Peroxidase activity found in the ribonucleoprotein particles from pea seedlings and rabbit liver

While studying the RNase and related enzymes in the RNP from pea seedlings, we found that the particles possessed peroxidase activity. Although nucleic acids have been reported to have peroxidase activity[1], the activity in the RNP was too strong to be attributed to the nucleic acids contained in the particles, suggesting that the enzyme peroxidase was present in the RNP. The RNP from rabbit liver also showed peroxidase activity.

RNP from pea seedlings

3-day-old pea seedlings were detached from their cotyledons, cut into small pieces, cooled to 5° and ground in a mechanical mortar with 2 vol. 0.5 *M* sucrose. The ground material was filtered through a cotton cloth and the filtrate was centrifuged at 50,000 × *g* for 30 min in a Spinco preparative centrifuge (with No. 30 rotor.). The supernatant was further centrifuged at 100,000 × *g* for 120 min and the precipitate of RNP (possibly containing small microsomes) was collected, suspended in water, and the suspension centrifuged at 10,000 × *g* for 15 min[2]. $MgCl_2$ was added to the supernatant to a concentration of 0.05 *M* and the precipitate was collected as a RNP preparation[3]. This preparation contained 151 μg RNA-phosphorus and 20 G.U. (guaiacol unit) and 965 B.U. (benzidine unit) of peroxidase/mg N (1 G.U. is defined as the amount of enzyme which produced 1 mg tetraguaiacol at 30° in 15 min[4]. 1 peroxidase unit[5] = 500 G.U.; 1 B.U. is defined as the amount of the enzyme which caused an increase of absorbancy at 600 mμ of 0.1 during the first 5 min of the reaction at 15° in the following reaction mixture: 5 ml 0.1 *M* acetate buffer, pH 5.3, 1 ml 0.05 % benzidine, 1 ml 0.05 % H_2O_2 and enzyme in a total volume of 8 ml.) The pH optimum of the peroxidase was found at 5.3 when guaiacol or benzidine was used as substrate. The enzyme also reacted equally well with other phenols and aromatic amines. It could be solubilized from the RNP by the compounds which combine with metal ions: citrate, phosphate and EDTA (see Table I), suggesting that the enzyme is combined on the particles through metals. Peroxidase is generally present in plant cells in a considerable amount, and in pea seedlings, about 1/3 of the total peroxidase activity was contained in the microsomes and RNP fractions. The pH-5-fraction also had some activity (see Table II). The absorption spectrum of the solubilized enzyme showed a maximum at about 410 mμ. This may be the same as cytochrome b_3 (see ref. 6) which was found in the microsomes from silver-beat petioles and wheat roots, and may also correspond to peroxidase *b* (see ref. 7) which was prepared from broad-bean leaves, because the peroxidase *b* has an absorption spectrum similar to that of the cytochrome *b* group.

RNP from rabbit liver

Rabbit liver was cut into small pieces and was washed with cold physiological saline in order to remove blood. The washed peaces were homogenized and the RNP was fractionated by the same procedure as applied to pea seedlings. The preparation

Abbreviations: RNA, ribonucleic acid; RNase, ribonuclease; RNP, ribonucleoprotein particles; EDTA, ethylenediaminetetraacetic acid.

TABLE I

SOLUBILIZATION OF THE PEROXIDASE FROM THE RNP

RNP was suspended in the indicated solutions. The pH of these solutions were 6.0 in the case of pea seedling and 5.3 in the case of rabbit liver. The suspensions were centrifuged at 10,000 × *g* for 30 min except in the case of sucrose, when 100,000 × *g* for 120 min was used. The peroxidase activities of both suspensions and supernatants were assayed. In the case of pea seedling, the activity was measured by reading the absorbancy at 470 mμ under the same condition as the B.U. determination except that guaiacol was used as substrate. In the case of rabbit liver, the activity was measured under the same condition as the B.U. determination. The peroxidase activity is expressed in percentage of the rate obtained with the suspension in water.

Treatment	*Pea seedling*		*Rabbit liver*	
	Suspension	*Supernatant*	*Suspension*	*Supernatant*
Water	100	8	100	67
Sucrose, 0.5 *M*			100	20
Acetate buffer, 0.1 *M*	111	22	77	63
EDTA, 0.001 *M* in acetate buffer	111	38	102	63
EDTA, 0.01 *M* in acetate buffer	100	47	97	66
EDTA, 0.1 *M* in acetate buffer	103	45		
Phosphate buffer, 0.1 *M*	106	48		
Citrate–phosphate buffer, 0.1 *M*	97	57	105	59

TABLE II

DISTRIBUTION OF PEROXIDASE IN THE CELL FRACTIONS

Cell debris and large particles (nuclei, mitochondria) were obtained as precipitate by centrifuging the homogenate at 20,000 × *g* for 15 min, and microsomes were separated from the mitochondrial supernatant by centrifuging at 50,000 × *g* for 30 min. Further centrifugation of the supernatant obtained at 100,000 × *g* for 120 min gave the RNP as precipitate. The pH-5 fraction was prepared from the microsomal supernatant by the addition of 0.1 *M* acetic acid to bring its pH to 5.2 and collecting the precipitate by centrifugation. The clear, final supernatant was also used for assay. Peroxidase activities of pea seedling and rabbit liver were assayed by the methods shown in Table I. The peroxidase activity is expressed as percentage of the rate obtained with the whole homogenate in the case of pea seedling and with microsomal supernatant in the case of rabbit liver.

Fractions	*Pea seedling*	*Rabbit liver*
Homogenate	100	
Cell debris and mitochondria	8	
Microsomes	10	
Microsomal supernatant		100
RNP	17	31
pH-5 fraction	18	46
Final supernatant	47	23

was pinkish and contained 21 μg RNA-phosphorus and 8 B.U. of peroxidase activity/mg nitrogen. The peroxidase in the RNP was not lost by washing with 0.5 *M* sucrose, but more than a half of the activity was solubilized by water or acetate and citrate buffers (see Table I). The pH 5-fraction also had peroxidase activity (see Table II). This enzyme from liver has very sharp specificity toward substrates, among which benzidine seems to be the best. The pH optimum was found at the same pH value as that of the pea-seedling enzyme. Although the test with benzidine leaves some room for ambiguity because this substrate can be oxidized even by haemoglobin or haematin, the fact that the peroxidase activity was eliminated by heating while the haematin-

catalysed oxidation is resistant to this treatment, was evidence that the peroxidase activity in the preparation was not due to free haematin. This was supported by the fact that the enzyme in the liver RNP was able to oxidize guaiacol. The absorption spectrum of the solubilized enzyme showed a maximum at about 410 mμ; the enzyme may correspond to cytochrome b_5 (see ref. 8, 9) which was found in the microsomes from rabbit liver, and which may also be responsible for the peroxidase activity in bovine liver[10] or rat uterus[11].

Since the amount of RNA in the RNP preparations, especially that from liver, is small, they probably contain other components of the microsomes. This seems to be due to the omission of deoxycholate and buffers which are ordinarily employed for these preparations. Deoxycholate is known not only to solubilize lipids but also to combine metal ions. Therefore, enzymes which are bound on RNP through metal ions are preserved on the RNP prepared without deoxycholate, while elimination of the metal ions by deoxycholate is likely to cause the loss of the enzymes through solubilization in the other preparations.

We have found previously a RNase[12] and related enzymes (3'-nucleotidase and purine nucleoside ribosidase)[13] in the RNP from pea seedlings. These facts suggest that nucleic acid is metabolized in RNP and also that it contains xanthine oxidase or uricase. In fact, the existence of uricase in liver microsomes has already been reported[14]. Both xanthine oxidase and uricase produce H_2O_2, which is necessary for peroxidase activity. When the RNP preparations from pea seedlings and rabbit liver were incubated with uric acid and guaiacol or benzidine at pH 5.3 or 7.5 at 37° for 30 min, coloured products were formed. This showed that H_2O_2 was produced by the uricase reaction. However, the colours were unlike those which appeared when H_2O_2 is added. With guaiacol, the reaction mixture was pink coloured, compared with a brownish yellow when H_2O_2 is added. With benzidine, the reaction mixture was yellow compared to blue. Although some peroxidase[15] can oxidize uric acid, they require the addition of H_2O_2 and the reaction product has no colour.

Thus, the present results seem to allow us to postulate the existence of the peroxidase system linked with nucleic acid metabolism in the RNP. It will be an interesting problem to identify the real substrate of peroxidase in the living cells.

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