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Toxicon 45 (2005) 389–393

TOXICON

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Mini-review

Cholera and Shiga toxin B-subunits: thermodynamic and structural considerations for function and biomedical applications

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Available online 29 January 2005

Abstract

The B-subunits of cholera and Shiga toxins are functionally and structurally related proteins with different chain lengths and no sequence similarity. They are responsible for toxin binding to specific glycosphingolipid receptors and intracellular toxin trafficking. Indeed, it is clearly established that B-subunits have the unique capacity of targeting the toxins to a poorly explored intracellular pathway, the retrograde route, allowing the transfer to the cytosol of the associated catalytic A-subunits, by retro-translocation from the endoplasmic reticulum. The B-subunits have also been used as vectors for antigen presentation in immunotherapeutic approaches. It is, however, not known if and how the B-subunits intervene in membrane translocation of the A-subunits and/or antigens to the cytosol. Therefore, it is important to characterise the driving force of pentamer formation, its conformational stability, and toxin–receptor interactions. This review summarises recent studies that have dealt with these topics.
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Keywords: Cholera toxin; Shiga toxin; Receptor binding; Structural stability

Contents

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0041-0101/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2004.12.014

1. Introduction: structure and function of cholera and Shiga toxins

Cholera toxin from *Vibrio cholerae*, Shiga toxin from *Shigella dysenteriae*, and related toxins, such as heat-labile toxins (LT) and Shiga-like toxins (also known as verotoxins), produced by certain strains of *Escherichia coli*, are members of the AB_5 class of bacterial protein toxins (Smith et al., 2004). They are implicated in several life-threatening diseases, such as cholera and verotoxin induced-hemolytic and uremic syndrome, the leading cause for pediatric renal failure. Understanding the biochemistry and cell biology of these toxins is crucial for the development of strategies to prevent or cure toxin related pathologies. Thus, toxin and receptor structures are explored in drug design, aiming at the identification of natural or synthetic small molecules inhibiting toxins directly or competing with toxin–receptor interactions. Cholera and Shiga toxins or individual subunits are also used to develop innovative biomedical applications, exploiting toxin properties that have evolved in interaction with their hosts. These strategies include cancer cell targeting for tumor detection and therapy, or dendritic cell targeting for immunotherapy of tumors and infectious diseases (Johannes, 2002).

The A-subunits have catalytic activities responsible for toxin-induced cytotoxicity. Cholera toxin activates cAMP production eventually leading to dehydration, while Shiga toxin inactivates ribosomes leading to protein biosynthesis inhibition and cell death. However, the A-subunits are unable to enter into cells alone. They need to be non-covalently associated to their respective B-subunits, responsible for binding to specific glycosphingolipid cell-surface receptors, expressed at variable levels depending on cell type. Available evidence suggests that A-subunit binding to B-subunit does not alter the structure and stability of the latter (Goins and Freire, 1988), and B-subunit trafficking is independent of the A-subunit (Sandvig et al., 1994).

The structures of cholera and Shiga toxins have been solved. Despite the absence of sequence similarity, the B-subunits of both toxins fold as homopentamers with superimposable structures. The monomeric B-fragments comprise 103 (11.6 kDa) or 69 residues (7.7 kDa) for cholera and Shiga toxin, respectively. They are among the smallest known lectins. Their three-dimensional structures consist of two three-stranded antiparallel β-sheets and an α-helix, with β-sheets from pairs of adjacent monomers forming six-stranded antiparallel β-sheets on the outer surface of the pentamer and the five α-helices delimiting a central pore (Fraser et al., 1994; Zhang et al., 1995). Cholera toxin B-subunit (CTxB) has an additional α-helix at its N-terminus, absent in the Shiga toxin B-subunit (STxB), and possesses longer secondary structure elements (Fig. 1). Superimposition of the two B-subunit structures reveals a similar fold in β-sheet monomer–monomer interfaces. The main differences are located in the protein surface exposed to the membrane, which allows them to interact with their receptors (Sixma et al., 1993).

2. Structural stability of CTxB and G_{M1} receptor binding

The structure of cholera toxin (and LT-1, which shares 80% sequence identity) alone, or in complex with its receptor, the pentasaccharide ganglioside G_{M1}, reveals the existence of one receptor binding site per monomer (Merritt et al., 1994; 1997). The apparent dissociation constant for the CTxB-G_{M1} complex depends on the way G_{M1} is presented, ranging from $\sim 10^{-12}$ M when G_{M1} is immobilized on solid surfaces, over $\sim 10^{-10}$ M for cell membrane localised G_{M1}, to $\sim 10^{-6}$ M when measured by isothermal

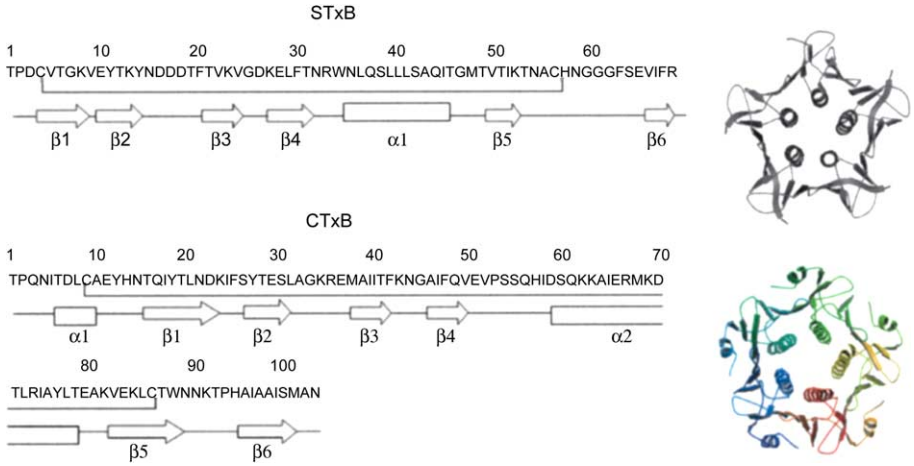

Fig. 1. Amino acid sequence, secondary structure elements, and three-dimensional structure of STxB and CTxB (PDB 1DMO and 1FGB, respectively, represented with Molscript (Kraulis, 1991)).

calorimetry using soluble G_{M1} analogues (Schön and Freire, 1989; Masserini et al., 1992; Kuziemko et al., 1996).

The structural stability of cholera toxin has been characterised by infrared spectroscopy and differential scanning calorimetry (DSC) in presence or absence of its receptor (Goins and Freire, 1988; Surewicz et al., 1990). Under these experimental conditions, CTxB has a reversible cooperative thermal transition centered at 74 °C that is not affected by A-subunit binding. However, interaction with G_{M1} increases the thermal transition of the complex by 20 °C and enhances the cooperativity of the unfolding process. Contradictory evidence exists on whether this stabilization effect is paralleled by conformational changes in the B-subunit. Comparison of amide I infrared spectra between free and ganglioside-bound B-subunit indicates no major secondary structure changes, whereas fluorescence and far-UV CD spectroscopy suggest the existence of such receptor-induced changes (Surewicz et al., 1990). It was also suggested that CTxB is capable of inducing ion channels in planar bilayers (Krasilnikov et al., 1991).

3. Structural stability of STxB and Gb_3 receptor binding

The initial crystal structure of Shiga toxin (or verotoxin-1, which has the same amino acid residues sequence except for one conservative change in the A-subunit) suggested a non-symmetrical organisation of the molecule. Later resolution of the solution structure of STxB revealed that the original observation was a crystallisation artifact (Stein et al., 1992; Fraser et al., 1994; Richardson et al., 1997). This conclusion was further confirmed by determining the STxB structure on lipid substrate using two-dimensional crystallisation and structure determination by electron diffraction (Hagnerelle et al., 2002).

As opposed to the CTxB, a controversy persists on the number of Gb_3 binding sites on STxB. By X-ray techniques, three receptor-binding sites per monomer were observed when a soluble Gb_3 analogue was complexed with STxB, two of which are localised in the monomer–monomer interface (Ling et al., 1998). On the contrary, NMR experiments showed that only one of these binding sites was significantly populated (Shimizu et al., 1998; Thompson et al., 2000). Indeed, results from mutagenesis studies indicated that mutations around Phe30 at the interface of site II were particularly deleterious to receptor binding and cell intoxication (Soltyk et al., 2002). However, for optimal binding, all three sites were required.

The apparent dissociation constant of the STxB interaction with soluble Gb_3 analogues is only $\sim 10^{-3}$ M, while it is about $\sim 10^{-9}$ M for interaction with whole cells (Saint Hilaire et al., 1994). Two major explanations have been put forward. On the one hand, lipid–protein interactions may contribute to binding. In line with this hypothesis, it has been described that STxB has variable affinity to Gb_3 molecular species with defined acyl chain length or saturation differences (Pellizzari et al., 1992; Arab and Lingwood, 1996). On the other hand, the two-dimensional organisation of Gb_3 in cell membranes may favour cooperative binding to STxB, a phenomenon that would be favored by the high number of Gb_3 binding sites per STxB pentamer (see above).

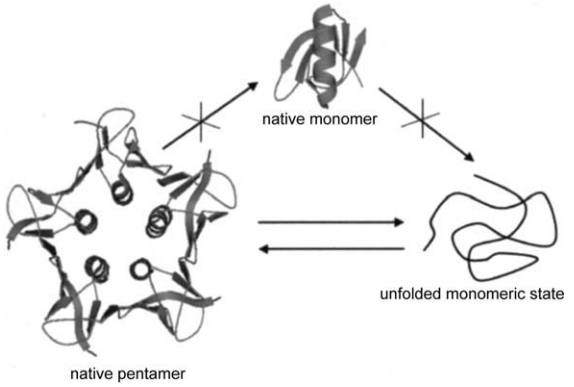

Fig. 2. Schematic representation of the STxB folding/unfolding process. Pentamer conformation unfolds directly into an unfolded monomer in aqueous solution. Native monomer is not detected under these conditions.

The structural stability of STxB has been recently reported (Pina et al., 2003). Thermal denaturation of STxB in aqueous solution monitored by DSC showed a protein concentration-dependent reversible two-state process, as expected for a reaction where oligomer dissociation and monomer unfolding occurs simultaneously, with only native pentamer and unfolded monomer states being experimentally detected (Fig. 2). The thermal transition of STxB unfolding is centered at 88 °C, ca. 10 °C above that of CTxB, under the same conditions. Theoretical analysis confirm that only the folded pentamer and unfolded monomers are significantly populated during the denaturation process, and that folded monomers are not stable in solution due to exposed hydrophobic surfaces when not associated into pentamer.

4. Perspectives

A series of studies has revealed that STxB can introduce antigenic proteins and peptides into the MHC class I restricted antigen presentation pathway of various cells such as human dendritic cells and B-lymphocytes, requiring antigen processing in the cytosol (Lee et al., 1998; Haicheur et al., 2000). These observations were somewhat unexpected since it was assumed that only the catalytic A-subunits of toxins translocate to the cytosol. Determining whether the hydrophobic interfaces between STxB monomers can interact with membranes, following binding to Gb_3, will be a critical step toward understanding the mechanism of STxB-dependent antigen presentation. The characterization

of putative membrane- or membrane mimic-induced conformations of STxB shall permit to correlate the physico-chemical state of the monomer with toxin binding to membranes, membrane translocation, and STxB-dependent antigen presentation.

Acknowledgements

We would like to thank P.D. Coureux for assistance, and K. Mitchell, C. Lamaze and P. England for critical reading of the manuscript. D.G. Pina is recipient of a post-doctoral fellowship from Fundação para a Ciência e a Tecnologia, Portugal (Ref. SFRH/BPD/14568/2003). Work on this subject area in the Traffic and Signaling Laboratory was supported by grants from Association pour la Recherche sur le Cancer (n° 3105 and 5177), by the Ligue Nationale contre le Cancer, and by the Fondation de France.

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