Activation of HMG-CoA reductase by microsomal phosphatase

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Abstract HMG-CoA reductase activity can be modulated by a reversible phosphorylation-dephosphorylation with the phosphorylated form of the enzyme being inactive and the dephosphorylated form, active. Phosphatases from diverse sources, including cytosol, have been shown to dephosphorylate and activate HMG-CoA reductase. The present study demonstrates phosphatase activity capable of activating HMG-CoA reductase that is associated with purified microsomes. The incubation of microsomes at 37°C for 40 min results in a twofold stimulation of HMG-CoA reductase activity, and this stimulation is blocked by sodium fluoride or phosphate. The ability of microsomes to increase HMG-CoA reductase activity occurs regardless of whether microsomes are prepared by ultracentrifugation or calcium precipitation. Additionally, phosphatases capable of activating HMG-CoA reductase are present in both the smooth and rough endoplasmic reticulum. Freeze-thawing does not prevent microsomes from activating HMG-CoA reductase but preincubation results in a significant decrease in the ability of microsomes to increase HMG-CoA reductase activity. Thus, the present study demonstrates that purified liver microsomes contain phosphatase activity capable of activating HMG-CoA reductase.—**Feingold, K. R., M. H. Wiley, A. H. Moser, S. R. Lear, and M. D. Siperstein.** Activation of HMG-CoA reductase by microsomal phosphatase. *J. Lipid Res.* 1983. **24:** 290–296.

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HMG-CoA reductase is the rate-limiting enzyme in the pathway of cholesterol synthesis. Moreover, cholesterol feeding and other dietary manipulations alter hepatic cholesterogenesis primarily by changing the activity of this enzyme (1–3). HMG-CoA reductase activity is dependent on two factors, the total quantity of enzyme present and the activation state of the enzyme. It is now well recognized that HMG-CoA reductase activity can be modulated by a reversible phosphorylation-dephosphorylation with the phosphorylated form of the enzyme being inactive and the dephosphorylated form, active (4–9). In normal hepatic tissue, it is believed that approximately 15% of the HMG-CoA reductase is present in the active form (dephosphorylated) and 85% exists in the inactive state (phosphorylated) (10). Furthermore, manipulations such as cholesterol or cholestyramine feeding, fasting, or varying the diurnal cycle alter enzyme activity but do not change the ratio of active to inactive HMG-CoA reductase in the liver, suggesting that the chronic regulation of HMG-CoA reductase in vivo is primarily dependent upon the quantity of enzyme present (10–12). However, the studies of Hardgrave et al. (13) have demonstrated that with chronic manipulations, in addition to changes in enzyme quantity, alterations in the catalytic efficiency play some role in determining total enzyme activity. Moreover, recent observations suggest that the short-term regulation of HMG-CoA reductase activity following both cholesterol and mevalonate feeding is modulated by changes in the activation state with the concentration of HMG-CoA reductase becoming a regulatory mechanism only following extended duration of treatment (14–16). Additionally, it has been demonstrated that glucagon decreases HMG-CoA reductase activity by stimulating phosphorylation of the enzyme (17).

Thus, it is apparent that the enzymes involved in the phosphorylation and dephosphorylation of HMG-CoA reductase potentially play an important role in the regulation of HMG-CoA reductase activity. Studies have demonstrated that a protein kinase that phosphorylates HMG-CoA reductase is present in both the microsomal and cytosolic fractions (6, 9, 18, 19). Various phosphatases are also present in the cytosol (20) and they have been shown to be capable of activating HMG-CoA reductase (6, 7, 9, 19, 21). It should be noted, though, that phosphatases from diverse sources (*E. coli* alkaline phosphatase, potato acid phosphatase) in vitro also have the ability to dephosphorylate and activate HMG-CoA reductase (10, 22) and thus the physiological importance of these cytosolic phosphatases in regulating microsomal

Abbreviation: HMG, 3-hydroxy-3-methylglutaryl.

HMG-CoA reductase is unclear. Only one report has previously described a microsomal phosphatase capable of activating HMG-CoA reductase, and in this study the addition of sodium sulfite, magnesium chloride, and EDTA were required for this phosphatase effect to occur (23). The purpose of the present report is to describe a phosphatase activity that is associated with purified microsomes and is capable of activating HMG-CoA reductase.

METHODS

Materials

Glucose-6-phosphate (monosodium salt), NADP (yeast β NAD), glucose-6-phosphate dehydrogenase (type XXIII from *Leuconostoc mesenteroides*), sodium EDTA, 2-mercaptoethanol, dl-isocitrate, *p*-nitrophenol phosphate, and *p*-nitrophenol standard were purchased from Sigma Chemical Co. [3-^{14}C]HMG-CoA (40–60 mCi/mmol) and RS-[5-^{3}H]mevalonolactone (5.7 Ci/mmol) were purchased from New England Nuclear. Sprague-Dawley rats (~180 g) and rat chow were purchased from Simonsen Laboratories. Ultrafluor scintillation fluid was purchased from National Diagnostics. Ion exchange resin AG-1-X8, 200–400 mesh (formate form) was purchased from Bio-Rad.

Microsomal preparation

Animals were maintained in a reversed light cycle room (light 3 PM to 3 AM, dark 3 AM to 3 PM) and fed Simonsen rat chow and water ad libitum. The animals were killed at the mid dark point (9 AM) in the light cycle by ethyl ether anesthesia and the liver was excised. The liver was homogenized in 0.3 M sucrose, 10 mM 2-mercaptoethanol, 10 mM sodium EDTA, and 50 mM sodium fluoride with a Dounce homogenizer (1 g/3 ml buffer). The homogenate was centrifuged at 10,000 *g* for 15 min at 5°C in a J-21B centrifuge (Beckman). The supernatant was next centrifuged at 100,000 *g* for 60 min at 5°C in an L5-50 ultracentrifuge (Beckman). The microsomal pellet was washed two times using 3 ml of homogenization buffer, followed by centrifugation at 100,000 *g*.

Microsomes were also prepared by the calcium precipitation method of Kamath and co-workers, as modified by Goodwin and Margolis (24). Briefly, the homogenate was prepared and centrifuged at 10,000 *g*, as described above. One ml of the 10,000 *g* supernatant was mixed with 10 ml of cold (4°C) 12.5 mM sucrose containing 8 mM calcium chloride and 50 mM sodium fluoride (pH 7.5). After 3 min, the microsomes were sedimented by centrifugation at 100 *g* for 10 min at 4°C. The supernatant was removed and the microsomal pellet was washed one time by resuspending the pellet in cold 12.5 mM sucrose containing 8 mM calcium chloride and 50 mM sodium fluoride, followed by centrifugation at 100 *g*.

The "smooth" and "rough" microsomal fractions were prepared by the Cs^{+} aggregation technique of Dallner (25). Briefly, after homogenization and 10,000 *g* centrifugation as described above, 1 M cesium chloride was added to the supernatant fraction to a final concentration of 15 mM. Four ml of this solution was then laid over 2.5 ml of 1.3 M sucrose containing 15 mM cesium chloride. Centrifugation for 120 min at 50,000 rpm in a Beckman type SW56 rotor resulted in a pellet (rough microsomal fraction) and a well-defined aggregate at the gradient interface (smooth microsomal fraction). These fractions were washed two times using 3 ml of homogenization buffer and centrifugation at 100,000 *g* for 60 min at 5°C.

Incubation procedures

Microsomal preparations were incubated at a final volume of 2 ml in a 37°C water bath for 40 min or the time specified. The basic incubation mixture was 0.3 M sucrose, 10 mM 2-mercaptoethanol, 10 mM sodium EDTA, and either 50 mM sodium chloride or 50 mM sodium fluoride. Additions of 0.1 ml of liver cytosol (which consists of the supernatant of the first 100,000 *g* fraction prepared in the absence of NaF) and 0.1 ml of 1 M potassium phosphate (pH 7.4) to make a final concentration of 50 mM potassium phosphate were made as specified. After incubation the microsomes were reisolated by 100,000 *g* centrifugation and then frozen at −70°C until assayed the following day.

HMG-CoA reductase assay

HMG-CoA reductase activity was determined by incubating 50–200 μg of microsomal protein in a 0.2 ml solution containing 0.1 M potassium phosphate, 20 mM glucose-6-phosphate, 2.5 mM NADP, 12.5 mM dithiothreitol, and 1.2 units of glucose-6-phosphate dehydrogenase (pH 7.4). The reaction was initiated by the addition of [3-^{14}C]HMG-CoA, and then the mixture was incubated at 37°C for 1 hr. The reaction was terminated by the addition of 0.02 ml of 10 N HCl, and an internal standard of [5-^{3}H]mevalonate (approximately 100,000 cpm) was added. The acidified reaction mixture was incubated at room temperature for 30 min to assure lactonization of the mevalonate. The [^{14}C]mevalonate was then separated from HMG-CoA by using a 5-cm Bio-Rad resin column (26). The eluent from the column was counted in Ultrafluor Scintillation Fluid with a Beckman Ls-330 Scintillation Counter. The gain and discriminator window settings were adjusted so that less than 0.01% of the tritium was counted in

TABLE 1. Activation of HMG-CoA reductase in microsomes

	Exp 1	Exp 2	Exp 3
	pmol mevalonate/mg protein per min		
No incubation	92	21	—
Incubated NaF	89	13	174
Incubated NaCl	199	45	313
Incubated NaF + PO_4	78	14	178
Incubated NaCl + PO_4	98	23	217

Microsomes were isolated and purified by centrifugation as described in the Methods section. They were then incubated at 37°C for 40 min in a mixture containing 0.3 M sucrose, 10 mmol 2-mercaptoethanol, and 10 mmol Na EDTA to which was added, as indicated, 50 mmol NaF, 50 mmol NaCl, and 50 mmol KPO_4^{2-}. After incubation, the microsomes were re-isolated by centrifugation and the HMG-CoA reductase activity was determined. The values given are the mean of duplicate samples.

the ^{14}C window and less than 15% of the ^{14}C counts were recorded in the ^{3}H window. Counts were corrected for spillover and background. Recovery of the tritiated mevalonate averaged approximately 60%. Enzyme activity was corrected by subtracting the activity of a zero time blank. HMG-CoA reductase activity was expressed as picomole mevalonate synthesized/mg protein per min. Protein was assayed by the method of Lowry et al. (27) using bovine serum albumin as the standard.

Phosphatase assay

Phosphatase activity was determined by modifying the method outlined in Sigma Technical Bulletin 104. Briefly, 50–200 μg of microsomal protein was incubated in an imidazole buffer (pH 7.4) containing *p*-nitrophenol phosphate (2 mg/tube) at 37°C in a water bath. After 30 min, the reaction was stopped by adding 10 ml of 0.02 N NaOH and the absorbance was determined with a Beckman Spectrophotometer at 400 nm.

Glucose-6-phosphate dehydrogenase assay

Glucose-6-phosphate dehydrogenase activity was determined by adding 0.5 mg–1 mg of microsomal protein to a cuvette containing 2.5 ml of 0.1 M glycylglycine buffer, pH 8, 0.1 ml of 0.03 M glucose-6-phosphate, 0.1 ml of 0.01 M NADP, and 0.2 ml of 0.15 M magnesium sulfate. The formation of NADPH at 30°C was continuously monitored in a Beckman Spectrophotometer at 340 nm (28).

5′-Nucleotidase assay

5′-Nucleotidase activity was determined by adding approximately 100 μg of microsomal protein to a tube containing glycine-NaOH buffer, pH 8.5, $MgCl_2$, and 3 μmol of 5-AMP. The tube was incubated at 37°C for 15 min and the reaction was stopped by the addition of TCA to a final concentration of 5%. The mixture was then centrifuged and an aliquot of the supernatant was analyzed for inorganic phosphate (29). Inorganic phosphate was measured by incubating a portion of the supernatant with one part 10% ascorbic acid and six parts 0.24% ammonium molybdate, 0.4 H_2O in 1 N H_2SO_4. The mixture was incubated at 37°C for 1 hr and the absorbance was determined at 820 nm with a Beckman Spectrophotometer (30).

Isocitrate dehydrogenase assay

Isocitrate dehydrogenase activity was determined by adding to a cuvette 0.3 ml of 0.25 M glycylglycine buffer (pH 7.4), 0.1 ml of 0.018 M $MgCl_2$, 0.1 ml of 0.00135 M NADP, 0.1 ml 0.006 M dl-isocitrate, 0.5 mg–1 mg of microsomal protein, and 2 ml of distilled water. The formation of NADPH at 30°C was continuously monitored in a Beckman Spectrophotometer at 340 nm (31).

RESULTS

The results in **Table 1** demonstrate that purified microsomes (microsomes that have been isolated by centrifugation and washed two times with homogenization buffer followed by centrifugation) contain a phosphatase that is capable of activating HMG-CoA reductase. In experiment 1, the activity of HMG-CoA reductase in liver microsomes isolated in the presence of sodium fluoride, a known inhibitor of phosphatase activity, is 92 pmol of mevalonate/mg protein per min. When these microsomes are incubated in a buffer containing sodium fluoride at 37°C for 40 min, the activity of HMG-CoA reductase is unchanged (89 pmol/mg per min). However, when microsomes are incubated in buffer in the absence of sodium fluoride, the activity of HMG-CoA reductase is increased 124% (199 pmol/mg per min). In contrast to the above results, the incubation of microsomes without sodium fluoride but in the presence of 50 mM potassium phosphate results in only a slight increase in HMG-CoA reductase activity (26%) when compared to microsomes isolated in buffer + sodium fluoride + phosphate. It should be noted that phosphate is an inhibitor of phosphatase activity. While the absolute magnitude of HMG-CoA reductase activity varied, similar results were observed in experiments 2 and 3. Thus these data demonstrate that the incubation of purified microsomes results in an increase in the activity of the enzyme, HMG-CoA reductase, and moreover this increase in activity can be prevented or blunted by either fluoride ion or phosphate, indicating that the increase in HMG-CoA reductase activity is due to phosphatase activity. Additionally, using *p*-nitrophenol phosphate as a substrate at pH 7.4, purified microsomes

formed 133 μg of *p*-nitrophenol/mg protein per 30 min, demonstrating the presence of phosphatase activity in purified microsomes. That this microsomal phosphatase activity does not represent cytosolic contamination is suggested by the absence of glucose-6-phosphate dehydrogenase activity in purified microsomes before and after incubation. Similarly, the low 5′-nucleotidase activity in the purified microsomes (0.0143 μmol/mg per min) suggests that contamination with plasma membranes is not the source of the phosphatase activity. The absence of isocitrate dehydrogenase activity suggests that our purified microsomes are not contaminated with mitochondria. Additionally, it should be noted that isocitrate dehydrogenase is also present in cytosol and thus the absence of this enzyme in purified microsomes further suggests the absence of cytosolic contamination of the microsomal fraction.

Fig. 1 demonstrates the time course of the increase in HMG-CoA reductase activity in microsomes incubated in the absence of sodium fluoride. HMG-CoA reductase activity increases progressively with time, reaching a maximum activity approximately 2 × control in 40 min.

To determine if microsomes prepared by a method other than ultracentrifugation also contain phosphatases capable of activating HMG-CoA reductase, we prepared microsomes using the calcium precipitation method. In experiments similar to those described in Table 1, the activity of HMG-CoA reductase in microsomes incubated in the absence of sodium fluoride is 59.1% and 148% greater than microsomes incubated in the presence of sodium fluoride (Exp. 1, NaF incubation 33 vs. NaCl 52.5; Exp. 2, NaF incubation 27 vs. NaCl 67 pmol/mg per min). This result demonstrates that the increase of HMG-CoA reductase activity occurring during incubation is not dependent on the method of preparation of the microsomal fraction.

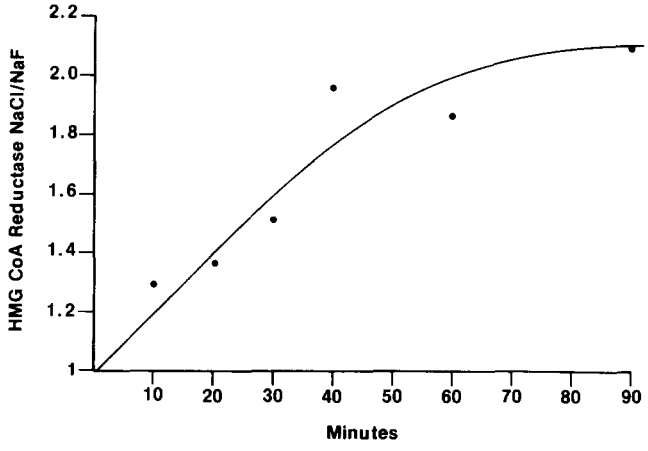

Fig. 1. Microsomes were prepared by centrifugation as described in the Methods section and were then incubated at 37°C for the time indicated, either in the presence of NaF or NaCl. After incubation, the microsomes were isolated by centrifugation and the HMG-CoA reductase activity was determined. The data are presented as the ratio of the HMG-CoA reductase activity in NaCl/activity in NaF.

TABLE 2. Effect of freeze-thawing on the activation of HMG-CoA reductase

	Exp 1	Exp 2
	pmol mevalonate/mg protein per min	
No incubation	94	
Freeze-thawed		
Incubated NaF	94	51
Incubated NaCl	204	73
Incubated NaF + PO_4	75	
Incubated NaCl + PO_4	102	
Not freeze-thawed		
Incubated NaF	89	47
Incubated NaCl	199	78

Microsomes were isolated by centrifugation. Prior to washing, a portion of the microsomes were rapidly frozen and then thawed two times. The microsomes were then washed as described in the Methods section and the incubation was performed as described in Table 1. After incubation, the microsomes were re-isolated by centrifugation and the HMG-CoA reductase activity was determined. The values given are the mean of duplicate samples.

The effect of prior freeze-thawing on the increase in HMG-CoA reductase activity with incubation of microsomes is shown in **Table 2.** As is apparent, the results are very similar to those observed in microsomes that were not freeze-thawed (Tables 1 and 2) and demonstrate that the incubation of microsomes results in a twofold stimulation of HMG-CoA reductase activity that is prevented by sodium fluoride and blunted by phosphate. Thus, freeze-thawing, which disrupts microsomal structure, does not result in either a loss or solubilization of microsomal phosphatase activity.

Table 3 demonstrates the effect of preincubation on the ability of microsomes to activate HMG-CoA reductase. In this experiment, purified microsomes were prepared in our usual manner and were then preincubated at 37°C for 40 min in a buffer containing sodium fluoride. After incubation, the microsomes were re-isolated by centrifugation and then incubated in the presence or absence of sodium fluoride. As described above, HMG-CoA reductase activity increases in microsomes when incubated in the absence of sodium fluoride (#1 vs. #2). In microsomes that are preincubated, the activity of HMG-CoA reductase is unchanged (#1 vs. #3) demonstrating that HMG-CoA reductase activity is not altered by the preincubation. However, the previously observed increase in HMG-CoA reductase activity that occurs when microsomes are incubated in the absence of sodium fluoride is markedly blunted (#3 vs. #4) compared to that seen without preincubation. That this re-

TABLE 3. Effect of pre-incubation on the activation of HMG-CoA reductase in microsomes

	Exp 1	Exp 2	Exp 3
	pmol mevalonate/mg protein per min		
No pre-incubation			
1) Incubated NaF	72	64	215
2) Incubated NaCl	152	137	375
Pre-incubation			
3) Incubated NaF	75	61	232
4) Incubated NaCl	100	71	234
5) Incubated NaF + 100 λ liver cytosol	92	67	242
6) Incubated NaCl + 100 λ liver cytosol	224	148	826

Purified microsomes were prepared by centrifugation as described in Methods. The microsomes were then preincubated at 37°C for 40 min in 0.3 M sucrose, 10 mmol mercaptoethanol, 10 mmol EDTA, and 50 mmol NaF. The microsomes were then re-isolated by centrifugation and incubated at 37°C for 40 min in the usual incubation mixture, either in the presence of NaF or NaCl. Liver cytosol consisting of the 100,000 *g* supernatant was added as indicated. After incubation,the microsomes were reisolated by centrifugation and the activity of HMG-CoA reductase was determined. The values given are the mean of duplicate samples.

sult is not simply due to a change in HMG-CoA reductase per se during preincubation is demonstrated by the ability of liver cytosol (which contains a variety of phosphatases) to increase greatly HMG-CoA reductase activity (#5 vs. #6). Thus, these results demonstrate that incubation at 37°C for 40 min results in the solubilization or loss of activity of phosphatase enzyme in the microsomal fraction. It should also be noted that, as observed previously by this laboratory, HMG-CoA reductase is not solubilized under these mild incubation conditions (32).

Table 4 demonstrates that the preincubation of microsomes results in the solubilization of microsomally

TABLE 4. Effect of supernatant derived from pre-incubated microsomes on the activity of HMG-CoA reductase activity

	pmol mevalonate/mg protein per min
1) NaF	182
2) NaCl	206
3) Supernatant–NaCl	470
4) Supernatant–NaF	170

Purified microsomes were prepared by centrifugation as described in Methods. The microsomes were then preincubated at 37°C for 40 min in 0.3 M sucrose, 10 mmol 2-mercaptoethanol, 10 mmol EDTA, and 50 mmol NaF. The microsomes were then re-isolated by centrifugation and incubated at 37°C in the usual incubation mixture either in the presence or absence of NaF or NaCl. The supernatant was derived from the 100,000 *g* centrifugation of purified microsomes that had been incubated in 0.3 M sucrose, 10 mmol 2-mercaptoethanol, 10 mmol EDTA, and 50 mmol NaCl for 40 min at 37°C. NaF to a concentration of 50 mmol was added to the supernatant where indicated. After incubation, the microsomes were re-isolated by centrifugation and the activity of HMG-CoA reductase was determined. The values given are the mean of duplicate samples.

associated phosphatase. As observed in Table 3, the activity of HMG-CoA reductase in preincubated microsomes is not markedly activated on incubation (#1 vs. #2) demonstrating the loss of phosphatase activation. However, the incubation of preincubated microsomes in supernatant derived from incubated microsomes results in a great increase in HMG-CoA reductase activity (#3) and most importantly, this activation of HMG-CoA reductase is blocked by NaF (#4) suggesting that the increase in activity is mediated by phosphatase. These data demonstrate that the preincubation of microsomes results in the solubilization of microsomally associated phosphatase activity.

Table 5 shows the effect of prior incubation on HMG-CoA reductase activity in "smooth" and "rough" microsomes. It is apparent that in both the smooth and rough fractions the incubation of microsomes in the absence of sodium fluoride results in an increase in HMG-CoA reductase activity (137% in smooth microsomes and 72% in rough microsomes). These data demonstrate that both the rough and smooth microsomal membranes contain phosphatase activity that activates HMG-CoA reductase.

DISCUSSION

The present study demonstrates that phosphatase that is capable of activating HMG-CoA reductase is associated with purified microsomes. As demonstrated in Table 1, the incubation of microsomes at 37°C for 40 min results in a twofold stimulation of HMG-CoA reductase activity. Furthermore, this stimulation could be blocked or blunted by either sodium fluoride or phosphate, compounds that are well known inhibitors of phosphatase activity, indicating that the increase in HMG-CoA reductase activity is due to phosphatase ac-

TABLE 5. Activation of HMG-CoA reductase in smooth and rough microsomes

	pmol mevalonate/mg protein per min
Smooth microsomes	
Incubated NaF	86
Incubated NaCl	204
Rough microsomes	
Incubated NaF	32
Incubated NaCl	55

Rough and smooth microsomes were prepared by cesium chloride gradients as described in the Methods section. The microsomal preparations were then incubated at 37°C for 40 min in a mixture containing 0.3 M sucrose, 10 mmol 2-mercaptoethanol, 10 mmol Na EDTA, and either 50 mmol NaF or 50 mmol NaCl, as indicated. After incubation, the microsomes were re-isolated by centrifugation and HMG-CoA reductase activity was determined. The values given are the mean of duplicate samples.

tivity located in the microsomes. It is very likely, therefore, that mirosomal phosphatases activate HMG-CoA reductase by a mechanism similar to that demonstrated for other phosphatases, i.e., by removing a phosphate from HMG-CoA reductase and thus converting the enzyme to its active, dephosphorylated form (4, 6, 8, 9). This stimulation of HMG-CoA reductase activity in microsomes occurs regardless of whether the microsomes are prepared by ultracentrifugation or calcium precipitation. Additionally, the incubation of either smooth or rough microsomal preparations results in an increase in HMG-CoA reductase activity, indicating that phosphatases capable of activating HMG-CoA reductase are present in both the smooth and rough endoplasmic reticulum.

That this phosphatase activity, capable of activating HMG-CoA reductase, is intimately related to the microsomal membrane and not due to the trapping of cytosol is demonstrated by the failure of freeze-thawing to result in its removal. Moreover, the absence of glucose-6-phosphate dehydrogenase, a known cytosolic enzyme, activity in purified microsomes before and after incubation indicates that cytosolic contamination of the microsomal fraction is not a likely explanation for our observations. Similarly, the low, nucleotidase specific activity in our purified microsomes (0.0143 μmol/mg per min in purified microsomes vs. $\sim$1 μmol/mg per min in purified plasma membranes (33)) suggests that plasma membranes are not the source of this phosphatase activity. Additionally, the absence of isocitrate dehydrogenase activity makes it unlikely that mitochondria contamination of our microsomes could account for the phosphatase activity. In contrast, preincubation of microsomes in homogenization buffer at 37°C for 40 min results in a significant decrease in the ability of microsomes to increase HMG-CoA reductase activity, suggesting that either the solubilization or inactivation of phosphatases occurs with such incubation. The ability of supernatant derived from preincubated microsomes to activate HMG-CoA reductase demonstrates that preincubation results in the solubilization of the microsomally associated phosphatase. Studies are currently in progress to purify and characterize this solubilized enzyme(s).

Thus the present study demonstrates that associated with purified liver microsomes is phosphatase activity that is capable of activating the enzyme, HMG-CoA reductase. It is possible that if phosphatases are physiological regulators of HMG-CoA reductase activity, the microsomally associated phosphatases will be of potential importance because of their location in the microsomes and their resulting proximity to the enzyme, HMG-CoA reductase.

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