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HUMAN HEPATIC 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE: EVIDENCE FOR THE REGULATION OF ENZYMIC ACTIVITY BY A BICYCLIC PHOSPHORYLATION CASCADE

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Microsomal human liver HMG-CoA reductase has been shown to exist in active (dephosphorylated) and inactive (phosphorylated) forms. Microsomal HMG-CoA reductase was inactivated in vitro by ATP-Mg in a time dependent manner; this inactivation was mediated by reductase kinase. Incubation of inactivated enzyme with phosphatase resulted in a time dependent reactivation (dephosphorylation). Polyacrylamide gel electrophoresis of purified HMG-CoA reductase incubated with reductase kinase and radiolabeled ATP revealed that the ^{32}P radioactivity and HMG-CoA reductase enzymic activity were localized in a single electrophoretic position. Partial dephosphorylation of the phosphorylated enzyme was associated with loss of ^{32}P and increase in HMG-CoA reductase activity. Human reductase kinase also exists in active and inactive forms. The active (phosphorylated) form of reductase kinase can be inactivated by incubation with phosphatase. Phosphorylation of inactive reductase kinase with ATP-Mg and a second kinase, reductase kinase kinase, was associated with a parallel increase in the enzymic activity of reductase kinase and the ability to inactivate HMG-CoA reductase. The combined results present initial evidence for the presence of human HMG-CoA reductase and reductase kinase in active and inactive forms, and the in vitro modulation of its enzymic activity by a bicyclic phosphorylation cascade. This bicyclic cascade system may provide a mechanism for short-term regulation of the pathway for cholesterol biosynthesis in man.

Several published reports have established that 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase, EC 1.1.1.34) is the key enzyme in the regulation of the pathway for cholesterol biosynthesis (1-3). Extensive data has now been acquired which suggests that the enzymic activity of rat liver HMG-CoA reductase is under precise biochemical control (1-6). Two separate general mechanisms involved in the regulation of the catalytic activity of HMG-CoA reductase have been identified (1-3). The first

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Abbreviations: 3-hydroxy-3-methylglutaryl coenzyme A reductase, HMG-CoA reductase; reductase kinase, RK; reductase kinase kinase, RKK; sodium dodecylsulfate, $NaDodSO_4$; sodium fluoride, NaF.

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mechanism involves short-term regulation which is achieved by (i) allosteric or isosteric effects and (ii) covalent modification involving reversible phosphorylation of HMG-CoA reductase and reductase kinase. The second mechanism for the regulation of HMG-CoA reductase activity, long-term regulation, is achieved by changes in enzyme concentration by modulation of HMG-CoA reductase synthesis and/or degradation.

To ascertain if the activity of hepatic HMG-CoA reductase in man is regulated by short-term mechanisms we have undertaken a systematic investigation of the human enzyme. In the present communication we describe the presence of human hepatic HMG-CoA reductase in active and inactive forms and the in vitro modulation of its enzymic activity by reversible phosphorylation. We also present evidence which suggests that the regulation of the enzymic activity of human HMG-CoA reductase involves a bicyclic cascade system of phosphorylation - dephosphorylation of HMG-CoA reductase and RK.

MATERIALS AND METHODS

Materials. Mevalonic acid was obtained from Sigma. Sephadex-G-25M (PD 10) columns were procured from Pharmacia; Bio-Rex 5 anion exchange resins were obtained from Bio-Rad Laboratories. HMG-CoA, ATP, and NADPH were purchased from P-L Biochemicals; [3-^{14}C]-HMG-CoA (54.4 mCi/mmol), [5-^{3}H]-mevalonic acid (2 Ci/mmol) were obtained from New England Nuclear; phosphocellulose was from Whatman.

Liver Samples. Human liver samples were obtained from fasting patients under a peer-reviewed protocol approved by a Clinical Research Committee (7).

Preparation of Microsomes. Human liver microsomes were prepared either by Ca^{++} precipitation or ultracentrifugation. Fresh human liver (.33 g/ml) was homogenized in 40 mM KH_2PO_4 (pH 7.2), 50 mM KCl, 30 mM EDTA, 0.1 M sucrose, 5 mM DTT, 1 mM sodium azide (buffer A), in the presence and absence of 50 mM NaF or NaCl (8) and centrifuged at 10,000 x g for 15 min. Aliquots of the 10,000 x g post-mitochondrial supernatants were utilized for isolation of microsomes by $CaCl_2$ (8 mM) precipitation (9). The microsomes isolated by this rapid procedure were suspended in 2 ml of buffer A containing either 50 mM NaCl or NaF, and stored frozen at -20°C. Separate aliquots of the 10,000 x g supernatant were centrifuged at 105,000 x g for 90 min. The cytosol was carefully removed and used for isolation of RK and RKK. The microsomal pellet (30 mg/ml) was either washed once in buffer A or used as an unwashed microsomal suspension. The microsomal pellet was stored frozen at -20°C. HMG-CoA reductase was purified to homogeneity as previously described (7).

Isolation of Active and Inactive Forms of Reductase Kinase. Microsomal suspensions or clear cytosol were freed from buffer A and lower molecular weight components by chromatography on Sephadex G-25M equilibrated in 50 mM imidazole (pH 7.4), 250 mM NaCl, 100 mM sucrose, 1 mM EDTA, 2 mM DTT and 1 mM Na azide (buffer B). The eluted fractions were used as a source for active RK.

Inactive (dephosphorylated) RK was prepared by incubating cytosolic RK in buffer B with partially purified phosphoprotein phosphatase as previously reported (5). The incubation was terminated by the addition of 50 mM NaF.

Microsomal HMG-CoA reductase which contained no RK activity was isolated by the precedures previously reported (10).

Isolation of Reductase Kinase Kinase. Human or rat liver cytosol was fractionated with ammonium sulfate. The protein which precipitated at 65% saturation was dissolved in 5 mM Tris-HCl (pH 7.5), 1 mM EDTA, 50 mM NaCl, 5 mM DTT, 10% glycerol, 50 mM phenylmethylsulfonyl fluoride (buffer C), dialyzed overnight in the same buffer, applied to a column of phosphocellulose (1.6 x 6 cm), and eluted with a gradient of 1 M KCl in buffer C (100 ml) and buffer C (100 ml). Fractions (4 ml) were collected and assayed for RKK activity. Fractions containing RKK activity were pooled, dialyzed in buffer C, concentrated and stored frozen at -20°C.

Phosphoprotein phosphatase was partially purified up to the DEAE Sephadex chromatographic step by the method of Brandt et al. (11).

Assay of HMG-CoA Reductase Activity. HMG-CoA reductase activity was routinely assayed following preincubation for 20 min at 37°C in a buffer containing 0.15 M potassium phosphate (pH 6.9), 0.2 M KCl, .01 M EDTA, 5 mg/ml BSA, and 10 mM DTT. Incubations were conducted at 37°C with NADPH (2 mM), DL-[3-^{14}C]-HMG-CoA (.05 mM, 300,000 dpm) and DL-[^{3}H]-mevalonic acid (400,000 dpm). The reaction was terminated by the addition of 50 ul of 10 N HCl, and labeled mevalonolactone was separated by column chromatography (Bio-Rex 5 anion exchange resin, 1 ml) as described (4,12). In the assay of RK, the interference by cytosolic mevalonate kinase was completely avoided by the addition of excess EDTA before HMG-CoA reductase assay as described elsewhere (10).

Analysis of Phosphorylated [^{32}P]-Labeled HMG-CoA Reductase. Partial phosphorylation of purified HMG-CoA reductase was carried out for 60 min at 30°C in a reaction mixture containing 15 mM Tris-HCl (pH 7.5), 5.7 mM Mg-acetate, .11 mM [γ -^{32}P]ATP (800 cpm/pmol), .27 mg/ml of partially purified RK and .2 mg/ml of homogeneous HMG-CoA reductase. Control samples lacked either ATP, RK, or HMG-CoA reductase. At the end of incubation, aliquots were analyzed for HMG-CoA reductase activity in order to determine the extent of inactivation. HMG-CoA reductase activity was also determined following partial dephosphorylation of control and phosphorylated (inactivated) HMG-CoA reductase. Phosphorylated and dephosphorylated samples from each incubation mixture were analyzed for ^{32}P-incorporation into HMG-CoA reductase protein by the chromatographic method of Huang and Robinson (13). ^{32}P radioactivity in the electrophoretic band of HMG-CoA reductase was also analyzed following $NaDodSo_4$ gel electrophoresis (14).

Aliquots from each reaction mixture were analyzed by polyacrylamide gel electrophoresis under non-denaturing conditions as described by Maurer (15) for gel system No. 6, except that the buffers and gels contained 1 mM DTT. After electrophoresis, two gels from each group were sliced into 3 mm segments, one used for assaying HMG-CoA reductase activity, the second one for counting protein-bound radioactivity. A parallel gel was used for staining with Coomassie blue.

Protein was determined by the Coomassic dye binding method (16) using bovine serum albumin as standard.

RESULTS

Inactivation and Reactivation of Microsomal HMG-CoA Reductase Activity.

The first evidence that human liver HMG-CoA reductase existed in active

and inactive forms was obtained from Ca^{++} precipitated microsomes isolated in the presence and absence of NaF which inhibits endogenous microsomal phosphatase(s). The expressed enzymic activity of microsomal HMG-CoA reductase was higher in the livers homogenized and assayed in the presence of NaCl (68 units) than was observed when the homogenization and assay were performed in the presence of NaF (21 units) (Table I). The addition of NaF inhibited the phosphatase(s) enzymes during homogenization, isolation of microsomes, and preincubation of the assay, thus preventing the activation (dephosphorylation) of inactive HMG-CoA reductase.

The additon of NaF during preincubation of the microsomes isolated with NaCl also reduced the enzymic activity of HMG-CoA reductase from 68 to 47 units (Table I). The addition of NaF inhibited the partial dephosphorylation of phosphorylated HMG-CoA reductase which occurred during preincubation prior to assay of HMG-CoA reductase enzymic activity. The expressed enzymic

TABLE I

Effect of Sodium Fluoride and Phosphoprotein Phosphatase on Active and Inactive Forms of Microsomal HMG-CoA Reductase

Microsomal fraction	Additions to Preincubation Mixture			HMG-CoA Reductase Activity (units)[c]
	NaCl[a]	NaF[a]	Phosphoprotein Phosphatase	
+ NaCl	+	-	-	68
	-	+	-	47
	+	-	+	94
+ NaF[b]	-	+	-	21
	+	-	-	25
	+	-	+[b]	91

Microsomes from human liver were isolated in the presence of 50 mM sodium chloride (+ NaCl) or sodium fluoride (+ NaF) and suspended in buffer containing either 50 mM NaF or NaCl. Ninety µg of microsomal protein from both microsomal preparations were preincubated for 45 min at 37°C with and without phosphoprotein phosphatase (15 µg) in a medium containing 50 mM imidazole (pH 7.5), 5 mM EDTA, and 2 mM DTT. At the end of pre-incubation HMG-CoA reductase activity was determined as described in Materials and Methods.

[a] 50 mM NaCl or NaF.

[b] NaF concentration during dephosphorylation was less than 0.1 mM.

[c] Units expressed as pmol/min/mg

activity of HMG-CoA reductase in the liver microsomes prepared with NaF did not differ significantly if the preincubation did not contain NaF indicating that the phosphatase enzyme(s) remained inhibited (Table I).

The treatment of microsomal HMG-CoA reductase prepared in the presence or absence of NaF with partially purified phosphatase fully reactivated (dephophorylated) HMG-CoA reductase and resulted in similar total HMG-CoA enzyme activity (Table I).

The inactivation and reactivation of human liver HMG-CoA reductase was confirmed in microsomes isolated by high speed ultracentrifugation. The enzymic activity of microsomal HMG-CoA reductase was rapidly inhibited in the presence of ATP-Mg (Fig. 1A). No inactivation occurred in the absence of ATP or Mg^{++}. The inhibition of enzymic activity was time (Fig. 1A) and ATP-Mg

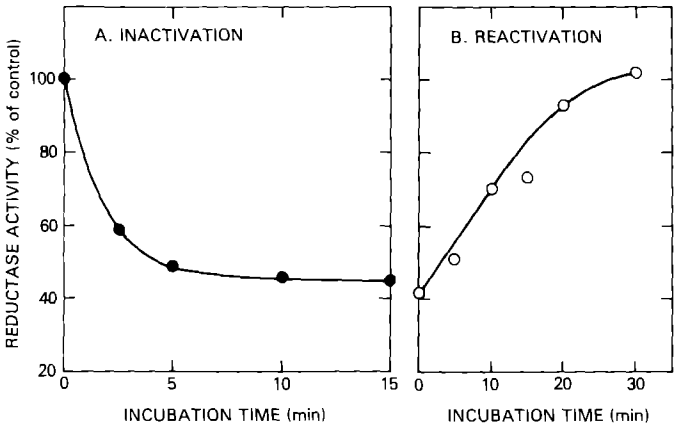


Figure 1. Inactivation and reactivation of human hepatic microsomal HMG-CoA reductase.

Panel A: INACTIVATION. Washed microsomal suspension was desalted in buffer B and aliquots (500 µl, 4.6 mg protein) were incubated at 30°C with ATP (2mM) and Mg^{++} (4 mM) or in control incubations containing only Mg^{++}. At indicated time intervals aliquots (140 µl) from control and experimental incubations were added to tubes containing 20 µl of .6 M EDTA to terminate the reductase kinase reaction, and HMG-CoA reductase enzymic activity was determined as described in Materials and Methods. The mean value ± S.D. of HMG-CoA reductase activity (100%) from control incubations was 29.3 ± .7 pmoles/min.

Panel B: REACTIVATION. Control and ATP-inactivated microsomes (30 min point) were prepared as described above. Aliquots (286 µg) from each microsomal preparation were incubated at 37°C either with 50 mM NaF + BSA in buffer B (283 µg) or 50 mM NaCl + phosphoprotein phosphatase (283 µg). At the indicated time intervals, the reaction was terminated by the addition of 30 µl of .5 M NaF and HMG-CoA reductase enzymic activity determined. The mean ± S.D. inhibited value of HMG-CoA reductase by ATP-Mg (40.6 ± 1.1%) was calculated by utilizing values from control incubations at each time interval (NaF + BSA in buffer B). Percent increase due to dephosphorylation (NaCl + phosphatase) of inactivated HMG-CoA reductase at the indicated times was calculated from control incubations containing NaCl + BSA in buffer B.

concentration dependent (data not shown). Incubation of ATP-Mg inactivated enzyme with partially purified phosphoprotein phosphatase resulted in a time dependent reactivation (dephosphorylation) of HMG-CoA reductase (Fig. 1B). The dephosphorylation reaction was completely blocked by NaF.

Phosphorylation-Dephosphorylation of HMG-CoA Reductase. To establish definitively that inactivation-reactivation of HMG-CoA reductase was due to phosphorylation-dephosphorylation, purified HMG-CoA reductase (7) was incubated with partially purified reductase kinase and .11 mM [γ^{32}P]ATP at 30°C. A sixty min incubation was associated with 48% reduction of HMG-CoA reductase activity (Table II). Analysis of the incubation mixture by polyacrylamide gel electrophoresis revealed that protein-bound ^{32}P radioactivity was present in the position corresponding to the enzymic activity and electrophoretic band of HMG-CoA reductase (Fig. 2). Partial dephosphorylation of ^{32}P-labeled HMG-CoA reductase with phosphatase was

TABLE II
Partial Phosphorylation and Dephosphorylation of Purified Human Liver HMG-CoA Reductase

Fraction[a]	HMG-CoA reductase activity (pmol/min/assay)	Activation (fold)	HMG-CoA Reductase-Bound Radioactivity (total dpm/assay x 10^{-3})[b]	Loss of Radioactivity (fold)
A. HMG-CoA Reductase (minus ATP)	19.0	--	--	--
B. HMG-CoA Reductase (plus ATP)	9.8	--	45.7	--
C. A + Phosphatase	25.4	1.3[c]	--	--
D. B + Phosphatase	20.4	2.1[c]	20.7	2.2[c]

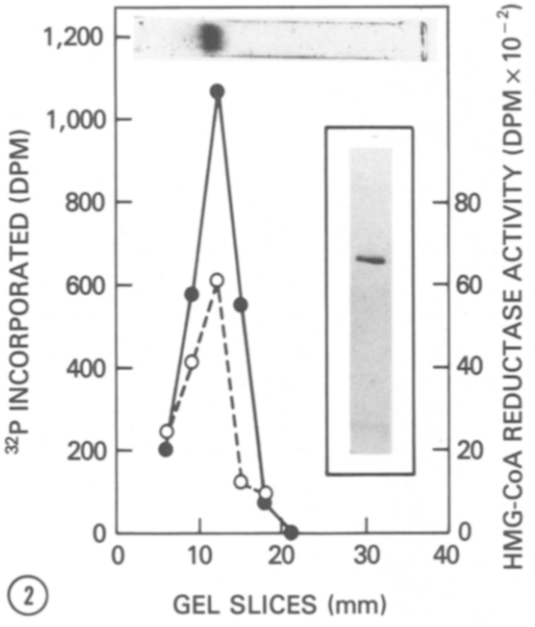
[a]Purified HMG-CoA was partially phosphorylated (48% inactivation of enzymic activity) with radiolabeled ATP and partially purified RK (B) as described under Materials and Methods. Control incubations (A) had all the components except radiolabeled ATP. The other two controls had either RK or HMG-CoA reductase plus all the other components. After 60 min of incubation, the reaction was terminated by the addition of 10 µl of .6 M EDTA. Aliquots from phosphorylated or control HMG-CoA reductase were incubated either with NaF-inactivated phosphatase (A and B) or with active phosphatase (C and D) for 30 min at 37°C. Control and partially dephosphorylated samples were analyzed for HMG-CoA reductase activity and protein-bound radioactivity by chromatography as previously described (13).

[b]Protein-bound radioactivity obtained in RK alone or HMG-CoA reductase alone (controls, above) were subtracted from B and D.

[c]Fold increase in enzymic activity or decrease in HMG-CoA reductase-bound radioactivity following partial dephosphorylation.

associated with a loss of the radioactivity and an increase in the enzymic activity (Table II). It is interesting to note that the increase in enzymic activity and loss of HMG-CoA reductase bound radioactivity following partial dephosphorylation was about two-fold (Table II). Partially phosphorylated and dephosphorylated aliquots containing labeled HMG-CoA reductase were analyzed by $NaDodSo_4$ gel electrophoresis. ^{32}P-radioactivity (2,218 dpm) was associated with the electrophoretic band of HMG-CoA reductase, with an apparent molecular weight of 53,000 which is consistent with the molecular weight of purified human liver HMG-CoA reductase (Fig. 2, inset; 7). Following partial dephosphorylation, ^{32}P-radioactivity in the electrophoretic band of HMG-CoA reductase was significantly reduced (1,170 dpm).

Interconversion of human cytosolic HMG-CoA reductase kinase. The active form of cytosolic RK can be inactivated by incubation with phosphatase. Completely inactivated (dephosphorylated) RK did not catalyze the phosphorylation (inactivation) of HMG-CoA reductase (Fig. 3). Incubation of inactivated (dephosphorylated) RK with ATP-Mg and a partially purified cytosolic protein kinase, designated reductase kinase kinase (RKK), was associated with complete activation (phosphorylation) of RK and an increase in



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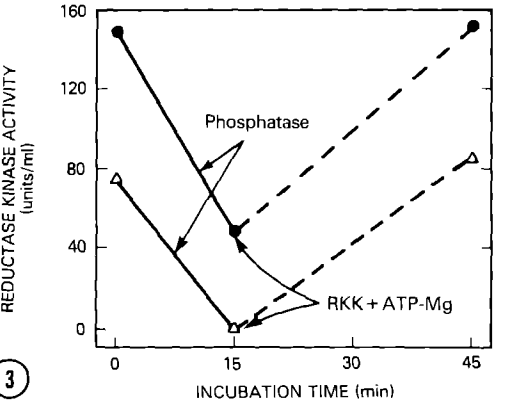

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the phosphorylation (inactivation) of HMG-CoA reductase (Fig. 3). RKK alone or with ATP-Mg had no effect on HMG-CoA reductase enzymic activity. Microsomal RK, like cytosolic RK, was also able to undergo reversible inactivation and reactivation (data not shown). Cytosolic RK (Figure 2, Δ-Δ) was totally inactive following dephosphorylation with phosphatase. However, the reductase kinase activity in the second cytosolic fraction (Figure 2, o-o) was reduced to only 33% of the initial activity by treatment with phosphatase due to the high level of initial RK activity and only partial dephosphorylation. Cytosolic (Fig. 3) inactivated RK was reactivated following incubation with RKK and ATP-Mg. The RK activity following reactivation with RKK and ATP-Mg was higher than the initial RK activity (Fig. 3, Δ--Δ) apparently due to the presence of inactive (dephosphorylated) RK at the time of isolation of the cytosol.

Figure 2. Polyacrylamide gel electrophoresis of [^{32}P]-HMG-CoA reductase phosphorylated in vitro.

Purified HMG-CoA reductase was partially phosphorylated (48% inactivation of enzymic activity) with labeled ATP, and analyzed by polyacrylamide gel electrophoresis as described under experimental procedures. The gels were sliced (3 mm segments), and protein-bound radioactivity (●) as well as enzymic activity of HMG-CoA reductase (0) were determined. Radioactivity data are corrected by subtraction of control values. A parallel gel was stained and denotes the position of the human liver HMG-CoA reductase. The gel in the inset represents the electrophoretic band of purified HMG-CoA reductase following $NaDodSO_4$ gel electrophoresis.

Figure 3. Inactivation (dephosphorylation) and reactivation (phosphorylation) of cytosolic reductase kinase.

Cytosols from human liver containing reductase kinase activity of two separate patients (o, and Δ) were desalted in buffer B, and 50 µl (42 µg, o; 16 µg,Δ) were assayed for reductase kinase activity in buffer containing 80 µl of 5 mM imidazole (pH 7.4), 50 mM NaCl, 1 mM DTT, 60 mM NaF, 1.25 mM ATP, 2.5 mM Mg acetate. The intrinsic reductase kinase activities of the cytosol from the two liver preparations are shown on the vertical axis.

INACTIVATION; 50 µl (228 µg, o; 88µg, Δ) of desalted cytosol were incubated in a total volume of 250 µl with either 50 mM NaCl + phosphatase (189 µg) or 50 mM NaF + phosphatase as a control for 15 min at 37°C. At the end of incubation, the reaction was terminated by the addition of 20 µl of .5 M NaF, or 20 µl of .5 M NaCl to the control samples. 50 µl aliquots from each tube were assayed for reductase kinase activity as outlined above.

REACTIVATION: Samples (50 µl) of phosphatase treated cytosols were incubated at 37°C for 15 min with reductase kinase kinase (152 µg), 1.25 mM ATP, 2.5 mM Mg acetate, 60 mM NaF, 50 mM NaCl and 80 µl of 5 mM imidazole buffer (pH 7.4). Control tubes had all the components except RKK. Reductase kinase free microsomal HMG-CoA reductase (178 µg protein, see Materials and Methods) was added to each incubation and the incubation was continued for an additional 15 min. The reaction was terminated by the addition of 23-fold excess of EDTA (in relationship to Mg^{++}) and HMG-CoA reductase enzymic activity determined. One unit of reductase kinase activity is defined as the quantity of kinase which produces a decrease of one pmol of HMG-CoA reductase activity under standard assay conditions.

DISCUSSION

Results described in this communication represent the initial demonstration of the presence of human hepatic HMG-CoA reductase in active and inactive (phosphorylated) forms. When microsomes were prepared under conditions that prevent interconversion of active and inactive forms (50 mM NaF and 30 mM EDTA) microsomal enzyme is isolated primarily in an inactive form (expressed enzyme activity). Treatment of these microsomal preparations with phosphatase converts HMG-CoA reductase to the fully active enzyme (total enzymic activity). It is likely that *in vivo* hepatic HMG-CoA reductase activity in man is modulated by short-term mechanism involving reversible inactivation. Depending upon the physiological state of the patients, the ratio of active and inactive forms of the enzyme may change significantly.

Data presented in this report suggest that the enzymic activity of microsomal human liver HMG-CoA reductase can be rapidly modulated *in vitro*. The inactivation of HMG-CoA reductase is catalyzed by a protein kinase, RK and ATP-Mg, while activation (dephosphorylation) requires phosphoprotein phosphatase.

In vitro phosphorylation of purified HMG-CoA reductase was established by analysis of [^{32}P]-labeled HMG-CoA reductase. A single peak of protein bound radioactivity coincident with the enzymic activity of purified HMG-CoA reductase was observed when the samples were analyzed by polyacrylamide gel electrophoresis under non-denaturing conditions. Analysis of [^{32}P]-labeled protein by chromatography and by NaDodSo$_4$ gel electrophoresis revealed that protein-bound radioactivity was associated with the purified HMG-CoA reductase. Partial dephosphorylation of partially phosphorylated HMG-CoA reductase was associated with the loss of the radioactivity and restoration of enzymic activity of HMG-CoA reductase. These results provide direct evidence for the *in vitro* phosphorylation-dephosphorylation of the human enzyme. In addition, if the assay of freshly isolated HMG-CoA reductase is a true reflection of the *in vivo* enzymic activity, it is probable that most of the interconvertible forms of HMG-CoA reductase *in vivo* is stored in a phosphorylated or inactive form. Thus, *in vivo* dephosphorylation of inactive

HMG-CoA reductase would increase the enzymic activity many fold (short-term control) in the absence of modulation of de novo protein synthesis and/or degradation (long-term control).

The present studies also provide evidence that human RK, the enzyme which catalyzes the phosphorylation and inactivation of the HMG-CoA reductase, also exists in active and inactive forms. Inactivated microsomal or cytosolic RK with or without ATP-Mg failed to inhibit the enzymic activity of microsomal or purified HMG-CoA reductase. Incubation of both microsomal and cytosolic inactivated (dephosphorylated) RK with ATP-Mg and a second kinase designated RKK restored (via phosphorylation) the ability of RK to inactivate (phosphorylate) the enzymic activity of HMG-CoA reductase.

Based on the combined results presented in this communication we conclude that the enzymic activity of human liver HMG-CoA reductase can be modulated in vitro by a bicyclic cascade system involving reversible phosphorylation of HMG-CoA reductase and RK. In the in vitro system the regulation of HMG-CoA reductase consists of an open type of bicyclic cascade (Fig. 4). The bicyclic cascade is composed of both a RK cycle and HMG-CoA reductase cycle. The

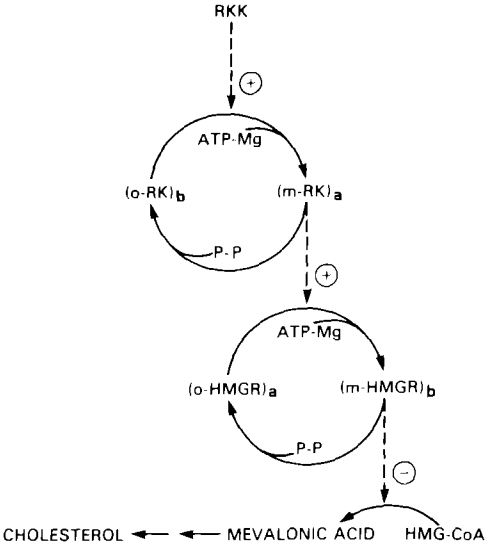

Figure 4. Schematic representation of the modulation of the enzymic activity of human liver HMG-CoA reductase. RKK, reductase kinase kinase; $(o\text{-}RK)_b$ and $(m\text{-}RK)_a$, unmodified inactive and modified (phosphorylated) active forms of reductase kinase, respectively; $(o\text{-}HMGR)_a$ and $(m\text{-}HMGR)_b$, unmodified active and modified (phosphorylated) inactive forms of HMG-CoA reductase, respectively; P-P, phosphoprotein phosphatase.

initial cascade involves the conversion of unmodified or less active RK $(o\text{-}RK)_b$ to its modified and more active form $(m\text{-}RK)_a$. This conversion is catalyzed by active RKK. The active form of RK $(m\text{-}RK)_a$ catalyzes the phosphorylation of HMG-CoA reductase which is associated with the conversion of an active form $(o\text{-}HMGR)_a$ to a less active form $(m\text{-}HMGR)_b$ of HMG-CoA reductase. The activation of $(m\text{-}HMGR)_b$ and inactivation of $(m\text{-}RK)_a$ are catalyzed by a phosphoprotein phosphatase (P-P) (Fig. 4). This bicyclic cascade system in human liver is similar to the systems which we have previously established in rat liver (3,5).

It is intriging to postulate that a similar regulation of HMG-CoA reductase and cholesterol biosynthesis may occur *in vivo* in man. Additional studies will be required to improve our understanding of the molecular mechanisms involved in the regulation of hepatic HMG-CoA reductase activity in man.

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