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Bacterial Protein Toxins

Third European Workshop
Überlingen, June 28 – July 3, 1987

Edited by
F.J. Fehrenbach, J.E. Alouf, P. Falmagne,
W. Goebel, J. Jeljaszewicz, D. Jürgens
and R. Rappuoli

131 figures and 48 tables

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Preface

This book contains the contributions presented at the 3rd European Workshop on Bacterial Protein Toxins, in Überlingen/F.R.G., June 29th - July 3rd 1987. The conference was organized and held in the tradition of the two preceeding conferences in Seillac, (France, 1983) and Wepion, (Belgium, 1985). The success of the three conferences completely justified the continuity of the European Workshops on Bacterial Protein Toxins as a series of conferences to be held every two years.

Research in the field of Bacterial Toxinology has necessarily become interdisciplinary and therefore attracts scientists from diverse fields of research. The continuing success of these conferences reflects the consistently high standard of the scientific contributions, their timeliness, relevance and breadth of appeal.

The conferences are always held in late June to early July in a pleasant area of the host european country. The selected locations are always remote from large and busy cities, and provide facilities conducive to a relaxed atmosphere, where accompanying families can also enjoy a holiday. The number of participants is limited to about 120 active scientists and ample time is provided between the lectures to allow the continuation of extended discussions.

It is also an important aim of these conferences to attract young scientists and to encourage their participation by providing them with some financial support. Already this policy has promoted considerable scientific cooperation between numerous laboratories and in particularly has promoted the exchange of visiting junior and senior scientists.

The proceedings of each workshop are published to ensure rapid distribution of the information provided at the conferences. The communications of the 3rd European Workshop on Bacterial Protein Toxins again covers major aspects of Bacterial Toxinology:

Molecular architecture of toxins in relation to function

Toxin/cell surface interactions

Genetic aspects of toxinogenesis

Toxin/secretion and internalization

Toxin-lipid interaction

Toxins as virulence factors

Applied toxinology

It is clear from these conferences that the pace of research in the various aspects of toxinology continues to increase. At the same time, new areas of investigation are unfolding and will become subjects for duscussion at forthcoming European Workshops on Bacterial Protein Toxins.

The organizers would like to express their sincere appreciation and profound thanks to all sponsors who provided financial support. It was largely through the contributions of the DEUTSCHE FORSCHUNGSGEMEINSCHAFT that a sound financial basis for the 3rd European Workshop on Bacterial Protein Toxins was established and the success of the conference assured.

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Contents

Third European Workshop on Bacterial Protein Toxins, June 28 - July 3, 1987
Überlingen - **Proceedings**

Molecular architecture of toxins in relation to function

Toxin cell surface interaction

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Genetic aspects of toxinogenesis

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Molecular Architecture of Toxins in Relation to Function

Fehrenbach et al. (Eds.), Bacterial Protein Toxins, Zbl. Bakt. Suppl. 17

Studies on the Synthesis, Assembly and Structure of the Heat-Labile Enterotoxin (LT) of Escherichia coli

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ABSTRACT

Enteropathogenic strains of Escherichia coli produce a heat-labile enterotoxin (LT), the subunits of which are synthesized on membrane-bound polysomes, exported through the cytoplasmic membrane and processed before being released into the periplasm.

LT-B is released rapidly (within 13-14 sec) following initiation of synthesis, and is immediately integrated into high-molecular weight aggregates. LT-A is released only after one to several minutes and binds to LT-B aggregates rather slowly to form holotoxin. While LT-B and LT-A are synthesized in equimolar amounts and LT-B is assembled into holotoxin quantitatively, part of the LT-A is degraded in the cytoplasm (before export) and in the periplasm (after release from the cytoplasmic membrane).

Isolated holotoxin crystallizes in different crystal forms, depending on the crystallization conditions. Form II contains only B_5, and shows five-fold symmetry. Form III contains holotoxin, also shows five-fold symmetry, and yields high resolution data in the rotating anode generator (2.3 A) and the synchrotron (<2.0 A). At least five heavy-atom derivatives show promising results and a $KAuCl_4$ derivative has been analyzed in the Hamburg synchrotron beam.

INTRODUCTION

The heat-labile enterotoxin (LT) produced by enterotoxigenic Escherichia coli strains is a multimeric protein which is closely related to cholera toxin (CT). LT binds to the membrane of target epithelial cells via its binding component LT-B_5, which consists of five identical subunits LT-B. The active component (LT-A) consists of a single polypeptide which, following binding of LT to target membranes (via binding of B_5 to membrane

gangliosides GM_1), is cleaved proteolytically into sequences A_1 (22.5 kD) and A_2 (5.2 kD). A_2 remains bound to B_5, while A_1 penetrates the target membrane to trigger a cascade of events culminating in increased Cl^- and water permeability of the target cells (Middlebrook and Durland, 1984).

Since LT as well as CT act directly on the target membranes, they must be exported by E. coli and Vibrio cholerae, respectively. In fact, CT can be produced extracellularly in large amounts (Mekalanos et al., 1978), while LT has also been reported in culture supernatants. Thus, LT as well as CT are considered to be exotoxins, although there is some doubt that LT is similar to CT in this respect, as we will show below.

Enteropathogenic E. coli strains generally produce very small amounts of LT, typically of the order of 0.01 -0.1 % of the total cell protein. Given a molecular weight of 85.000 for the holotoxin, this amounts to about 100-1000 copies of toxin per cell. Most of these toxin molecules are found not in the culture medium, but in the cell envelope, where they appear to associate closely with the bacterial outer membrane (Wensink et al., 1978; Gankema et al; 1980). Gram-negative bacteria normally shed outer membrane fragments or blebs during growth (Hoekstra et al., 1976; Mug-Opstelten and Witholt, 1978), and in the case of enterotoxigenic E. coli, such fragments are enriched with toxin (Wensink et al., 1978). When the cells are endowed with adhesins, which are also localized in or on the outer membrane, the resulting fragments contain adhesins as well as toxin (Middeldorp and Witholt, 1981, 1983).

While E. coli releases only small amounts of outer membrane material (ca. 5%) under laboratory conditions, considerably more outer membrane may be released under in vivo conditions in the gut. E. coli cells adhering to porcine gut surfaces show characteristic "granules" and "blebs" (Nagy et al. 1976) suggesting that gut enzymes might loosen the interactions between the bacterial outer membrane and peptidoglycan, resulting in massive release of outer membrane material.

Summarizing the above, in contrast to Vibrio cholerae, which produces large amounts of extracellular CT, E. coli generally produces modest amounts of LT, which accumulate in the cell envelope, and may bind to the outer membrane, most likely to its inner surface. Adhesins, when made, are localized on the external surface of the outer membrane. When membrane fragments are released, a normal process for growing E. coli and one which may be accelerated during bacterial adhesion to the porcine, human or other gut, such fragments may function as toxin delivery systems. They bind to epithelial gut surfaces via the adhesins, and thus deliver toxin to its site of action (Middeldorp and Witholt, 1981).

Given the above, LT is really an endotoxin; it is part of the cell envelope, rather than an exported protein. CT, on the other hand, is a classical exotoxin, accumulating as a free protein in the culture supernatant. These differences are not likely to be due to the toxins themselves, which closely resemble one another. Instead, they may be related to quantitative effects: thus, a small amount of toxin - LT - binds to the