THE EFFECTS OF RIBOSOMES ON THE ACTIVITY OF A MEMBRANE BOUND ENZYME CATALYSING THIOL-DISULPHIDE INTERCHANGE

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1. Introduction

An enzyme is present in the microsome fraction of rat liver, which catalyses disulphide interchange in a variety of proteins [1]. The enzyme has also been found in various other mammalian tissues [2,3] and in the microsome fractions of baker's yeast, cabbage and garden peas [4]. In *E. coli*, the enzyme is found in the "cell-wall–membrane" fraction after sonication [4]. To obtain maximal activity when assaying this enzyme in membrane preparations, both β-mercaptoethanol and EDTA need to be present in the incubation mixture [5]. These studies were initiated in an attempt to elucidate the role of these activators.

2. Materials and methods

Microsomes were prepared from the livers of 100 g male, albino rats by differential centrifugation [6]. "Rough" and "smooth" sub-fractions were isolated by a modification of the method of Bloemendal et al. [7]. Post-mitochondrial supernatant was layered onto a discontinuous sucrose gradient consisting of 3.5 ml of 1.3 M sucrose and 3.5 ml of 2.0 M sucrose. All sucrose solutions were made up in Tris-HCl buffer (50 mM, pH 7.5) containing KCl (25 mM) and $MgCl_2$ (5 mM) [TKM]. Centrifugation at 90,000 × *g* for 3 hr produced two bands of microsomal material at the solution interfaces. The first band at the supernatant–1.3 M sucrose interface was shown to be "smooth" and the second, at the 1.3–2.0 M sucrose interface, "rough" by an estimation of the RNA/protein ratio and an examination of electron-micrographs of the harvested pellets of each band. Harvesting was effected by centrifuging the separated bands (diluted into 0.25 M sucrose-TKM) at 105,000 × *g* for 1 hr. For assay the pellets were resuspended by gentle action in a small Potter-Elvehjem homogeniser. Enzyme activity was measured by its effect on the rate of reactivation of "randomly-reoxidised" ribonuclease [8]. The activity of the RNase was determined by a pH-stat method, employing RNA as substrate [9], protein estimations were made by the method of Lowry et al. [10] and RNA by the method of Schmidt and Thannhauser [11], using the extinction coefficient of Fleck and Begg [12].

3. Results and discussion

5 ml samples of a microsome preparation, resuspended in 0.1 M Tris-HCl, pH 7.5, were dialysed against 1 litre portions of the same buffer containing either EDTA (5 mM) or β-mercaptoethanol (5 mM). A third sample was dialysed against the Tris buffer without additions and a fourth sample was left undialysed. The dialysis was carried out at 3–5°C. 0.5 ml aliquots were removed after dialysis for 5 and 20 hr; these were assayed for disulphide interchange activity and the concentrations of microsomal protein estimated. Dialysis against the Tris buffer alone gave rise to significant, time-dependent activation. This activation was greatly enhanced by the presence of EDTA but β-mercaptoethanol had no effect.

The effect of EDTA was further investigated by dialysing microsomes against buffers containing 0, 1, and 5 mM EDTA. The dialysis buffers were analysed for K, Mg, Ca, Pb, Zn, Cu, Mn and Fe, using a Techtron model AA-100 Atomic Absorption spectrophoto-

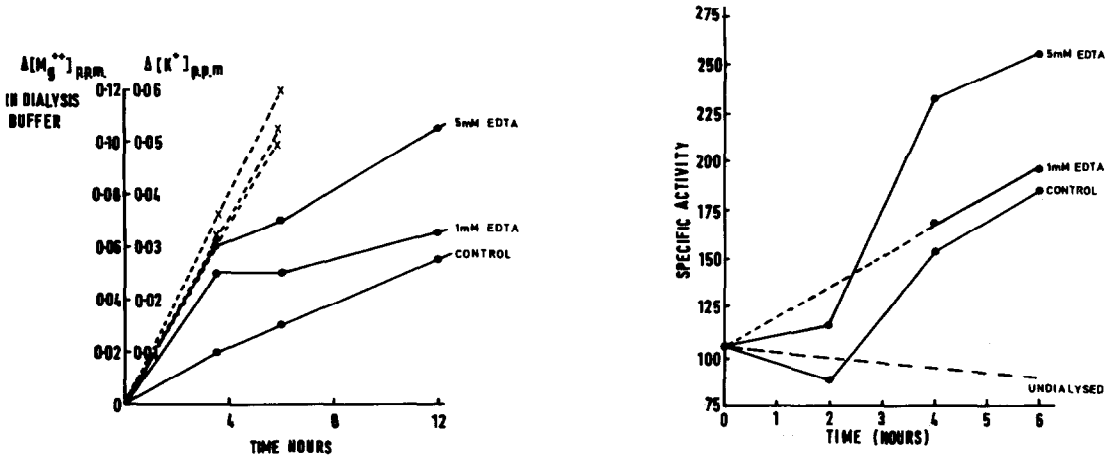

Fig. 1. (a) The increase in the concentration of magnesium (———) and potassium (– – – – –) in the dialysis buffers during the dialysis of microsomes as described in the text. (b) The increase in disulphide interchange activity of microsomes during dialysis against buffers containing EDTA.

meter. Only the levels of Mg and K were found to increase with time of dialysis; the rate of removal of Mg appearing to be a function of the EDTA concentration (fig. 1a). The increase of disulphide interchange enzyme activity during dialysis was followed and plotted as a function of time. This is shown in fig. 1b where the enzyme activity per mg of microsomal protein has been plotted as specific activity.

The activation was presumably brought about by the removal of Mg by chelation with EDTA but this effect could not be reversed by adding back the Mg^{++} to the microsomes. The magnesium in the samples before dialysis is that retained by the microsomal pellet during preparation since the final pellet was resuspended in magnesium-free buffer. The small inhibition obtained by including 2 mM $MgCl_2$ in the assay incubation mixture (about 20%) does not correspond to the great activation (250–300%) following removal of the metal. The activation cannot be due to the removal of loosely bound magnesium from the enzyme as might have been expected [13,14]. These results indicated that removal of magnesium causes a change which is not immediately reversible but which in turn gives rise to the observed enzyme activation. The most obvious change that takes place in the microsomes upon lowering the concentration of magnesium is the detachment of ribosomes from the membrane surface [15], and for this reason the activity of the enzyme in both "rough" and "smooth" subfractions of the microsomal preparation was investigated. The activities of the two fractions were very different, the "smooth" having about three times the activity of the "rough". However, after dialysis against an EDTA-containing buffer (5 mM EDTA in Tris-HCl, pH 7.5), the activities were virtually identical. The increase in activity was accompanied by a shedding of ribosomes, clearly shown by electron-micrographs and in accordance with the findings of Sabatini et al. [15].

To verify the coupling of the two processes, samples were prepared after incubation of microsomes in

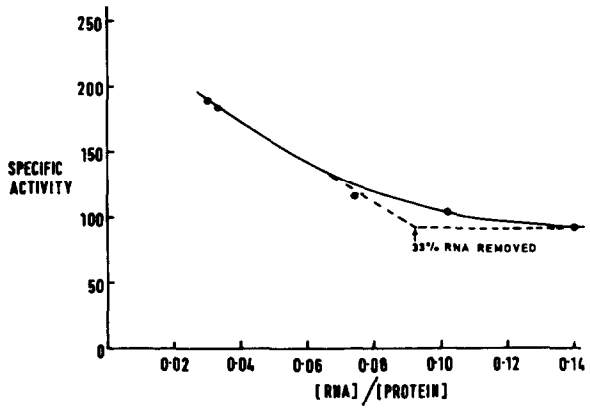

Fig. 2. Variation of disulphide interchange activity with the RNA content of EDTA treated microsomes.

various concentrations of EDTA (5–50 mM) for varying times. The samples were centrifuged at 105,000 × *g* after layering over 2.0 M sucrose-TKM. The ribosomes sedimented through the sucrose and formed a pellet at the bottom of the tube. The loss of nucleic acid from the interfacial band of membrane material ceased after 5 hr of centrifugation. The bands were harvested and resuspended in TKM as above. The "depleted" microsomes were assayed for disulphide interchange activity and the concentrations of both RNA and protein estimated. The specific activity was found to be a smooth function of the RNA/protein ratio (fig. 2). The region which shows a significant decrease in this quotient without increase in enzyme activity could be explained by the finding of Sabatini et al. [15] that EDTA treatment causes a stepwise release of the ribosomal assembly. The small sub-unit, furthest from the membrane, is lost first, leaving the large sub-unit still intact on the membrane surface. Tashiro et al. [16] have shown that the small sub-unit contributes about one third of the total ribosomal RNA. The amount of RNA that can be lost before activation begins can be estimated by extrapolation. The graph in fig. 2 suggests that this is 30–35% of the starting value.

Thus it would seem that the activity of the disulphide interchange enzyme is masked by the large ribosome sub-unit when it is attached to the microsomal membrane. This strongly suggests that the ribosomal assembly and the disulphide interchange enzyme interact on the endoplasmic reticulum of the cell. Further experiments are in progress to define the nature of such interactions.

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