



Essays in Biochemistry

Edited by D.K. Apps and
K.F. Tipton

P

PORTLAND PRESS



volume 29 1995

Essays in Biochemistry

Edited by D.K. Apps and
K.F. Tipton

Advisory board

H.S. Bachelard (U.K.)
D.J. Bowles (U.K.)
C.M. Cuchillo (Spain)
K.R.F. Elliott (U.K.)
J. Mowbray (U.K.)
T. Nagatsu (Japan)
A.E. Pegg (U.S.A.)



PORTLAND PRESS



Essays in Biochemistry is published by Portland Press Ltd on behalf of the Biochemical Society

Portland Press Ltd
59 Portland Place
London W1N 3AJ
U.K

Copyright © 1995 by the Biochemical Society, London

All rights reserved. Apart from any fair dealing for the purposes of research or private study, or criticism or review, as permitted under the Copyright, Designs and Patents Act, 1988, this publication may be reproduced, stored or transmitted, in any forms or by any means, only with the prior permission in writing of the publishers, or in the case of reprographic reproduction in accordance with the terms of the licences issued by the Copyright Licensing Agency. Inquiries concerning reproduction outside those terms should be sent to the publishers at the above-mentioned address.

Although, at the time of going to press, the information contained in this publication is believed to be correct, neither the authors nor the publisher assume any responsibility for any errors or omissions herein contained. Opinions expressed in these Essays are those of the authors and are not necessarily held by the Biochemical Society, the editors or the publishers.

British Library Cataloguing-in-Publication Data

A catalogue record for this book is available from the British Library

ISBN 1-85578-017-8

ISSN 0071-1365

Typeset by Portland Press Ltd and printed in Great Britain by Henry Ling Ltd, Dorchester

Essays in Biochemistry

The Authors

Jean-Marie Frère graduated from the University of Liège in 1964 with a degree in Chemistry and obtained a Ph.D. in Biochemistry from the University of Montréal in 1969. After holding a postdoctoral position for one year at the Massachusetts Institute of Technology, he returned to Liège in 1970 and was appointed Professor of Enzymology in 1979. Since 1971, his research interests have mainly centred on the enzymes that interact with β -lactam antibiotics: penicillin-sensitive DD-peptidases and β -lactamases. **Marc Jamin** graduated in 1988 in Biochemistry from the University of Liège. He obtained a Ph.D. in Biochemistry in 1993 under the supervision of J. M. Frère. He currently holds a postdoctoral position in the Department of Biochemistry at Liège as a Chargé de Recherches of the Belgian National Research Foundation. **Jean-Marc Wilkin** obtained his B.Sc. in Biochemistry from the University of Liège in 1989 and his Ph.D. (under the supervision of J.M. Frère) in 1993. He is currently working as a postdoctoral fellow at the Research School of Biological Sciences, The Australian National University, Canberra.

Kunihiko Suzuki, M.D., graduated in History and Philosophy of Science from Tokyo University, Japan, in 1955 and obtained his M.D. from the Faculty of Medicine in 1959. During the 1960s he held academic positions at Albert Einstein College of Medicine, New York, and the University of Pennsylvania School of Medicine. In 1972 he was appointed Professor of Neurology and Neuroscience at the Albert Einstein College of Medicine. He has held positions as Director of the Brain and Development Research Center, and Professor of Neurology and Psychiatry at the University of North Carolina School of Medicine, since 1986. His scientific interests have included brain lipids and their metabolism, and genetic neurological disorders, particularly those involving lysosomal hydrolases. He has actively participated in the evolution of the field through the phases of analytical biochemistry, enzymology and molecular biology.

Catherine Rice-Evans is Professor of Biochemistry at UMDS, Guy's Hospital, and Director of Free Radical Research. She obtained her B.Sc. degree in Chemistry at the University of London and her Ph.D. undertaking research at the National Institute for Medical Research. Her major research interests are in the involvement of free radicals in the pathogenesis of disease and the role of antioxidants in the maintenance of health and disease prevention. She is currently President of the Society for Free Radical Research (Europe) and a member of UNESCO Global Network for Molecular and Cell Biology. **Vimala Gopinathan** is Senior Registrar in Paediatrics at St Thomas's Hospital with a special interest in neonates. She became a member of the Royal College of

Physicians of London in 1989. Her major research interests are in plasma antioxidant status in preterm infants.

Ed Wood has a D.Phil. from the University of Oxford and has worked at the University of Leeds since 1971, where he is now Reader and Head of the Department of Biochemistry and Molecular Biology. His research interests are in human skin and its diseases, and in wound healing. He is also Editor of *Biochemical Education* and Chairman of the Professional and Education Committee of the Biochemical Society. **Ian Harris** graduated in Biochemistry at the University of York, 1990, and received a Ph.D. from the Department of Biochemistry and Molecular Biology at the University of Leeds in 1994. His interests in using keratinocyte sheets as a treatment for patients with leg ulcers have continued his work at the Yorkshire Regional Tissue Bank.

Edward (Ted) Maden is Johnston Professor of Biochemistry at the University of Liverpool, where he has recently introduced an Honours Module course on Specific Eukaryotic Genes. This course complements a standard course on gene structure and expression, by showing for a hand-picked selection of eukaryotic genes how the cloning of these genes arose from the contemporary state of the art, and what has been learnt from their detailed and continuing analysis. Genes chosen for this kind of in-depth treatment include ribosomal RNA genes, on which Ted Maden has carried out most of his research, globin genes, in which many fundamentals of eukaryotic genes were first discovered, and opsin genes, which underlie our visual perception of the external world, as well as several other exemplary gene systems. The seeds for Ted Maden's interest in opsins and visual perception were sown in his final year at Cambridge University where, as a Physiology undergraduate, he attended a series of lectures on vision by the late W.A.H. Rushton.

Peter Lund is a lecturer in the Microbial Genetics and Cell Biology Research Group, in the School of Biological Sciences at the University of Birmingham. His current research interests encompass several areas of molecular chaperone and heat shock protein biology, in particular the biological role of the hsp60 proteins and their potential medical and biotechnological importance. He has published work in several different research areas, including protein secretion, gene targeting, and regulation of gene expression.

Anthony R. Clarke obtained his B.Sc. from the University of Sheffield in 1980, and his Ph.D. from the University of Bristol in 1983. He is currently a lecturer and Lister Institute Fellow at the University of Bristol. He has published extensively in the field of enzyme catalysis and protein engineering and, more recently, on protein folding and the mechanism of chaperonins. **Steven G. Burston** obtained a B.Sc. in 1989 and a Ph.D. in 1992 from the University of Bristol, where he is currently a postdoctoral associate in the Biochemistry Department. His initial work centred on identifying equilibrium and kinetic

intermediates during spontaneous protein folding, and he has recently entered the field of molecular chaperones.

Jane Irwin obtained her degree in Biochemistry from the University of Dublin in 1987 and her Ph.D. in 1994. She is currently employed in the Department of Biochemistry in a project concerned with the diagnosis of Alzheimer's disease. **Keith Tipton** graduated with a degree in Biochemistry from the University of St Andrews in 1962, and obtained his Ph.D. from the University of Cambridge in 1966. He is currently Professor of Biochemistry at Trinity College, Dublin.

Corinne Smith is a postdoctoral scientist working in the group of Dr Tony Clarke in the Department of Biochemistry at the University of Bristol. She is a visiting worker with the Prion Disease Group. **John Collinge** is a Wellcome Senior Research Fellow in the Clinical Sciences and leads the Prion Disease Group at the Department of Biochemistry and Molecular Genetics at St Mary's Hospital Medical School, Imperial College, London.

Phil Turner is a Senior Lecturer in the Department of Biochemistry, University of Liverpool, where his research group is involved in studying various aspects of gene expression in eukaryotes. These include studies of transcription factors, in both animal viruses and their hosts, the function of the U7 small nuclear RNP particle in processing histone pre-mRNA and the use of ribozymes to inhibit gene expression. He is a graduate of the Biochemistry Department at Leeds University and obtained his Ph.D. in the Department of Molecular Biology, University of Edinburgh, in 1978. Before being appointed to his present position, he was a member of the Department of Biological Sciences, University of Warwick, where he carried out postdoctoral work on histone gene expression in the Developmental Biology Group. **Helen James** graduated in 1990 from the University of Wales, Cardiff, with a B.Sc. Joint Honours in Biochemistry and Chemistry. She went on to study ribozymes and modified snRNAs for a Ph.D. at Liverpool University, in the laboratory of Dr Phil Turner, and is currently a postdoctoral research associate in the School of Biological Sciences, University of East Anglia. Her research involves the design and optimization of ribozymes against mRNAs associated with chronic myeloid leukaemia with a view to a potential therapy.

Don Cowan graduated with a Ph.D. from the University of Waikato in Hamilton, New Zealand, in 1980. He stayed with the newly formed Thermophile Research Unit, then funded by Shell Ventures, for a further 5 years, working on the enzymology of a number of thermophilic and hyperthermophilic Bacteria and Archaea. In 1985 he moved to the U.K. to take up a lectureship in the Department of Biochemistry at University College, London, where he currently holds a Senior Lectureship, supervises a research group (still working on thermophiles), administers a B.Sc. degree in Biotechnology, runs a small biotechnology company and seldom suffers from boredom.

Abbreviations

A ₂ pm	diaminopimelic acid
Ac ₂ KAA	N ^α , N ^ε -diacetyl-L-lysyl-D-alanyl-D-alanine
AcKAA	N ^α -acetyl-L-lysyl-D-alanyl-D-alanine
ADA	adenosine deaminase
ADH	horse liver alcohol dehydrogenase
ADRP	autosomal dominant retinitis pigmentosa
apo E	apolipoprotein E
ASVB	Avocado sun-blotch virus
a.t.r.-f.t.i.r.	attenuated total reflection Fourier transform infrared spectroscopy
BH	protonated base
bis(NAD ⁺)	N ₂ , N ₂ '-(adipodihydrazide)-bis(N ⁶ -carbonylmethyl)NAD ⁺
BPD	bronchopulmonary dysplasia
BSE	bovine spongiform encephalopathy
CAT	chloramphenicol acetyltransferase
c.d.	circular dichroism
CJD	Creutzfeld–Jakob disease
CLD	chronic lung disease
cpn	chaperonin
DD-peptidase	D-alanyl-D-alanine peptidase
DMSO	dimethylsulphoxide
ECM	extracellular matrix
EGF	epidermal growth factor
ER	endoplasmic reticulum
GDH	glutamate dehydrogenase
GPI	glycosylphosphatidylinositol
GSS	Gerstmann–Sträussler syndrome
HDV	hepatitis delta virus
h.p.l.c.	high-performance liquid chromatography
H ₂ O ₂	hydrogen peroxide
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HMM	high molecular mass
hsp	heat-shock protein
IgG	immunoglobulin G
IVH	intraventricular haemorrhage
IVS	intervening sequence

LCR	ligase chain reaction
LDH	lactate dehydrogenase
LDL	low-density lipoprotein
LMM	low molecular mass
MHC	major histocompatibility complex
n.m.r.	nuclear magnetic resonance
NEC	necrotizing enterocolitis
NIPAM	<i>N</i> -isopropyl acrylamide
NO	nitric oxide
nt	nucleotide
O ₂ ⁻	superoxide radical
PBP	penicillin-binding protein
PCR	polymerase chain reaction
PEG	polyethylene glycol
PFK	phosphofructokinase
PrP	prion protein
RNase P	ribonuclease P
ROP	retinopathy of prematurity
SAP	sphingolipid activator protein
snRNA	small nuclear RNA
snRNP	small nuclear ribonucleoprotein
STRSV	satellite RNA of tobacco ringspot virus
ss	single stranded
TRiC	TCP ring complex
VLBW	very low birth weight
YADH	yeast alcohol dehydrogenase

Contents

Authors.....	ix
---------------------	-----------

Abbreviations.....	xiii
---------------------------	-------------

Bacterial DD-transpeptidases and penicillin

Marc Jamin, Jean-Marc Wilkin and Jean-Marie Frère

Introduction: the bacterial DD-transpeptidases and the peptidoglycan.....	1
How penicillin kills bacteria.....	2
The physiological DD-transpeptidases	4
The DD-peptidases of <i>Streptomyces</i> R61, <i>Streptomyces</i> K15 and <i>Actinomadura</i> R39.....	4
DD-Peptidases and β -lactamases.....	7
Ester and thioester substrates.....	9
Modification of the <i>Streptomyces</i> R61 enzyme by site-directed mutagenesis.....	13
The search for a general base	17
Penicillin-resistant PBPs	20
Conclusion: does penicillin behave as a substrate analogue?	21
References.....	22

2 Sphingolipid activator proteins

Kunihiko Suzuki

Introduction.....	25
Brief history	27
Nomenclature	28
Molecular genetics.....	28
Activator function <i>in vitro</i>	30
Physiological activator function <i>in vivo</i> and genetic disorders	31
Other possible functions	33
Future investigations.....	34
References.....	36

3 Oxygen toxicity, free radicals and antioxidants in human disease: biochemical implications in atherosclerosis and the problems of premature neonates

Catherine A. Rice-Evans and Vimala Gopinathan

Introduction.....	39
Antioxidants in the maintenance of health and protection against disease	43
Atherosclerosis: oxidants and antioxidants	47
Hyperoxia and oxygen toxicity: oxygen radical injury in the newborn	55
Plasma antioxidant status in neonates	59
References.....	62

4 Reconstructed human skin: transplant, graft or biological dressing?

Edward J. Wood and Ian R. Harris

Introduction.....	65
The structure of human skin: regeneration and grafting.....	66
Skin immunology.....	69
Growing keratinocyte sheets	70
Is allografting with keratinocyte sheets successful?	72
Skin banking and the HIV problem.....	73
Cryopreservation	74
Dermal equivalents and skin equivalents	74
The future	80
Conclusions	82
References.....	82

5 Opsin genes

B. Edward H. Maden

Introduction.....	87
Rhodopsin function and structure in outline	88
Bovine opsin cDNA.....	91
Bovine and human opsin genes	93
Human colour vision	93
Red and green abnormalities and the absolute red-green map.....	95
<i>Drosophila</i> opsins and opsin phylogeny	97
The seven transmembrane helix superfamily of receptors.....	101

Exploring the details of structure and function of opsins.....	101
Concluding comments	108
References	108

6 The roles of molecular chaperones *in vivo*

Peter A. Lund

Introduction.....	113
The hsp70 proteins: multi-functional and ubiquitous chaperones.....	114
The hsp60 proteins: archetypal molecular chaperones	116
The cytosolic TCPI-like proteins.....	118
Other molecular chaperones: probable and possible.....	118
Summary.....	119
References	122

7 Molecular chaperones: physical and mechanistic properties

Steven G. Burston and Anthony R. Clarke

Introduction.....	125
The hsp70 molecular chaperone	126
Hsp70 as part of a larger molecular chaperone complex.....	128
The role of hsp70 in maintaining a translocation-competent protein conformation.....	128
The actions of chaperonins in assisted protein folding.....	129
TCPI ring complex: a eukaryotic chaperone in the cytosol.....	133
Hsp90: molecular chaperone or protein regulator?	134
Summary.....	134
References	135

8 Affinity precipitation: a novel approach to protein purification

Jane A. Irwin and Keith F. Tipton

Introduction.....	137
Affinity precipitation with bis-ligands	138
Affinity precipitation with heterobifunctional ligands.....	151
Conclusion.....	154
References	155

9 Molecular pathology of prion diseases

Corinne Smith and John Collinge

Introduction.....	157
-------------------	-----

Molecular genetics of the human prion diseases	159
A model of prion propagation	165
Effect of disruption of PrP gene in mice	166
Analysis of prion structural elements.....	167
Summary of theories of prion propagation.....	170
Conclusions	171
Further reading.....	172
References	172

10 Ribozymes

Helen A. James and Philip C. Turner

Introduction.....	175
RNA catalysis	175
Self-splicing RNAs	177
Ribonuclease P	180
Self-cleaving RNAs	181
Hammerhead ribozymes	182
Hairpin ribozymes	187
Hepatitis delta virus	188
<i>Neurospora</i> mitochondrial VS RNA.....	189
Ribozyme delivery.....	189
Suggestions for further reading.....	190
References	191

11 Protein stability at high temperatures

D.A. Cowan

Introduction.....	193
Thermophilic organisms	194
Thermostable proteins	195
Mechanisms of protein thermostability	200
Inactivation at high temperatures.....	201
Consequences of hyperstability	201
Protein engineering for thermostability.....	204
Biotechnological implications	204
Future applications.....	205
Summary.....	206
References	206

Subject index	207
----------------------------	------------

Bacterial DD-transpeptidases and penicillin

Marc Jamin, Jean-Marc Wilkin and
Jean-Marie Frère*

*Laboratoire d'Enzymologie and Centre d'Ingénierie des Protéines,
Institut de Chimie, B6, Université de Liège, B-4000 Sart-Tilman, Liège
1, Belgium.*

Introduction: the bacterial DD-transpeptidases and the peptidoglycan

D-Alanyl-D-alanine peptidases (DD-peptidases) are membrane-bound enzymes involved in the synthesis and remodelling of the peptidoglycan (or murein), a macromolecular sacculus composed of linear glycan chains cross-linked by short peptides (Figure 1), which completely surrounds the cytoplasmic membrane of bacterial cells and is responsible for their shape and mechanical resistance to their own osmotic pressure¹.

The peptidoglycan is a dynamic structure that is continuously remodelled during the cell cycle under the regulated control of two conflicting synthetic (transpeptidase and transglycosylase activities) and hydrolytic (endopeptidase, carboxypeptidase and glycosidase activities) machineries. While the external face of the murein shell is eroded by these autolytic enzymes, disaccharide-peptide precursors linked to an isoprenoid lipid carrier are formed in the cytoplasm, either by *de novo* synthesis or by recycling the liberated peptides. These building blocks are translocated across the cell membrane and incorporated into the peptidoglycan by reactions which occur in the extracellular compartment; disaccharide-peptide units are added to the growing glycan chains and the peptide bridges are subsequently formed by transpeptidation between the peptide chains of adjacent strands. The R-D-alanyl group

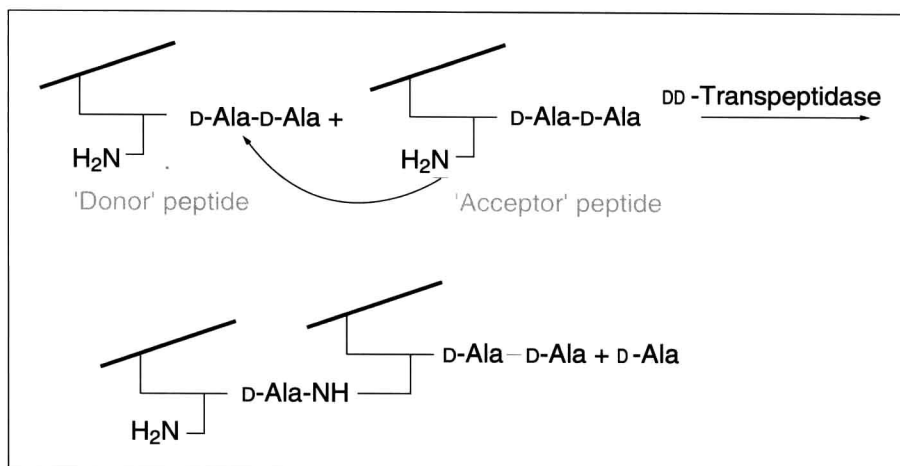
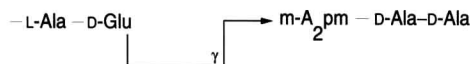


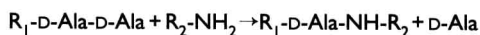
Figure 1. Formation of the peptide bridge in peptidoglycan synthesis

The heavy lines represent the glycan chains composed of alternating *N*-acetylglucosaminyl and *N*-acetylmuramyl residues. The peptide moiety attached to the lactyl side-chain of the latter exhibits specific variations in the different bacterial genera. In Gram-negative bacteria, a



sequence is found, where the free 'acceptor' amino group is on the D centre of meso-diaminopimelic acid (m-A₂pm; a detailed structure of the C-terminal tetrapeptide can be found in Figure 3b). For more details, see references 1 and 2 and references therein.

The reaction can also be represented by the simple scheme:



where $R_1\text{-D-Ala-D-Ala}$ and $R_2\text{-NH}_2$ are the donor and acceptor peptides, respectively.

of a D-alanyl-D-alanine-terminated 'donor' precursor is transferred to the amino-group of a neighbouring 'acceptor' peptide and the C-terminal D-alanine of the former is released (Figure 1). The equilibrium of that reaction is displaced to the formation of the murein by the insolubilization of the polymer and the diffusion of D-alanine away from the reaction site².

How penicillin kills bacteria

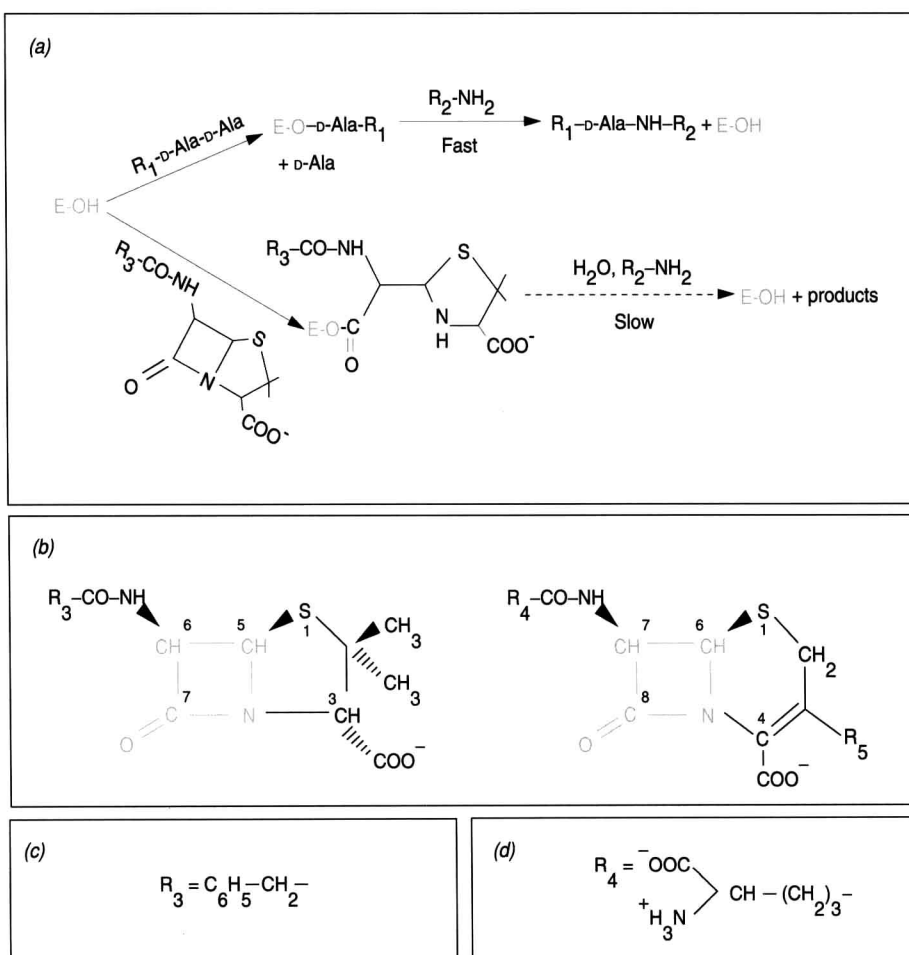
All DD-transpeptidases discovered so far are active-site serine enzymes whose catalytic pathways involve transient acylenzyme adducts (Figure 2a) where the penultimate D-alanine residue of the 'donor' substrate is ester-linked to the active-site serine side-chain.

Penicillins, cephalosporins (Figure 2b) and other β-lactam antibiotics inhibit peptidoglycan biosynthesis by inactivating the DD-transpeptidases; they

form a covalent, stable acylenzyme with the same residue (Figure 2), thus blocking the bacterial growth and division^{3,4}. Uncross-linked peptidoglycan is unable to resist the cell osmotic pressure and lysis most often occurs, but a triggering of the bacterial autolytic system also appears to play an important role in this phenomenon, at least in some species. The specificity of β -lactams as antibacterial agents results from the fact that the peptide moiety of peptidoglycan is unique to the bacterial world, and that no similar transpeptidation reaction involving D-alanyl-D-alanine-terminated peptides exists in eukaryotic organisms.

Figure 2. Catalytic pathway of DD-transpeptidases (E-OH) and inactivation by penicillins (a) and structures of penicillins and cephalosporins (b)

(a) Hydrolysis or aminolysis of the penicilloyl-enzyme is so slow that this reaction is generally devoid of physiological importance. It can also involve an additional breakdown of the penicilloyl moiety. (b) The structures of penicillins and cephalosporins are shown in detail. The β -lactam ring is shown in blue. Typical examples are (c) benzylpenicillin and (d) cephalosporin C. In 6-amino-penicillanate, the amino group of the side-chain is unsubstituted (R_3 -CO- is replaced by H).



The physiological DD-transpeptidases

The physiologically important DD-transpeptidases are membrane-bound proteins which can be labelled with radioactive or fluorescent penicillins and separated by SDS/PAGE. These techniques reveal the presence of several 'penicillin-binding proteins' (PBPs) in the membrane of all eubacteria⁵. The role of these various enzymes in peptidoglycan synthesis and cell division is presently best understood in *Escherichia coli* (Table 1). The position of the active-site serine in the sequence allowed the PBPs to be divided into low- and high-molecular-mass enzymes. The low-molecular-mass PBPs (LMM-PBPs) exhibit carboxypeptidase (PBPs 4, 5 and 6) activities which seem to be dispensable to the survival of the bacteria but which take part in the regulation of the cell cycle. The high-molecular-mass PBPs (HMM-PBPs) are two-domain proteins with a C-terminal, penicillin-binding domain responsible for the transpeptidase activity. Genetic and morphological approaches highlighted the roles of the HMM-PBPs, and of the products of additional genes, in cell wall elongation, septum formation during the cell division and in shape determination^{2,5-7}. Intimately associated with the autolysins in the regulation of the cell cycle, the different DD-transpeptidases undergo activation and deactivation in the different steps of this cycle⁸. However, the catalytic and regulation mechanisms of these enzymes remain poorly understood, since large quantities of purified proteins have never been available.

The cloning and sequencing of the genes coding for various PBPs of several species have supplied detailed information on the primary structures of the corresponding proteins and, more recently, the elimination of the DNA region coding for the membrane-anchoring peptide has allowed the production of some apparently functional, soluble penicillin-binding domains of these enzymes. Nevertheless, most of the information which has been accumulated on the catalytic properties of penicillin-sensitive DD-transpeptidases, and on their interactions with β -lactams, has been obtained with soluble DD-peptidases synthesized by some members of the Actinomycetales order.

The DD-peptidases of *Streptomyces* R61, *Streptomyces* K15 and *Actinomadura* R39²

The DD-peptidases of *Streptomyces* R61 and *Actinomadura* R39 are secreted in the extracellular medium as soluble proteins, and that of *Streptomyces* K15 is loosely bound to the cytoplasmic membrane and can be solubilized in the presence of 0.5 M NaCl. The purified proteins catalyse the cleavage of the C-terminal D-alanine of simple synthetic peptides, such as N^α , N^ϵ -diacetyl-L-lysyl-D-alanyl-D-alanine (Ac_2KAA) and N^α -acetyl-L-lysyl-D-alanyl-D-alanine (AcKAA). In the presence of acceptor compounds exhibiting a suitably located amino group, they also perform transpeptidation reactions according to the scheme depicted in the legend of Figure 1. The *Streptomyces* K15 enzyme is so efficient as a transpeptidase that it only hydrolyses a minor proportion of the