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Phosphatidate phosphohydrolase activity in liver cell surface membranes

Phosphatidate phosphohydrolase (L-α-phosphatide phosphohydrolase, EC 3.1.3.4) activity has been observed in most subcellular fractions derived from homogenized tissues[1–8]. It appears in several forms (i) membrane-bound, (ii) readily solubilized[6,7] and (iii) soluble. A large proportion of the membrane-bound activity sediments in microsomal fractions.

The rapid turnover of phosphatidic acid and the response of this turnover to acetylcholine[9] suggest that phosphatidate phosphohydrolase may be responding in a parallel fashion to transport phenomena. These transport phenomena are probably located in the surface membrane and the recent demonstration of the localization of high (Na^+-K^+)-ATPase activities in a purified surface membrane preparation[10], tends to support this view. Membrane-bound phosphatidate phosphohydrolase activity may also be localized in the surface membrane. Indeed, phosphatidate phosphohydrolase activity has already been reported in erythrocyte surface membranes[11]. Part or all of the activity in the microsomal fractions from many tissues may therefore be due to the presence in these fractions of fragmented surface membranes.

The ready availability of a purified surface membrane fraction from rat liver[12] allowed an investigation of the possible localization of this enzyme in the surface membrane of cells in this tissue.

Purified surface membrane fractions were prepared in 0.3 M sucrose from the perfused livers of 6–8-week-old rats according to previously published methods from this laboratory[12]. Other cellular fractions were prepared as by-products from the same fractionation series[12]. Fractions were monitored for content and purity by enzymatic

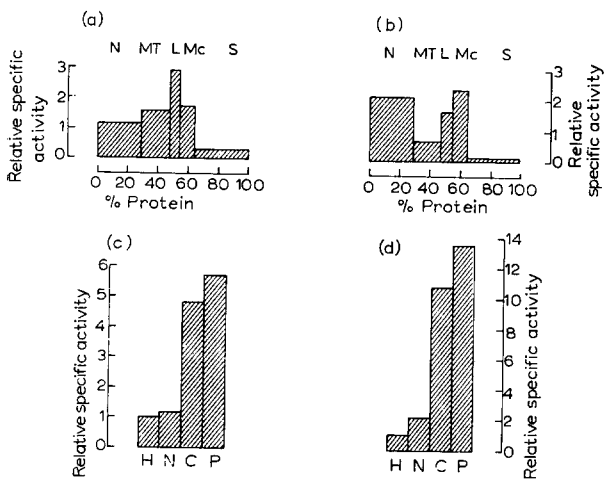

Fig. 1. Distribution of phosphatidate phosphohydrolase activity (a, c) and 5′-nucleotidase (b, d) during subfractionation of liver homogenate. a and b: Results from fractionation of homogenate. c and d: Results from subfractionation of crude nuclear fraction obtained during initial fractionation. Relative specific activity, *i.e.* specific activity of fraction divided by specific activity of homogenate. H, homogenate; N, crude nuclear fraction; MT, mitochondrial fraction; L, lysosomal fraction; Mc, microsomal fraction; S, supernatent fraction; C, crude surface membrane fraction; P, purified surface membrane fraction. Conditions of assay as in text. Duplicate determinations gave good agreement and recoveries of both enzymes throughout the procedures were 105–110%.

TABLE I

INCREASE IN SPECIFIC ACTIVITY OF PHOSPHATIDATE PHOSPHOHYDROLASE ACTIVITY DURING PURIFICATION OF SURFACE MEMBRANE

Specific activities are expressed as μmoles substrate hydrolysed per h per mg protein at 37°. Assay system described in text.

Prep. No.	*Specific activity*		*Relative specific activity*
	Purified surface membrane	*Homogenate*	*Surface membrane/homogenate*
1	1.27	0.23	5.5
2	0.79	0.17	4.65
3	1.30	0.25	5.2
4	2.17	0.37	5.85

markers and by electron microscopy[12]. Phosphatidate phosphohydrolase activity was measured at pH 6.0 using highly purified sodium phosphatidate as substrate[5,7].

Simple fractionation into conventional subcellular fractions gave a distribution of phosphatidate phosphohydrolase activity resembling that reported for rat liver by SEDGWICK AND HÜBSCHER[7]. The enrichments observed in mitochondrial and lysosomal fractions reflect, in part, their content of readily solubilized[6,7] activity (Fig. 1a). At this stage, there was little parallelism of phosphatidate phosphohydrolase activity with 5′-nucleotidase (Fig. 1b). Once the bulk of mitochondria, lysosomes and endoplasmic reticulum had been removed, refractionation of the crude nuclear pellet gave a purified plasma membrane fraction, which showed a striking enrichment of the enzyme (Fig. 1c). At this stage a close parallelism in behaviour between phosphatidate phosphohydrolase activity and 5′-nucleotidase was apparent (Fig. 1d). The relative purification of the enzyme was consistent in successive preparations (Table I).

In the purified plasma membrane fraction the content of mitochondrial (succinate dehydrogenase) and lysosomal (acid phosphomonoesterase) enzymes has been shown previously to be very low[12] and therefore the phosphatidate phosphohydrolase activity in the fraction must be regarded as that due to the contribution of membranes, principally the surface membrane. It would appear, therefore, that in liver, phosphatidate phosphohydrolase activity has a wide subcellular distribution. The contribution of the surface membrane to the membrane-bound activity is considerable and calculations based upon the enrichment relative to 5′-nucleotidase show that up to 37% of the total phosphatidate phosphohydrolase activity of the liver may be localized in the surface membrane.

The presence of this activity in the microsomal fraction in this tissue may therefore be explained, at least in part, by a contribution from fragmented surface membrane, and the question of the levels of phosphatidate phosphohydrolase activity in fragmented endoplasmic reticulum must await detailed and definitive results from submicrosomal fractionation. In this context, however, it is to be noted that phosphatidate phosphohydrolase activity has been observed in all submicrosomal fractions from intestinal mucosa, but it was preferentially concentrated in a fraction which was reduced in glucose-6-phosphatase and glyceride biosynthetic ability[8].

The role of phosphatidate phosphohydrolase activity in its various forms in the different morphological compartments of the cell is not known. Work with rat

liver, cat and hamster intestinal mucosa has indicated that it may be the soluble (*i.e.* cell sap) form of phosphatidate phosphohydrolase activity which is preferentially involved in lipid biosynthesis[13–15]. Thus the role of the membrane-bound enzyme is unexplained[13]. When this is coupled with the observation of AGRANOFF[16] that other monoesterified phosphate compounds with lipid-like properties could act equally well as substrate for this enzyme activity, one may speculate that this enzyme and its substrate may play an important role in the metabolism of the surface of the cell.

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Department of Medical Biochemistry and Pharmacology,
University of Birmingham,
Birmingham (Great Britain)

R. COLEMAN

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