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Review

Lipid requirements for entry of protein toxins into cells

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ABSTRACT

The plant toxin ricin and the bacterial toxin Shiga toxin both belong to a group of protein toxins having one moiety that binds to the cell surface, and another, enzymatically active moiety, that enters the cytosol and inhibits protein synthesis by inactivating ribosomes. Both toxins travel all the way from the cell surface to endosomes, the Golgi apparatus and the ER before the ribosome-inactivating moiety enters the cytosol. Shiga toxin binds to the neutral glycosphingolipid Gb3 at the cell surface and is therefore dependent on this lipid for transport into the cells, whereas ricin binds both glycoproteins and glycolipids with terminal galactose. The different steps of transport used by these toxins have specific requirements for lipid species, and with the recent developments in mass spectrometry analysis of lipids and microscopical and biochemical dissection of transport in cells, we are starting to see the complexity of endocytosis and intracellular transport. In this article we describe lipid requirements and the consequences of lipid changes for the entry and intoxication with ricin and Shiga toxin. These toxins can be a threat to human health, but can also be exploited for diagnosis and therapy, and have proven valuable as tools to study intracellular transport.

Contents

Abbreviations: Cer, ceramide; CE, cholesterol esters; DAG, diacylglycerol; ELISA, enzyme-linked immunesorbant assay; ER, endoplasmic reticulum; Gb3, globotriaosylceramide; GlcCer, glucosylceramide; HG, hexadecylglycerol; LacCer, lactosylceramide; MS, mass spectrometry; PA, phosphatidic acid; PDMP, DL-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol; PI3P, phosphatidylinositol 3-phosphate; PLA2, phospholipase A2; PS, phosphatidylserine; TLC, thin layer chromatography; TGN, trans Golgi network; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

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1. Introduction

A number of protein toxins found in plants, such as ricin and viscumin, and bacterial toxins, such as Shiga toxin and diphtheria toxin, target components of the protein synthesis machinery, meaning that they have to enter the cytosol (for review, see [12,138,140,141,151]. These toxins have one moiety that binds to the cell surface, and another enzymatically active moiety that are translocated from an intracellular organelle to the cytosol where it exerts its effects. Many of these toxins inhibit protein synthesis by inactivating the 60S subunit of the ribosome, which is the case for Shiga toxins [36] and ricin [35] that we focus on in this article (Fig. 1). This will eventually lead to cell death, in some cases after additional stress-induced signalling and apoptosis [157,158]. Shiga toxins are produced upon infection with *Shigella dysenteriae* and by several types of *Escherichia coli*, and such bacteria can be found in water and food supplies. Outbreaks with serious disease as a result occur even in industrialized countries, and children and the elderly are most susceptible to disease [161,168]. However, in 2011 there was a large outbreak in Europe where also adults became sick, especially women [15,66,76,78,96]. Shiga toxins may cause kidney failure and neurological problems, and this European outbreak lead to almost 4000 symptomatic cases, including 50 deaths [76]. The European outbreak turned out to be caused by contaminated vegetables, most probably by organically grown sprouts. However, although the protein toxins are a problem in connection with infectious disease and their possible use in bioterrorism [2,120], they can also be exploited for positive purposes. They have proven to be valuable tools to study basic processes in cell biology. Since ricin and Shiga toxin start their journey into the cell at the cell surface, then they are endocytosed and transferred to the Golgi apparatus, and then they use retrograde transport to reach the ER before they are finally translocated to the cytosol (Fig. 2), they can be used to study all of these transport steps [12,61–63,138,151]. For instance, studies of abrin, a toxin quite similar to ricin revealed more than thirty years ago that an endocytosed molecule can be recycled [126], and investigations of ricin endocytosis clearly showed that there are several mechanisms for endocytosis [106,131]. Importantly, Shiga toxin was the first molecule shown to be transported all the way from the cell surface to the Golgi, the ER and the nuclear envelope [127].

Protein toxins can also be exploited in medicine, for instance by targeting the enzymatically active part to cancer cells (immunotoxins or other toxin conjugates) [6,25,33,55,75,94,146]. Moreover, their binding moiety or enzymatically inactive variants of the toxins can be used in connection with diagnosis, vaccination, and gene transfer [1,27,34,37,39,63,95,111,166]. In order to obtain optimal use of toxins for various purposes one needs to increase the

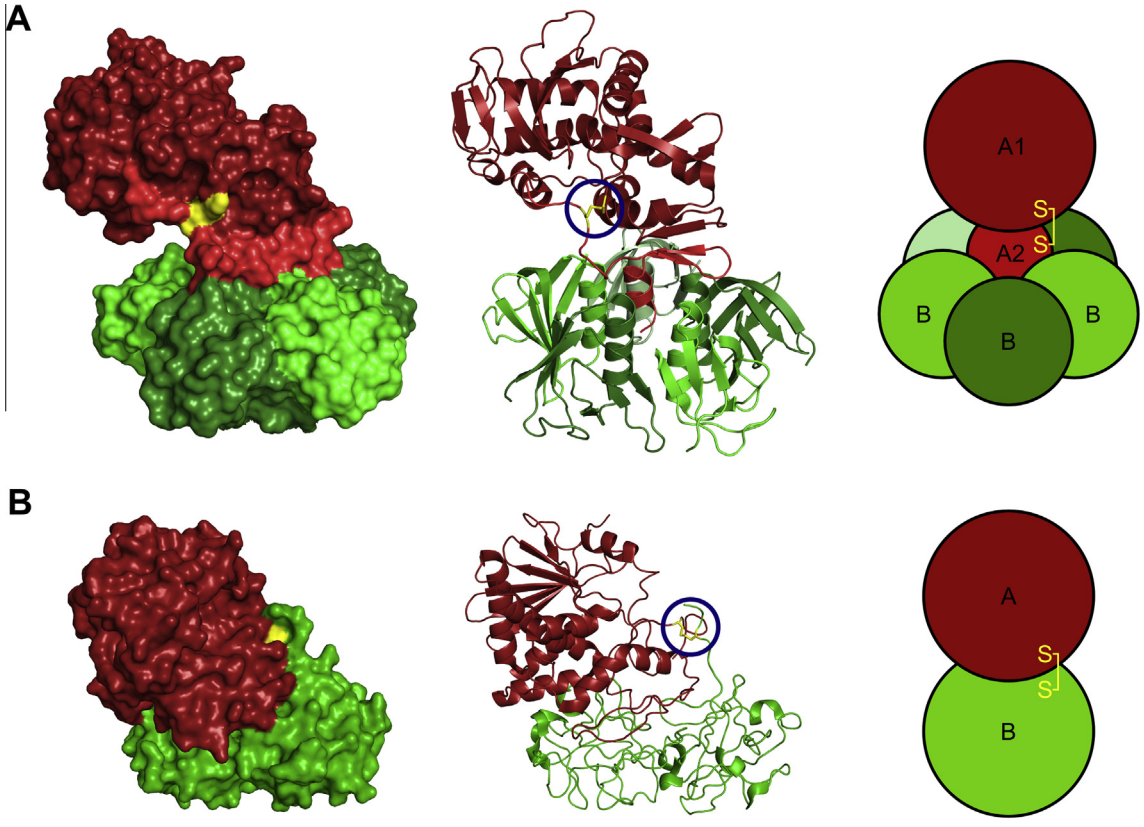

Fig. 1. The structure of (A) Shiga toxin (PDB ID: 1DM0, [45]) and (B) ricin (PDB ID: 2AAI, [124]) as determined by X-ray crystallography. The enzymatically active A moieties are colored red and the B moieties green. For Shiga toxin the A1 is in dark red and A2 in a lighter shade. The disulfide bonds linking the enzymatically active moieties to the rest of the toxin are indicated in yellow and marked with circles in the ribbon structures.