INTRODUCTION

Protein toxins which efficiently kill eukaryotic cells are found in plants and produced by bacteria. Examples of such toxins are the plant toxins ricin, abrin, modeccin, viscumin and volkensin, and the bacterial toxins diphtheria toxin and Shiga toxin (for review, see Olsnes and Sandvig 1988). Schematic structures of toxins are shown in Fig. 1. All these toxins kill cells in the following manner: They bind to cell surface receptors by their B-chains, they are internalized by endocytosis, and then an enzymatically active part of the molecule, the A-chain, enters the cytosol where it inhibits protein synthesis, either by inactivation of the 60 S subunit of the ribosome or by inactivation of elongation factor 2. In spite of their structural similarities, these protein toxins enter the cytosol from different intracellular compartments, and they have different requirements for entry.

Studies of the entry mechanisms of protein toxins are of interest for several reasons. First of all, studies of the entry of the A-chains through the membrane may give information of relevance to transport of other proteins. Due to the high toxicity, transport of a few molecules across the cell membrane is sufficient to monitor an inhibition of protein

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synthesis. Secondly, the protein toxins have during the last few years been used to make so-called "immunotoxins", i.e. molecules consisting of the enzymatically active part of the toxin (the A-chain) or a larger part of the toxin molecule coupled to an antibody directed against certain types of cancer cells (Olsnes et al 1989). In order to construct efficient immunotoxins it is useful to know as much as possible about the normal mechanism of toxin transport. The third point is that the protein toxins have proven useful as tools in the study of endocytosis and intracellular transport (van Deurs et al 1989; Sandvig et al 1989). The toxin ricin binds to both glycoproteins and glycolipids with terminal galactose and can thus serve as a marker for surface molecules in general. It is bound even when exposed to low intracellular pH and can therefore be used to follow the transport of endocytosed molecules. In contrast to ricin, the bacterial toxin Shiga toxin binds to a much more limited number of molecules at the cell surface, it binds to glycolipids with the sequence galα1-4gal (Lindberg et al 1987). This toxin can therefore be used to study routes taken by glycolipids.

We will in the present paper concentrate on studies of the uptake, intracellular transport and the intoxication of cells with ricin and Shiga toxin. We will present evidence that ricin is endocytosed by two different mechanisms, and we will show quantitative data on the amount of Shiga toxin transported to the Golgi apparatus in polarized cells. Furthermore, evidence that the transport of these toxins to the Golgi apparatus is required for intoxication of cells is shown.