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REGULATION OF 3-HYDROXY-3-METHYLGLUTARYL COENZYME A
REDUCTASE BY REVERSIBLE PHOSPHORYLATION

by

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Submitted to the Faculty of the Graduate School in partial fulfillment of the requirement for the Degree of Doctor of Philosophy in the Department of Biochemistry, Indiana University School of Medicine, June 1979.

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ABSTRACT

Rat liver microsomal HMG-CoA reductase is inactivated in a time dependent manner by incubation with ATP and Mg^{+2} . Incubation of the inactive reductase with purified rat liver phosphorylase phosphatase fully restored reductase activity. During purification of the phosphatase, the reductase activating activity and the phosphorylase phosphatase activity copurified quite closely. Both activities were inhibited in a parallel fashion by sodium flouride and by the spontaneously active heat stable phosphatase inhibitor protein. These studies show that a phosphorylation-dephosphorylation event is associated with interconversion of the reductase.

The reductase inactivating enzyme (reductase kinase) was extracted from the microsomes by repeatedly washing the membranes with neutral buffer. After concentration by ultrafiltration, this extract was used as a source of soluble reductase kinase. The washed microsomes were used to assay the soluble kinase. Both microsomal and soluble reductase kinase were inactivated in a time dependent fashion by incubation at 37° . Inactivation of the kinase was inhibited by both flouride and phosphate. Incubation with purified phosphorylase phosphatase greatly enhanced the rate of inactivation of the soluble kinase.

Reductase kinase activity was restored by a second incubation in low ionic strength buffer in the presence of ATP and Mg^{+2} . Reactivation did not take place in the high ionic strength buffer routinely used to assay reductase and reductase kinase activities. The rate of reactivation of the kinase was not enhanced by cAMP. The rate of

activation of the reductase kinase was markedly enhanced by the particulate glycogen-protein complex in a dose dependent fashion. The reductase kinase activating enzyme (reductase kinase kinase) was also found in the cytosol. The cytosolic enzyme was bound to a phosphocellulose column and was then eluted with .75M sodium chloride.

These data are consistent with a model in which both HMG-CoA reductase and reductase kinase are regulated by reversible phosphorylation. The active form of the reductase is dephosphorylated while that of the reductase kinase is phosphorylated. Reductase kinase is activated by a cAMP-independent protein kinase which is present in the soluble reductase kinase preparation, in the cytosol and in the particulate glycogen-protein complex. Both the reductase and the reductase kinase are dephosphorylated by phosphorylase phosphatase.