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Inhibition of protein synthesis by a toxic lectin from *Viscum album* L. (mistletoe)

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1. The haemagglutinating and toxic lectin from *Viscum album* L. (mistletoe) inhibits protein synthesis in a lysate of rabbit reticulocytes, with an ID_{50} (concentration giving 50% inhibition) of 2.6 μg/ml. This effect is enhanced (ID_{50} 0.21 μg/ml) if the lectin is reduced with 2-mercaptoethanol. 2. The lectin inhibits protein synthesis also in BL8L cells in culture. Inhibition occurs after a lag time of 3h. The ID_{50} is 7ng/ml, and increases after reduction of the lectin. 3. This and the gross lesions observed in rats poisoned with *V. album* lectin indicate this is a toxin very similar to ricin.

Several lectins are known that inhibit protein synthesis. Among these, ricin and abrin (reviewed by Olsnes & Pihl, 1977) and modeccin (Refsnes *et al.*, 1977; Stirpe *et al.*, 1978) are highly toxic to animals and inhibit protein synthesis in cells as well as in cell-free systems. Other inhibitory lectins are much less toxic to animals and inhibit protein synthesis in cell-free systems, but only at much higher concentrations in cells, presumably because they cannot easily enter them (Saltvedt, 1976; Barbieri *et al.*, 1979, 1980). A common feature of these inhibitory lectins, either toxic or non-toxic, is their specificity for D-galactose. This prompted us to study the galactose-specific lectin from *Viscum album* L. (mistletoe), which indeed was a potent inhibitor of protein synthesis in both a cell-free system and in whole cells. This may well account for the toxicity of *V. album* lectin (Lutsik, 1975; Luther *et al.*, 1977), which in this respect and in other properties seems to be a toxin very similar to ricin.

Experimental

Materials

Viscum album (mistletoe) lectin was prepared as described by Ziska *et al.* (1978). L-[^{14}C]Leucine (sp. radioactivity 339mCi/mmol) was from The Radiochemical Centre, Amersham, Bucks., U.K. Reagents for protein synthesis were purchased from Sigma, Poole, Dorset, U.K. All other chemicals were of analytical grade.

Methods

Protein synthesis was determined as described by Gasperi-Campani *et al.* (1978) with a lysate of rabbit reticulocytes prepared as described by Allen & Schweet (1962), or with BL8L cells (Judah *et al.*, 1977). Experimental details are given in the legends to the appropriate Figures. In either case, protein was precipitated with 10% (w/v) trichloroacetic acid, and was collected and washed with 5% (w/v) trichloroacetic acid by filtering through Whatman GF/A glass-fibre filters. Filters were transferred to counting vials with 10ml of Insta-Gel (Packard) and the radioactivity was determined in a Searle liquid-scintillation spectrometer with an external standard, with an efficiency of approx. 80%.

Results and discussion

Viscum album lectin inhibits protein synthesis by a rabbit reticulocyte lysate (Fig. 1). This was increased by reduction of the lectin with 2-mercaptoethanol; the ID_{50} (concentration giving 50% inhibition) was lowered from 2.6 μg/ml before to 0.21 μg/ml after reduction.

The lectin also inhibited protein synthesis in BL8L cells cultured *in vitro*, with an ID_{50} of 7ng/ml

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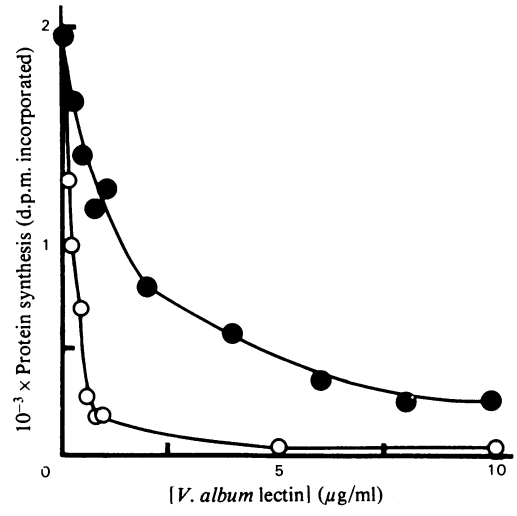

Fig. 1. *Effect of V. album lectin on protein synthesis by a rabbit reticulocyte lysate*

Reaction mixtures contained, in a final volume of 125μl: 10mM-Tris/HCl buffer, pH 7.4, 100mM-ammonium acetate, 2mM-magnesium acetate, 1mM-ATP, 0.2mM-GTP, 15mM-phosphocreatine, 6μg of creatine kinase, 0.05mM-amino acids (minus leucine), 0.19μCi of L-[^{14}C]leucine and 50μl of a lysate of rabbit reticulocytes. Lectin, when present, was dissolved in 0.14M-NaCl containing 5mM-sodium phosphate buffer, pH 7.2, and was added in its native form or after reduction at 37°C for 1h in the presence of 1% 2-mercaptoethanol. Incubation was at 27°C for 5min and the radioactivity incorporated into protein was determined on 25μl samples as described by Gasperi-Campani *et al.* (1978). It was shown that protein synthesis was not affected by 2-mercaptoethanol at the highest final concentration (0.01%) present in the mixtures with the reduced lectin. Symbols: ●, Native lectin; ○, reduced lectin.

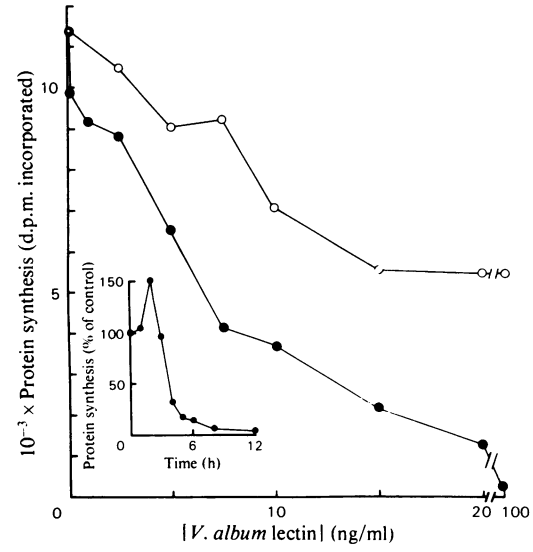

Fig. 2. *Effect of V. album lectin on protein synthesis by BL8L cells*

Each test was performed on a confluent monolayer of BL8L cells (approx. 2×10^6 cells), growing in a well of an eight-well Multiplate (Lux Scientific Corp., Newbury Park, CA, U.S.A.), in 2ml of Williams's Medium E (Flow Laboratories Ltd., Irvine, Ayrshire, Scotland, U.K.) supplemented with 5% (v/v) foetal-calf serum, 2mM-glutamine and gentamycin (50μg/ml). The appropriate amount of lectin was added dissolved in 10μl of phosphate-buffered saline. Cells were incubated for 18h at 37°C in a humidified atmosphere of air/CO_2 (19:1), then the medium with the toxin was removed and replaced with 2ml of medium without serum. [^{14}C]Leucine (0.5μCi/well) was added, and the cells were incubated for a further 1h before adding 0.2ml of 1M-NaOH to each well. The content of each well was transferred to test tubes with 1ml of 0.1M-NaOH used to wash the wells. After 30min at room temperature, protein was precipitated by adding 0.3ml of 100% trichloroacetic acid and treated as described in the Experimental section. Time-course experiments (inset) were performed with the lectin at the concentration of 100ng/ml, other details being as described above except for time, and for the toxin, which was present also in the medium without serum. Data are mean values for duplicate wells. Symbols: ●, Native lectin; ○, reduced lectin.

after 18h (Fig. 2). The inhibition decreased markedly after reduction of the lectin. Inhibition of protein synthesis in cells by 100ng of lectin/ml occurred after a time lag of 3h; a stimulation of [^{14}C]leucine incorporation was observed at 2h (Fig. 2, inset).

Rats poisoned with the lectin given by intraperitoneal injection at the dose of 100 or of 10μg/100g body wt. died after 20–22h or after 3–4 days respectively, with lesions (ascites, congested intestine, haemorrhages in the pancreas) very similar to those observed in rats poisoned with 1–10μg of ricin/100g body wt. (Derenzini *et al.*, 1976).

These results demonstrate that *V. album* lectin is a toxin that inhibits protein synthesis in a cell-free system as well as in whole cells. This may account for the known toxicity of this lectin to animals and cells (Lutsik, 1975; Luther *et al.*, 1977).

The effect on the lysate system was increased and that on cells was decreased if the lectin was reduced with 2-mercaptoethanol. A similar phenomenon was

observed with ricin and abrin (Olsnes & Pihl, 1972) and with modeccin (Refsnes *et al.*, 1977; Gasperi-Campani *et al.*, 1978), and was due to separation of the A-chain (which inhibits protein synthesis) from the B-chain (which binds to cells). *V. album* lectin has a mol.wt. of approx. 115000 (Ziska *et al.*, 1978), as opposed to approx. 60000 for ricin and related toxins, and consists of subunits of mol.wts. 29000 and 34000 (similar to those of ricin). Thus it seems that *V. album* lectin consists of four chains (possibly A_2B_2), presumably having the functions of the A and B chains of the other toxins consisting of two subunits only (AB). This and the similar effect on protein synthesis indicate that *V. album* lectin is very similar to ricin, which, however, is a more potent toxin, having an LD_{50} for mice of 3μg/kg (Olsnes, 1978), whereas the LD_{50} of *V. album* lectin is 80μg/kg (Lutsik, 1975).

In addition to the effect of protein synthesis, *V. album* lectin has some other properties in common with ricin. Thus both toxins bind to plasma proteins [ricin (Olsnes & Pihl, 1977); *V. album* lectin (Franz *et al.*, 1977; Ziska & Franz, 1979)] and have some anti-neoplastic activity [ricin (Lin *et al.*, 1970); *V. album* lectin (Lutsik, 1975; Luther *et al.*, 1977)], and bring about similar macroscopic lesions in rats [Derenzini *et al.* (1976) and the present observations].

V. album lectin is the fourth toxin of plant origin inhibiting protein synthesis, and is present in the leaves of the plant, whereas ricin and abrin are in the seeds, and modeccin is in the seeds and in the roots, of the respective plants. This suggests that toxins of this kind can be present in many parts, and possibly in any part, of different and unrelated plants.

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