PREPARATION OF UNDAMAGED HYPOCOTYL MICROSOMES BY Ca^{++}-AGGREGATION TECHNIQUE

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ABSTRACT

Microsomes from legume hypocotyls may be prepared by treating the post-mitochondrial supernatant fraction with 8 mM calcium chloride. Chemical degranulation detaches over 70% ribosomes from such microsomes as measured by RNA/protein ratios, showing thereby the intactness of ribosomes on reticular membranes. Microsomes seem to suffer substantial losses of phospholipids on degranulation. The microsomal preparation contained glucose-6-phosphatase, Na^+-K^+-Mg^{++} ATPase, alkaline phosphatase and 5 Nucleoticase.

INTRODUCTION

THE ultracentrifugal technique for the preparation of microsomes is quite time consuming and detrimental for ribosome-membrane interactions[1,2] Degranulation of rough endoplasmic reticulum has been reported to occur in an ultracentrifuge due to *high g forces, high hydrostatic* pressures, *lipid* peroxidation[3] and wall effects. In order to avoid these defects the microsomes were prepared from cowpea and gram hypocotyls by ca^{++}-aggregation technique at low '*g*' values. Detachment of ribosomes from these microsomal preparations by various degranulatory techniques showed that the ribosomes were intact on the reticular membranes and these membranes could safely be used for experiments on *in vitro* protein biosynthesis. Degranulation of hypocotyl microsomes resulted in a substantial loss of phospholipids, not reported in the case of microsomes from animal tissues. The presence of glucose-6-phosphatase (microsomal marker enzyme) Na^+-K^+-Mg^{++} ATPase, alkaline phosphatase and 5′-nucleotidase in these microsomal preparations further demonstrated the nativity of this organelle.

MATERIALS AND METHODS

Cowpea (*Vigna sinensis*) and gram (*Cicer urietinum*) seeds were surface sterilised in 5% copper sulfate solution and were soaked overnight in running tap water. They were then allowed to germinate in petri dishes at 28° C in the seed germinator for about 96 hr. The hypocotyls (about 2 cm long) were collected in ice-cold 0·225 M ST buffer (0·225 M sucrose and 25 mM tris at pH 7·4). The homogenate was prepared in 2·5 W/V of ice-cold 0·225 M ST buffer using Potter-Elvenjem homogeniser by rotating its pestle at about 3000 rpm at 0-4° C. The homogenate was filtered through three layers of cheese cloth and was used for the preparation of microsomes. The clear homogenate was centrifuged at *10,000 g in a fixed* angle Janetzki 6 × 26 ml rotor for 20 min at 4° C. The microsomes from the post-mitochondrial supernatant fraction were prepared according to Gupta and Dani[4] The *microsomal pellets obtained were washed* twice with 0·225 M ST buffer and resuspended in the same buffer before freezing at —20° C. Degranulation of microsomes using EDTA[5], citrate plus pyrophosphate[6] and lithium chloride[7] was achieved by standard methods. Protein concentrations were measured by the method of Lowry *et al.*[8]. RNA concentrations were assayed according to a slightly modified method of Munro and Fleck[9] after the original method of Schmidt and Thannhauser[10]. Phospholipids were determined by extracting and purifying membrane lipids by the method of Folch *et al.*[11] followed by phosphorus estimation by the method of Ames[12]. *Values for phosphorus* were converted to phospholipids using a factor of 25. Glucose-6-phosphatase[13], Na^+-K^+Mg^{++}ATPase[14], alkaline phosphatase[15], acid phatase[16], 5′-nucleotidase[17] and succinic dehydrogenase[18] were assayed according to standard procedures.

RESULTS AND DISCUSSION

Data presented in Table I show that RNA/protein ratios of microsomes prepared from cowpea and gram hypocotyls at low *g* forces are quite high inspite of the fact that the microsomes prepared contain both rough and smooth vesicles. Very little use of calcium aggregation technique has been made for the preparation of microsomes from plant tissues. The attachment of ribosomes to the reticular membranes has been suggested to be important in secretory protein synthesis, particularly in animal cells[19]. It is therefore surprising to find the attachment of ribosomes to such a great extent in our microsomal preparations where secretion of proteins may not be of a very high order. It thus seems possible that the attachment of ribosomes to reticular membranes has another role which is not clearly understood at present. The microsomal samples analysed for these studies could contain free ribosomes as well, but it seems highly improbable as the free *ribosomes do not sediment at 10,000 g and it has* been shown in microsomal preparations from animal cells that free ribosomes do not aggregate in the presence of calcium[20],

TABLE I

Analysis of microsomes prepared from cowpea and gram hypocotyls at low 'g' force before and after degranulation

	Cowpea microsomes				Gram microsomes			
		Degranulated with				Degranulated with		
	Control	EDTA	Cit + PPi	LiCl	Control	EDTA	Cit+PPi	LiCl
RNA/ Protein	0·22 ±0·02*	0·05 ±0·001 (76·8)	0·06 ±0·001 (72·6)	0·06 ±0·003 (74·4)	0·29 ±0·03	0·05 ±0·002 (82·4)	0·07 ±0·006 (76·2)	0·06 ±0·005 (80·6)
RNA/ PL	0·17 ±0·03	0·12 ±0·014 (32·35)	0·12 ±0·002 (30·58)	0·11 ±0·01 (33·52)	0·27 ±0·05	0·10 ±0·001 (61·1)	0·09 ±0·02 (66·6)	0·10 ±0·004 (61·48)
PL/ Protein	1·24 ±0·27	0·39 ±0·06 (68·6)	0·51 ±0·02 (58·8)	0·55 ±0·02 (55·6)	1·03 ±0·09	0·50 ±0·02 (51·45)	0·78 ±0·21 (24·3)	0·52 ±0·05 (49·2)

Cit + PPi = Citrate + pyrophosphate.
* ± S D. of 3 experiments.
PL = Phospholipid
Per cent decreases are shown in parentheses.

It is clear from the data that RNA/protein ratios of degranulated microsomes as compared to those of microsomes without degranulation show from 72·6 to 82·4% loss of ribosomes with various degranulatory techniques. Comparison of RNA/protein and RNA/phospholipid ratios in Table I shows that degranulation on the basis of RNA/phospholipid ratios ranges from 31 to 67%. This fall in degranulation on the basis of RNA/phospholipid ratio shows that membranes lose phospholipids on degranulation. This observation is further corroborated by a substantial fall in phospholipid/protein ratios after degranulation of membrane samples. However, loss of phospholipids by degranulatory techniques in case of plant microsomes seems to be a curious phenomenon which has not been reported in case of microsomes from animal tissues. It is possible that the assembly of phospholipids in plant cell microsomes involves such physical and chemical forces which are disturbed by degranulating reagents leading to chaotropic effects on the membranes.

Data presented in Table II show that hypocotyl microsomes contain glucose-6-phosphatase, Na^+-K^+-Mg^{++} ATPase, alkaline phosphatase and 5'-nucleotidase. Na^+-K^+-Mg^{++} ATPase activity is about 5-folds in microsomes from cowpea as compared to that prepared from gram hypocotyls. Alkaline phosphatase activity was comparatively much higher in cowpea as compared to that in gram microsomes. Our microsomal preparations were free from lysosomes and mitochondria as indicated by complete absence of acid phosphatase and succinic dehydrogenase activities.

TABLE II

Assays of different phosphatases in microsomal membranes prepared from cowpea and gram hypocotyls

	Specific activity* ($n = 3$)	
	Cowpea microsomes	Gram microsomes
Glucose-6-phosphatase	3·24±0·002	4·56±0·07
Na^+-K^+-Mg^{++}ATPase	25·82±0·05	5·20±0·06
Alkaline phosphatase	0·52±0·01	0·02±0·005
Acid phosphatase	Nil	Nil
5'-Nucleotidase	7·28±0·05	5·20±0·09

* Specific activity : μmoles of Pi liberated/hr of incubation at 37° C/mg protein.

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REGIONAL DIFFERENCES IN THE HISTOCHEMICAL SITE AND PATTERN OF DISTRIBUTION OF ACID PHOSPHATASE IN THE ADRENAL GLAND OF *PTEROPUS GIGANTEUS GIGANTEUS* BRUNNICH (MEGACHIROPTERA : MAMMALIA)

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ABSTRACT

Pronounced differences were observed in the histochemical site and pattern of distribution of acid phosphatase (AcPase) in the cortical zones, as well as between cortex and medulla of the adrenal gland of *Pteropus giganteus giganteus*. Intense enzyme activity was noticed in the zona glomerulosa and reticularis but the fasciculatis cells displayed moderate AcPase activity. Medullary cells and blood vessels displayed intense enzyme activity. These differences in the site and pattern of distribution of AcPase have been discussed in relation to lysosomal activity gradient, kinetics of hydrolysis for releasing precursors and sustained, albeit variable, transport of material across the cell membrane.

INTRODUCTION

MAMMALIAN adrenal gland barring that of *Tachyglossus aculeatus*[1] is distinctly zonated into cortex and medulla. The cortex is differentiated into an outer glomerulosa, an intermediate fasciculata and a small inner reticularis. These differ not only in their cytological and biochemical characteristics but also in the nature of hormones elaborated. The medulla is embryologically of different origin; and secretes catecholamines which act as 'stress hormones'[1–3].

Histoenzymology of the chiropteran adrenal gland has been described for a few species[4–8]. The present report aims to deal with the regional differences in the histochemical site and pattern of distribution of a key lysosomal "marker" enzyme-acid phosphatase—in the adrenal cortical zones and medulla of *Pteropus giganteus giganteus*.

MATERIAL AND METHODS

Males of *P. g. giganteus* were netted shot from their roosting sites. Surgical procedures for recovery of adrenal gland were as described earlier. Tissues were fixed in chilled neutral formalin (10% at 4° C) for 6–8 hr. Frozen sections (10 μM) were incubated in the freshly prepared substrate according to Gomori's lead method[9] for 30–40 min (at 37° C). Incubated sections were washed in distilled water and treated with yellow ammonium sulphide for 1-2 min. Sections were washed in water and mounted in glycerine