Inhibition of Protein Synthesis *in vitro* by a Lectin from *Momordica charantia* and by other Haemagglutinins

By Luigi BARBIERI, Enzo LORENZONI and Fiorenzo STIRPE
Istituto di Patologia generale dell'Università di Bologna, I-40126 Bologna, Italy

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Protein synthesis by a rabbit reticulocyte lysate is inhibited by the haemagglutinating lectins from *Momordica charantia* and *Crotalaria juncea* seeds and from the roe of *Rutilus rutilus*, and by a commercial preparation of the mitogenic lectin from *Phytolacca americana*. The haemagglutinins from the seeds of *Ricinus communis* and of *Vicia cracca* acquired inhibitory activity after their reduction with 2-mercaptoethanol.

Lectins are proteins or glycoproteins with binding sites for specific carbohydrate groups (Lis & Sharon, 1973; Liener, 1976; Brown & Hunt, 1978), which have interesting biological properties, in that many of them agglutinate erythrocytes or other cells, and some are mitogenic to lymphocytes. Among lectins there are three potent toxins from plants, namely ricin, abrin (review by Olsnes & Pihl, 1977) and modeccin (Refsnes *et al.*, 1977; Stirpe *et al.*, 1978), which are potent inhibitors of protein synthesis in cells and in cell-free systems.

Various plant materials contain proteins which are non-toxic or scarcely toxic to animals and which inhibit protein synthesis in cell-free systems but not in whole cells, presumably because they cannot enter into cells (Obrig *et al.*, 1973; Irvin, 1975; Stirpe *et al.*, 1976; Gasperi-Campani *et al.*, 1977; Stewart *et al.*, 1977; A. Gasperi-Campani, L. Barbieri, P. Morelli & F. Stirpe, unpublished work). Some of these proteins were purified (Obrig *et al.*, 1973; Irvin, 1975) or semi-purified (Stirpe *et al.*, 1976; Sperti *et al.*, 1976), and in these cases it was ascertained that they inhibit protein synthesis through the same mechanism as do ricin and the other toxins mentioned above, i.e. by inactivating enzymically the 60S ribosomal subunit, and making it unable to bind the elongation factor 2.

Lin *et al.* (1978) reported two lectins (mol.wts. 32000 and 24000) from the seeds of a Cucurbitacea, *Momordica charantia* (bitter pear melon), the latter inhibiting protein synthesis by Ehrlich ascites cells at concentrations relatively high (from 100 µg/ml) as compared with the toxic lectins.

We report now that a haemagglutinating lectin from the seeds of *Momordica charantia* is a potent inhibitor of protein synthesis in a cell-free system (a lysate of rabbit reticulocytes). Inhibition of protein synthesis in the same system was obtained also with lectins from other seeds and from a fish roe.

Experimental

Materials

Seeds of *Momordica charantia*, originally from India, were obtained from Mr. F. G. Celo, Zweibrücken, West Germany, and the haemagglutinating lectin was purified by affinity chromatography on Sepharose 4B as described by Tomita *et al.* (1972) with minor modifications. *Ricinus communis* agglutinin was prepared as described by Nicolson & Blaustein (1972), and other lectins were obtained from the sources listed in Table 1. Other chemicals were from the same sources as described by Gasperi-Campani *et al.* (1978).

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 Yoshida AH-130 ascites cells.

Protein was determined by the method of Lowry *et al.* (1951) or spectrophotometrically (Kalb & Bernlohr, 1977).

Results and Discussion

The lectin purified by affinity chromatography from *Momordica charantia* seeds gave a single band on polyacrylamide-gel electrophoresis in either the presence or the absence of sodium dodecyl sulphate, had mol.wt. 115000, and agglutinated human erythrocytes (group O), the lowest active concentration being 24 ng/ml.

This agglutinin inhibited protein synthesis by a lysate of rabbit reticulocytes: the ID₅₀ (concentration giving 50% inhibition) was 1.74 µg/ml, i.e. similar to those of the toxic lectins mentioned above (e.g.

modeccin; see Stirpe *et al.*, 1978). The inhibition was unchanged in the presence of 100 mM-galactose, which inhibits haemagglutination by the lectin, as shown by Tomita *et al.* (1970) and confirmed with our preparation. This indicates that the inhibitory effect on protein synthesis is independent of the haemagglutinating property of the lectin.

The lectin at 100 µg/ml inhibited by 30% protein synthesis by Yoshida ascites cells: 50 µg/ml had no effect. The fact that relatively high concentrations, as compared with the toxic lectins, are required to affect protein synthesis by whole cells suggests that *Momordica charantia* lectin enters with difficulty into cells, and this in turn could account for its low toxicity to animals: it did not cause any apparent harm to rats when injected intraperitoneally at the dose of 1 mg/100 g body wt.

Momordica charantia lectin is the second example of a non-toxic lectin inhibiting protein synthesis *in vitro*, after the *Ricinus communis* agglutinin (distinct from ricin), as shown by Saltvedt (1976) and by Cawley *et al.* (1978). This led us to the hypothesis that other lectins could have the same property. Therefore 27 lectins were examined, before and after reduction with 2-mercaptoethanol, a treatment which greatly enhances the inhibitory effects of ricin and

abrin (Olsnes & Pihl, 1972) and of modeccin (Refsnes *et al.*, 1977; Gasperi-Campani *et al.*, 1978). Six lectins were inhibitory (Table 1), and among these the already known agglutinin from *Ricinus communis*, the *Momordica charantia* lectin prepared by a different method (Hořejší & Kocourek, 1978) in another laboratory, and the pokeweed mitogen. The inhibitory activity of the latter was observed before (Gasperi-Campani *et al.*, 1977), and was attributed to contamination by the powerful pokeweed antiviral peptide (Irvin, 1975). It was confirmed that commercial preparations of pokeweed mitogen contain 0.3% of pokeweed antiviral peptide, which is sufficient to account for the inhibition (J. D. Irvin, personal communication). It is noteworthy that a lectin from the roe of the fish *Rutilus rutilus* was also inhibitory. Reduction with 2-mercaptoethanol enhanced greatly the effects of the lectins from *Ricinus communis* and from *Vicia cracca*, and abolished the effect of the agglutinin from *Rutilus rutilus*.

It is not known whether all these lectins inhibit protein synthesis through the same mechanism. However, our results demonstrate that the capacity of inhibiting protein synthesis is common to several lectins, presumably to many others besides those identified by the present experiments, and thus should

Table 1. *Effect of various lectins on protein synthesis in vitro*

Reaction mixtures contained, in a final volume of 0.25 ml: 10 mM-Tris/HCl buffer, pH 7.4, 100 mM-ammonium acetate, 2 mM-magnesium acetate, 1 mM-ATP, 0.2 mM-GTP, 15 mM-phosphocreatine, 12 µg of creatine kinase, 0.05 mM-amino acids (minus leucine), 0.75 µCi of L-[¹⁴C]leucine (specific radioactivity 354 mCi/mmol) and 0.1 ml of a lysate of rabbit reticulocytes. Lectins, when present, were added at a final concentration of 100 µg/ml, and were used in their native form or after reduction at 37°C for 2 h in the presence of 1% 2-mercaptoethanol. Incubation was at 27°C for 5 min. Radioactivity incorporated into protein was determined as described by Gasperi-Campani *et al.* (1978). The following lectins were inactive at a concentration of 100 µg/ml: *Arachis hypogaea* (3, 4), *Canavalia ensiformis* (concanavalin A) (5), *Coregonus lavaretus maraena* (3), *Datura stramonium* (3), *Dolichos biflorus* (3), *Erythrina indica* (3), *Glycine max* (3, 6), *Helix pomatia* (3), *Lens culinaris* (4), *Ononis spinosa* (3), *Perca fluviatilis* (3), *Phaseolus coccineus* (3), *Phaseolus lunatus* (3), *Phaseolus vulgaris** (7), *Pisum sativum* (3), *Robinia pseudoacacia* (3), *Sarothamnus scoparius* (8), *Triticum vulgare* (wheat germ) (6), *Ulex europaeus* (3, 8), *Vicia sativa* (9), *Wistaria floribunda* (10). Sources of lectins: (1) Dr. B. Ersson, Uppsala, Sweden; (2) Grand Island Biological Co., Grand Island, NY, U.S.A.; (3) Dr. J. Kocourek, Praha, Czechoslovakia; (4) U.S. Biochemical Corporation, Cleveland, OH, U.S.A.; (5) Miles Laboratories, Elkhart, IN, U.S.A.; (6) Pharmacia Fine Chemicals, Uppsala, Sweden; (7) Dr. F. Serafini-Cessi of this Institute; (8) Dr. L. G. Gürtler, Munich, West Germany; (9) Dr. A. Falasca, Instituto di Chimica biologica, Bologna; (10) Dr. T. Kurokawa, Osaka, Japan.

Expt. no.	Lectin added	Protein synthesis (d.p.m. incorporated)	
		With native lectin	With reduced lectin
1	None (control)	2235	2214†
	<i>Crotalaria juncea</i> (1)	220	507
	<i>Phytolacca americana</i> (2)	23	4
	(pokeweed mitogen)		
	<i>Ricinus communis</i>	1379	2
2	None (control)	1981	2021†
	<i>Momordica charantia</i> (3)	348	85
	<i>Rutilus rutilus</i> (3)	392	1766
	<i>Vicia cracca</i> (3)	1873	22

* Phytohaemagglutinin (purified haemagglutinin factor, Serafini-Cessi *et al.*, 1979).

† Containing the same amount of 2-mercaptoethanol (14 µl of a 1% solution) as the samples with reduced lectins.

be considered another general property of lectins, like the mitogenic activity.

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