu***-homolog) (Ho *et al*., 1993; Kulaeva *et al*., 1995); and *rulAB* (**r**esistance to **U**V-**l**ight) (Sundin *et al*., 1996).

Activity and Specificity

The ability of *E. coli* UmuD (Burckhardt *et al*., 1988; Shinagawa *et al*., 1988) and several of its orthologs, including *Salmonella typhimurium* UmuD (Woodgate *et al*., 1991; McDonald *et al*., 1998b), the R-plasmid-encoded MucA (Shiba *et al*., 1990; Hauser *et al*., 1992) or RumA (Kulaeva *et al*., 1995) proteins to undergo post-translational cleavage *in vitro* and *in vivo* has been reported. In all cases, processing is greatly stimulated by RecA protein. *In vivo*, moderately efficient cleavage occurs in cells that have been exposed to cellular DNA damage and in which RecA is believed to be in a so-called 'activated' filamentous state bound to single-stranded DNA. Constitutive cleavage occurs in *E. coli* strains expressing mutant RecAs that are in the activated state in the absence of exogenous DNA damage (Shinagawa *et al*., 1988; Woodgate & Ennis, 1991; Ennis *et al*., 1995; Konola *et al*., 1998).

In vitro, the RecA-mediated reaction requires single-stranded DNA and magnesium for efficient nucleoprotein filament formation. In the absence of RecA, *E. coli* UmuD, MucA and RumA all undergo autoproteolysis at alkaline pH. The rate of autoproteolysis varies considerably between the orthologs, with the fastest being MucA ($t_{1/2}$ at pH 10 ~75 min) (Hauser *et al*., 1992) and the slowest being *E. coli* UmuD ($t_{1/2}$ at pH 10 >10 h) (Burckhardt *et al*., 1988; Hauser *et al*., 1992). Both of which are considerably slower than that of *E. coli* LexA ($t_{1/2}$ at pH 10 ~8 min) (Little, 1984; Hauser *et al*., 1992).

The UmuD-like proteins exist as dimers in solution (Woodgate *et al*., 1989; Battista *et al*., 1990) and cleavage is believed to occur via an intermolecular reaction in which the substrate tail of one protomer is cleaved in the active site of a dimer-mate (McDonald *et al*., 1998a, 1999). While both proteins form homodimers in solution, when $UmuD_2$ and $UmuD'_2$ are mixed together *in vitro*, they preferentially associate to form UmuD/UmuD′ heterodimers (Battista *et al*., 1990).

Structural Chemistry

The *E. coli* UmuD protein is 139 amino acids in length and has a calculated molecular mass of 15 063 Da and a pI of 4.5. The 115 residue UmuD′ protein has a molecular mass of 12 285 Da and also has a pI of 4.5 (Kitagawa *et al*., 1985; Perry *et al*., 1985).

The crystal structure of the $UmuD'_2$ protein was solved at 2.5 Å resolution, revealing an extended N-terminal tail and a globular C-terminal catalytic and dimerization domain containing a mostly beta protein fold (Peat *et al*., 1996a,b) (Figure 616.1A). NMR spectroscopy has been used to map the UmuD′ dimerization interface (Ferentz *et al*., 1997); to solve the UmuD′ solution structure (Ferentz *et al*., 2001) (Figure 616.1B); and to propose a structure for the UmuD/UmuD′ heterodimer (Ferentz *et al*., 2001; Sutton *et al*., 2002).

UmuD utilizes a serine–lysine dyad mechanism. Site-directed mutagenesis (Nohmi *et al*., 1988) and structural studies (Peat *et al*., 1996a) are consistent with Ser60 serving

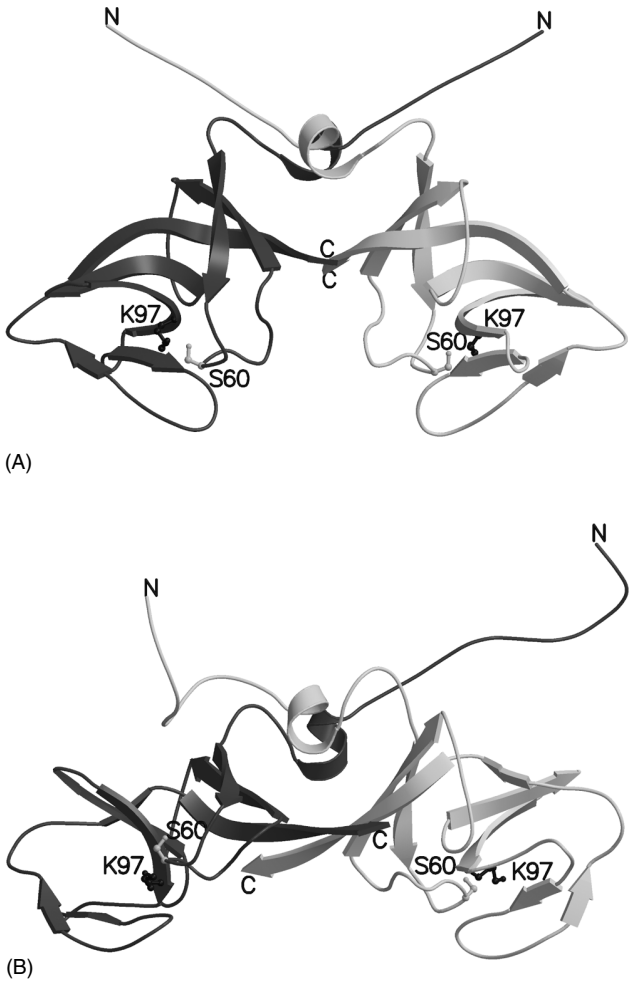


Figure 616.1 (A) The crystallographic structure of the $UmuD'_2$ dimer. The protein fold is shown as a ribbon with the side chains of the nucleophilic Ser60 and general base Lys97 shown in ball-and-stick. The atomic coordinates 1UMU (pdb code) were used to produce this figure after generating the symmetry-related molecules. (B) The NMR-determined structure of the $UmuD'_2$ dimer. Molecules A and B from the atomic coordinates 1I4V (pdb code) were used to produce this figure.

as the nucleophile and Lys97 the general base. A structural alignment of UmuD′ with the acyl-enzyme of signal peptidase (another member of the clan SF) suggested an orientation for the cleavage site in the UmuD-binding site. These studies also revealed that the nucleophilic Ser60 hydroxyl of UmuD attacks the scissile bond (located between Cys24┼Gly25 of UmuD) from the *si*-face rather than the *re*-face as seen in most serine proteases (Paetzel & Strynadka, 1999) (Figure 616.2).

NMR analysis of the UmuD/UmuD′ dimer (Ferentz *et al*., 2001) and crystallographic analysis of the analogous protein LexA (Luo *et al*., 2001) are consistent with the proposed substrate orientation. The crystal structure of LexA with its bound cleavage site suggests that the main-chain amide hydrogens from Ser60 and Asp59 in UmuD could serve as the oxyanion hole (Luo *et al*., 2001) (Figure 616.2B). Crystal structures are now available for four members of the clan SF: UmuD′, λCI repressor, LexA repressor, and signal peptidase (Peat *et al*., 1996a; Bell *et al*., 2000; Luo *et al*., 2001; Paetzel *et al*., 1998). A superposition of their active sites reveals that the N^{ζ} of the lysine general base is coordinated by three hydrogen bonds. In the case of UmuD′, the neutral ε-amino group of Lys97 (the deprotonated state is a requirement for it serving as the general base) would have two hydrogen bond acceptors (Val96 O and Thr95 O^{γ}) and one hydrogen bond donor (Ser60 $O^{\gamma}H$). It is proposed that the pK_a of the ε-amino group of Lys97 is depressed by its burial upon binding of the cleavage site in an energetically unfavorable position (Luo *et al*., 2001) (Figure 616.2).

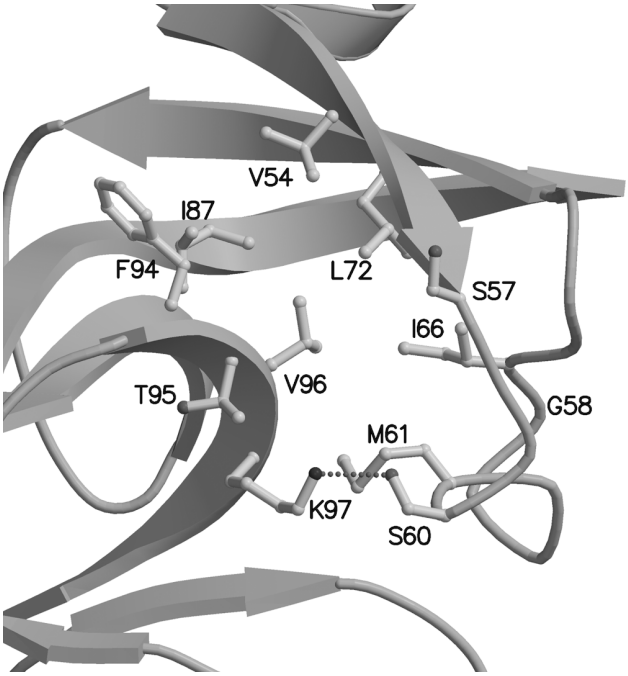
The NMR solution structure suggests that the $UmuD'_2$ dimer is structurally dynamic and that Ser60 and Lys97 are not within hydrogen bonding distance in solution (Ferentz *et al*., 2001) (Figure 616.1B). In contrast, all crystal structures of the clan SF proteases so far have shown the nucleophilic serine and general-base lysine to be within hydrogen bonding distance. Ferentz and colleges suggest that the crystal packing forces may, therefore, result in the stabilization of the catalytically competent conformation and that an interaction with a RecA nucleoprotein filament *in vivo* facilitates the cleavable conformation (Ferentz *et al*., 2001).

Preparation

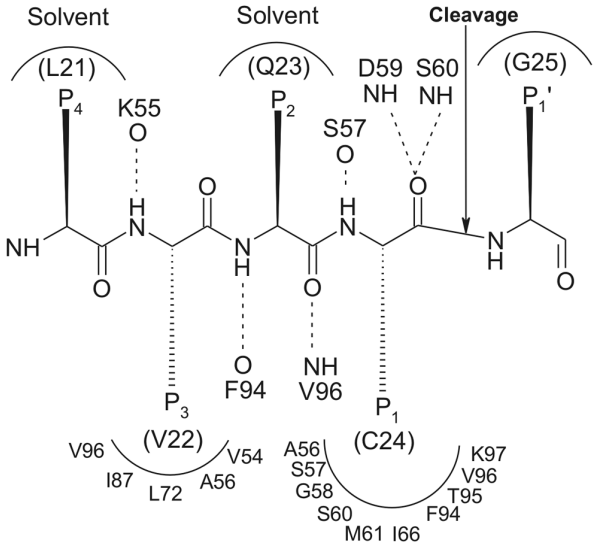
The UmuD protein was initially overexpressed in *E. coli* from a temperature-inducible λP_L promoter (Burckhardt *et al*., 1988). Homodimeric UmuD′ can be purified from the same UmuD overproducing strain if the cells are also exposed to the DNA-damaging agent mitomycin C, so as to promote *in vivo* conversion of UmuD to UmuD′ (Woodgate *et al*., 1989). Both UmuD and recombinant UmuD′ are now routinely expressed from an IPTG-inducible T7 promoter (Frank *et al*., 1993; Ferentz *et al*., 1997). Purification is relatively simple and involves ammonium sulfate precipitation, ion-exchange and gel-filtration chromatography. Under these conditions, up to 10 mg of highly purified UmuD or UmuD′ protein can be isolated from 1 liter of an induced *E. coli* culture.

Biological Aspects

Since their discovery, the Umu proteins have been hypothesized to participate in damage-induced mutagenesis. For



(A)



(B)

Figure 616.2 (A) The UmuD active site. The side chains of residues in the S1 and S3 binding pockets are shown in ball-and-stick. The crystal structure of UmuD′ shows that Ser60 O^{γ} and Lys97 N^{ζ} are within hydrogen bonding distance (Peat *et al*., 1996a). The atomic coordinates 1UMU (pdb code) were used to produce this figure. (B) A schematic of the possible interactions between the UmuD cleavage site region in the binding site of its dimer mate. The residues involved in forming the S1 and S3 binding sites are indicated. The cleavage site residues are in parenthesis. Potential hydrogen bonding interactions between the extended cleavage site region and the β strands that line each side of the binding sites are shown. The main-chain amide hydrogens of Ser60 and Asp59 would make up the oxyanion hole.

many years it was believed that the Umu proteins somehow modified the cell's main replicase, so that it would traverse otherwise replication-blocking lesions. However, in the past few years, the *E. coli* UmuC protein has been shown to possess intrinsic DNA polymerase activity (Tang *et al*., 1998, 1999; Reuven *et al*., 1999) and it is considered one of the 'founding members' of the recently described Y-family of DNA polymerases (Ohmori *et al*., 2001). *In vitro* studies suggest that UmuD′ stimulates the catalytic activity of the UmuC protein (Reuven *et al*., 1999), to which it is normally complexed (Woodgate *et al*., 1989; Bruck *et al*., 1996; Tang *et al*., 1998). This large family of lesion-bypassing DNA polymerases is found in all three kingdoms of life, yet interestingly, UmuD-like orthologs have only been identified in gram-negative bacteria, their self-transmissible R-plasmids or bacteriophages. Even more intriguing is the fact that the P1 and N15 bacteriophage orthologs actually encode for a preprocessed UmuD′-like protein and do not undergo post-translational cleavage to become biologically active, nor are they associated with a cognate *umuC*-like gene (McLenigan *et al*., 1999). Together, these observations have led to the suggestion that the UmuD and UmuD′-like proteins may participate in other biochemical pathways unique to gram-negative bacteria. One such role might be in a 'cell cycle' DNA damage-checkpoint pathway (Opperman *et al*., 1999; Sutton & Walker, 2001; Ferentz *et al*., 2001). Whatever their role(s) in addition to translesion replication, it is clear that *E. coli* has gone to great lengths to minimize the cellular concentrations of both the UmuD and UmuD′ proteins *in vivo* (Woodgate & Ennis, 1991). In addition to being tightly regulated at the transcriptional level by LexA, UmuD protein is rapidly degraded by the Lon protease (Frank *et al*., 1996a; Gonzalez *et al*., 1998). Some molecules of UmuD that escape Lon-mediated proteolysis are nevertheless converted to UmuD′. Instead of forming homodimers which are resistant to proteolysis (Frank *et al*., 1996a,b), the UmuD′ protomers preferentially associate with intact UmuD to form a UmuD/UmuD′ heterodimer, where the UmuD′ protomer becomes a substrate for another serine protease, ClpXP (Frank *et al*., 1996a; Gonzalez *et al*., 2000).

Distinguishing Features

Polyclonal rabbit antibodies have been produced against both UmuD and UmuD′ (Woodgate *et al*., 1989; Frank *et al*., 1996b) that can detect endogenous levels of the chromosomally encoded *E. coli* proteins (Woodgate & Ennis, 1991; Ennis *et al*., 1995).

Related Peptidases

The C-terminal proteolytic/dimerization domain of the UmuD protein (residues 50–136) has sequence and structural similarity to the proteolytic/dimerization domain of the large family (family S24) of λCI (Bell *et al*., 2000) and LexA-like repressors (Luo *et al*., 2001). It is also structurally related to the central catalytic domain of bacterial signal peptidase (family S26) (Paetzel *et al*., 1998, 2002; Paetzel & Strynadka, 1999). The families S24 and S26 both belong to the clan SF of serine proteases.

Further Reading

A structural analysis and comparison of the Ser/Lys protease has recently been performed (Paetzel *et al*., 2002). For a general discussion on serine-lysine proteases see Paetzel & Dalbey (1997). Recent reviews on UmuD and its role in the SOS response include those of Goodman & Woodgate (2000), Sutton *et al*. (2000, 2002) and Gonzalez & Woodgate (2002). There have been numerous reviews on the Y-family DNA polymerases, among which are Woodgate (1999), Goodman & Tippen (2000), Friedberg *et al*. (2000), Livneh (2001) and references therein.

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Handbook of Proteolytic Enzymes 2nd Edn
ISBN 0-12-079610-4

617. Signal peptidase I

Databanks

MEROPS name: Signal peptidase I
MEROPS classification: clan SF, family S26A, peptidase S26.001
IUBMB: EC 3.4.21.89
CAS registry: 65979-36-4
Species distribution: *Eubacteria*
Sequence known from: *Agrobacterium tumefaciens*, *Aquifex aeolicus*, *Azotobacter vinelandii*, *Bordetella pertussis*, *Bradyrhizobium japonicum*, *Brucella melitensis*, *Brucella suis*, *Buchnera aphidicola*, *Buchnera* sp. APS, *Escherichia coli*, *Haemophilus influenzae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Ralstonia solanacearum*, *Rhodobacter capsulatus*, *Salmonella enterica*, *Salmonella typhimurium*, *Shewanella oneidensis*, *Shigella flexneri*, *Sinorhizobium meliloti*, *Treponema pallidum*, *Vibrio cholerae*, *Wigglesworthia brevipalpis*, *Xanthomonas axonopodis*, *Xanthomonas campestris*, *Xylella fastidiosa*, *Yersinia pestis*
Tertiary structure: Available