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Changes in sinusoidal plasma membrane enzyme activities during the pre-replicative phase of liver regeneration

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Changes in a range of plasma membrane enzyme activities during the early period of liver regeneration are thought to be related to the initiation of DNA synthesis and the triggering of cellular activation. The sinusoidal plasma membrane was isolated from control and partially hepatectomized animals at various intervals during the pre-replicative phase. The specific activities of 5′-nucleotidase, $(Na^+ + K^+)$-ATPase, Ca^{2+}-ATPase, Mg^{2+}-ATPase showed that after partial hepatectomy changes in the enzyme activities at the sinusoidal plasma membrane region occur. These changes are probably related to the remodeling of the cell-surface that occurs before the division of hepatocytes.

The pre-replicative phase of liver regeneration is characterized by a series of molecular and morphological changes constituting an ordered sequence of complex reactions leading to the initiation of DNA synthesis (at 15–18 h) and subsequent cell division (at 24–30 h). Changes in soluble enzymes or in intracellular molecules in regenerating liver have been well documented [1,2] but not much work has been focussed on plasma membrane changes in this early period of the regenerative process. Furthermore, the few studies done with plasma membrane [3] have used subcellular fractions derived mainly from the whole hepatic cell-surface, and such studies do not take into account the fact that in the first few hours of the cell activation the sinusoidal face of the hepatocyte is the most important and metabolically active region of the cell surface. Regeneration is accompanied by changes in the plasma membrane of hepatocytes [4–7] and a study of regenerating liver in the early stages, provides an opportunity to examine the sequence of events leading to the initiation of DNA synthesis.

The plasma membrane of the sinusoidal pole of the hepatocyte contains the greatest concentration of hormone and metabolite receptors of the cell [8] and therefore is positioned to play a crucial role in the reception and/or transduction of the early signals that trigger cell activation.

Very recently we have characterized the glycoproteins from the functional domains of the hepatocyte plasma membrane [9]; at the present, in this work we studied the changes occurring in sinusoidal plasma membrane enzymes.

Isolated plasma membrane fractions derived from the sinusoidal domain of the hepatocyte [10] were first tested for purity and yield (Table I).

Fig. 1 shows changes in specific activities of a range of plasma membrane enzymes from livers obtained at various intervals from 2 to 15 h after

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TABLE I

BIOCHEMICAL CHARACTERIZATION OF THE RAT LIVER SINUSOIDAL PLASMA MEMBRANE FROM CONTROL ANIMALS

Numbers in parentheses indicate number of experiments. Values are presented as means ± S.D. (a) The recovery ± S.D. of each marker is the percent of the homogenate total activity present in the final membrane fraction. (b) The enrichment is defined as the ratio of specific activities in the plasma membrane to specific activities in the homogenate. The recovery of protein was: 0.46 ± 0.14 mg per g wet weight of liver (c).

Enzyme activity	Recovery (a)	Relative enrichment (b)
Succinate dehydrogenase [12]	0.22 ± 0.07 (3)	0.83 ± 0.02 (3)
Glucose-6-phosphatase [13]	0.31 ± 0.10 (4)	0.11 ± 0.02 (4)
Acid phosphatase [14]	0.85 ± 0.03 (3)	2.98 ± 0.01 (3)
5′-Nucleotidase [15]	4.48 ± 0.19 (4)	20.55 ± 4.69 (4)
Alkaline phosphatase [16]	2.63 ± 1.71 (4)	9.32 ± 4.56 (4)
Phosphodiesterase I [17]	4.12 ± 1.56 (5)	14.47 ± 5.50 (5)
$(Na^+ + K^+)$-ATPase [18]	2.88 ± 0.64 (3)	10.08 ± 2.83 (3)
Mg^{2+}-ATPase [18]	2.36 ± 0.49 (5)	8.26 ± 1.73 (5)
Ca^{2+}-ATPase [19]	2.11 ± 0.86 (3)	7.38 ± 3.01 (3)
Adenylate cyclase [3]	0.26 ± 0.07 (2)	0.93 ± 0.27 (2)
Protein [20]	0.28 ± 0.08 (24)	(c)

partial hepatectomies [11]. The figure also shows enzyme activities for sham-operated animals.

5′-Nucleotidase, Fig. 1(a), is a transmembrane ectoenzyme that showed a biphasic decrease in the specific activity a few hours before the initiation of the DNA synthesis suggesting that the enzyme may be involved in several mechanisms in the regulation of this process. Firstly, the enzyme could elevate the concentration of nucleotides available for nucleic acid synthesis or the concentration of UDPglucose available for plasma membrane glycoprotein biosynthesis. Secondly, 5′-nucleotidase as well as nucleotide pyrophosphatase (phosphodiesterase I) both can act as ribonucleases and therefore the lowered activities of these enzymes may contribute to an accumulation of mRNAs necessary for the synthesis of proteins involved in cell activation. Thirdly, assuming that the two cAMP surges observed in this pre-replicative period are actually involved in the DNA synthesis [21] then the decreased 5′-nucleotidase activity would produce a diminution of adenosine, an inhibitor of adenylate cyclase in the liver [22]. The fact that no changes in the specific activity of 5′-nucleotidase were found in sham operated animals throughout this period indicates that the enzyme modifications now described are intrinsic to this pre-replicative process.

$(Na^+ + K^+)$-ATPase couples the vectorial transport across the membrane of Na^+ and K^+ to the hydrolysis of ATP. The significant specific activity increase observed at 8–10 h (Fig. 1(b)) can be considered important from the viewpoint of regulation in the regenerative period. In the first place, as described in other experimental systems [23], increased $(Na^+ + K^+)$-ATPase activities have been considered characteristic of proliferative activation. Also Koch and Leffert [24] have demonstrated that the $(Na^+ + K^+)$-ATPase inhibition by ouabain can account for the blockage of DNA synthesis observed after partial hepatectomy. This increased cellular efflux of Na^+ might be the response to an initial intracellular accumulation which may be a starting signal for cellular activation [24]. The fact that the specific activity of $(Na^+ + K^+)$-ATPase in membranes of sham-operated animals did not change during this period, indicates that the changes now observed in the hepatectomized animals are intrinsic to the regenerative process and therefore are probably involved in the regulation of cell activation.

It has been suggested that Ca^{2+}-ATPase, Fig. 1(c), is involved in calcium transport [19] but the enzyme is unlikely to be involved in calcium mobilization because its specific activity is measured at high ATP (3–5 mM) and calcium (5–10 mM) concentrations. ATPases actually involved in calcium transport show their optimum activity at micromolar calcium amounts and at the most at 1 mM [25]. The enzyme is mainly located at the canalicular plasma membrane [26], but significant activity is also detected at the sinusoidal fraction. Although its biological function is unknown there is evidence suggesting an involvement in growth and differentiation [19]. The fact that the lowest specific activity of Ca^{2+}-ATPase coincided with the two waves of cAMP intracellular accumulation at 2–4 h and 10–15 h, points to the possibility that cAMP may inhibit the activity of the enzyme. This possibility was tested with membranes de-

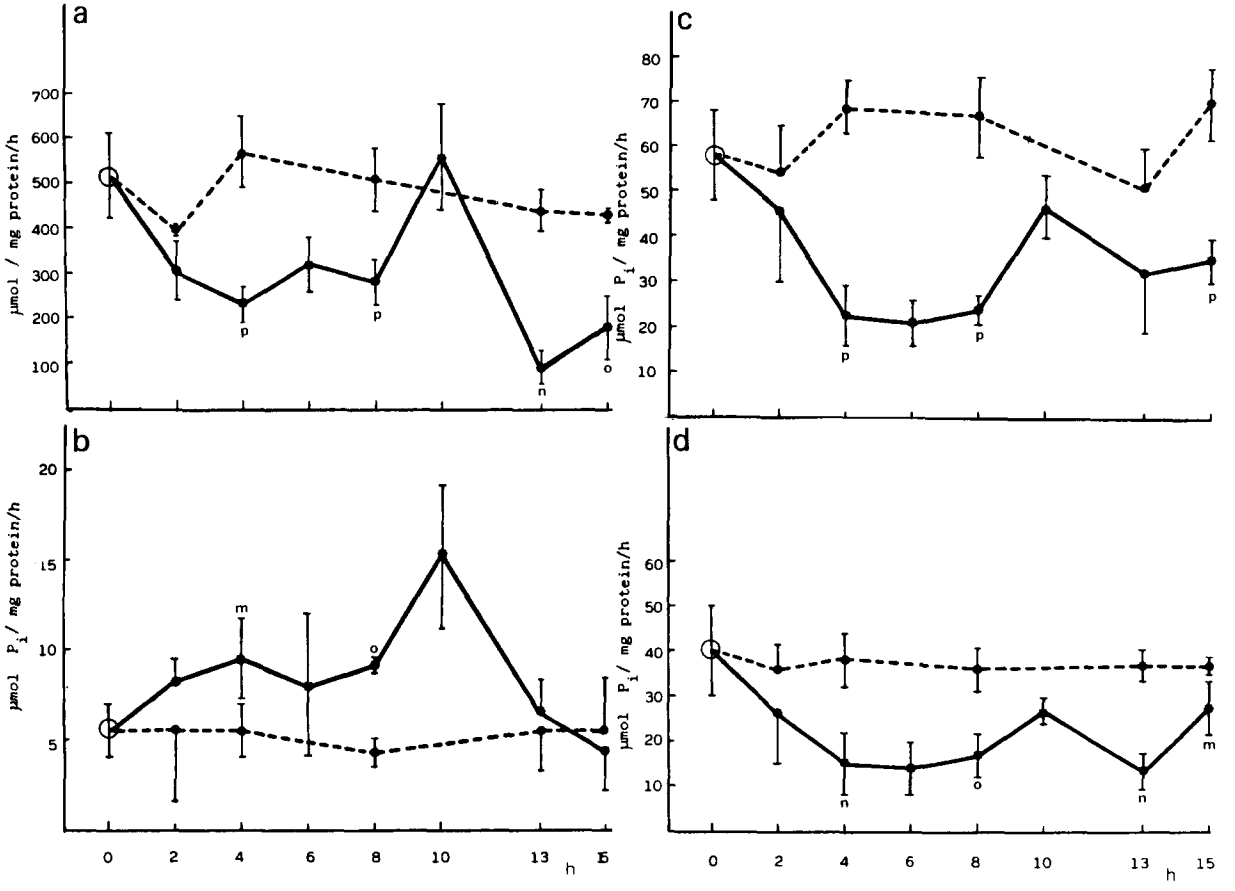

Fig. 1. Activities of enzymes of liver sinusoidal plasma membranes isolated at various times after partial hepatectomy. Sinusoidal plasma membranes were isolated from livers of unoperated control animals (○), sham operated animals (- - - - - -) and partially hepatectomized rats (———). Specific activities are expressed as µmol of product formed per mg protein per h. Each point represents the mean of six different experiments. (a) 5′-Nucleotidase, (b) $(Na^+ + K^+)$-ATPase, (c) Ca^{2+}-ATPase and (d) Mg^{2+}-ATPase. $^m P < 0.05$, $^n P < 0.01$, $^o P < 0.001$, $^p P < 0.0001$.

rived from the bile canalicular plasma membrane region, where high specific activity was found and these also showed the biphasic pattern of activity during the 15 h of hepatic regeneration; when 5 mM cAMP was present in the assay a 30% inhibition occurred [26].

In regard with Mg^{2+}-ATPase, Fig. 1(d) shows that the specific activity remains diminished throughout this pre-replicative phase. The enzyme is associated and conditioned to the membrane lipids and is assumed to be involved in control of the fluidity of the plasma membrane in the sense that a decreased activity implies an increase of fluidity which is consistent with a permeability modulation [27].

All the enzymes analyzed have been related with transport processes and all of them exhibit significant modifications during this period. Furthermore, it is interesting to point out that all the enzymes (alkaline phosphatase and phosphodiesterase I and the content of sialic acid bound to the plasma membrane too, not shown) behave in such a way that at 10 h an inflexion point is found that coincides with the second wave of cAMP and other intracellular changes that are crucial for the transition to G_1. These differences are now shown to occur at a specific plasma membrane domain that is subject to high metabolic activity because it forms an interface of the hepatocyte and circulating blood. Furthermore, these changes are a super-

ficial indication of underlying modulations of membrane biogenesis and turnover.

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