MOLECULAR ACTION OF TOXINS AND VIRUSES

Edited by

OF THE REPORT OF THE

PHILIP COHEN and SIMON VAN HEYNINGEN



1982

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Research over the past few years has greatly advanced our understanding of cellular control mechanisms, and is now linking areas of biology that were previously thought of as being quite separate. Molecular Aspects of Cellular Regulation is an occasional series of books on multidisciplinary topics which are designed to illustrate general principles of cellular regulation. The first volume of this series, Recently Discovered Systems of Enzyme Regulation by Reversible Phosphorylation dealt with the role of protein phosphorylation as the major general control mechanism by which intracellular events in mammalian tissues are controlled by neural and hormonal stimuli. This second volume, The Molecular Action of Toxins and Viruses, might at first sight appear to be about an unrelated subject. In fact the book demonstrates that the actions of neural and hormonal stimuli are strikingly similar to those of bacterial toxins and viruses. These similarities stem from the fact that each of these agents must influence intracellular functions from extracellular locations. Their actions are initiated by interaction with receptors on the outer membranes of target cells which determine the specificity of the effects. These interactions can either trigger the activation of membranebound enzymes, or cause a change in membrane permability, or lead to the uptake into the cell of the agent or at least the active part of it.

The extraordinary potency of bacterial toxins and viruses is frequently caused by the covalent modification of intracellular proteins or nucleic acids. Many of the toxins are enzymes, so that a single molecule can, in principle, catalyse the modification of all the available substrate in a cell. Some bacterial, avian and mammalian viruses code for protein kinases which phosphorylate proteins of the host cell thereby modifying their activities (Chapters 7 and 9). Their actions therefore resemble the mechanisms by which many neural and hormonal stimuli control normal cell functions. Other bacterial viruses and some toxins cause the ADP-ribosylation rather than

the phosphorylation of host proteins (Chapters 1, 2 and 8). Although ADP-ribosylation was first discovered in the investigation of these nonphysiological effectors, evidence is accumulating that ADP-ribosylation is also involved in the normal control of cell metabolism. Another highly potent class of compound secreted by a bacterial cell to act on another cell is the colicins, some of which are also enzymes that cleave the DNA or RNA of their target cell at specific points (Chapter 5). Several toxins also act by damaging components of the transcription or translational machinery of cells (Chapters 1, 3, 5 and 6). Other toxins and some of the colicins work directly on the cell membrane (Chapters 4 and 5).

The following chapters present an overview of the rapid progress that has been made in this important area of research, and highlight the similarities in the mechanisms by which different toxins and viruses so fundamentally affect the metabolism of cells. We hope that one result of this book will be to put these pathological agents, some of them infamous for their effect on man, more firmly into the mainstream of biochemistry and cell biology.

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CHAPTER 1

Diphtheria toxin: biological activity

In 1951. Feering Adiscovered that dight light toxin was produced only by distributions blocks that are two sens for playe beta or closely reliabed bacte-

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Tsuyoshi Uchida [1] sanis adi lo noi sellari

relationship to phage multiplication in host bacteria. The beta phage has double stranged DNA with a molecular weight of $2.3 \times 10^{\circ}$ [9]. In 1971,

munitarities produce aftered toxin were isolated to

1. Introduction

It is well established that diphtheria toxin catalyses the ADP-ribosylation of protein synthesis elongation factor-2 (EF-2) and that this reaction plays an important role in the lethal effect of the toxin on susceptible animals. This enzymic activity, which is located in the N-terminal portion of the molecule (fragment A), is not unique however, since *Pseudomonas aeruginosa* exotoxin has also been found to catalyse the same ADP-ribosylation of EF-2. Similarly, cholera toxin (Chapter 2) and LT-toxin of *Escherichia coli* (Chapter 6) catalyse the ADP-ribosylation of GTP binding proteins that regulate the activity of adenylate cyclase (Chapter 2). Although excellent review articles are available on diphtheria toxin [1-3], several important questions have not been resolved. The receptor for the toxin has not been isolated, and little is known about its mechanism of entry into the cell cytoplasm. This chapter includes recent information about the biosynthesis and activity of diphtheria toxin, toxin-resistant mutant cells, the entry of toxin into cells and fragment A in the cytoplasm of living cells.

2. Biosynthesis of diphtheria toxin

Diphtheria toxin is synthesized and secreted extracellularly by diphtheria bacilli Corynebacterium diphtheriae which have been lysogenized or infected with certain corynephages carrying the toxin gene which produces a single polypeptide chain with a molecular weight of 62,000. Toxin synthesis is inhibited by addition of iron to the culture medium. Two main topics, the toxin gene itself and the regulation of its expression, are described below.

2.1. Toxin gene

In 1951 Freeman discovered that diphtheria toxin was produced only by diphtheria bacilli that are lysogenic for phage beta or closely related bacteriophage [4]. Groman, and Barksdale and Pappenheimer confirmed and extended this work [5,6]. A non-toxinogenic strain was converted to a lysogenic or toxinogenic state by lysogenization with a suitable phage carrying the toxin gene. The toxin gene is also expressed early during vegetative replication of the phage [7] and is expressed in the non-integrated and nonreplicated states of the phage DNA [8]. Thus synthesis of toxin has no relationship to phage multiplication in host bacteria. The beta phage has double stranded DNA with a molecular weight of 2.3 × 10⁷ [9]. In 1971, mutants that produce altered toxin were isolated to determine whether the toxin gene of the phage contains the structural information for toxin synthesis, or whether the toxin gene acts indirectly to permit the expression of a host structural gene for the toxin, and studies were made to determine whether the character producing altered toxin was carried by the phage or by the host bacteria. Uchida et al. [10] isolated beta phages carrying mutated toxin genes by treatment with the mutagen nitrosoguanidine. Lysogens with the mutant phage can produce non-toxic proteins with little or no toxicity that are serologically related to diphtheria toxin (cross reacting material: CRM) [11] (Table 1). The character producing CRMs is carried by these mutant phages. Therefore, the phage carries structural information for the toxin. This result was later confirmed by Murphy et al. [12]. The toxin can be synthesized in a cell-free protein synthesizing system from E. coli using DNA from beta phage. Some CRMs were also isolated in other laboratories [13-15].

The toxin gene has been mapped in a single region of the phage genome using ts mutant beta phage [16,17], host range and beta-gamma hybrid phage [18]. A large number of ts mutants of virulent beta phage were isolated and mapped [19]. Laird and Groman reported prophage maps obtained using heteroimmune double lysogens and also determined the orientation of the toxin gene [20,21]. Holmes reported the orientation of the toxin gene in a vegitative map [15]. The two results were identical. The corynephage beta is functionally similar to lamda phage of E. coli. The beta phage genes involved in formation and assembly of phage heads are in one cluster, and those involved in tail formation are in another (Fig. 1). Beta phages with mutations affecting toxin yields were isolated [22]. The mutant phage has a gene that does not code for a protein that affects toxin yield but that regulates the expression of the toxin gene. This gene might be a mutated

TABLE 1.

Some properties of CRMs*

	M _r	Toxicity ^a	Enzymic activity ^b	Binding to receptor ^c	Propo		Main tryptic products
Toxin	62,000	25-30	100	100	-ş	\$-\$	şH 22,000
Fragment A	22,000	0	100	0	H		
CRM22	22,000	0	100	0	H_x-		\$H 22,000
CRM30	30,000	0	100	0 9	-s -		şH 10,000
CRM45	45,000	2.5×10-5	100	0 _ 5	-3 ×-		şH 22,000 şH 21,000
CRM176	62,000	0.1-0.2	8-10	100 _x	ş—ş	ş-ş	şH 22,000 şH Hş şH 40,000
CRM197	62,000	0	0	100 s	s-ş	ş-ş	şH 22,000 şH Hş şH 40,000
CRM228	62,000	0	0	15-20 _{-x} -5	s—ş	\$-\$ _x -	şH Hş şH 40,000
A45-B176°	62,000	25-30	100	100			Intern (Paradole)
A45-B197	62,000	25-30	100	100			
A45-B228	62,000	5	100	20			

* Modified from Uchida et al. [11], Gill et al. [137] and Pappenheimer [1].

a Minimum lethal dose per µg in Guinea pigs.

b NAD:EF-2-ADPr-transferase activity per mole after activation by trypsin and reduction as a percentage of that of fragment A.

c Ability to inhibit the action of toxin on HeLa cells competitively. The blocking activity of CRM197 is taken as 100.

d X is presumed mutation site.

 A45 was obtained from CRM45, and B176 from CRM176. Hybrid toxin was formed from A45 and B176.

form of the promoter gene of the toxin operon. Mapping of beta phage DNA by restriction enzymes will be reported in the near future.

2.2. Synthesis, secretion and regulation

Diphtheria toxin is synthesized and secreted extracellularly by diphtheria bacilli as a single polypeptide chain. The toxin is then accumulated in the culture medium reaching a final concentration of about 400–500 µg per ml of the medium by the Park Williams number 8 (PW 8) strain under optimal conditions for toxin production. The toxin protein amounts to about 75–90% of the total extracellular proteins and about 5% of the total bacterial proteins. The average time required for the whole process of synthesis of toxin protein from amino acids and its release into the medium is about 3 min [23] (Fig. 2). The toxin is synthesized in the cell at a site closely associated with