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DISTINGUISHABLE ATPASE ACTIVITIES OF CELL WALL AND PLASMA MEMBRANE

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Abstract—Basal and Na^+–K^+ stimulated ATPase (ATP phosphohydrolase, E.C. 3.6.1.3) are both present in isolated preparations of purified cell wall and plasma membrane from cotyledon tissue of *Phaseolus vulgaris*. A comparison of the enzymes in the two fractions has revealed that the specific activities of basal and cation-sensitive ATPase are markedly higher in isolated cell wall than in the plasma membrane fraction. In addition, enrichments of both enzymes calculated on a protein basis relative to corresponding homogenates were considerably higher for cell wall than for plasma membrane. Thus, while part of the ATP-hydrolyzing activity of the wall may be attributable to the enzymatic properties of imbedded plasma membrane, there must also be additional non-membranous ATPase in the protein complement of the wall itself.

INTRODUCTION

THE PRESENCE of ATPase (ATP phosphohydrolase, E.C. 3.6.1.3) in isolated cell wall fractions has been documented for several tissues including maize coleoptiles,[1] roots of *Avena sativa*[2] and cotyledons of *Phaseolus vulgaris*.[3] Enzymatic hydrolysis of ATP is also a characteristic property of microsomal membrane fractions from a variety of plant tissues and in most cases microsomal ATPase displays cation sensitivity, particularly to the ions Mg^{2+}, Na^+ and K^+.[2,4,5] Microsomal fractions are heterogeneous with respect to membrane type but do normally contain some vesicles which have been derived from the plasma membrane.[6] It seems quite clear that the Mg^{2+}-dependent Na^+–K^+ stimulated ATPase found in microsomal fractions of mammalian tissue is attributable to the presence of fragmented plasma membrane.[6,7] The same may hold true for the cation sensitive ATPases found in microsomal fractions from plant tissue.

There is some morphological evidence to indicate that portions of plasma membrane are imbedded in plant cell walls.[8,9] Assuming that ATPase may be one of the enzymes associated with plant plasma membrane, it is possible that the ATPase activities of the cell wall are bound to plasma membrane imbedded in the wall rather than being part of the non-membranous protein complement of the wall.[9] Using a recently reported procedure

[1] A. KIVILAAN, T. C. BEAMAN and R. S. BANDURSKI, *Plant Physiol.* **36**, 605 (1961).
[2] J. FISHER and T. K. HODGES, *Plant Physiol.* **44**, 385 (1969).
[3] Y. F. LAI and J. E. THOMPSON, *Phytochem.* **9**, 1017 (1970).
[4] H. D. BROWN and A. M. ALTSCHUL, *Biochem. Biophys. Res. Commun.* **15**, 479 (1964).
[5] G. HANSSON and A. KYLIN, *Z. Pflanzenphysiol.* **60**, 270 (1969).
[6] R. COLEMAN, R. H. MICHELL, J. B. FINEAN and J. N. HAWTHORNE, *Biochim. Biophys. Acta* **135**, 573 (1967).
[7] H. M. BERMANN, W. GRAM and M. A. SPIRTES, *Biochim. Biophys. Acta* **183**, 10 (1969).
[8] W. G. WHALEY, H. H. MOLLENHAUER and J. H. LEECH, *Am. J. Bot.* **47**, 401 (1960).
[9] J. L. HALL, *Planta* **85**, 105 (1969).

for isolating and purifying fragments of plasma membrane from cotyledon tissue of *Phaseolus vulgaris*,[10] we have examined the possibility that the ATPase activity of cell walls reflects the presence of imbedded plasma membrane.

RESULTS AND DISCUSSION

The preparation and properties of a purified cell wall fraction from this tissue have been previously described.[3] Starch grains are characteristically present in the isolated fraction, but otherwise it is relatively free of cytoplasmic contamination. This is indicated by the degree of enrichment of cellulose and a phospholipid content equivalent to only about 1% of the level in the homogenate.

Partially purified plasma membranes[10] are enriched in basal ATPase activity (that measured in the absence of added cations) by 3–6-fold relative to homogenate on a specific activity basis and in Na^+–K^+ stimulated ATPase activity by 6–9-fold. Contamination by microsomal and mitochondrial membranes, as monitored by measuring levels of marker enzymes in the isolated fractions, was found to be minimal. The increased specific activity of the basal enzyme and in particular that of the Na^+–K^+ stimulated ATPase, therefore, was interpreted as indicating that the fraction is a preparation of partially purified plasma membrane.

These cell wall and plasma membrane fractions each possess basal and Na^+–K^+ stimulated ATPase activities (Table 1). The pH optimum for the cation-sensitive enzyme was found to be near 8·0 for each fraction and assays were routinely carried out at this pH value. It was necessary to measure the Na^+–K^+ sensitive and basal ATPase activities at the same pH because net Na^+–K^+ sensitive activity was computed by subtracting the basal activity from that measured in the presence of the added cations.

TABLE 1. ATPASE ACTIVITIES OF ISOLATED CELL WALL AND PLASMA MEMBRANE FROM 4-DAY-OLD COTYLEDON TISSUE OF *Phaseolus vulgaris*

		Cell wall			Plasma membrane		
		mg P/mg protein/hr			mg P/mg protein/hr		
Expt.	Activities	Homogenate	Cell wall	Enrichment	Homogenate	Plasma membrane	Enrichment
A	Basal	3·6	65·0	18	0·9	3·9	4·3
	Net Na^+–K^+	4·5	77·0	17	1·3	9·5	7·3
B	Basal	3·7	43·0	11·6	0·6	1·6	2·7
	Net Na^+–K^+	4·5	57·0	12·6	1·0	6·2	6·2
C	Basal	2·9	43·5	15	0·8	4·6	5·7
	Net Na^+–K^+	3·7	66·5	18	1·2	10·4	8·6

Basal activity is that measured in the absence of added cations. Net Na^+–K^+ activity is the total activity measured in the presence of added Na^+ and K^+ minus the basal activity. Enrichment is the ratio of the specific activity in the fraction to that in the homogenate.

A comparison of the cell wall and plasma membrane enzymes has revealed that they are distinguishable. The isolated cell wall showed much higher enrichments for both types of

[10] Y. F. LAI and J. E. THOMPSON, *Biochim. Biophys. Acta* **233**, 84 (1971).

ATPase activity, relative to homogenate on a specific activity basis, than were obtained for plasma membrane (Table 1). Enrichments of from 11- to 18-fold were obtained for cell wall yet corresponding figures for plasma membrane ranged from 3- to 8-fold (Table 1). A further distinction is that for plasma membrane, enrichment of the Na^+–K^+ sensitive enzyme was always significantly greater than that for the basal enzyme within experiments. Such was not the case for cell wall. For this fraction, enrichments of the two types of ATPase activities relative to homogenate were of comparable magnitudes.

It would appear, therefore, that the ATPase activity detectable in cell walls is not totally due to membranes intimately associated with the wall, as has been previously suggested.[9] The specific activities of both the basal and Na^+–K^+ stimulated ATPase are much higher in isolated cell wall than in purified plasma membrane. If the ATPase activities of the wall were entirely attributable to imbedded membrane, the specific activities should have been equal to or lower than corresponding values for isolated plasma membrane, because the cell wall possesses some non-membranous protein. Presumably part of the ATP-hydrolyzing activity of the wall is attributable to the enzymatic properties of imbedded plasma membrane, but there must also be additional non-membranous enzyme. In fact, a comparison of the specific activities indicates that there is a much greater prevalence of ATPase in the cell wall than on the plasma membrane, particularly for the basal enzyme. It seems reasonable that the ATPases of the cell wall could be quite closely situated to imbedded membrane, yet not be part of it, and thus still participate in energy-dependent transport across the membrane.

EXPERIMENTAL

Seeds of *Phaseolus vulgaris*, variety Kinghorn, were germinated and grown in moist vermiculite at 28° under conditions producing etiolation. Cotyledons were harvested 4 days after planting. Purified cell wall and plasma membrane were isolated as previously reported.[3,10] Basal and cation-sensitive ATPase were assayed according to the procedure described by Lai and Thompson.[10] For determinations of pH profiles, the assay medium was adjusted to the required pH values.

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