Enzymes linked to phosphatidylinositol in plasma membranes

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Treatment of tissues, isolated cells, and cell homogenates and fractions with a phosphatidylinositol-specific phospholipase C has demonstrated a selective release of several enzymes from plasma membranes. The purified phospholipase has no detectable action on mammalian phospholipids other than phosphatidylinositol and although this phospholipid appears to form only a very small proportion of the outward-pointing phospholipid in plasma membrane its hydrolysis closely parallels the selective release into the bathing medium of substantial proportions of some enzymes.

Alkaline phosphatase is readily released in very high proportions from slices, homogenates or microsomal fractions prepared from a variety of tissues (Stein & Logan, 1965; Ikezawa et al., 1976; Low & Finean, 1977a) and also from isolated cells such as lymphocytes (Low & Finean, 1978) and hepatocytes (S. D. Shukla & J. B. Finean, unpublished work). In some cases the release has been estimated at 100% as related to the total activity detectable in suspensions of unincubated samples (Low & Finean, 1977a, 1978), but in the case of isolated hepatocytes the release has been observed to approach a limit of about 70% as related to the unincubated but detergent-treated cell suspension (S. D. Shukla & J. B. Finean, unpublished work).

5'-Nucleotidase has also been reported to be released from a variety of preparations by the phosphatidylinositol-specific phospholipase C (Low & Finean, 1978; Taguchi & Ikezawa, 1978), but recent work has emphasized that, in some systems at least, this enzyme is less easily released and at a much lower level than in the case of alkaline phosphatase. As illustrated in Fig. 1 this level is, nevertheless, significantly higher than the release of a number of other recognized plasma membrane enzymes which appear to be completely unaffected by the phosphatidylinositol-specific phospholipase C (Low & Finean, 1978; S. D. Shukla & J. B. Finean, unpublished work).

Studies of the nature of the released enzyme activities have indicated that both alkaline phosphatase and 5'-nucleotidase are released as soluble protein molecules (Low & Finean, 1978). Acetylcholinesterase has been totally released from intact pig erythrocytes by the phosphatidylinositol-specific phospholipid C without inducing any haemolysis, but similar treatment of human erythrocytes released only a very small percentage of this enzyme activity (Low & Finean, 1977b).

The observations so far made emphasize the selectivity of enzyme release from plasma membranes as a result of hydrolysis of phosphatidylinositol by the specific phospholipase C and also the variability with respect to the levels of release of different enzymes from the same cell and of nominally the same enzymes from different cell types or even from the same cell in different species. These observations would suggest that, although the association between a particular enzyme and phosphatidylinositol in a membrane is specific and possibly direct, accessibility to the link between them is probably influenced by other features of the membrane composition and structural organization. At the molecular level there are problems in visualizing the mode of access of the specific phospholipase (mol.wt. approx. 30000) to a phosphatidylinositol which is accommodated in a lipid layer, but also linked directly to a relatively large enzyme protein such as alkaline phosphatase (mol.wt. 100000-200000).

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Effects upon UDP-glucuronyltransferase of phospholipase treatment or of phospholipid depletion during isolation

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UDP-glucuronyltransferase* (EC 2.4.1.17) is an enzyme or a group of integral enzymes, embedded in the endoplasmic-reti-

* Abbreviation used: GT, UDP-glucuronyltransferase.

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cular membranes of liver and some extra-hepatic tissues. The principal reaction catalysed is the following:

\[ \text{Aglycone} + \text{UDP-glucuronic acid} \xrightarrow{\text{GT}} \text{glucuronic acid} + \text{UDP} \]

In vivo, glucuronyltransferase conjugates, inactivates and assists in the excretion of biologically active amphiphats of endogenous or xenobiotic origin (Dutton & Burchell, 1977). In vitro, the transferase can also perform some reverse reactions, UDP