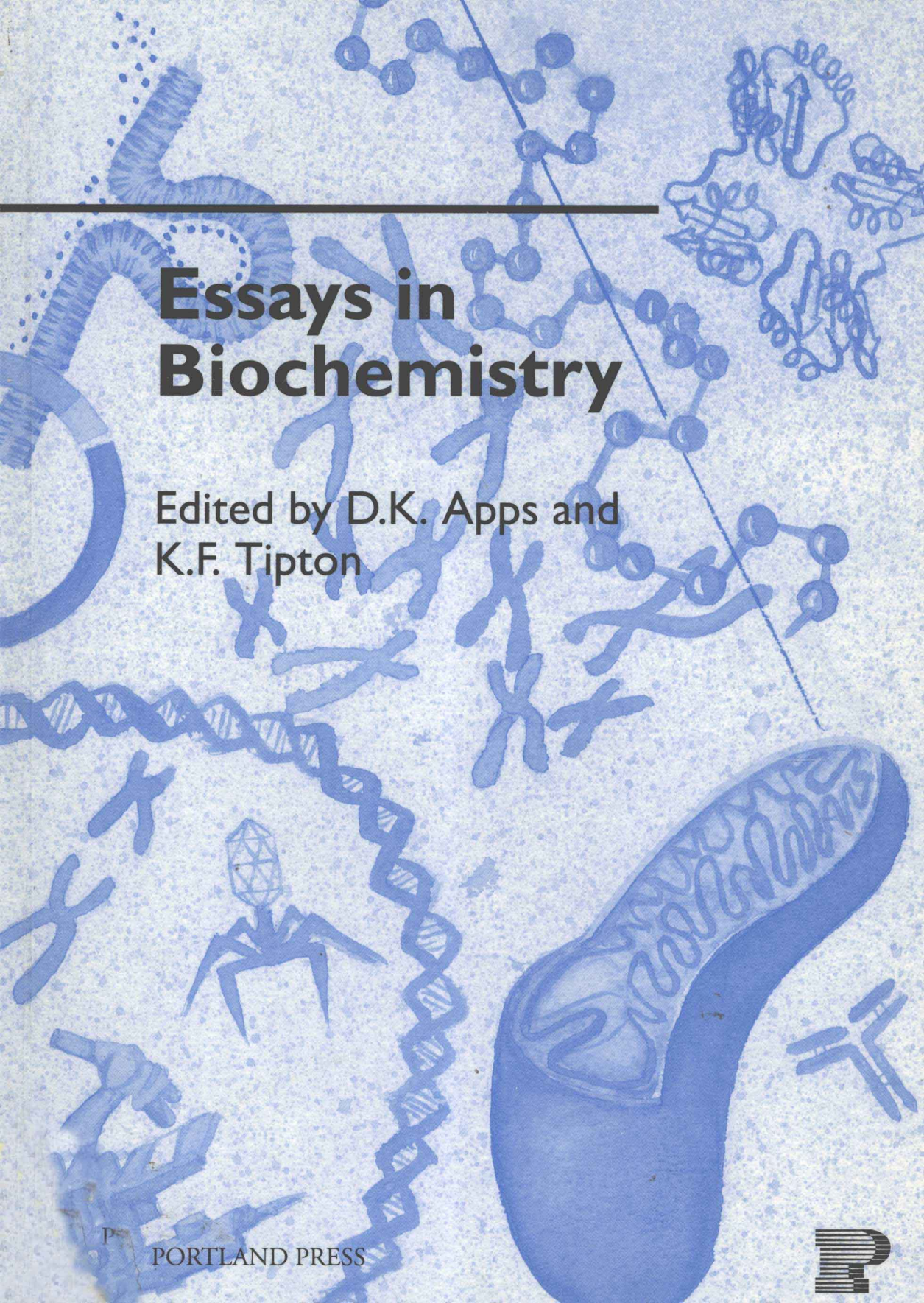

Essays in Biochemistry

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

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Essays in Biochemistry

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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_2pm	diaminopimelic acid
Ac_2KAA	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_2O_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	*N*-isopropyl acrylamide
NO	nitric oxide
nt	nucleotide
$O_2^{-\cdot}$	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

Bacterial DD-transpeptidases and penicillin

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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 pressure[1].

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

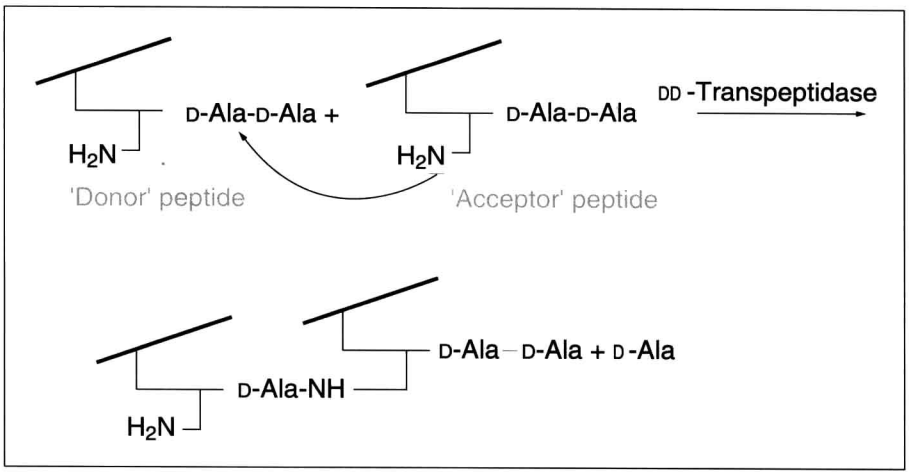
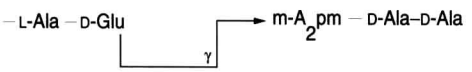


Figure 1. Formation of the peptide bridge in peptidoglycan synthesis
The heavy lines represent the glycan chains composed of alternating *N*-acetylglucosaminyl and *N*-acetylmuramyl residues. The peptide moiety attached to the lactyl side-chain of the latter exhibits specific variations in the different 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-A_2pm; a detailed structure of the *C*-terminal tetrapeptide can be found in Figure 3*b*). For more details, see references 1 and 2 and references therein.

The reaction can also be represented by the simple scheme:

$$R_1\text{-D-Ala-D-Ala} + R_2\text{-NH}_2 \rightarrow R_1\text{-D-Ala-NH-}R_2 + \text{D-Ala}$$

where R_1-D-Ala-D-Ala and R_2-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 site[2].

How penicillin kills bacteria

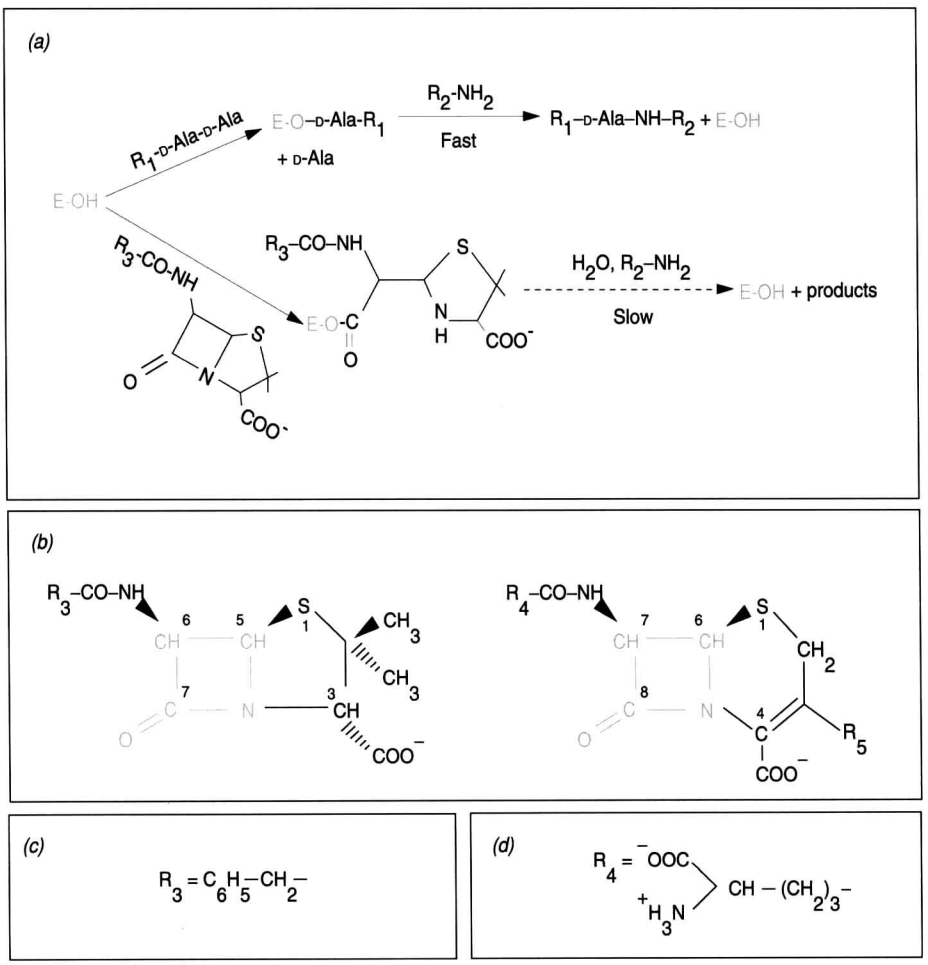
All DD-transpeptidases discovered so far are active-site serine enzymes whose catalytic pathways involve transient acylenzyme adducts (Figure 2*a*) where the penultimate D-alanine residue of the 'donor' substrate is ester-linked to the active-site serine side-chain.

Penicillins, cephalosporins (Figure 2*b*) 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 eubacteria[5]. 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 cycle[8]. 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* R39[2]

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