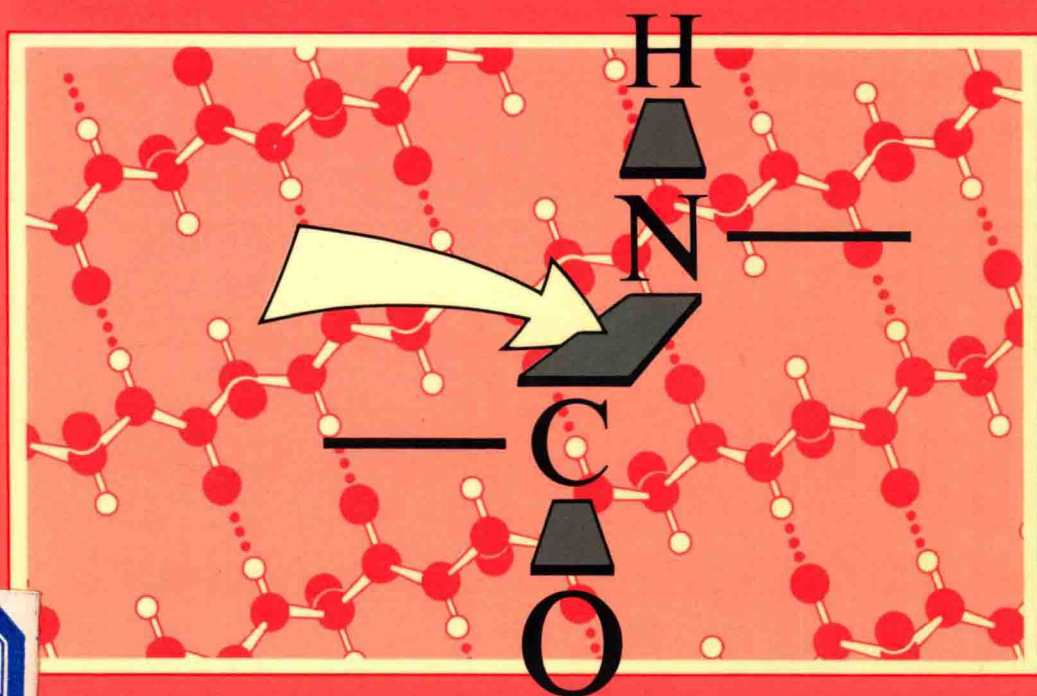


Proteolytic enzymes

a practical approach

Edited by

R J Beynon & J S Bond



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Preface

Proteolytic enzymes are of widespread interest to the scientific community because they can be used as tools, and because they play critical roles in biological systems. For the protein chemist, synthetic chemist, membrane biologist and clinician, proteases are tools or probes to study polypeptides and macromolecular structures, or to interfere with pathological processes. For the biochemist, physical chemist, cell biologist and molecular biologist, they are of interest for the intrinsic properties and for the irreversible modifications they effect in physiological systems. Proteases are involved in a multitude of important physiological processes that range from the functional activation or inactivation of proteins by single proteolytic events, to the complete dissolution of proteins to their constituent amino acids. They have become the focus of a wide range of basic and applied research, and are targets for intervention, experimentally and therapeutically.

The focus of this book is on practical aspects of the handling, characterization, inhibition and use of this class of enzymes. Because of the diversity of the proteases, authors have provided general advice to the reader along with some specific examples. Our hope is that the book will provide ideas and protocols for scientists studying a wide variety of systems and problems.

A note on terminology: the term *protease* is synonymous with the term *peptide hydrolase*; these terms include all enzymes that cleave peptide bonds. Proteases are further subdivided into *exopeptidases*, enzymes that cleave peptide bonds at the amino- or carboxy-terminus, and *endopeptidases*, those that cleave peptide bonds internally in a polypeptide. The term *proteinase* is synonymous with *endopeptidase*.

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Abbreviations

-AA-	amino acyl group
ABz or oABz	<i>o</i> -aminobenzoyl
Ac	acetyl
α 2-M	α 2-macroglobulin
APMSF	4-amidinophenylmethanesulphonyl fluoride
APH	aminophthalhydrazide
ATEE	<i>N</i> -acetyl-L-tyrosine ethyl ester
azoprotein	protein covalently dyed with sulphanilamides
BAEE	<i>N</i> -benzoyl-L-arginine ethyl ester
BISTRIS	bis(2-hydroxyethyl)imino-tris(hydroxymethyl)methane
BSA	bovine serum albumin
BOC or tBOC	<i>t</i> -butyloxycarbonyl
BTEE	<i>N</i> -benzoyl-L-tyrosine ethyl ester
Bz	benzoyl
CBZ	carbobenzoxo, benzyloxycarbonyl, Z
DCI	3,4-dichloroisocoumarin
DFP, DIFP, DIFP or Dip-F	diisopropyl fluorophosphate
DMSO	dimethyl sulphoxide
DNS	dimethylaminonaphthalenesulphonyl, dansyl
DPCC	diphenyl carbamyl chloride
DTT	dithiothreitol
E-64	<i>L-trans</i> -epoxysuccinyl-leucylamido(4-guanidino)butane
EDTA	ethylenediamine tetraacetic acid
ELISA	enzyme-linked immunosorbent assay
EtOH	ethanol
FA	furylacryloyl
FITC	fluorescein isothiocyanate
F ₃ MCA	4-trifluoromethylcoumaryl-7-amide
GHCl	guanidinium hydrochloride
HEPES	<i>N</i> -2-hydroxyethylpiperazine- <i>N</i> -2-ethane-sulphonic acid
HFBA	heptafluorobutyric acid
HOAc	acetic acid
HPLC, hplc	high performance liquid chromatography
IAA	iodoacetic acid
MBNA	4-methoxy- β -naphthylamide
MCA or NHMec	4-methylcoumaryl-7-amide
Me ₂ SO	dimethyl sulphoxide
MeO	methoxy-
MeOH	methanol
MMP	matrix metallo-proteinases; includes MMP1 (collagenase), MMP2 (gelatinase) and MMP3 (stromelysin)
MSH	melanocyte stimulating hormone
NA or NHNan or pNA	4-nitroanilide or <i>p</i> -nitroanilide
NEDD	<i>N</i> -(1-naphthyl) ethylenediamine dihydrochloride
NEM	<i>N</i> -ethyl maleimide

NHNap	2-naphthylamide
Nph	<i>p</i> -nitrophenylalanine
OMe	methyl ester
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCMB	<i>p</i> -chloromercuribenzoate and <i>p</i> -hydroxymercuribenzoate
PMSF	phenylmethanesulphonyl fluoride
POMC	pro-opiomelanocortin
PrOH	propanol
PVDF	polyvinylidene difluoride
RER	rough endoplasmic reticulum
RP-HPLC	reverse-phase HPLC
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
STI	soybean trypsin inhibitor
SUC	succinyl
SV	secretory vesicles
TCA	trichloroacetic acid
TES	<i>N</i> -tris[hydroxymethyl]methyl-2-aminoethane sulphonic acid
TFA	trifluoroacetic acid
TIMP	tissue inhibitor of metallo-proteases
TLC	thin layer chromatography
TLCK	tosyl-lysine chloromethyl ketone
Tos-	tosyl, (4-toluenesulphonyl)
t-PA	tissue plasminogen activator
TPCK	tosylamido-2-phenylethyl chloromethyl ketone
Tris	tris-(hydroxymethyl)-aminoethane
uPA	urinary plasminogen activator
Z	benzyloxycarbonyl, carbobenzoxy (= CBZ)

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ABBREVIATIONS

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