INHIBITION OF PROTEIN SYNTHESIS BY PR TOXIN, A MYCOTOXIN FROM *PENICILLIUM ROQUEFORTI*

Yvonne MOULÉ, Mongi JEMMALI* and Nicole DARRACQ

Institut de Recherches Scientifiques sur le Cancer, Boîte Postale 8, 94800 Villejuif and **Service des Mycotoxines de l'INRA, 75013 Paris, France*

Received 9 January 1978

1. Introduction

Some strains of *Penicillium roqueforti* synthesize PR toxin (PRT), a compound which produces toxic effects in various animal species [1,2]. The LD_{50} for male rats and mice is about 7 mg/kg by intraperitoneal injection. PRT induces short-term alterations in liver metabolism [3–5]. In the experiments presented here, we studied the inhibition of protein synthesis by PRT in an attempt to elucidate the mechanism of this effect.

2. Materials and methods

A PRT-producing strain of *Penicillium roqueforti* originally isolated from cheese, was grown in the medium used in [2]. The mycotoxin was isolated and crystallized as in [2]; its purity was checked by thin-layer chromatography.

2.1. *Determination of in vivo protein synthesis*

Adult male Wistar rats (Commentry strain) 280–300 g, were starved overnight before sacrifice. They were injected intraperitoneally with varying doses of PRT dissolved in DMSO; control rats received the vehicle alone. The animals were killed by decapitation after the intervals of time indicated in fig.1. Ten minutes before killing, they received an intraperitoneal injection of 12.5 μCi DL-[*carboxyl*-^{14}C]leucine. Each experiment consisted of 4 PRT-treated rats and 4 controls.

The livers were quickly removed, rinsed and homogenized in 4 vol. cold water. The radioactivity of the liver proteins labelled in vivo were determined as in [6]. The proteins were measured as in [7] and the radioactivity was counted in a liquid scintillation spectrometer (Intertechnique, Paris). The results were expressed in terms of the relative specific radioactivity (dpm per mg protein/dpm acid soluble pool).

2.2. *Amino acid incorporating system*

The standard assay contained (in 0.25 ml): 50 mM Tris–HCl, pH 7.5, 0.5 mM ATP, 0.1 mM GTP, 3 mM phosphoenol-pyruvate, 6.25 μg pyruvate kinase (350–500 units/mg), 10 mM Mg $(CH_3COO)_2$, 100 mM KCl, 40 mM NaCl, 0.05 mM each of the 19 unlabelled aminoacids, 0.5 μCi L-[U-^{14}C]leucine (280 mCi/mmol), about 1 mg pH 5 enzyme fraction (unless otherwise stated) prepared as in [8], 25 μl DMSO (control) or 25 μl DMSO containing PRT. The reaction was started by adding a suspension of rat liver polysomes corresponding to about 2–3 A_{260} units (unless otherwise stated). After incubation at 37°C for 45 min, three 50 μl samples were pipetted on Whatman 3 MM filter paper discs. The radioactivity of the incorporated labelled leucine was measured as in [9].

Polysomes were isolated from the livers of fasted rats according to a technique using a soluble RNase inhibitor preparation in each step of the cell fractionation procedure [10–12].

Abbreviations: PRT, PR toxin; DMSO, dimethylsulfoxide

3. Results and discussion

3.1. *In vivo effect of PR toxin*

A single injection of PRT inhibited in vivo protein synthesis in rat liver (fig.1). The effect culminated 7 h after toxin dosing; then, a progressive recovery of the synthetic activity of the liver tissue was observed up to 24–30 h for most doses. Doses of 10 mg/kg caused death of the animals about 18 h after toxin administration. Similar time-course curves were obtained in rat kidney (not shown).

It must be pointed out that PRT did not modify the uptake of amino acids in the liver cells; however, in order to exclude any possible individual variability in the acid soluble pool, the present results have been expressed in terms of the relative specific radioactivity (see section 2). The results obtained under these conditions support the view that PRT blocks the translational process itself.

Although a dose of 7 mg/kg corresponds to the LD_{50} of PRT for the rat (50% of the treated animals die within 48–60 h), the toxic effect due to the transient inhibition of protein synthesis may not be responsible for the irreversible lesions leading to the death of the rat; however, a possible secondary effect due to the lack of some specific proteins should be taken into consideration.

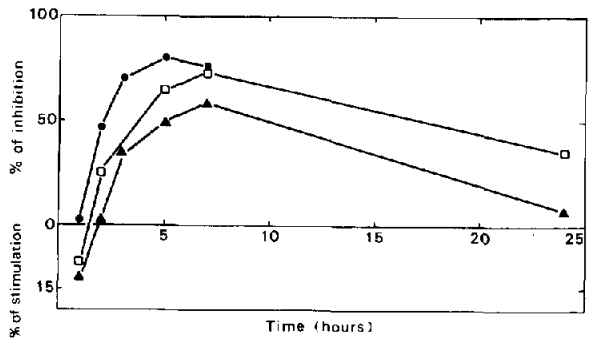

Fig.1. Time-course inhibition of protein synthesis by PR toxin in rat liver. Rats were killed after administration of PRT at the time intervals indicated on the graph; 10 min before killing, they received an injection of 12.5 μCi DL-[*carboxy*-^{14}C]leucine. The % inhibition was calculated with regard to the values obtained for controls receiving DMSO alone during the same interval of time. Doses of PRT: (▲) 5 mg/kg; (□) 7 mg/kg; (●) 10 mg/kg. Each value corresponds to 2 or 3 expt (2 groups of 4 rats each per expt) expect for a 1 h interval which included 4 expt.

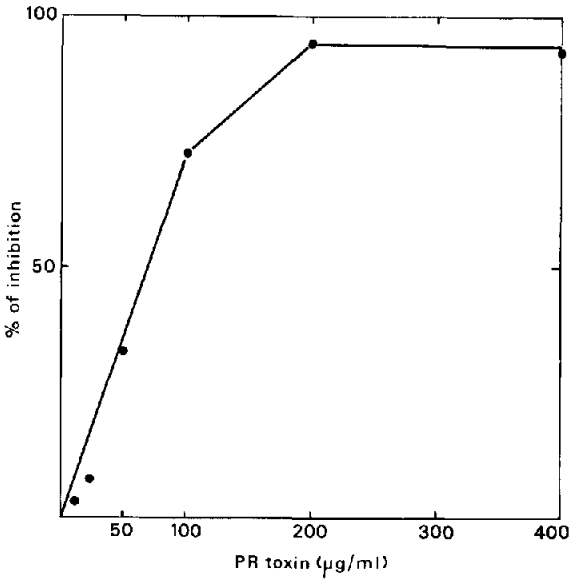

Fig.2. Dose response of the inhibition induced by PR toxin on amino acid incorporation by isolated liver polysomes. The experimental conditions are in section 2.

The slight but significant stimulation of the in vivo protein synthesis 1 h after toxin injection of 5 mg/kg at 7 mg/kg is still unexplained.

3.2. *In vitro effect of PR toxin*

PRT inhibited the in vitro incorporation of amino acids mediated by a polysomal standard system (fig.2). These results demonstrate that PRT acts at the level of the translational process. In addition, they confirm earlier experiments which show that PRT is directly active on cell metabolism [4].

The following experiments were performed in an attempt to elucidate the mechanism of PRT-induced inhibition of translation. Figure 3 shows that the extent of inhibition was not significantly affected by adding increasing amounts of polysomes to the assay (2–44 A_{260} units/ml), whatever the amount of PRT present in the system (50–200 μg/ml). By contrast, the addition of increasing concentrations of pH 5 enzyme (0.2–7.6 mg protein/ml) progressively relieved the inhibitory effects of PRT on protein synthesis (fig.4). These data suggest that PRT impairs in vitro translation by alteration of the pH 5 enzyme fraction rather than by impairment of polysomes. The

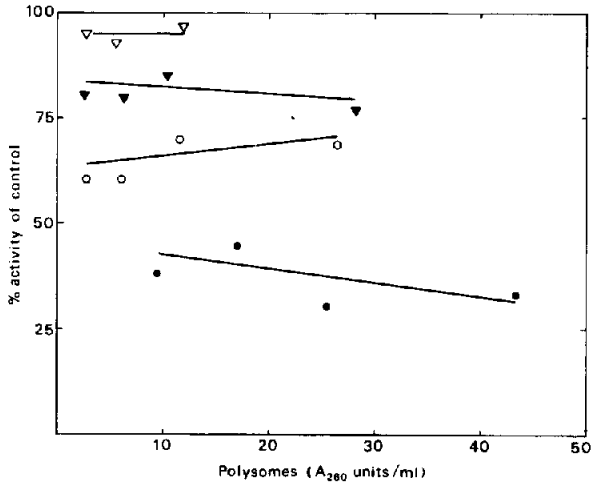

Fig.3. Effect of increasing amounts of polysomes on the inhibition of in vitro translation by PR toxin. Concentrations of PRT/ml incubation: (●) 200 μg; (○) 100 μg; (▼) 50 μg; (▽) 12.5 μg. For each experiment, the % activity was calculated with regard to controls containing the same amounts of pH 5 enzyme and of polysomes added in the assay performed in the presence of PR toxin.

extent of the recovery depended upon the relative amounts of PRT and pH 5 enzyme. High doses of PRT (400 μg/ml) probably saturated the toxin-sensitive sites of some component(s) of the pH 5

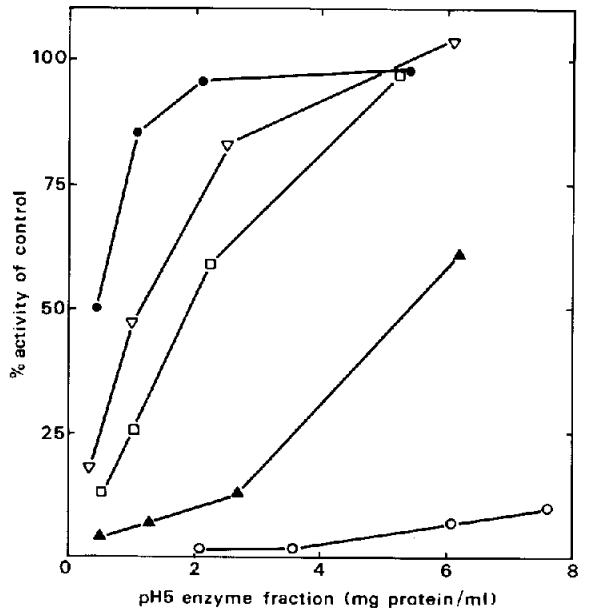

Table 1
Effect of NH_4^+ on PRT in the presence of pH 5 enzyme fraction or of polysomes

Experimental procedure	No salt		100 mM $(NH_4)_2SO4$			
	1	2	3	4	5	6
Preincubation of PRT:						
mixture A[a]	+	+	+	+	+	+
PRT[b]	+	+	+	+	+	+
NH_4^+	–	–	+	–	+	–
pH 5 enzyme	+	–	+	+	–	–
polysomes	–	+	–	–	+	+
Incubation:						
NH_4^+	–	–	–	+	–	+
pH 5 enzyme	–	+	–	–	+	+
polysomes	+	–	+	+	–	–
% Inhibition	57	66	7	61	6	23

[a] Mixture A contained ATP, GTP, Tris–HCl, pH 7.5, phosphoenolpyruvate, pyruvate kinase, $Mg(CH_3COO)_2$, KCl, NaCl and amino acids as indicated in section 2

[b] The concentration of PRT in the assay was 200 μg/ml

Preincubation was at 0°C for 1 min except in sample 6 which was preincubated for 6 min. The remaining ingredients were then added and the translational test was pursued at 37°C for 45 min

fraction. However, when the highest concentrations of pH 5 enzyme were used, the activity restarted. Moderate doses of PRT allowed a total recovery of the enzyme reaction if a sufficient amount of the pH 5 fraction was added; these results show that pH 5 enzyme proteins were in a non-limiting amount as compared with those of the control system without PRT.

In order to get more information on this point, we studied the action of NH_4^+ on PRT-induced effect on translation. In earlier experiments, we showed that the toxin was completely inactivated when brought in contact with NH_4^+ before the in vitro assays were performed [4] (table 1, samples 3, 5). However, when

Fig.4. Effect of increasing amounts of the pH 5 enzyme fraction on the inhibition of in vitro translation by PR toxin. Concentrations of PRT/ml incubation: (○) 400 μg; (▲) 200 μg; (□) 50 μg; (▽) 12.5 μg; (●) 3.12 μg. For each experiment, the % activity was calculated with regard to controls containing the same amounts of pH 5 enzyme and of polysomes added in the assay performed in the presence of PR toxin.

PRT was allowed to react with its cellular target first, it was not inactivated by subsequent addition of NH_4^+ [4]. The results reported in table 1 show that when PRT and the pH 5 enzyme were mixed together before the addition of $(NH_4)_2SO_4$, the extent of inhibition was equal to that of the controls (sample 4). By contrast, a previous contact between PRT and polysomes produced only a partial recovery of the PRT activity in the presence of NH_4^+ (sample 6). The data confirm the view that PRT acts predominantly on the pH 5 enzyme fraction.

Preliminary experiments show that PR toxin has no effect on the formation of aminoacyl-tRNAs (assays performed with either L-[U-^{14}C]leucine or a mixture of 15 L-[U-^{14}C]amino acids). Further experiments now in progress are aimed at studying the mechanism of the interaction between PR toxin and pH 5 enzyme component(s).

Acknowledgements

The authors wish to thank Agnès Clolus for the preparation of the manuscript. This work was partly supported by contract from the 'Ministère de la Qualité de la Vie (Section: Contamination des Chaînes Biologiques)' and by a grant from the 'Commissariat à l'Energie Atomique' (Paris).

References

[1] Still, P. E., Wei, R. D., Smalley, E. B. and Strong, F. M. (1972) Fed. Proc. Fed. Am. Soc. Exp. Biol. 31, 733.
[2] Wei, R. D., Still, E. B., Smalley, E. B., Schnoes, H. K. and Strong, F. M. (1973) Applied Microbiol. 25, 111–114.
[3] Wei, R. D., Schnoes, H. K., Smalley, E. B., Lee, S. S., Chang, Y. N. and Strong, F. M. (1973) in: Symposium on Mycotoxins (Tokyo).
[4] Moulé, Y., Jemmali, M. and Rousseau, N. (1976) Chem.–Biol. Interact. 14, 207–216.
[5] Moulé, Y., Moreau, S. and Bousquet, J. F. (1977) Chem.–Biol. Interact. 17, 185–192.
[6] Sarasin, A. and Moulé, Y. (1975) Eur. J. Biochem. 54, 329–340.
[7] Hartree, E. F. (1972) Anal. Biochem. 48, 422–427.
[8] Wettstein, F. O. and Noll, H. (1965) J. Mol. Biol. 11, 35–53.
[9] Mans, R. J. and Novelli, G. D. (1961) Arch. Biochem. Biophys. 94, 48–53.
[10] Blobel, G. and Potter, V. R. (1967) J. Mol. Biol. 28, 539–542.
[11] Demarle, A. and Moulé, Y. (1971) Int. J. Cancer 8, 86–96.
[12] Moulé, Y., Jemmali, M., Rousseau, N. and Darracq, N. (1977) Chem.–Biol. Interact. 18, 153–162.