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Inhibition of Protein Synthesis by Ricin: Experiments with Rat Liver Mitochondria and Nuclei and with Ribosomes from *Escherichia coli*

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1. Ricin, a toxic protein from the seeds of *Ricinus communis* which inhibits poly(U)-directed polyphenylalanine synthesis by rat liver ribosomes (Montanaro *et al.*, 1973), does not affect protein synthesis by isolated rat liver mitochondria. 2. The toxin is ineffective also on poly(U)-directed polyphenylalanine synthesis in reconstituted systems with ribosomes isolated from rat liver mitochondria or from *Escherichia coli*. 3. Ricin inhibits protein synthesis by isolated rat liver nuclei, but at concentrations much higher than those affecting rat liver ribosomes.

Ricin, a toxic protein from *Ricinus communis* seeds, is a powerful inhibitor of protein synthesis in cell-free systems (Olsnes & Phil, 1972). It has been reported that this toxin brings about an impairment of the translocation step of polypeptide chain elongation (Montanaro *et al.*, 1973), due to irreversible damage of ribosomes (Montanaro *et al.*, 1973; Olsnes *et al.*, 1973) and more precisely of the 60S ribosomal subunit (Sperti *et al.*, 1973). No inhibitory effect was observed on protein synthesis by ribosomes isolated from *Escherichia coli* (Olsnes *et al.*, 1973).

In the present paper we report that ricin does not affect protein synthesis by rat liver mitochondria or mitochondrial ribosomes, and has a limited inhibitory effect on protein synthesis by isolated rat liver nuclei.

Experimental

Preparation of subcellular fractions and supernatant enzymes. Ribosomes from *E. coli* were prepared as described by Nirenberg & Matthaei (1961). Rat liver mitochondria were isolated under sterile conditions as described by Saccone *et al.* (1969). Mitochondrial ribosomes were prepared as described by Greco *et al.* (1973) with 1mM-dithiothreitol added to TMK buffer (10mM-Tris–HCl, pH7.4, 10mM-magnesium acetate, 100mM-KCl) throughout the preparative procedure; 55S ribosomes were isolated by sucrose-gradient centrifugation (Greco *et al.*, 1973). Nuclei were isolated from rat liver as described by Laval & Bouteille (1973*a*). For some experiments nuclei were disrupted by sonication for 45s in an MSE sonicator at peak power.

Supernatant enzymes from *E. coli* were prepared as described by Wood & Berg (1962), with some modification. *E. coli* cells were ground with alumina and were centrifuged at 20000*g* for 10min. The supernatant was treated with deoxyribonuclease (3μg/ml) for 5min and was centrifuged at 20000*g* for 30min and then at 105000*g* for 4h. $(NH_4)_2SO_4$ (0.43g/ml) was added to the upper two-thirds of the supernatant; after centrifugation at 30000*g* for 30min the pellet was resuspended in 20mM-Tris–HCl buffer, pH7.8, containing 60mM-KCl, 14mM-magnesium acetate and 6mM-2-mercaptoethanol, and was dialysed overnight against the same liquid.

Mitochondrial supernatant enzymes were prepared by treating the isolated organelles with digitonin (0.1mg/ml of protein) for 2min at 0°C; 2.5vol. of TMK buffer (containing 1mM-dithiothreitol) was then added, and the suspension was centrifuged twice at 12000*g* for 10min. The pellet was resuspended in the same buffer and sonicated at 70W for 1min. Further processing was as described by Richter & Lipmann (1970).

Polyphenylalanine synthesis by ribosomes from E. coli and from rat liver mitochondria. This was measured as described by Greco *et al.* (1973). Mixtures were preincubated for 5min without poly(U), which was then added to start the reaction. After 15min of incubation at 37°C the reaction was stopped by adding trichloroacetic acid (5%, w/v, final concentration) and 100μg of bovine serum albumin. The samples were kept at 90°C for 15min and the hot-acid-insoluble radioactivity was collected on glass-fibre filters (Whatman GC/C), washed with 5% trichloroacetic acid and measured in a liquid-scintillation spectrometer.

Protein synthesis by isolated mitochondria and isolated nuclei. Isolated mitochondria (2.5mg of protein) were kept at 0°C for 30min and then incubated at 37°C in 1ml of the reaction mixture described by Kroon & Vries (1971). The reaction

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Table 1. *Effect of ricin on poly(U)-directed polyphenylalanine synthesis by ribosomes from E. coli or by 55S mitochondrial ribosomes*

Reaction mixtures contained, in a final volume of 0.25ml: 10mM-Tris–HCl buffer, pH7.8, 50mM-KCl, 10mM-magnesium acetate, 25μM-L-tyrosine, a mixture of 17 amino acids each at 50μM concentration, 30μM-GTP, 6mM-2-mercaptoethanol, 1mM-dithiothreitol, 5mM-phosphoenolpyruvate, 20μg of pyruvate kinase/ml, 1mg of tRNA from *E. coli*/ml, 1mg of poly(U)/ml, 5nmol of [^{14}C]phenylalanine/ml (sp. radioactivity 450Ci/mol), supernatant enzymes (1mg of protein/ml), and ribosomes from *E. coli* (4 E_{260} units/ml) or from mitochondria (3.2 E_{260} units/ml).

Ribosomes	Supernatant	Ricin added (μg/ml)	[^{14}C]Phenylalanine incorporated (pmol/15min per mg of RNA)	Change (%)
E. coli	*E. coli*	None	3415	
		10	3095	−10
		64	2940	−14
		128	2865	−17
Mitochondria	*E. coli*	None	1080	
		10	943	−16
		64	1005	−7
		128	868	−20
Mitochondria	Mitochondria	None	44	
		10	46	+5
		64	48	+10
		128	49	+10

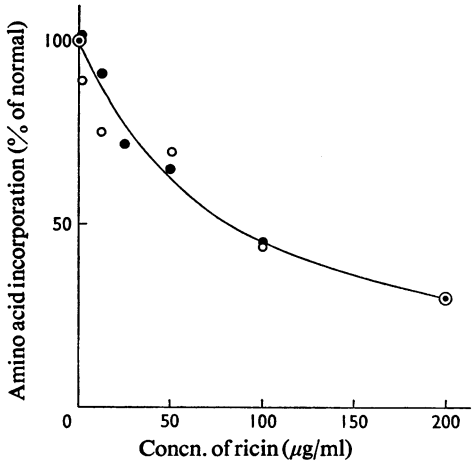

Fig. 1. *Effect of ricin on amino acid incorporation into protein by isolated rat liver nuclei*

Experiments were performed with intact (●) or sonically disrupted (○) nuclei. The reaction mixtures contained, in a final volume of 1ml: 10mM-Tris–HCl buffer, pH7.5, 100mM-NaCl, 10mM-$MgCl_2$, 5mM-2-mercaptoethanol, 1μCi of *Chlorella* U-^{14}C-labelled protein hydrolysate (sp. radioactivity 57mCi/mg-atom of C), and nuclei (0.68mg of protein). Values obtained without ricin added were taken as 100% values, and were 9377 and 9222c.p.m./h respectively for intact and for sonically disrupted nuclei.

was stopped and the hot-acid-insoluble radioactivity was measured as described above.

Protein synthesis by isolated nuclei was measured as described by Laval & Bouteille (1973*b*), with minor modifications. The reaction was stopped with 3ml of 10% (w/v) trichloroacetic acid containing a 2mM mixture of amino acids and the suspension was centrifuged. The sediment was resuspended with 3ml of 5% trichloroacetic acid containing a 2mM mixture of amino acids, kept at 90°C for 15min and the hot-acid-insoluble radioactivity was measured as described above.

General. Ricin was purified by the method of Moulé (1951). Protein was determined by the method of Gornall *et al.* (1949). The concentration of isolated RNA was determined from the E_{260}, by assuming E_{260} (1mg/ml) = 25. Chemicals were obtained from the same sources as indicated in a previous paper (Montanaro *et al.*, 1973).

Results

When ricin was added to a protein-synthesizing system consisting of ribosomes and soluble factors from *E. coli*, it brought about only a slight inhibitory effect (Table 1) even at concentrations much higher than those effective in a similar system from rat liver (Montanaro *et al.*, 1973). A similarly limited inhibition, or no inhibition at all, was observed on protein synthesis by isolated whole rat liver mitochondria (results not shown). This was not due to lack of penetration of the toxin into the organelles, since similar results were obtained with isolated mitochondrial ribosomes (Table 1) in the presence of soluble factors, either from rat liver mitochondria or from *E. coli*, which bring about a much higher

poly(U)-directed incorporation of [^{14}C]phenylalanine into protein, as already reported (Greco *et al.*, 1974).

Protein synthesis by isolated rat liver nuclei was inhibited by ricin (Fig. 1) to the same extent whether whole or sonically disrupted nuclei were used. Protein synthesis was linear for at least 1h either in the absence or in the presence of ricin (50μg/ml), although it proceeded at a lower rate in the latter case. However, the inhibition was less marked than that exerted by ricin in systems containing cytoplasmic ribosomes, 50% inhibition being caused by a concentration of ricin 25 times higher than that giving maximum inhibition with ribosomes (Montanaro *et al.*, 1973).

Discussion

Our results confirm that ricin does not affect the protein-synthesizing capacity of *E. coli* ribosomes (Olsnes *et al.*, 1973). In view of this, it is not surprising that the toxin does not affect protein synthesis by mitochondria or by mitochondrial ribosomes, since it is known that the protein-synthesizing system of mitochondria is more similar to bacterial than to mammalian cytoplasmic systems in several respects, including the sensitivity to various inhibitors (Kroon *et al.*, 1972). Therefore it seems conceivable to consider ricin as a specific inhibitor of 80S-ribosome-supported protein synthesis.

Ricin inhibits protein synthesis in isolated nuclei, although at higher concentrations than those effective with ribosomes (Montanaro *et al.*, 1973). This effect is difficult to interpret since there is a lack of agreement about the effect of various inhibitors on nuclear protein synthesis (for review see Laval & Bouteille, 1973*b*). Further, the nuclear system for protein synthesis has not been defined, and consequently a difficult accessibility of ricin cannot be excluded, even with sonically disrupted nuclei.

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