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Fatty acyl-CoA esters and the permeability of rat liver microsomal vesicles

A characteristic feature of several enzymes (e.g. glucose-6-phosphatase, UDP-glucuronosyltransferase) of endoplasmic reticulum is their latency: their activity is low in native membrane, whilst agents altering the membrane structure activate them. This phenomenon can be explained by two different models. According to the compartmentation hypothesis, the active site of these enzymes has intraluminal orientation, and the transport of substrate(s) through the membrane is rate-limiting [1,2]. Agents disrupting the membrane barrier allow free entrance of substrates to the active sites. The conformational model states that the activity of these enzymes is limited by their membranous environment and can be increased by agents affecting protein–lipid interactions [3].

Fatty acyl-CoA esters, the obligatory intracellular intermediates of fatty-acid-metabolizing pathways, have been reported to modulate several cellular functions. We have shown previously that fatty-acyl-CoAs inhibit glucose-6-phosphatase activity in rat liver microsomes by acting on substrate transport into microsomal vesicles, but that high acyl-CoA to microsomal protein concentrations can disrupt microsomal vesicles, leading to increased activity due to decreased latency [4]. More recently, Mithieux and Zitoun have reported that long-chain-fatty-acyl-CoA esters do not inhibit, but activate, glucose-6-phosphatase within a given range of concentrations [5]. They concluded that the effect was due to a conformational change of the enzyme, i.e. to the release of its mannose-6-phosphatase activity.

Acyl-CoAs, like other amphipathic molecules, can behave as detergents (see [4] for references). This effect depends on the detergent/membrane mass ratio. To minimize the adverse consequences, acyl-CoAs are presumably bound to specific binding proteins inside the cell (see [4] for references). We have also observed that acyl-CoAs decrease the latency of another enzyme of endoplasmic reticulum, namely UDP-glucuronosyltransferase [6]. Therefore the effect of palmitoyl-CoA on the permeability of rat liver microsomal vesicles has been investigated.

The possible acyl-CoA-dependent alterations of the permeability of rat liver microsomes were detected by two different approaches. First, it was estimated on the basis of microsomal β-glucuronidase latency measured using native and alamethicin-permeabilized vesicles [7]. β-Glucuronidase is a luminal protein of the endoplasmic reticulum containing no cytosolic or membrane-crossing domains [8,9]. Water-soluble glucuronides present in the external space therefore cannot interact with it unless microsomal vesicles are disrupted or permeabilized to allow access of the substrate to the active site of the enzyme. The latency of the enzyme was investigated using phenolphthalein glucuronide, a compound which has poor membrane permeability [10], as a substrate. Secondly, osmotically induced

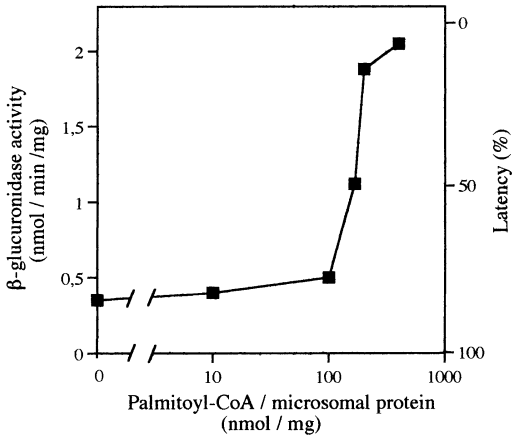

Figure 1 Effect of palmitoyl-CoA on the latency of β-glucuronidase activity in rat liver microsomes

Native and alamethicin-permeabilized microsomes (1–5 mg of protein/ml) prepared and stored as described in [4] were incubated in the presence of 0–2000 μM palmitoyl-CoA and 1 mM phenolphthalein glucuronide in a medium containing 100 mM KCl, 20 mM NaCl, 3.5 mM $MgCl_2$ and 20 mM Mops, pH 7.2, at 37 °C for 20 min. Activity was detected by measuring the amount of aglycone liberated. Activity is shown as a function of palmitoyl-CoA/protein ratio. Maximal activity obtained in alamethicin-permeabilized vesicles was 2.17 ± 0.08 nmol/min per mg of protein (0% latency). Data are means for four separate experiments; S.D. values were less than 10% of the corresponding means.

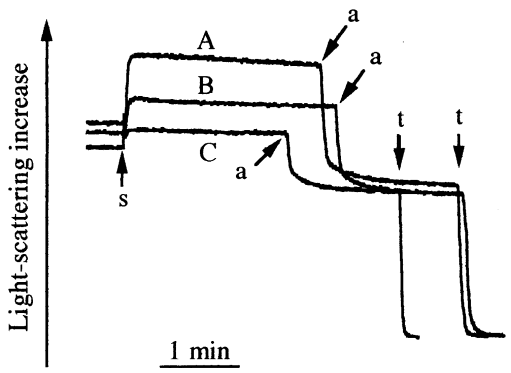

Figure 2 Effect of palmitoyl-CoA on the osmotically induced shrinking of rat liver microsomal vesicles induced by sucrose

Upon addition of the non-permeant sucrose (s; 50 mM) native microsomes (100 μg of protein/ml) shrunk (trace A). Preincubation (2 min) with 10 (trace B) or 20 μM palmitoyl-CoA (trace C) (100 or 200 nmol/mg of protein respectively) proportionally decreases the extent of shrinking, indicating the partial permeabilization of vesicles. Light-scattering measurements were performed as described in [4]. Alamethicin (a; 10 μg/ml) and Triton X-100 (t; 50 μg/ml) were added at the end of each trace to respectively allow the complete permeabilization and solubilization of vesicles [7]. A typical experiment out of five is shown.

changes, caused by non-permeant compounds, in the size of vesicles was measured by a light-scattering technique in the absence or in the presence of palmitoyl-CoA [4].

It was found that palmitoyl-CoA decreased the latency of β-glucuronidase activity in microsomes. As expected, the effect was dependent on the palmitoyl-CoA/microsomal protein ratio rather than the absolute palmitoyl-CoA concentration. Half-maximal effect was reached between 100 and 200 nmol palmitoyl-CoA/mg of protein ratio (Figure 1). Similar effects were observed for the effect of palmitoyl-CoA on UDP-glucuronosyltransferase latency (results not shown).

Light-scattering measurements revealed that the preincubation of microsomal vesicles (0.1 mg of protein/ml) with 10 or 20 μM palmitoyl-CoA (100 or 200 nmol/mg of protein) made them permeable to the non-permeant sucrose (Figure 2), citrate, phenolphthalein glucuronide, UDP-glucuronic acid or mannose 6-phosphate (results not shown), indicating a profound disruption of the membrane barrier.

These results imply that the detergent effect of acyl-CoAs must be taken into account when investigating their effects. The range in which Mithieux and Zitoun observed the stimulation of glucose-6-phosphatase activity [5] is similar to that which permeabilizes the vesicles in our measurements. Although other direct interactions between the glucose-6-phosphatase enzyme and acyl-CoAs in disrupted microsomes cannot be excluded, the effect of acyl-CoAs on microsomal membrane permeability cannot be ignored.

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