Klaus Aktories (Ed.)

Bacterial Toxins

Tools in Cell Biology and Pharmacology

Laboratory Companion

With 42 Figures

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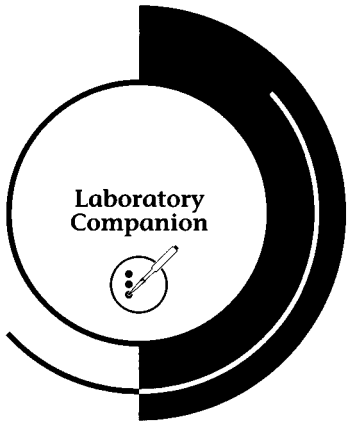

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Preface

Remarkable progress has been made in the field of bacterial protein toxins: the molecular mechanisms of several toxins (e.g., clostridial neurotoxins and cytotoxins) whose modes of action were obscure until recently, have been elucidated during the last years. This progress in "cellular and molecular toxinology" not only provides insights into the mode of action of important virulence factors and pathomechanisms of diseases, but also has a major impact on the understanding of regulation and mechanisms of eukaryotic cell functions which are disturbed by the toxins. These spectacular advances mainly depend on a bidirectional approach: cell biological methods are successfully applied for elucidation of the molecular mechanisms of bacterial toxins and, on the other hand, the bacterial toxins are used as powerful, extremely valuable tools to unravel mechanisms in molecular cell biology.

The reasons for using toxins as tools are evident. First, they are very potent agents, a fact which is often based on their enzymatic activity (e.g., clostridial neurotoxins, which act as endoproteases, are the most potent agents known). Second, the high selectivity and specificity of the toxins are most important for their use as tools. Cell selectivity may be due to specific receptor binding (e.g., selectivity of neurotoxins). The high specificity of action depends on the extremely specific recognition of target proteins. Finally, bacterial protein toxins seem to be maximally efficient agents. In most cases the toxins strike the eukaryotic cell at a crucial site indicating modification of "important" eukaryotic components or signaling pathways. Therefore, the toxins are excellent tools to recognize the biological importance of a cellular component, or the biological significance of a signal pathway altered by the toxins.

This book is intended to establish and facilitate the use of bacterial protein toxins as tools in cell biology and pharmacology. The volume provides a review and an update of recent developments in the field of those bacterial protein toxins which are most often used as cell biological and pharmacological tools. Moreover, the volume gives detailed methodological protocols and advice for application of the toxins, and is intended to introduce these bacterial protein toxins as cell biological tools to a broad audience of scientists. Therefore, it was decided to have for each toxin or toxin family one chapter as a special review to give the general properties of the toxins, and up to three

additional chapters which focus on methodological aspects of toxin usage. Seven toxins or toxin families are covered by this volume. These include the "classical" G-protein-ADP-ribosylating toxins cholera toxin and pertussis toxin, which are established tools to study signal transduction processes (chapters 1-4). Properties and application of C3-like transferases, which specifically ADP-ribosylate Rho proteins, are described in chapters 5-7. Chapters 8-11 deal with the actin-ADP-ribosylating toxins, which are the most effective agents to depolymerize cellular F-actin and to inhibit actin polymerization. The Rho-glucosylating *C. difficile* toxins, whose molecular mechanism has been elucidated only recently, are discussed in chapters 12 and 13. Reviews of recent studies on the basic properties of clostridial neurotoxins and their application in cell and neurobiology are given in chapters 14 to 16. Chapters 17 and 18 describe in detail the general properties, new developments and methodological aspects of pore-forming toxins. In chapter 19, the application of bacterial protein toxins as transporting tools is discussed and application protocols are given. Finally, chapter 20 is a brief safety guide, which should help to deal with general problems in handling of biological toxins.

Last not least, I would like to thank all the authors for their outstanding effort. I am convinced that their excellent contributions will inspire research in the field of protein toxins and facilitate the use of toxins as tools in biological sciences.

February 1997 Klaus Aktories

A note on the layout of this book:

In order to facilitate the use of this book as a methodological source for your bench work, a wide page format has been chosen.
Due to the type of durable binding used, the book has the advantage of lying flat on your bench top for convenient use. In addition, a wide margin leaves room for your own notes and provides some key notes and pictograms to assist you in finding the relevant information:

a pipette symbol marks the start of a step by step protocol section, a grey bar runs down the margin of the whole protocol section

this symbol draws your attention to potential hazards and safety suggestions

comments on the key steps in methodology are highlighted by a key symbol

a "good idea" symbol marks useful hints for optimization of methodology

this pictrogram indicates discussions of alternative approaches

the tool indicates troubleshooting guides that should help you in finding out what could or did go wrong and in solving and avoiding problems

suggestions for monitoring quality and reliability of the experimental procedure are highlighted by the magnifying glass

Contents

Abbreviations

The following is a list of the most common abbreviations used throughout the book.

5-HT	5-hydroxytryptamine
ACh	acetylcholine
ACTSK	actin cytoskeleton
ADP	adenosine diphosphate
ARF	ADP-ribosylation factor
ATP	adenosine triphosphate
BFA	brefeldin A
BHI	brain-heart infusion
BME	2-mercaptoethanol
BoNT	botulinum neurotoxin
BSA	bovine serum albumin
C2T	botulinum C2 toxin
CB	cellubrevin
CNT	clostridial neurotoxin
CPE	cytopathogenic effect
CROP	combined repetitive oligopeptides
CT	cholera toxin
CTL	cytotoxic T lymphocyte
DEPC	diethyl pyrocarbonate
DMPC	dimyristoylphosphatidylcholine
DT	diphtheria toxin
DTT	dithiothreitol
EDIN	epidermal differentiation inhibitor
EDTA	ethylenediaminetetraacetic acid
EF-2	elongation factor 2
ER	endoplasmic reticulum

ETA	exotoxin A from *Pseudomonas aeruginosa*
EXAFS	X-ray absorption fine structure
FCS	fine calf serum
FGF	fibroblast growth factor
GABA	γ-aminobutyric acid
GDI	guanine nucleotide dissociation inhibitor
GDP	guanosine diphosphate
GDS	guanine nucleotide dissociation stimulator
GERL	Golgi-endoplasmic recticulum-lysosome
GPI	glycophosphoinositol
GST	glutathione S-transferase
GTP	guanosine triphosphate
GTPase	guanosine triphosphatase
HIC	hydrophobic interaction chromatography
HlyA	*E. coli* hemolysin
HT	hemorrhagic toxin
ICE	interleukin converting enzyme
IL	interleukin
IMAC	immobilized-metal-ion-affinity chromatography
IPTG	isopropyl-β-D-thiogalactopyranoside
LDCV	large dense core vesicles
LF	lethal factor
LPA	lysophosphatidic acid
LT	*E. coli* heat-labile enterotoxin
MHC	major histocompatibility class
NAD	nicotinamide adenine dinucleotide
NMJ	neuromuscular junction
NSF	*N*-ethylmaleimide-sensitive factor
PA	protective antigen
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate-buffered saline
PDGF	platelet-derived growth factor
PFT	pore-forming toxins
PGE_2	prostaglandin E_2

PLD	phospholipase D
PMSF	phenylmethylsulfonyl fluoride
PT	pertussis toxin
RBL	rat basophilic leukemia
RTX	repeats in toxin
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SDS	sodium dodecyl sulfate
SLMV	synaptic-like microvesicles
SLO	streptolysin O
SNAP-25	synaptosomal-associated protein of 25 kDa
SNAPs	soluble NSF attachment proteins
SNARE	SNAP receptor
SSV	small synaptic vesicles
t-SNARE	target SNAP receptor
TCA	trichloroacetic acid
TeNT	tetanus neurotoxin
TeTx	tetanus toxin
Tox A	toxin A
Tox B	toxin B
UDP-Glc	uridine disphosphate glucose
UDP-GlcNAc	uridine diphosphate *N*-acetylglucosamine
v-SNARE	vesicle SNAP receptor
VAMP	vesicle-associated membrane protein (= synaptobrevin)

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CHAPTER 1

Cholera Toxin: Mechanism of Action and Potential Use in Vaccine Development

G.-F. ZHANG, W. A. PATTON, J. MOSS and M. VAUGHAN

1.1 Introduction

Cholera results from an intestinal infection with the pathogenic bacterium *Vibrio cholerae* that causes a debilitating, and even deadly, diarrhea. Successful treatment of cholera requires effective rehydration with solutions of glucose and salts (Kaper *et al.*, 1995). Administration of antibiotics decreases the duration of disease (Kaper *et al.*, 1995); vaccines are only partially effective. Koch, who first described *Vibrio cholerae* as the causative agent of cholera, suggested that it was a toxin-mediated disease (Koch, 1884). Over a half-century later, the existence of cholera toxin (CT) was demonstrated in cell-free culture filtrates (De, 1959; Dutta *et al.*, 1959); a decade later, purification of the protein toxin was achieved (Finkelstein and LoSpalluto, 1969). Since then, it has been found that CT is just one member of a family of bacterial toxins that are ADP-ribosyltransferases, which catalyze transfer of the ADP-ribose portion of nicotinamide adenine dinucleotide (NAD) to a nucleophilic acceptor (Moss and Vaughan, 1988a,b; Burnette, 1994; Merritt and Hol, 1995). CT specifically modifies arginine, either free or as part of a protein. In all cells, including intestinal epithelial cells, the major substrate is the guanine nucleotide-binding subunit of the stimulatory regulator of adenylyl cyclase, $G_{s\alpha}$ (Moss and Vaughan, 1988b). ADP-ribosylation of arginine-201 in $G_{s\alpha}$ (Robishaw *et al.*, 1986) leads to persistently high intracellular levels of cAMP (Moss and Vaughan, 1988b), perhaps other effects on arachidonate metabolites (Peterson *et al.*, 1994; Reitmeyer and Peterson, 1990), and the massive fluid and electrolyte flux that are characteristic of cholera (Kaper *et al.*, 1995).

action of cholera toxin

Cholera toxin and the very similar (in structure and mechanism of action) heat-labile enterotoxin from *E. coli* (LT-1 or LT, which is responsible for the syndrome of traveler's diarrhea) have been widely applied as molecular tools to facilitate understanding of signalling systems. Examples of molecules besides $G_{s\alpha}$ that were identified because of work on CT are the ADP-ribosylation factors (ARFs), now known to play a critical role in intracellular vesicular transport (Moss and Vaughan, 1995), and the mammalian ADP-ribosyltransferases (Zolkiewska *et al.*, 1994). As more is learned about CT structure and biochemistry, modified or mutant CT and LT molecules are being gen-

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erated for use as targeting molecules to direct covalently attached ligands to specific cells, or as adjuvants for use in vaccine development (Holmgren *et al.*, 1994). This chapter is a review of recent studies on CT and LT structure, mode of cell entry, mechanism of action, and their use as tools in biochemical studies.

1.2 Molecular Aspects of Cholera Toxin Action

1.2.1 Structure and Relationship to Other Toxins

related toxins

Cholera toxin and *E. coli* heat-labile enterotoxins, members of the AB_5 family of toxins (Burnette, 1994; Merritt and Hol, 1995) consist of a single catalytic A subunit and five B subunits (Spangler, 1992). Another oligomeric toxin is pertussis toxin (PT), a secretory product of *Bordetella pertussis* (for review and primary references, see Moss and Vaughan, 1988b; Burnette, 1994; Merritt and Hol, 1995). CT, LT, and PT are ADP-ribosyltransferases that use NAD as an ADP-ribose donor and α subunits of the heterotrimeric guanine nucleotide-binding (G) proteins as an acceptor (Moss and Vaughan, 1988b). CT and LT ADP-ribosylate an arginine (Robishaw *et al.*, 1986) in $G_{s\alpha}$, causing its activation (Gill and Meren, 1978; Johnson *et al.*, 1978; Northup *et al.*, 1980), whereas PT ADP-ribosylates a cysteine in $G_{\alpha i}$, resulting in uncoupling from its receptors (Bokoch *et al.*, 1983, 1984; West *et al.*, 1985). Diphtheria toxin (DT) from *Corynebacterium diphtheriae* and the closely related exotoxin A (ETA) from *Pseudomonas aeruginosa* are also ADP-ribosylating toxins, but are monomeric proteins that ADP-ribosylate elongation factor 2 (EF-2), leading to inhibition of protein synthesis and cell death (Wilson and Collier, 1992).

structure of cholera toxin

CT is an oligomeric protein of 84 kDa composed of one A subunit (CTA, approx. 29 kDa) and five B (CTB, approx. 11.6 kDa) subunits (Ohtomo *et al.*, 1976). CTA is synthesized as a single protein that is later proteolytically nicked to produce two polypeptides (Mekalanos *et al.*, 1979), CTA_1 and CTA_2, which remain covalently linked by a single disulfide bond near the carboxyl end of CTA_1. Both the proteolytic cleavage, in a short sequence between the two cysteines, and reduction of the disulfide bond are required for generation of active CTA_1 (Mekalanos *et al.*, 1979, Tomasi *et al.*, 1979). Both CT and LT holotoxins have a "doughnut-shaped" pentameric B subunit into which amino acids of the A_2 subunit are anchored (Spangler, 1992). One face of the B subunit contains five binding sites for ganglioside G_{M1} (Sixma *et al.*, 1991), the cell-surface binding site for CT and LT. Each binding site is composed (for the most part) of residues from a single individual monomer, but binding to G_{M1} occurs only when CTB is in the pentameric form (Fishman, 1982). CTB anchors the holotoxin to the membrane in an orientation such that the G_{M1} binding sites face and interact with the membrane, whereas the portion of CTA that does not penetrate the center of the B pentamer is above the opposite face of the pentamer (Sixma *et al.*, 1991, 1993).