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DESENSITIZATION TO ATP-Mg INHIBITION OF 3-HYDROXY-3-METHYLGLUTARYLCoA REDUCTASE BY HEAT TREATMENT OF MICROSOMES

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Summary:- HMGCoA reductase is found to be inhibited by palmitylCoA and free CoA. The inhibition of this enzyme by ATP-Mg, but not by palmityl CoA, is lost on preincubation of microsomes at 50°C for 15 min.

3-Hydroxy-3-methylglutaryl (HMG)CoA reductase, the key enzyme in cholesterol biosynthesis, is subject to regulation by a variety of factors *in vitro* and conditions *in vivo* (1,2). The activity of this enzyme was reported to increase during isolation of microsomes (3), on incubation of liver homogenates at 37° before separating microsomes (4), or by incubation of microsomes with soluble supernatant fraction (5), sodium sulfite, magnesium chloride or EDTA (6). We were not able to reproduce some of these effects as maximum activity was probably already attained in our microsomal preparations. Addition of sodium flouride to the homogenizing medium was reported to give microsomal preparations of low activity which could then be increased by adding the "activating protein" isolated from cytosol (3). The differences in activity levels in microsomes in different laboratories may indeed be a reflection of preventing inactivation or aiding activation to different degrees occurring during homogenization and isolation.

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The activity of HMGCoA reductase can be inhibited on incubation of microsomes with ATP-Mg (7-10) and this can be prevented by chelating Mg^{++} with EDTA (3) or by competition of ATP-Mg with AMP, adenosine or Ca^{++} (11). A set of activating and inactivating proteins have been described for this enzyme in different sources (3,7,10,12). The need for ATP-Mg, the apparent catalytic nature of the inactivation process (10), and the fluoride-sensitivity of the activation system are strongly suggestive of phosphorylation-dephosphorylation cycle known to operate for a number of regulatory enzymes. However, no definitive evidence is yet available and mutually opposite claims have been made (3,8). Addition of protein kinase inhibitor had no effect on the ATP-Mg inhibition of HMGCoA reductase (unpublished data).

It was found that addition of fatty acids (13) and palmitylCoA, arachidonylCoA and free CoA (14) inhibited incorporation of acetate into sterols in a system containing microsomes and soluble supernatant. Since incorporation of mevalonate was unaffected, it was conjectured that HMGCoA reductase must have been the site of inhibition. Fatty acids alone, however had no effect on the activity of the enzyme in isolated microsomes (13), and acylCoA compounds were the obvious inhibitors of the enzyme but were not tested.

Arising out of these observations we sought answers to the following questions: Will the heat treatment of microsomes affect the ATP-Mg inhibition of the enzyme? Will palmitylCoA inhibit the enzyme? Will the formation of acylCoA explain the ATP-Mg inhibition?

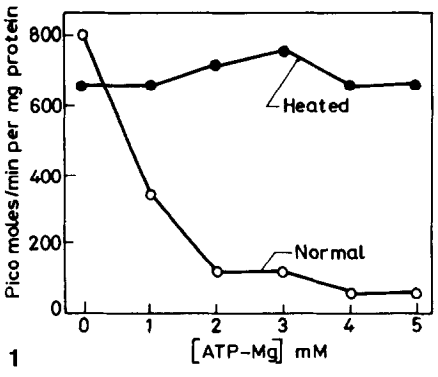
Microsomes were prepared from livers of rats killed at midnight to obtain activity at the peak of the rhythm and assayed for HMGCoA reductase. All the materials and methods employed are the same as described before (15). In the first set of experiments, (15 mg

protein/ml) were suspended in buffer of pH 7.4 (EDTA, 30 mM; NaCl, 70 mM; potassium phosphate, 50 mM; dithiothreitol, 10 mM) and incubated at 50°C for 15 min. The mixture was cooled in an ice-bath and the enzyme activity was measured at 37°C in the absence and presence of ATP-Mg (1-5 mM). Heat treatment decreased the activity by about 20% whereas the capacity for inhibition by ATP-Mg in such preparations was completely lost (Fig 1).

This effect is dependent on time and reached completion in 15 min. at 50°C. At higher temperatures the enzyme activity was also lost. This finding of heat desensitization offers a ready method of preparing HMGCoA reductase, insensitive to ATP-Mg, that can be used as the substrate for characterizing the "ATP-Mg dependent inactivating enzyme". Whenever preincubation was employed in the experiments reported earlier, the activity tested subsequently in presence of ATP-Mg in the reaction mixtures would be higher compared to others without the preincubation because of lack of inhibition and therefore will give an apparent activation. The less extensive inactivation by ATP-Mg in microsomes prepared by special procedures (3,4,10) which involved heating before isolation of microsomes is also explained by the desensitization effect.

In the second set of experiments, the enzyme activity in microsomes was tested in presence of palmitylCoA upto 2 mM concentration. As expected, it showed considerable inhibition (Fig 2). Free CoA also showed inhibition but free palmitate had no effect. The concentrations required for 50% inhibition were approximately 0.8 mM and 1.8 mM for palmitylCoA and CoA, respectively. The inhibition by palmitylCoA was also found with heat-treated microsomes (Fig 2).

Treatment of ATP-Mg inactivated microsomes with hydroxylamine, which splits CoA from acylCoA, had no effect. In view of this and



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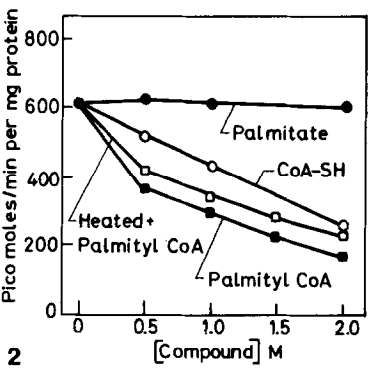

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Fig 1. Effect of ATP-Mg on heated and normal microsomal HMGCoA reductase Midnight microsomes (15 mg protein/ml) prepared from rat liver were suspended in buffer of pH 7.4 (EDTA, 30 mM, potassium phosphate 50 mM, NaCl, 70 mM., and dithiothreitol, 10 mM). Heated microsomal HMGCoA reductase was obtained by incubating the microsomes at 50°C for 15 min in a water-bath. HMGCoA reductase assay was carried out as described previously (15). ATP-Mg was added as a neutral solution. All values represented in the figure are mean of duplicate measurements.

Fig 2. Effect of palmityl CoA, coenzyme A and palmitic acid on heated and normal microsomal HMGCoA reductase Palmityl CoA and coenzyme A were added to assay mixture as neutral solutions. Palmitic acid was added as suspension in the buffer. Other conditions are same as given under Fig 1.

the differential effect of the two inhibitors to heat treatment the requirement of ATP-Mg in the inactivation is probably not for producing acylCoA. Other results are indicative of a possible competition by free CoA with the substrate, HMGCoA. If a kinase or acyl activation type of reaction is implicated, it will be a special one using ADP, since both ATP and ADP are effective, (3,10), and equally in our experiments (11), and ATP is hydrolyzed to ADP by microsomes. One possibility of using ADP is by polynucleotide phosphorylase yielding polyadenylic acid but using this product in assay of HMGCoA reductase showed no inhibition (unpublished data).

Neither addition of ATP-Mg nor heat treatment had changed the small rate of hydrolysis of added substrate, HMGCoA. Therefore the observed changes are not due to relative decrease in substrate con-

centration. PalmitylCoA is known to inhibit glucose-6-phosphate dehydrogenase, added in the reaction mixture to regenerate NADPH (16). Under conditions of the present experiment 55% inhibition was obtained in its activity by the added palmitylCoA. But since an excess of this enzyme was added, the residual amount was still sufficient to sustain NADPH throughout the reaction period in the control and experimental samples and therefore this does not account for the reduction of HMGCoA reductase activity.

The present experiments add two interesting regulatory properties to the list of effects observed for HMGCoA reductase. The heat desensitization of ATP-Mg inhibition appears to be a case of denaturation of the microsomally bound inactivating system but it cannot be ruled out whether a conformational change in HMGCoA reductase itself had occurred thus making it insensitive, as in the case of isoleucine inhibition of threonine deaminase (17). One can doubt whether inhibition by palmitylCoA will have physiological significance especially since high cholesterol and high fat go together. There are a large number of examples of effects of palmitylCoA on glycolytic pathway, citric acid cycle enzymes and enzymes involved in lipid metabolism. The present experiments introduce the isoprene pathway into the list.

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