The *in vitro* effect of ribonuclease on latent acid phosphatase of rat liver mitochondria

Numerous enzyme activities in freshly prepared rat liver mitochondria are lower than in aged or damaged preparations[1,2,3,4,8]. Means are known to release these latent mitochondrial enzymes in active form[1,2,3,4]; but they are non-specific and do not permit any clear understanding of the phenomenon.

As a working hypothesis, latent enzymes of mitochondria have been considered as an inactive complex resulting from a native combination of the enzyme with some mitochondrial components; possibly ribonucleic acid. Indication that some mitochondrial enzymes exist as a ribonucleoprotein complex is found in the literature[5,6].

To test this hypothesis, the release of latent acid phosphatase of "resting" mitochondria[3], following treatment with ribonuclease was investigated.

Fresh rat liver mitochondria isolated in 0.25 *M* sucrose were prepared by the method of SCHNEIDER AND HOGEBOOM[7]. The ribonuclease, described as proteinase-free (Worthington Biochemical Laboratory, Freehold, N. J.) was employed. The acid phosphatase activity was measured essentially as described previously[8], except that the incubation period was reduced from one hour to ten minutes. This was necessary to avoid mitochondrial damage during the enzyme assay[1,2].

In the first experiment 0.5 ml of a 20% mitochondrial suspension (100 mg wet weight of mitochondria) in 0.25 *M* sucrose was pre-incubated at 37° C for 30 minutes with various concentrations of ribonuclease dissolved in 0.25 *M* sucrose. The acid phosphatase activity was then measured. Fig. 1 shows that latent acid phosphatase was liberated progressively with increasing concentration of ribonuclease. A plateau was reached at about 0.5–1.0 mg of ribonuclease. Thus, in the following experiments the concentration of ribonuclease was adjusted to 1 mg per 100 mg (wet weight) of mitochondria. It was observed that upon addition of ribonuclease to the mitochondrial suspension, the pH dropped from 6.8 to 6.4 and the mitochondria agglutinated.

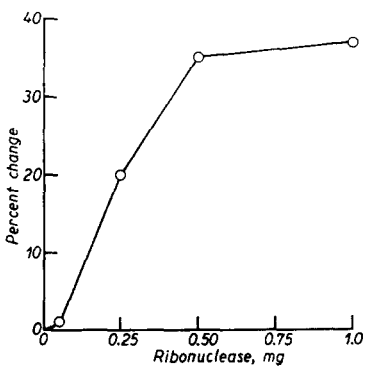

Fig. 1. Release of latent acid phosphatase activity of mitochondria following 30 minutes preincubation at 37° C with various concentrations of ribonuclease. 0.5 ml of a fresh 20% mitochondrial suspension in 0.25 *M* sucrose, were treated with various amounts of ribonuclease. The acid phosphatase activity was measured in fresh and ribonuclease-treated mitochondria. The acid phosphatase activity released was expressed as the percent increase above the activity of the fresh mitochondria.

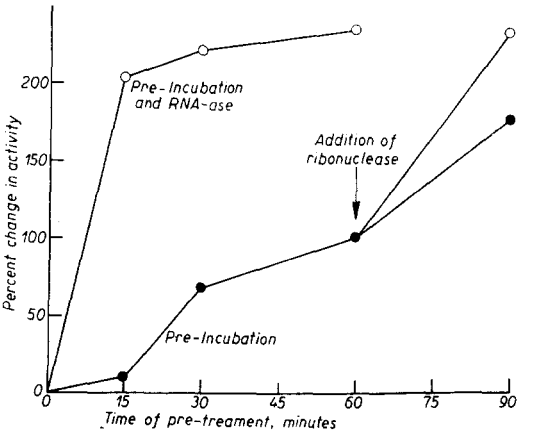

Fig. 2. Release of acid phosphatase during various period of pre-incubation at 37° C with or without ribonuclease. 0.5 ml of a fresh 20% mitochondrial suspension in 0.25 *M* sucrose were pre-treated with 1 mg of ribonuclease. The percent change was calculated as in Fig. 1.

When mitochondria were pre-treated at 37° C with ribonuclease, latent acid phosphatase was released very rapidly and almost entirely during the first 15 minutes (Fig. 2). The effect of pre-incubation without ribonuclease was negligeable during that period. This lag phase during pre-incubation was followed by a higher and rather constant rate of liberation of active acid phosphatase. It is of interest to note that treatment of mitochondria with ribonuclease during 30 minutes, following a 60 minutes period of pre-incubation alone, raised the activity to a level comparable to a continuous pre-treatment with ribonuclease.

When the same type of experiments were conducted with mitochondria of animals fasted

during 24 and 48 hours, the level of latent enzyme released by pre-incubation with or without ribonuclease was 50 to 60% lower than in fed animals. The same phenomenon was observed with latent adenosinetriphosphatase[3]. Since fasting is known to decrease the RNA content of rat liver[9], a certain correlation might be established between the RNA content of mitochondria and the amount of latent enzymes. This is being investigated further.

Fig. 3 indicates that apparently the ribonuclease effect was not a non-specific protein effect. In fact, pre-treatment of mitochondria with albumin did not influence the rate of release of latent acid phosphatase. Fig. 3 shows also that the agglutination of mitochondria by addition of copper ions (the addition of $CuSO_4$ sucrose solution at a final concentration of $5.0 \cdot 10^{-4}$ *M* to mitochondrial suspension lowered the pH to 6.3 and the mitochondria agglutinated as with ribonuclease or Ca ions[11]) did not influence the release of latent enzyme.

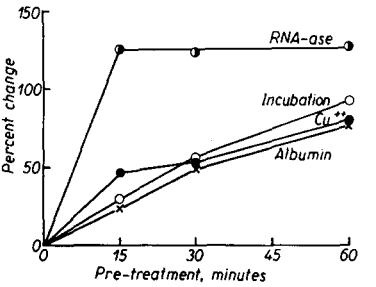

Fig. 3. Effect of various agents on latent acid phosphatase activity of rat liver mitochondria. 0.5 ml of a fresh 20% mitochondrial suspension in 0.25 *M* sucrose were pre-treated at 37° C during various periods of time with ribonuclease (1 mg), albumin (1 mg), Cu^{+2} ($5.0 \cdot 10^{-4}$ *M*, final concentration). The percent change in activity was calculated as in Fig. 1. For the Cu^{+2} experiment actual corrections were made for the slight activation of acid phosphatase activity by Cu^{+2}.

It remains to be proven that the ribonuclease effect on latent acid phosphatase is specifically due to the splitting of an inactive ribonucleoenzyme complex or to an unspecific damage caused by removal of ribonucleic acid from mitochondria. It is of interest in this respect that acid phosphatase of fresh liver microsomes—which is probably not present in latent form[1,2]—is not affected by ribonuclease[12].

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Heterogeneity of hypertensin preparations*

Several investigators have attempted the purification of hypertensin[1-9].

The purity of the various preparations reported varies widely and many discrepancies exist about the properties of the final product. For instance, KUETHER AND HANEY[8] and BUMPUS AND PAGE[7] have purified the hypertensin obtained from hog hypertensinogen and hog renin. The specific activity of KUETHER AND HANEY preparation is 24 times greater than BUMPUS AND PAGE product but on total hydrolysis gives one amino acid more (threonine) and no detectable N-terminal residue while BUMPUS AND PAGE report aspartic acid as N-terminal residue.

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