INHIBITION OF PROTEIN SYNTHESIS BY MODECCIN, THE TOXIN OF *MODECCA DIGITATA*

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1. Introduction

Modecca digitata Harv. (synonyms: *Adenia digitata* Burtt-Davy, *Adenia senensis* Engl.) is a passifloracea growing in austral Africa, the fruits and roots of which are highly poisonous to man and animals [1]. Green and Andrews [2,3] observed that the toxicity of the large roots of this plant was due to a cyanogenetic glycoside and to a highly toxic protein they called modeccin 'by analogy with ricin'. The pathological changes observed by these investigators [3] in animals poisoned with partially purified modeccin seem to be very similar to those brought about by ricin [4–6], and this prompted us to ascertain whether modeccin inhibits protein synthesis as do ricin and the related toxin abrin [7].

With the experiments to be described in this paper modeccin was purified to homogeneity from the roots of *Modecca digitata*. It was observed that this toxin is indeed a powerful inhibitor of protein synthesis in cells and in a cell-free system.

2. Experimental

2.1. *Materials*

Roots of *Modecca digitata* were obtained from the Department of Botany, University of Pretoria, South Africa. All chemicals were obtained from the same sources as [8].

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2.2. *Biochemical determinations*

Protein synthesis was determined as described [8] with a lysate of rabbit reticulocytes, prepared as described [9], or with Ehrlich ascites cells.

RNAase activity was estimated spectrophotometrically as described [10]. Protein was determined by the method [11] with bovine serum albumin as a standard, or by the method [12].

2.3. *Toxicity experiments*

The toxicity of modeccin was evaluated in male Swiss mice weighing 20–25 g, supplied with food and water ad libitum. The toxin, dissolved in 0.9% NaCl, was injected intraperitoneally at seven doses varying from 4.0 to 0.1 μg/100 g body wt, to groups of 6 animals/dose. LD_{50} was evaluated by the method of Spearman-Kärber as described [13].

3. Results and discussion

3.1. *Purification of modeccin*

Roots of *Modecca digitata* were minced and homogenized with 10 ml/g 0.2 M NaCl containing 0.005 M Na-phosphate buffer, pH 7.2. The resulting suspension was stirred at room temperature under an aspirated fume cupboard for 2–3 h; during this time the cyanogenetic glycoside contained in the roots was hydrolysed and HCN was liberated. Stirring was continued overnight in a cold room and a clear, almost colourless extract could be obtained by filtration through filter paper on a Büchner funnel. To this filtrate solid ammonium sulphate was added slowly under constant

stirring to 60% saturation. The precipitate was collected by centrifugation, redissolved in phosphate-buffered saline, and dialysed against the same solution for at least 24 h. This solution (crude modeccin) was then applied to a column of Sepharose 4B, previously equilibrated with phosphate-buffered saline. On elution with 0.2 M galactose a very sharp peak was obtained, which gave a single protein band on polyacrylamide gel electrophoresis. This protein is hereupon referred to as modeccin.

3.2. *Properties of modeccin*

Modeccin was highly toxic when given to mice by the intraperitoneal route. Animals died at a variable time, depending upon the dose administered, but never before 8 h of injection: for this reason an acute LD_{50} and a delayed LD_{50} were calculated at 48 h and at 10 days and were 0.53 and 0.23 μg/100 g body wt, respectively. At post-mortem examination alterations consistent with those described [3] were observed. Lesions were similar, although not identical, to those brought about by ricin [4–6], the main differences being a less-marked intestinal damage and a severe hepatic damage in the animals poisoned with modeccin (Derenzini, M., unpublished observations).

Modeccin had a strong inhibitory effect on protein synthesis by a lysate of rabbit reticulocytes (fig.1), with an ID_{50} (concentration giving 50% inhibition) of 4 μg/ml, i.e., of the same order of magnitude of ricin and abrin [7] and of other seed proteins which, although less toxic to animals, are potent inhibitors of protein synthesis in cell-free systems [8,14]. Inhibition occurred also, and to the same extent, when modeccin was added to the reaction mixture 5 min or 10 min after starting the incubation, i.e., when protein synthesis was already in progress (results not shown). Modeccin had no RNAase activity, and its inhibitory effect on protein synthesis was abolished by boiling.

The toxin also inhibited protein synthesis by Ehrlich ascites cells in vitro (fig.2), synthesis being

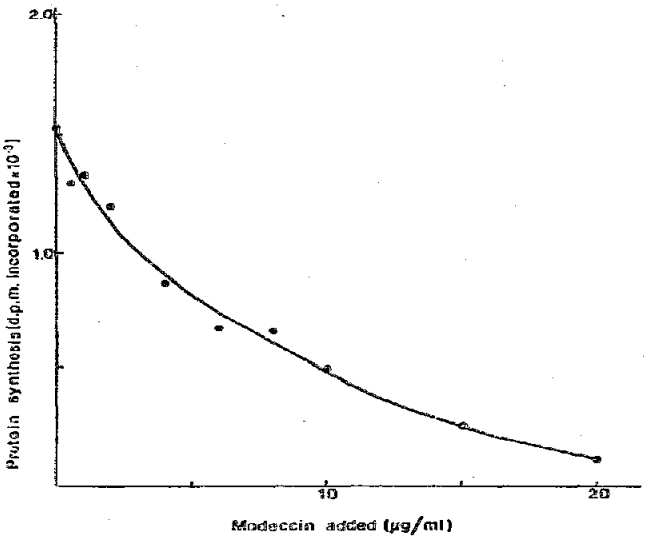

Fig.1. The effect of modeccin on protein synthesis by a reticulocyte lysate. The reaction mixture contained, in final vol 0.25 ml: 10 mM Tris–HCl buffer, pH 7.4, 100 mM ammonium acetate, 2 mM magnesium acetate, 1 mM ATP, 0.2 mM GTP, 15 mM creatine phosphate, 12 μg creatine kinase, 0.05 mM amino acids (minus leucine), 0.75 μCi L-[^{14}C]leucine (spec. act. 348 mCi/mmol), the appropriate amount of modeccin and 0.1 ml rabbit reticulocyte lysate. Incubation was at 27°C for 5 min. Acid-insoluble, alkali-resistant radioactivity was measured on 25 μl samples.

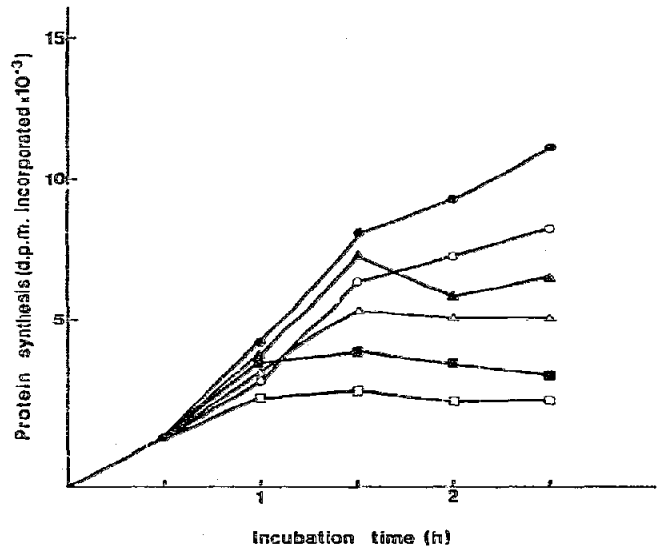

Fig.2. The effect of modeccin on protein synthesis by Ehrlich ascites cells. Cells (3.6×10^6/ml) were incubated at 37°C in 1 ml medium E 2a [15] containing 5% calf serum and 1 μCi L-[^{14}C]leucine, without (●) or with modeccin at the concentration of 0.01 (○), 0.1 (▲), 1 (△), 10 (■) and 50 (□) μg/ml. Data are mean values of duplicate results. Other details are as in fig.1. Values at 30 min were very close to each other, and only the control value is given, for the sake of clarity.

completely arrested by as little as 0.1 μg modeccin/ml. Inhibition occurred after a time-lag which varied between 30 min and 90 min, depending upon the concentration of the toxin.

The pathological changes observed in the animals poisoned with modeccin and the inhibition of protein synthesis in cells and in a cell-free system indicate that modeccin is a very potent toxin, with properties similar although not identical to those of ricin and abrin.

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References

[1] Watt, J. N. and Breyer-Brandwijk, M. G. (1962) in: The Medicinal and Poisonous Plants of Southern and Eastern Africa, pp. 826–827, Livingstone, Edinburgh, London.
[2] Green, H. H. and Andrews, W. H. (1923) S. Afr. J. Sci. 20, 273.
[3] Green, H. H. and Andrews, W. H. (1923) Rep. Vet. Res. S. Afr. 9/10, 381–392.
[4] Flexner, S. (1897) J. Exp. Med. 2, 197–216.
[5] Waller, G. R., Ebner, K. E., Scroggs, R. A., Das Gupta, B. R. and Corcoran, J. B. (1966) Proc. Soc. Exp. Biol. Med. 121, 685–691.
[6] Derenzini, M., Bonetti, E., Marinozzi, V. and Stirpe, F. (1976) Virchows Arch. B Cell Path. 20, 15–28.
[7] Olsnes, S. and Pihl, A. (1977) in: Receptors and Recognition, Series B, Vol. 1: The Specificity and Action of Animal, Bacterial, and Plant Toxins (Cuatrecasas, P. ed) pp. 129–173, Chapman and Hall, London.
[8] Gasperi-Campani, A., Barbieri, L., Lorenzoni, E. and Stirpe, F. (1977) FEBS Lett. 76, 173–176.
[9] Allen, E. H. and Schweet, R. S. (1962) J. Biol. Chem. 237, 760–767.
[10] Gianfranceschi, G. L., Amici, D. and Guglielmi, L. (1975) Biochim. Biophys. Acta 414, 9–19.
[11] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[12] Warburg, O. and Christian, W. (1941) Biochem. Z. 310, 384–421.
[13] Finny, D. J. (1964) Statistical Method in Biological Assay pp. 524–530, Griffin, London.
[14] Stirpe, F., Pession-Brizzi, A., Lorenzoni, E., Strocchi, P., Montanaro, L. and Sperti, S. (1976) Biochem. J. 156, 1–6.
[15] Puck, T. T., Cieciura, S. J. and Fisher, H. W. (1957) J. Exp. Med. 106, 145–157.