Phosphorylation of HMG-CoA reductase induced by mevalonate accelerates its rate of degradation in isolated rat hepatocytes

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Incubation of rat hepatocytes with 10 mM mevalonate produces a decrease in HMG-CoA reductase activity and in the rate of synthesis of both monomeric and dimeric HMG-CoA reductase, and an increase in the rate of degradation of the monomeric form without significant change in that of the dimeric form. Since mevalonate promotes a short-term phosphorylation of the monomeric form without affecting the dimeric form, it is suggested that the mechanism of degradation of reductase is controlled by its phosphorylation state.

Hydroxymethylglutaryl-CoA reductase *Enzyme degradation* *Mevalonate* *Phosphorylation*

1. INTRODUCTION

3-Hydroxy 3-methylglutaryl-CoA reductase (EC 1.1.1.34), the rate-limiting enzyme of cholesterol and isoprenoid biosynthetic pathways [1], is bound to the endoplasmic reticulum of animal cells through 7 membrane-spanning regions [2], with its catalytic active center projected into the cytoplasm. We have proposed that there is an equilibrium between a monomeric form of 97 kDa [3] and a dimeric form composed of two monomers linked through a carbohydrate chain [4]. The dimeric form with an approximate molecular mass of 200 kDa has been recently confirmed using a radiation mechanism for inactivation [5]. The different rates of degradation of the monomeric and dimeric forms suggest that dimerization can be a mechanism of control of the enzymic activity [4].

In several papers, mevalonate, the product of the reductase-catalyzed reaction, has been shown to decrease the enzymatic reductase activity in different cell lines [6–8] and whole rat liver [9]. Here, we present evidence that, in rat hepatocytes, mevalonate changes the rate of degradation of the monomeric form of reductase without significantly altering that of the dimeric form. Since phosphorylation of the reductase monomer is increased with respect to the control, while the dimer remains at the same phosphorylation level, we suggest that mevalonate changes the rate of degradation of HMG-CoA reductase through a phosphorylation mechanism.

2. MATERIALS AND METHODS

Hydroxymethyl[^{14}C]glutaric acid,CoA,NADP$^+$, glucose-6-phosphate dehydrogenase and silica gel plates were obtained from the sources described in [10]. Mevalonate was from Sigma and ^{32}P (carrier-free) from Amersham. Other chemicals were obtained from the sources indicated in [4].

The techniques used here, namely, isolation of rat hepatocytes, incubation of hepatocytes with

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Abbreviations: PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethylsulfonyl fluoride; HMG-CoA, hydroxymethylglutaryl-CoA

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[^{35}S]methionine, immunoprecipitation of the solubilized HMG-CoA reductase, SDS-PAGE and fluorography have been reported [4].

The phosphorylation experiments were performed by incubating 5 × 10^6 cells for 90 min in a final volume of 1.5 ml of low-phosphate (0.1 mM) Hank's buffer supplemented with amino acids and cofactors as described by Eagle [11] to which 1 mCi carrier-free ^{32}P was added. Thereafter, aliquots of cells with or without 10 mM mevalonate were incubated for several periods of time. After solubilization, immunoprecipitation and SDS-PAGE, the radiolabeled bands detected by autoradiography were cut and the radioactivity incorporated into protein quantitated taking advantage of the Cerenkov effect.

HMG-CoA reductase activity was assayed as in [12] in a preparation of hepatocytes obtained as follows: cells (15 × 10^6) were solubilized for 30 s in 1 ml buffer (1% Triton X-100, 5 mM EDTA, 5 mM EGTA, 0.1 M NaCl, 0.1 mM leupeptin, 0.01 mM antipain, 15 μg/ml aprotinin, 2 mM PMSF, 5 mM DTT, 0.01 M phosphate; pH 7.5). The suspension was then centrifuged at 12000 × *g* for 15 min and the supernatant incubated for 150 min at 37°C.

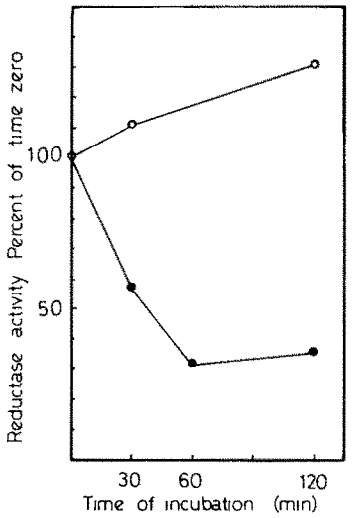

Fig.1. Influence of mevalonate on HMG-CoA reductase activity. Isolated rat hepatocytes were incubated with (●) or without (○) 10 mM mevalonate. At different times, HMG-CoA reductase activity was determined and is shown as a percentage of the reductase activity at zero time (25 pmol/min per mg protein).

3. RESULTS

Incubation of rat hepatocytes with 10 mM mevalonate produced a time-dependent decrease in HMG-CoA reductase activity reaching the maximum value at 60 min (fig.1). Using immunoprecipitation of a biologically radiolabelled enzyme, we investigated whether mevalonate affected the rate of synthesis of the monomeric and dimeric forms of reductase at the same level. In order to do so, we incubated rat hepatocytes with 10 mM mevalonate and [^{35}S]methionine for different periods of time, and the reductase was then immunoprecipitated as described in section 2. The results are shown in fig.2. Mevalonate appeared to have produced a decrease in the rate of synthesis of the monomeric and dimeric forms. In addition, the newly synthesized monomeric and dimeric reductase incubated without mevalonate (controls) remained at the same ratio for 40 min.

The effect of mevalonate on the degradation of radiolabeled reductase was determined in pulse-chase experiments. Cells were incubated with [^{35}S]methionine for 60 min. The cells were then pelleted and resuspended in a medium without labeled methionine and with 2 mM unlabeled methionine and incubated with or without 10 mM mevalonate for different periods of time. At the times indicated in fig.3, the enzyme was immuno-

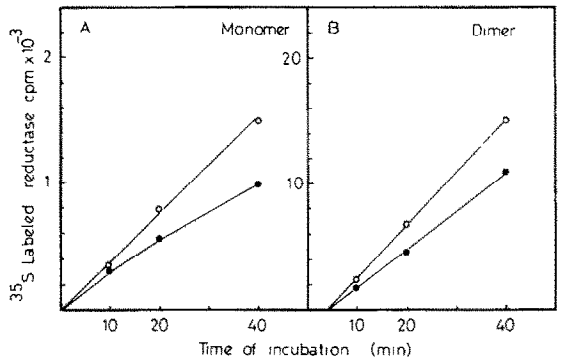

Fig.2. Effect of mevalonate on the synthesis of the monomer and dimer forms of HMG-CoA reductase. Rat hepatocytes were incubated with [^{35}S]methionine (○) or with a combination of [^{35}S]methionine plus mevalonate 10 mM (●). At the indicated times, reductase from 3 × 10^6 cells was immunoprecipitated and the radioactivity present in the 104 and 180 kDa peptides was measured. (A) Monomer, (B) dimer.

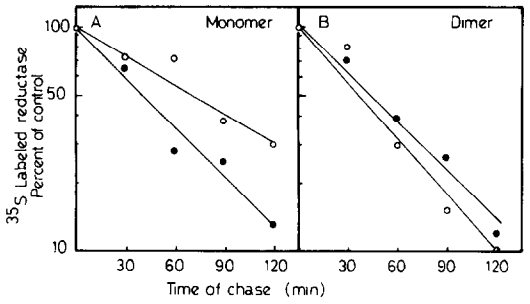

Fig.3. Influence of mevalonate on the degradation of monomeric and dimeric forms of HMG-CoA reductase. Rat hepatocytes were incubated for 60 min in the presence of 25 μCi [^{35}S]methionine 10^6 cells, then washed and resuspended in a medium containing unlabeled 2 mM methionine and incubated in the absence (○) or presence (●) of 10 mM mevalonate. At the indicated times, the enzyme from 4 × 10^6 cells was immunoprecipitated and the ^{35}S content of the 104 kDa (A) and 180 kDa (B) polypeptides was measured as described in section 2. The percentage of radioactivity corresponding to zero time (3634 cpm, monomer; 24150, dimer) is represented on the ordinate.

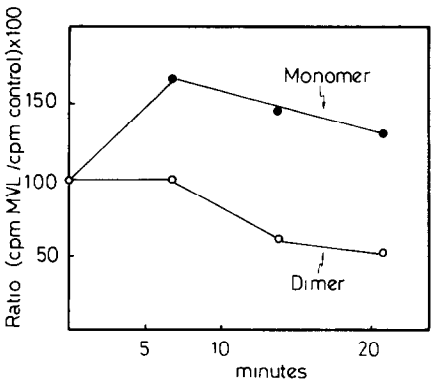

Fig.4. Effect of mevalonate on the phosphorylation of the monomeric and dimeric forms of HMG-CoA reductase. Rat hepatocytes (5 × 10^6 cells) were preincubated for 90 min in the presence of 1 mCi $^{32}PO_4$, and further incubated at different times in the absence or presence of 10 mM mevalonate. At the indicated times, the enzyme was immunoprecipitated from an equal aliquot of cells (5 × 10^6) and the ^{32}P content of the 104 kDa (●) and 180 kDa (○) polypeptides was measured as described in section 2. The ordinate represents the percentage of the ratio of cpm of the immunoprecipitated polypeptide in the presence of mevalonate vs cpm in its absence. Radioactivity corresponding to zero time: 485 cpm, monomer; 500 cpm, dimer.

precipitated and the radioactivity corresponding to the 180 and 104 kDa bands was quantitated. As can be seen in fig.3A, mevalonate decreased the half-life of the monomer (40 vs 70 min). Degradation in the dimer was slightly stabilized by mevalonate (fig.3B). Since mevalonate had no effect on the degradation of total radiolabeled proteins (not shown), our results indicated a specific effect of mevalonate on the degradation of the monomeric form of HMG-CoA reductase without significantly affecting the dimeric form.

In addition to investigating the effect of mevalonate on the half-life of both monomeric and dimeric forms, we assayed the effect of this compound on the phosphorylation state of each of two reductases. Accordingly, we incubated isolated rat hepatocytes with 1 mCi $^{32}PO_4$ for 90 min. Thereafter, aliquots of cells were incubated for different periods of time with or without 10 mM mevalonate, and processed as described in section 2. Fig.4 shows that mevalonate increases the phosphorylation state of the monomeric reductase by 65% over the control in 7 min, while phosphorylation decreased over longer periods of time. Unlike the monomer, the dimer practically did not change its phosphorylation state in 7 min, and over longer periods of time decreased to lower levels than the control. By comparing these results with those of fig.3, it was observed that an increase in phosphorylation of the monomeric reductase correlated with a more active rate of degradation (shorter half-life). The same applied to the dimer: small decreases in phosphorylation of the dimer produced by mevalonate caused significant small increases in the half-life of this form of HMG-CoA reductase.

4. DISCUSSION

Mevalonate has been shown to be an effective repressor of HMG-CoA reductase activity in several cell lines and whole rat liver [6–9]. This metabolite, together with LDL, completely represses the expression of the gene of HMG-CoA reductase in compactin-treated cells [6]. However, the mechanism of this enhanced decrease of enzyme activity after a few minutes of administration of mevalonate has not been explained in previous papers.

Our results show that mevalonate appears to increase the rate of degradation of the monomeric form, and to a lesser extent, to decrease degradation of the dimeric form. The observation that the increase of phosphorylation of the monomeric form caused by mevalonate correlates with its augmented rate of degradation, and that small decreases of phosphorylation of the dimer with respect to the control induced by mevalonate also correlate with a small enhancement of the stability of this dimeric form, suggests that there is a mechanism for the degradation of HMG-CoA reductase controlled by its phosphorylation state. These results agree with those of Parker et al [13]. These authors showed that in in vitro experiments phosphorylated microsomal reductase was much more easily degraded by the calcium-dependent protease calpain II than the unphosphorylated reductase. The correlation between phosphorylation of enzymes and accelerated rate of degradation has also been shown for pyruvate kinase [14] and fructose-1,6-bisphosphatase [15].

While it has been reported that 25-hydroxycholesterol increases the degradation of reductase without changing its phosphorylation state [16], it has also been shown that cholesterol influences reductase degradation when its NH_2 terminus is bound to the endoplasmic reticulum through 7 spanning regions. The absence of binding of reductase to the endoplasmic reticulum, as a consequence of a mutation of the reductase gene, prevents cholesterol-induced degradation [17,18]. Independently of the fact that alternative mechanisms of control of reductase degradation may exist, the phosphorylation–dephosphorylation cycle appears to be a control mechanism for reductase degradation, particularly when mevalonate is present in excess in the incubation medium.

ACKNOWLEDGEMENTS

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