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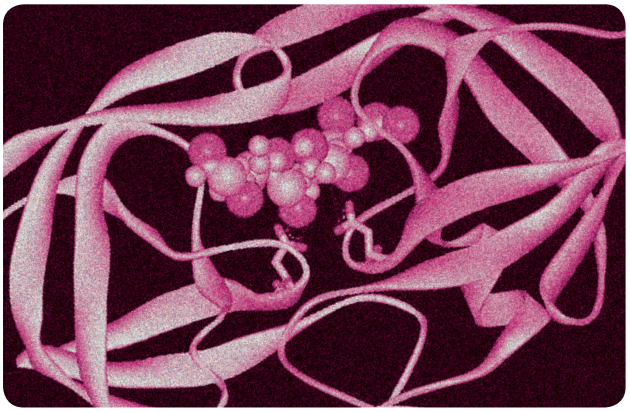
Protease Inhibitors

Introduction

Proteases are key enzymes in the regulation of cellular processes, they are found everywhere in all cells and tissues. Upon cell lysis proteases are released into the lysate. Some of the proteases pose a significant impediment to the analysis of biochemical processes. They can generate erroneous results concerning the activity, structure, or location of proteins. Within only a few minutes protease activity can destroy the preparations that took several days of work. Protease inhibitors are employed in order to prevent involuntary protein degradation. They may be synthesized in the laboratory or purified from natural sources.

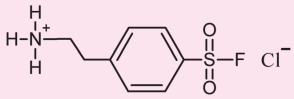
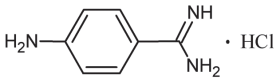
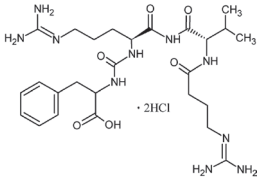
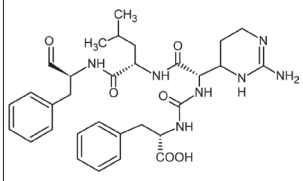
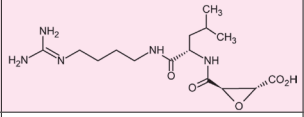
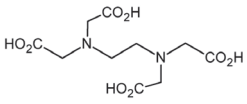
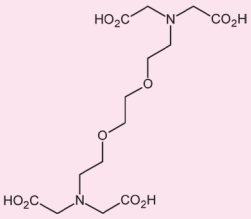
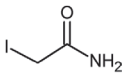
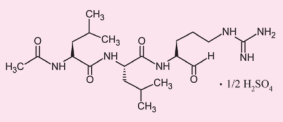
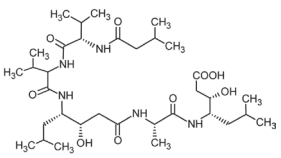
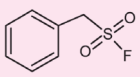


Keywords

- Protease activity
- Protein isolation
- Specific protease inhibitor
- Competitive inhibition

Proteases (also termed proteinase or peptidase) catalyze the hydrolysis of peptide bonds. Exopeptidases remove amino acids from the C- or N-terminus, whereas endopeptidases are capable of cleaving peptides within the molecule. Proteolytic enzyme activity largely depends on the active center of the enzyme. The main components involved in the enzymatic reaction are the amino acids serine, cysteine, and aspartic acid. A fourth group employs metal ions, leading to the classification of the metallo, serine, cysteine and aspartic proteases. In all eukaryotic cells and bacteria a large number of proteases are located in various compartments, the cytosol, mitochondria, vacuoles, lysosomes, ER, or in the extracellular space. Intracellular proteases are essential regulators in the synthesis, activation and degradation of proteins. Extracellular or secreted proteases are most prominent in the intestinal tract of animals or as a part of the blood-clotting cascade. Accordingly, different tissues or organisms contain different sets of proteases. Knowledge of the protease set of a particular expression system enables researchers to combat protease activity throughout the procedure of purification and analysis of proteins.
As proteases evolved, specific natural inhibitors co-evolved, targeting the active center of the enzymes. Protease inhibitors are common in nature, where they have protective and regulatory functions. For instance, about 20 of the nearly 200 proteins of blood serum are protease inhibitors. Various mechanisms are characterized including chemical modification of proteases, competitive binding to the active site or competitive binding to cofactors. For example: (i) TLCK irreversibly inhibits trypsin by alkylating the histidine residue in the active site of the enzyme, (ii) Trypsin inhibitor from soybean forms a strong protein-protein interaction to the active site of trypsin and related serine proteases, (iii) 2-Macroglobulin traps endopeptidases inside of the inhibitor, (iv) bestatine resembles a Phe-Leu substrate dipeptide, but the first residue contains a α-hydroxy group resulting in competitive active site-directed inhibition. Protease inhibitors bind to their target proteins reversibly or irreversibly. Reversibly binding inhibitors may be lost during dialysis or other purification steps. So it is of practical importance to know the mode of action for selecting the appropriate protease inhibitors and preparing solutions and buffers.
Protease inhibitors are supposed to provide specificity so that proteases are blocked but other proteins stay unaffected. Other desired characteristics include solubility and stability. Ideally, the substances are also non-toxic and easy to handle. Scientists at research institutions have relied on the quality of PanReac AppliChem's protease inhibitors for many years. Our protease inhibitors are available as individual substances to target specific proteases (Tab. 1) or more convenient, as cocktails specifically designed to inhibit proteases of the most common expression systems (Tab. 2).

Tab. 1: Individual Protease Inhibitors

Code	Description	M g/mol	Structure	Target Protease Class. Target Enzymes	Mechanism	Recommended Working Concentration	Stock Solution
A1421	AEBSF hydrochloride	239.69		serine proteases, thrombin, chymotrypsin, kallikrein, plasmin, proteinase K, Trypsin	Irreversible inhibition by sulfonylation of a functional group in the active center	0.1 - 2 mM	20 - 100 mM in buffer
A2126	p-Aminobenzamidine dihydrochloride	208.09		serine proteases, trypsin, plasmin, thrombin	Competitive inhibitor	1 mM	100 mM in water
A2266	6-Aminohexanoic acid	131.18		serine proteases		5 mM	500 mM in buffer
A2129	Antipain dihydrochloride	677.63		serine/cysteine proteases, trypsin, papain, cathepsin B		10 - 50 µg/ml	10 mg/ml in water, DMSO, MeOH
A2132	Aprotinin	6511.52	basic protein, consists of 58 amino acids	serine proteases, trypsin, chymotrypsin, kallikrein, plasmin		2 - 10 µg/ml	10 mg/ml in water
A2144	Chymostatin	607.70		serine/cysteine proteases. α-, β-, γ-, δ-chymotrypsin, papain, cathepsin A, B and D	Reversible inhibitor	6 - 60 µg/ml (10 - 100 µM)	20 mg/ml in DMSO, acetic acid
A2157	E-64	357.40		cysteine proteases, papain, bromelain, calpain, cathepsin B, H, L, tumor cathepsin, Streptococcus protease, ficin	Irreversible inhibitor	10 µM	1 - 10 mM in DMSO, 50 % EtOH
A1103	EDTA	292.25		metallo proteases.	Chelating agent, deactivates metal dependent enzymes	1 - 10 mM	500 mM in water
A0878	EGTA	380.35		metallo proteases, KEX 2, calcium-dependent proteases	Calcium specific chelator	1 - 10 mM	in aqueous solution
A1666	Iodoacetamide*	184.96		serine proteases, cerastocytin		1 - 5 mM (185 - 925 µg/ml)	in aqueous solution
A2183	Leupeptin hemisulfate	475.60		serine/cysteine proteases, plasmin, trypsin, papain, cathepsin B, thrombin, calpain	Reversible inhibitor	5 - 50 µg/ml (10 - 100 µM)	1 mg/ml in aqueous solutions
A2205	Pepstatin A	685.91		acid proteases, aspartic proteases, pepsin, cathepsin D, renin, HIV- and MMTV-proteases		1 - 5 µM (0.7 – 3.5 µg/ml)	in MeOH, EtOH, acetic acid solution, DMSO
A0999	PMSF *	174.19		serine/cysteine proteases, trypsin, chymotrypsin, thrombin, papain	Irreversible inhibitor	0.1 - 1 mM	10 -100 mM in EtOH 1.74 - 17.4 mg/ml

* Inactivation by reducing agents

Tab. 2: Protease Inhibitor Cocktails

Code	Description	Composition	Target Protease Class and Application
A7779	Protease Inhibitor Cocktail 5 MammCell/Tissue	E-64 ..... 1 µM AEBSF · HCl ..... 500 µM Aprotinin ..... 150 nM Leupeptin hemisulfate ... 1 µM	Inhibits serine, cysteine and trypsin-like proteases, as well as esterases. Suited for preparation of extracts from mammalian cells and tissue. Lyophilized mixture to make up a 100X solution.

Abbreviations

AEBSF	4-(2-Aminoethyl)-benzolsulfonylfluoride
E-64	N-(trans-Epoxysuccinyl)-L-leucine-4-guanidinobutylamide
EDTA	Ethylenediaminetetraacetate
EGTA	Ethyleneglycol-bis-(2-aminoethyl)-tetraacetate
EtOH	Ethanol
MeOH	Methanol
PMSF	Phenylmethanesulfonylfluoride

Related products

A1086 Tris ultrapure
A1069 HEPES for buffer solutions
A1360 Urea BioChemica
A1499 Guanidine Hydrochloride BioChemica
A1073 Imidazole for buffer solutions
A1101 DTT BioChemica
A1390 Tween® 80 BioChemica
A1112 SDS ultrapure
A0962 Acrylamide 4K solution (40 %)
A1142 Ammonium Persulfate BioChemica
A1148 TEMED
A5243 PVDF-Star Transfer Membrane 0.45 µm
A5237 Reprobe Nitrocellulose supported 0.22 µm Transfer Membrane
A5239 Pure Nitrocellulose unsupported 0.45 µm Transfer Membrane
A5242 Reprobe Nitrocellulose supported 0.45 µm Transfer Membrane

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