Mike Lord

Ricin

Castor oil seeds contain a compound that is so toxic to mammalian cells that it has been used in bioterrorism. This article explains the mechanism of toxicity and how this has a potential use in pharmaceuticals to combat parasites and cancer.

Key words

Ricin
Ribosomes
Protein synthesis
Tumour

The commercial value of the seeds of the castor oil plant *Ricinus communis* has long been appreciated. These seeds store fat, rather than the carbohydrate used by many seeds such as those of cereal plants. The oil stored in *Ricinus* seeds has been used in the production of pharmaceutical products such as zinc and castor oil cream, and its properties as a lubricant have been used in other commercial products, such as the motor oil 'Castrol' — a trade name derived from 'castor oil' (see Figure 1).

However, *Ricinus* seeds also produce a more sinister compound — the poisonous protein ricin. This protein is classified as an agent of bioterrorism.

Figure 1 (A) Ricin is synthesised in the seeds of *Ricinus communis*. (B) Commercial uses of castor oil include pharmaceuticals and lubricants.

Its toxicity was demonstrated by the assassination of the dissident Bulgarian journalist Georgi Markov, allegedly by a small metal pellet containing ricin introduced into his leg by expulsion from the tip of an umbrella (see Figure 2).

Discovery of ricin

Ricin was identified in the nineteenth century. It had been known for a long time that extracts from *Ricinus* seeds were toxic to mammals. Hermann Stillmark, working at Dorpat University in Estonia in 1888, sought to identify the toxic component of these seed extracts. At the time, the blood was widely regarded as the essential life-giving component, so Stillmark mixed blood with *Ricinus* seed extract. He saw that the *Ricinus* extract caused the red blood cells to stick together (agglutinate). He then discovered that the component in the seed extract that caused the agglutination was a protein, which he termed ricin. Stillmark had discovered the first lectin, now known as a large family of sugar-reacting proteins that cause blood cells to agglutinate. Stillmark concluded that the toxicity of *Ricinus* extracts was due to the protein ricin causing blood cells to clump together. We now know that this is an oversimplification. While the toxicity of *Ricinus* extracts is indeed due to the protein ricin, agglutination is actually caused by a different protein, namely *Ricinus communis* agglutinin (RCA), a protein that is closely related to ricin.

Properties of the *Ricinus* lectins

RCA is a potent **agglutinin** but is only weakly toxic to intact mammalian cells. Its relative ricin is a weak agglutinin that is extremely toxic to cells. The reason for this difference is connected to the **quaternary structures** of the two lectins. Ricin is a protein comprised of two different polypeptide chains (i.e. it is heterodimeric). It has an A chain and a sugar-binding B chain, whereas RCA is made up of four polypeptides (two A chains and two sugar-binding B chains) (see Figure 3). The presence of two B chains allows RCA to crosslink adjacent red blood cells that expose sugar (galactose)-containing proteins (called glycoproteins) at their surface membranes, thus clumping them together. This agglutination ties up

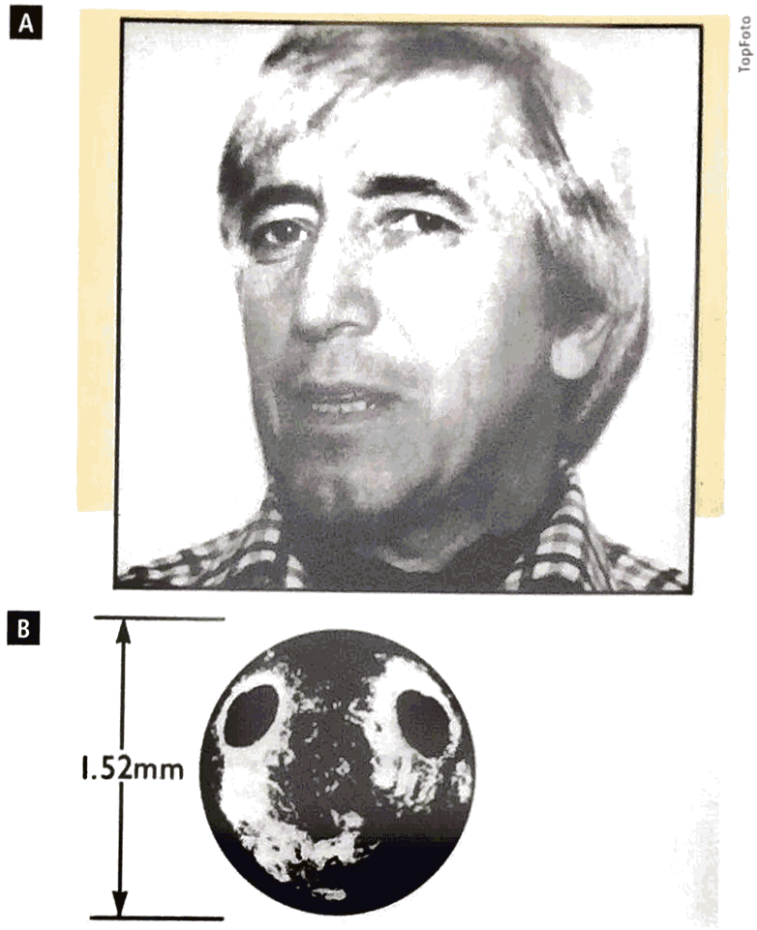

Figure 2 Ricin was allegedly used for the assassination of the journalist Georgi Markov (A), being contained in a small pellet (B) introduced into his leg by an umbrella.

RCA within the clump so that it is no longer free to bind and enter other cell types. Ricin, on the other hand, contains only one sugar-binding B chain. This does not allow efficient crosslinking of red blood cells, so that ricin remains free to circulate and to wreak havoc after it binds and enters into other cell types.

The biological target of ricin

So, what makes the protein ricin so toxic to mammalian cells? It is now known that the A chain of ricin (RTA, ricin toxin A) is an enzyme that damages an essential component of the protein synthesis machinery — the ribosome (see Box 1). Ribosomes are made up of more than 50 proteins and several RNA molecules — ribosomal RNAs

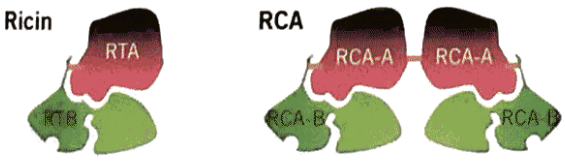

Figure 3 Diagrammatic representations of the ricin and RCA structures. Ricin is a heterodimer in which the A (red) and B (green) chains are covalently joined by a disulphide bond. RCA is a tetramer composed of two ricin-like heterodimers, which themselves are joined by a disulphide bond between a cysteine residue of the RCA A chain (this cysteine residue is not present in RTA).

Box 1 Ribosome basics

Ribosomes are the cellular sites of protein synthesis. Each ribosome can interact in specific ways with mRNA, tRNAs carrying amino acids and various accessory proteins to allow the efficient and accurate polymerisation of amino acids into a polypeptide (protein) chain. The order in which amino acids are joined up is dictated by the sequence of codons in the attached mRNA. In other words, the sequence of codons is converted into a sequence of amino acids, hence this process is known as translation. Translation occurs on ribosomes at a rate of around four amino acids per second. A typical polypeptide 300 amino acid residues long is therefore synthesised from its component amino acids in just over 1 minute. Assembled polypeptides are then released from ribosomes to function in the cell.

(rRNAs). Ribosomes are organised in two subunits — large and small — each with a set of unique proteins and rRNAs (see Box 2). All types of RNA are polymers of ribonucleotides (made up of a phosphate group, ribose and any of the bases adenine, uracil, guanine and cytosine). The various rRNAs provide a structural scaffold for ribosomal proteins, offer sites for interacting molecules (mRNA, tRNAs, accessory proteins) and participate in crucial activities such as peptide bond formation.

RTA is so toxic because it removes one particular base (adenine) in a crucially important section of the ribosome RNA. Only one base out of 5000+ bases in the large subunit of the ribosome is altered, but this small change renders the ribosome unable to synthesise proteins. Because RTA is an enzyme, every molecule can modify thousands of ribosomes per minute. The resulting inhibition of protein synthesis causes stress and eventually cell death.

Box 2 Ribosomes and RNA

The ribosomes, their subunits and individual rRNAs are described by Svedberg units (S), which reflect their sedimentation rate during centrifugation. The heavier the particle, the faster it sediments when centrifuged, and the higher its Svedberg value is. Eukaryotic ribosomes are therefore said to be 80S, composed of 60S and 40S subunits. In mammalian cells, within each large ribosome subunit are 28S, 5.8S and 5S rRNAs and in the small subunit is a single 18S rRNA.

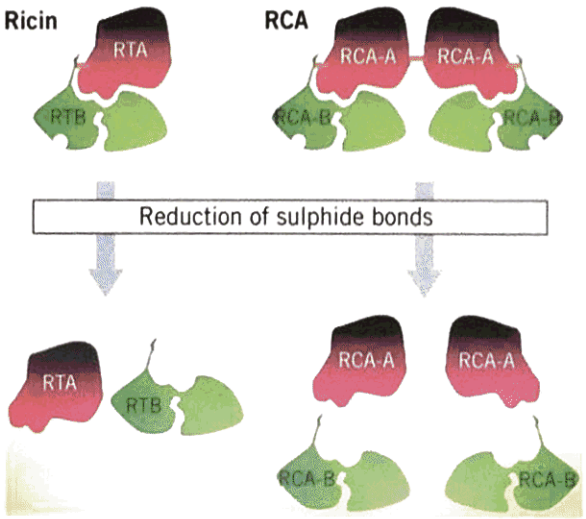

Figure 4 Reducing the interchain disulphide bonds of the *Ricinus* lectins generates their individual A and B chains.

Structure and function of ricin

Stretch and challenge

Ricin consists of two polypeptides — the ribosome-inactivating enzyme RTA joined to a B chain (abbreviated to RTB, for ricin toxin **B** chain) (see Figure 3). RTB has two binding sites specific for galactose. This enables RTB to bind to cell surface components that contain exposed galactose. It so happens that galactose is particularly common on mammalian glycoproteins and glycolipids (sugar-containing proteins and lipids), such that most animal cells have millions of potential binding sites for ricin. RTB-mediated binding to the plasma membrane is the first step of the cellular intoxication pathway described below.

The two polypeptides — RTA and RTB — are covalently linked by a disulphide bond between two cysteine residues. In the castor oil seed where ricin is made, there is an oxidation reaction between these two R groups leading to the formation of an S–S bond. If the environment favours the opposite process (reduction), the S–S bond will yield two SH groups, thus breaking the covalent linkage between RTA and RTB (see Figure 4). Only after it is separated from RTB does RTA exhibit catalytic activity. This is because in intact ricin the RTB polypeptide hides the catalytic site of RTA. This feature is undoubtedly important in the plant that produces it because the seed cells that make ricin have ribosomes. To protect plant ribosomes from the effects of RTA, ricin is made as an inactive protein and it is stored in the safe haven of plant vacuoles, well away from its substrates. The reduction of ricin into RTA and RTB is also necessary to allow RTA access to the cytosol, as described below.

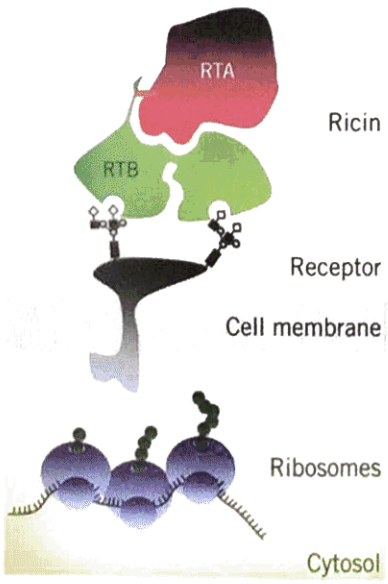

Figure 5 Ricin binds to galactose-containing receptors at the cell surface. The substrate for ricin is the ribosome in the cell cytosol.

Terms explained

Agglutinin A protein that binds to sugar molecules on the surface of cells causing them to clump together.

Endocytosis Uptake of extracellular materials by infolding of the plasma membrane, followed by pinching off of a membrane-bound vesicle containing extracellular fluid and materials.

Monoclonal antibody An antibody that is directed specifically against a single antigen, produced by a cloned population of antibody-producing cells.

Quaternary structure Level of protein structure involving interactions between two or more polypeptide chains to form a single multimeric protein.

Further reading

Lord, J. M. and Hartley, M. R. (2010) *Toxic Plant Proteins*, Springer-Verlag.

Ricin entry into cells

Stretch and challenge

Ricin binds its target cells through interactions of RTB with specific receptors. RTB has two related domains, each of which contains a galactose-binding site. Therefore ricin can bind two galactose residues exposed on cell surface molecules, provided they are close together on the plasma membrane (see Figure 5). This binding is followed by toxin uptake, a process that does not involve direct penetration of the surface membrane but instead requires uptake into the cell by **endocytosis**. This delivers the toxin to the endoplasmic reticulum (ER), via the Golgi complex. Here ricin is cleaved into its two subunits and then RTA crosses the ER membrane to reach its substrates in the cytoplasm. This is a complete reversal of the secretory pathway, a process whereby proteins synthesised by ER-bound ribosomes are transported and released from the cell (see Figure 6).

While it is understood that the 'toxic fraction' of internalised ricin has to reach the ER lumen, it is not known how the toxin accomplishes the final step of membrane translocation and escapes from degradation. However, it is known that ricin must be reduced before it leaves the ER. Reduction of ricin into its subunits is catalysed by an ER enzyme protein disulphide isomerase, which breaks the intrachain disulphide bond (this enzyme is required because the environment in the ER is oxidising).

Some applications of ricin

The UK and US governments classify ricin as a weapon for bioterrorism. A more constructive potential use for ricin is as a component of pharmaceuticals known as immunotoxins (ITs). These are protein-based drugs in which the RTB component of ricin, responsible for its binding to cells, is replaced with a **monoclonal antibody** that only interacts with a particular type of cell. So, for example, if the antibody component of an IT is directed towards a surface protein present only on tumour cells or parasites, then the IT should only bind to and kill these cells. Antibodies and other cell-surface binding components are currently being linked to potent toxins such as RTA, offering promising candidates for pharmaceuticals of the future.

Although this 'magic bullet' approach is appealing, there are technical problems to overcome before ITs can be use therapeutically. For example, if the IT contains native RTA, the toxin will be glycosylated. This means the sugar receptors on liver cells will remove the IT from the circulation, thus reducing the dose that might reach the target cells. Also, because RTA is a 'foreign' protein it will stimulate a host immune response — producing antibodies that can bind and inactivate the IT. The most serious limitation, as far as therapeutic applications in humans is concerned, is the lack of specificity of tumour cell antigens (the cell surface targets of the IT). While some antigens are expressed in high amounts on tumour cells, they may also be expressed at a much lower level on normal cells, leading to serious side effects caused by the lack of absolute tumour cells specificity. Clearly more research is required before the full potential of ITs can be realised.

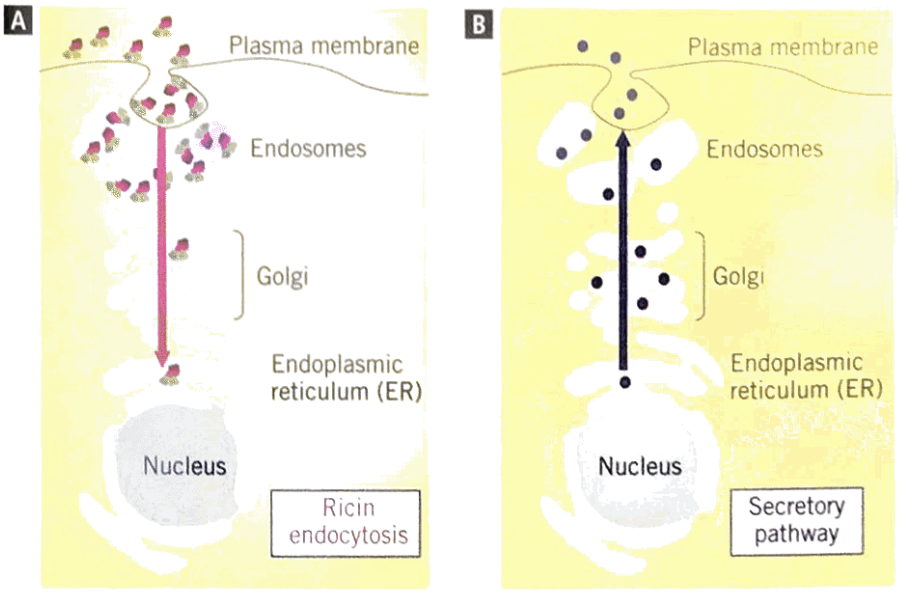

Figure 6 Ricin enters target cells by endocytosis from the plasma membrane to the ER (A), a process that is the reverse of the normal secretory pathway (B).

Mike Lord is professor of molecular cell biology in the School of Life Sciences at the University of Warwick. His research focuses on how protein toxins, such as ricin, enter and kill mammalian cells.

Professor Lynne Roberts contributed to the writing of this review. The illustrations used were produced by **Dr Robert Spooner**.

Key points

- Ricin is a protein toxin that damages essential processes in cells.
- Ricin is highly toxic to mammalian cells because it inactivates protein synthesis.
- Ricin is a lectin that binds to galactose residues on the surface of target cells and subsequently enters by endocytosis.
- Ricin is transported in membrane-bound vesicles until it reaches the endoplasmic reticulum (ER).
- Ricin crosses the ER membrane to reach its ribosomal substrates in the cytoplasm.