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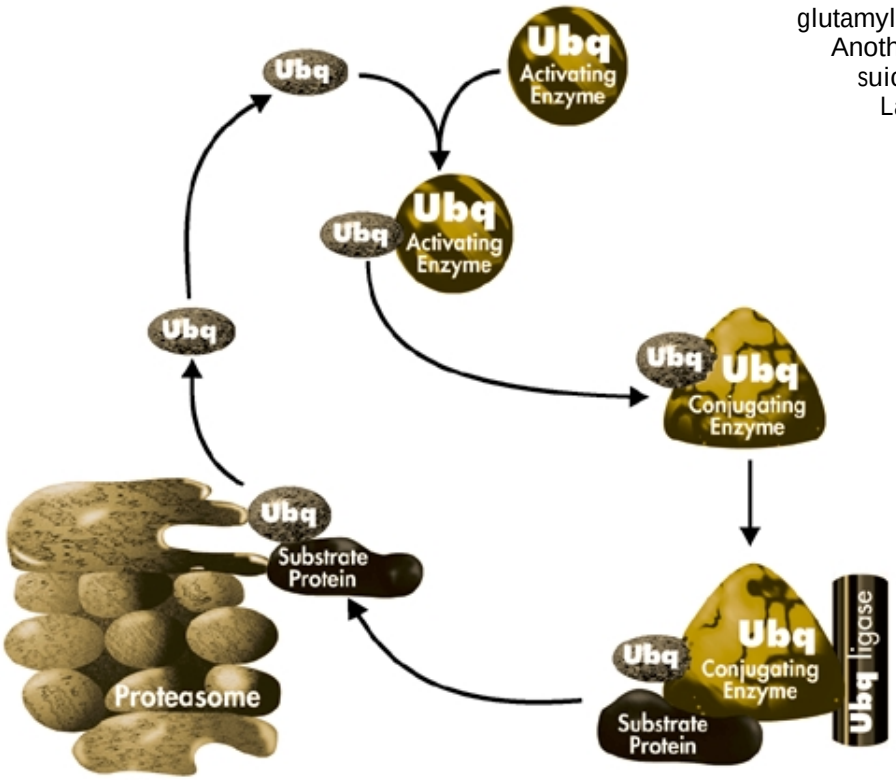
Proteasomes and Related Products

Proteasomes are large multi-subunit protease complexes that selectively degrade intracellular proteins. A vast majority of short-lived proteins are degraded by the ubiquitin-proteasome pathway. A protein marked for degradation is covalently attached to multiple molecules of ubiquitin, a 76-amino acid protein, which escorts it for rapid hydrolysis by the multi-component enzymatic complex known as the 26S proteasome. The proteolytic core of this complex, the 20S proteasome, contains multiple peptidase activities. This core is composed of 28 subunits arranged in four heptameric, tightly stacked, rings (α_7, β_7, β_7, α_7) to form a cylindrical structure. The α-subunits make up the two outer rings and the β-subunits the two inner rings of the stack. The entrance to the active site of the complex is guarded by the α-subunits that allow access only for the unfolded and extended polypeptides. The regulatory unit of the 26S proteasome is known as the 19S particle consisting of about 17 subunits that include ATPases, a de-ubiquitinating enzyme, and polyubiquitin-binding subunits.

This pathway plays a major role in the breakdown of abnormal proteins resulting from oxidative stress and mutations that may otherwise disrupt normal cellular homeostasis. The reactive oxygen species can promote partial unfolding of the protein which exposes its hydrophobic domains to the proteolytic enzymes of the 20S complex.



In the ubiquitin-proteasome degradation pathway, ubiquitin is first activated by an activating enzyme and is transferred to the conjugating enzyme. The conjugation is then performed by a series of enzymes known as E1, E2, and E3. The E1 forms a high-energy thioester bond with ubiquitin that is transferred to a reactive cysteine residue of the E2 enzyme. The final transfer of ubiquitin to an ε-amino group of a reactive lysine residue of the substrate protein is brought about by E3, the ubiquitin ligase enzyme. Ubiquitinated protein is escorted to the proteasome where it undergoes final degradation and the ubiquitin is recycled. The unique and distinguishing feature of the proteasome is the presence of multiple peptidase activities that include chymotrypsin-like activity (cleavage after hydrophobic side chains), postglutamyl peptidase activity (cleavage after acidic side chains), and trypsin-like activity (cleavage after basic side chains). More recently, a new peptidase, tripeptidyl peptidase II (TPPII), was identified that copurifies with the 26S proteasome. TPPII is believed to participate in the degradation of extra-lysosomal polypeptides and may substitute for some metabolic functions of the proteasome, particularly in the absence of normal proteasome function.

Several distinct groups of compounds, designed to act as proteasome inhibitors, have helped immensely in understanding the biological role and importance of the ubiquitin-proteasome pathway. These compounds block proteasome function without affecting the normal biological processes in the cell. The tripeptide aldehyde compounds are known to be reversible inhibitors of chymotrypsin-like, post-glutamyl, and trypsin-like activities of the proteasome. Another class of compounds, vinyl sulfones, act as suicide substrates for the active site nucleophiles. Lactacystin, a third group compound, is a covalent inhibitor of the chymotrypsin-like and trypsin-like activities of the proteasome. Its action is thought to be due to the action of its β-lactone form that is produced upon incubation in the aqueous medium. Some of these inhibitors also have a significant inhibitory effect on the activity of tripeptidyl peptidase II.

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Proteasome Inhibitors

Inhibitor	Cat. No.	Description	Solubility	Size	Refs.
Aclacinomycin A, *Streptomyces galilaeus* M.W. 811.9	**112270**	An anthracycline antitumor agent that inhibits the degradation of ubiquitinated proteins by blocking the chymotrypsin-like activity of the 20S proteasome. Also inhibits DNA topoisomerase I and II.	DMSO or ethanol	50 mg	1
Calpain Inhibitor I (MG-101) M.W. 383.5	**208719**	Ubiquitin-dependent inhibitor of proteolysis of IκB-α and IκB-β by the ubiquitin-proteasome complex. Also inhibits calpain I, calpain II, cathepsin B, and cathepsin L (K_i = 150 - 500 nM). Sequence: Ac-Leu-Leu-Norleucinal	DMSO or ethanol	5 mg 25 mg	2,3
Calpain Inhibitor II M.W. 401.6	**208721**	Inhibits calpains I and II, cathepsin B, and cathepsin L (K_i = 120 - 600 nM) but does not inhibit proteasome activity. Serves as a negative control for Calpain inhibitor I (Cat. No. 208719). Sequence: Ac-Leu-Leu-Methional	DMSO or ethanol	25 mg	2
Lactacystin M.W. 376.4	**426100**	Cell-permeable, irreversible, specific inhibitor of the trypsin-like and chymotrypsin-like activities of the 20S proteasome (IC_{50} ~1 µM). Blocks proteasome activity by targeting the catalytic β-subunit of the proteasome by covalently attaching to the N-terminal Thr of subunit X (MB1). Induces apoptosis in human monoblastic U937 cells.	DMSO	200 µg	4-6
clasto-Lactacystin β–Lactone M.W. 213.2	**426102**	Cell-permeable, active component of Lactacystin (Cat. No. 426100). Irreversible and highly specific inhibitor of the 20S proteasome. About 10-fold more sensitive than its precursor, lactacystin, *in vitro*.	DMSO	100 µg	6,7
MG-115 M.W. 461.6	**474780**	Potent reversible proteasome inhibitor (K_i = 21 nM and 35 nM for 20S and 26S proteasome, respectively). Blocks the assembly of class I molecules by inhibiting generation of peptides presented on MHC class I molecules. Sequence: Z-Leu-Leu-Norvalinal	DMSO or methanol	5 mg	8,9
MG-132 M.W. 475.6	**474790**	Cell-permeable, potent, reversible inhibitor. Reduces the degradation of ubiquitin-conjugated proteins by the 26S complex without affecting its ATPase or isopeptidase activities. Effective at micromolar concentrations. Sequence: Z-Leu-Leu-Leucinal	DMSO	1 mg 5 mg	8,10, 11
NLVS M.W. 722.6	**482240**	Cell-permeable, irreversible inhibitor of the trypsin-like, chymotrypsin-like, and peptidyl-glutamyl peptidase activities of proteasomes. Acts by covalently modifying the NH_2-terminal Thr of the catalytically active β subunit.	DMSO	500 µg	12,13
NP-LLL-VS M.W. 596.7	**492025**	An intermediate that can be used to prepare ^{125}I-radiolabeled NLVS (Cat. No. 482240) for proteasome inhibition studies.	DMSO	500 µg	12
Proteasome Inhibitor I M.W. 618.6	**539160**	Cell-permeable inhibitor of the chymotrypsin-like activity of the 20S proteasome. Causes accumulation of ubiquitinated proteins. Blocks activation of NF-κB in macrophages. ID_{50} = 250 nM *in vitro*; 15 µM in cells. Sequence: Z-Ile-Glu(OtBu)-Ala-Leucinal	DMSO or ethanol	1 mg 5 mg	14-16
Proteasome Inhibitor II M.W. 509.7	**539162**	Potent, cell-permeable proteasome inhibitor. Inhibits the chymotrypsin-like activity (K_i = 460 nM), but not the peptidylglutamyl-hydrolyzing activity. Also blocks the decay of IκB-α and IκB-β proteins in WEHI 231 cells. Sequence: Z-Leu-Leu-Phenylalaninal	DMSO	1 mg 5 mg	17,18
Ubiquitin Aldehyde M.W. 8500.0	**662056**	Stabilizes endogenous or *in vitro*-synthesized ubiquitin-protein conjugates. Potent, specific inhibitor of ubiquitin hydrolases and ubiquitin-protein isopeptidases involved in intracellular modification or turnover.	Water or aqueous buffers	50 µg	19,20

CALBIOCHEM's Proteasome Inhibitor Set (Cat. No. 539164). This convenient set contains 1 mg Proteasome Inhibitor I (Cat. No. 539160), 1 mg MG-132 (Cat. No. 474790), and 200 µg Lactacystin (Cat. No. 426100).

Proteasome Substrates

Substrate	Cat. No.	Sequence	M.W.	Solubility	Size	Refs.
Proteasome Substrate I, Fluorogenic	**539140**	Z-Leu-Leu-Leu-AMC	648.8	DMSO	5 mg	21
Proteasome Substrate II, Fluorogenic	**539141**	Z-Leu-Leu-Glu-AMC	664.8	DMSO	5 mg	22
Proteasome Substrate III, Fluorogenic	**539142**	Suc-Leu-Leu-Val-Tyr-AMC	763.9	DMSO	5 mg	8, 21, 23
Proteasome Substrate IV, Fluorogenic	**539143**	Z-Val-Lys-Met-AMC	667.8	DMSO	5 mg	24
Ubiquitin-AMC, Fluorogenic	**662075**	Ub-AMC	8500	DMSO	25 µg	25

Excitation max: ~380 nm, **Emission max:** ~460 nm

Antibodies

Antibody	Cat. No.	Comments	Application	Size	Refs.
Anti-20S Proteasome, α-Subunit, *Methanosarcina thermophila* (Rabbit)	**539153**	Specific for the α-subunit of the 20S proteasome. Cross-reacts with most mammalian tissues.	IB	100 µl	26
Anti-20S Proteasome, β-Subunit, *Methanosarcina thermophila* (Rabbit)	**539156**	Specific for the β-subunit of the 20S proteasome. Cross-reacts weakly in several mammalian tissues.	IB	100 µl	26
Anti-Ubiquitin, Bovine Erythrocyte (Mouse)	**662097**	Reacts with both ubiquitinated proteins and ubiquitin monomers.	IB	100 µg	27,28
Anti-Ubiquitin-Activating Enzyme E1A, N-Terminal, Human (Rabbit)	**662102**	Recognizes an N-terminal peptide sequence of human E1A. Does not cross-react with E1B.	IB, IP, IH	200 µl	29
Anti-Ubiquitin-Activating Enzyme E1B, N-Terminal, Human (Rabbit)	**662104**	Recognizes both E1A and E1B. Exhibits slight cross-reactivity with unknown ~200 kDa and 50 kDa proteins in Western blots.	IB, IP, IH	200 µl	29
Anti-Ubiquitin-Activating Enzyme E1A/E1B, C-Terminal, Human (Rabbit)	**662106**	Recognizes a common C-terminal peptide in human E1A and E1B.	IB, IP, IH	200 µl	29

IB: Immunoblotting; **IH:** Immunohistochemistry; **IP:** Immunoprecipitation

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Enzymes and Related Products

Product	Cat. No.	Description	Size	Refs.
20S Proteasome, *Methanosarcina thermophila,* Recombinant, *E. coli*	**539150**	A large multi-subunit protease complex of the 26S proteasome that degrades intracellular proteins. Has properties closely resembling the mammalian enzyme including the $\alpha_7\beta_7\beta_7\alpha_7$ structure in four stacked rings. M.W. 645,000.	500 µg	1-3
20S Proteasome, α-Subunit, *Methanosarcina thermophila,* Recombinant, *E. coli*	**539152**	Can be combined *in vitro* with the β-subunit to produce a fully assembled and catalytically active 20S proteasome. M.W. 27,000.	1 mg	1,2
20S Proteasome, Rabbit	**539154**	Can be activated by PA28 (Cat. No. 506280) or SDS. Fully functional using Proteasome Substrate III, Fluorogenic substrate (Cat. No. 539142) following SDS activation. M.W. 700,000.	10 µg	4,5
Isopeptidase T, Rabbit	**419700**	Involved in ubiquitin recycling and the hydrolysis of isopeptide linkages of polyUb chains. Fully functional using Ub-AMC (Cat. No. 662075) as substrate. M.W. 100,000.	25 µg	6,7
Ubiquitin-Activating Enzyme E1, Rabbit	**662070**	Catalyzes the first step in the transfer of ubiquitin to the target protein. M.W. 110,000.	10 µg	8,9
Ubiquitin C-Terminal Hydrolase, Rabbit	**662090**	Hydrolyzes the small C-terminal derivatives of ubiquitin that form non-specifically during protein ubiquitination. Fully functional using Ub-AMC (Cat. No. 662075) as substrate. M.W. 30,000.	10 µg	6,7
PA28 Activator, Rabbit	**506280**	Mixture of the α– and β–subunits. Enhances peptidase activity of the 20S proteasome. Fully functional using Proteasome Substrate III, Fluorogenic substrate (Cat. No. 539142). M.W. 170,000.	10 µg	10,11
Ubiquitin, GST-Tagged, Recombinant, *E. coli*	**662057**	Fully functional ubiquitin with an N-terminal GST-tag. Useful for glutathione affinity purification of ubiquitinated molecules or for immunodetection of conjugates using GST antibodies. M.W. 38,500.	1 mg	—
Ubiquitin, His-Tagged, Recombinant, *E. coli*	**662060**	Fully functional ubiquitin with an N-terminal His-tag. Useful for metal chelate affinity purification of ubiquitinated molecules or for immunodetection of conjugates using polyHis-tag antibodies. M.W. 8500.	1 mg	—
Ubiquitin K48R, Bovine, Recombinant, *E. coli*	**662062**	Mutant ubiquitin featuring a Lys^{48}-Arg^{48} mutation that prevents the formation of polyUb chain. Useful to determine lysine position linkage, to mono-ubiquitinate proteins, and to reduce polyUb chain length and conjugation rates. Fully functional as tested by its ability to charge the E1 enzyme (Cat. No. 662070). M.W. 8500.	1 mg	12
Ubiquitin, Methylated, Bovine	**662063**	Methylated ubiquitin that cannot form polyUb linkages with other ubiquitin molecules. May be used to mono-ubiquitinate proteins and reduce polyUb chain length and conjugation rates. M.W. 8500.	1 mg	13
Ubiquitin-Agarose	**662080**	Ubiquitin covalently linked to agarose beads. Useful for affinity binding of ubiquitin-activating enzyme (E1), ubiquitin conjugating enzyme (E2), ubiquitin ligase (E3), ubiquitin C-terminal hydrolases (UCHs), and other proteins with an affinity for ubiquitin.	500 µl	14,15

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