Regulation of HMG-CoA Reductase

Edited by

Benjamin Preiss

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Preface

The need for a volume dedicated solely to the regulation of HMG-CoA reductase is evidenced by the growth of interest in this enzyme during the past five years. This interest is due in part to newly developed methodologies that have provided efficient antibody and nucleic acid probes to assess the synthesis, degradation, and state of activity of the enzyme. The objective of this volume is to present recent developments in the field and to summarize the current state of knowledge on the regulation of this enzyme.

The book is addressed to investigators and graduate students interested in the regulation of HMG-CoA reductase and cholesterol biosynthesis, offering them an integrated view of subjects outside their specialized fields. As an introduction to this important area of research, the book is also addressed to those involved in medical research as well as to specialists in cardiology, endocrinology, and nutrition. Finally, as a model of an important regulatory enzyme, this volume should be of special interest to biochemists, cell biologists, and physiologists working in unrelated areas.

The first chapter deals with the role of cholesterol in the regulation of its own biosynthesis. Cholesterol feedback regulation of reductase is discussed with reference to the recent redefinition of the enzyme as an integral membrane protein. The second chapter reviews work involving compactin and related competitive inhibitors of HMG-CoA reductase. These compounds have become indispensable research tools. They have also been shown to lower plasma LDL cholesterol in different species, including man. Chapter 3 describes reversible phosphorylation of HMG-CoA reductase in hormonal and other short-term physiological changes. Phosphorylation of reductase is discussed as a possible first step in the degradation of the enzyme. Chapter 4 discusses the structure and properties of HMG-CoA reductase, including evidence for modulation of enzyme activity and structure unrelated to phosphorylation. Chapter 5 reviews the regulation of reductase in the biosynthesis of ubiquinone, dolichols, and isopentenyl-tRNA. Chapter 6 reviews regulation of reductase activity in extrahepatic tissues. Chapter 7 describes the contributions made through the use of cultured cells and their genetic manipulation, emphasizing the great potential of this approach. Chapter 8 addresses the current status of the LDL pathway and related mechanisms, and Chapter 9 deals with the regu-

lation of human hepatic HMG-CoA reductase and the special problems inherent in work with human tissues.

I wish to thank all of the contributors for their collaboration, with a special word to Dr. David M. Gibson for his continued encouragement. Thanks are also due to Drs. Johann van Lier and Gordon Fisher of Université de Sherbrooke, to Dr. Donald J. McNamara of the Rockefeller University, to Dr. C. Pandian, and to Sandra Forster Preiss, my wife.

Benjamin Preiss

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Membrane-Mediated Control of Reductase Activity

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I. INTRODUCTION

Gould (1951) was the first to show that incorporation of [^{14}C]acetate into cholesterol in livers of dogs or rabbits is considerably reduced when the animals are fed a cholesterol-supplemented diet. This effect has been confirmed since then in a wide variety of mammals tested, and there is complete agreement that the inhibition of hepatic cholesterogenesis under these conditions is brought about by a parallel change in the activity of 3-hydroxy-3-methylglutaryl-Coenzyme A (HMG-CoA) reductase. In most mammals, the effect of dietary cholesterol is limited to the liver, and since

the liver is quantitatively important in the economy of cholesterol in the body as a whole, this model is popular for investigations into the mechanisms of regulation of cholesterogenesis. Recently, however, the incubation of isolated cells or various cells in culture in the presence of cholesterol has demonstrated uptake of cholesterol by the cells and a similar reduction in cellular cholesterol synthesis and in the activity of HMG-CoA reductase. These last studies have enormously contributed to our knowledge of the influx and efflux of cellular cholesterol and the transport of lipoprotein cholesterol to extrahepatic tissues as well as to the liver.

To describe the effect of cholesterol on cellular cholesterogenesis, the term "feedback inhibition" was introduced; but at the present time, the molecular mechanism by which cholesterol controls the activity of HMG-CoA reductase is still the subject of intense investigation. The term feedback inhibition is borrowed from the microbial regulation of certain metabolic pathways, and is defined as inhibition of the rate of a metabolic pathway by direct allosteric suppression of the activity of the rate-limiting enzyme by the end product of the pathway (Monod and Jacob, 1961). Accordingly, Siperstein and Fagan (1964) suggested that HMG-CoA reductase is an allosteric enzyme and that dietary cholesterol causes allosteric inhibition of the enzyme. The concept of feedback inhibition implies that there is a pool of nonesterified cholesterol in the immediate environment of HMG-CoA reductase, and that changes in the size of this pool following the administration of dietary cholesterol or in other experimental conditions directly modulate the activity of the enzyme. However, earlier attempts to show inhibition of HMG-CoA reductase in subcellular fractions by the addition of cholesterol in various physical forms were unrewarding (Linn, 1967; Kandutsch and Packie, 1970; Shapiro and Rodwell, 1971; Brown *et al.*, 1974). This led Rodwell and colleagues (1973) to suggest that dietary feedback inhibition is mediated by repression of enzyme synthesis rather than by direct inhibition of preexisting enzyme. This mechanism of regulation of HMG-CoA reductase at the transcriptional or translational level would require that the relation between enzyme concentration and enzyme activity in the microsomal fraction from rats in various experimental conditions, including administration of dietary cholesterol, is the same.

The demonstration that cholesterol feeding produces changes in the temperature-induced kinetics of liver microsomal HMG-CoA reductase that are consistent with a decrease in the fluidity of the membrane to which the enzyme is attached (Sabine and James, 1976) focused attention on the role of the membrane in the regulation of HMG-CoA reductase activity. The possibility that changes in membrane fluidity brought about

by changes in the concentration of nonesterified cholesterol are responsible for direct modulation of HMG-CoA reductase activity is certainly an attractive one, especially in view of the physiological role common to all cells of cholesterol as a component of cellular membranes and as such an important factor in adjusting membrane fluidity. Consistent with this possibility, a recent report (Ness *et al.*, 1982) indicated that HMG-CoA reductase is an intrinsic membrane protein. Previously, the facile solubilization of an active enzyme by freezing and thawing or its facile extraction by buffers under certain conditions had been taken as evidence that the enzyme is a loosely bound extrinsic protein. However, recent evidence suggested that proteinases can be involved in such solubilization of HMG-CoA reductase (Ness *et al.*, 1981; Liscum *et al.*, 1983; Chin *et al.*, 1982a) to yield fragments in the 50,000–62,000-dalton range, whereas enzyme solubilized in the presence of inhibitors of proteinases has a subunit molecular weight of 90,000.

The molecular mechanism by which the concentration of nonesterified cholesterol modulates the activity of HMG-CoA reductase in endoplasmic reticular membrane is subject to intense investigation. However, the thermotropic properties of this enzyme, like those of a number of other membrane-associated functions, are influenced by the lipid composition of the membrane. Therefore, HMG-CoA reductase can be used as an intrinsic probe of endoplasmic reticular membrane to study the relationship between enzyme activity and the concentration of nonesterified cholesterol in the environment of the enzyme. It is impossible at present to obtain a direct measure of the concentration of nonesterified cholesterol interacting with the enzyme using an alternative approach.

II. REGULATION OF HMG-CoA REDUCTASE ACTIVITY

The concentration of HMG-CoA reductase protein associated with the endoplasmic reticular membrane can depend on the rate of enzyme synthesis and the rate of enzyme degradation. In mammalian systems that show relatively stable mRNA (Ochoa and de Haro, 1979), protein synthesis can be controlled at both the transcription and the translation levels. Regulation only at the transcriptional or translational level would imply that the relation between HMG-CoA reductase activity and enzyme concentration is the same in microsomal fractions from animals in various experimental conditions (including animals that received dietary cholesterol).

A. Regulation at the Transcriptional and Translational Levels

Earlier studies on the diurnal rhythm of HMG-CoA reductase supported the concept that changes in the activity of liver microsomal fraction, prepared from rats killed at various times during the light cycle, are due to changes in the concentration of the enzyme (Rodwell *et al.*, 1973, 1976). The changes in enzyme concentration have been attributed to changes in the rate of enzyme synthesis rather than to changes in the rate of enzyme degradation (Higgins *et al.*, 1974). An increase in the concentration of nonesterified cholesterol associated with chromatin that has been observed prior to the diurnal decrease in enzyme activity in rat liver (Erickson *et al.*, 1975) provided the evidence for regulation at the transcriptional level. Moreover, the use of protein synthesis inhibitors in studies of the effect of low density lipoprotein (LDL) on the activity of the enzyme in cultured human fibroblasts suggested that loss of enzyme activity due to LDL cholesterol can be attributed to a decrease in the rate of enzyme synthesis (Brown *et al.*, 1974). These studies have estimated a half-life for the enzyme of ~4 h.

Studies using oxysterols to suppress HMG-CoA reductase activity indicate that changes in the rate of enzyme degradation may play a role in the concentration of enzyme protein on the membrane. Thus, it was shown (Bell *et al.*, 1976; Erickson *et al.*, 1978; Chang *et al.*, 1981) that the half-life of the enzyme calculated from the decline in activity due to 25-hydroxycholesterol is shorter than that due to cycloheximide. The possibility that 25-hydroxycholesterol inhibits preexisting enzyme together with its effect on enzyme synthesis was suggested, and was supported in one study by immunotitration studies (Beirne *et al.*, 1977). However, other studies have failed to show a dissociation between enzyme activity and enzyme concentration following suppression of enzyme activity by 25-hydroxycholesterol (Faust *et al.*, 1982; Sinensky *et al.*, 1982). Consistent with this, Cavenee *et al.* (1981) showed that 25-hydroxycholesterol failed to inhibit HMG-CoA reductase in enucleated cells. To explain the short half-life of the enzyme following suppression by 25-hydroxycholesterol, it was suggested that enzymes responsible for the degradation of HMG-CoA reductase can have a short half-life (Chen *et al.*, 1982). The treatment of cells with 25-hydroxycholesterol inhibits enzyme synthesis, and the preexisting enzyme continues to be degraded at a rapid rate. In contrast, treatment of the cells with cycloheximide influences both synthesis and degradation of enzyme protein, thus providing a half-life that is longer.

The increase in enzyme activity seen after the treatment of cells with compactin or delipidated serum is also associated with an increased half-

life of the enzyme (Chang *et al.*, 1981; Alberts *et al.*, 1982), and it has been suggested that under these conditions there is an inhibition of degradation.

The regulation of the enzyme concentration at the level of transcription has been recently investigated by direct assay of mRNA for the enzyme using cDNA probes (Chin *et al.*, 1982b). These studies showed that suppression of HMG-CoA reductase protein concentration in UT-1 cells, following treatment of the cells with LDL or oxysterols, corresponds to a decreased concentration of mRNA that coded for a protein immunoprecipitable by anti-HMG-CoA reductase antibody. Moreover, monoclonal antibody directed against HMG-CoA reductase and cDNA to the reductase mRNA, prepared from these cultured hamster cells, were used successfully to study regulation of the enzyme mRNA in rat liver (Liscum *et al.*, 1983). Thus, hepatic reductase activity was increased 45-fold when rats were fed cholestyramine and mevinolin (Tanaka *et al.*, 1982; Liscum *et al.*, 1983). Under these conditions, the amount of immunodetectable reductase protein rose by 33-fold, and the reductase mRNA became visible by blot hybridization. In the livers of control rats, no reductase mRNA was detectable, whereas when the mevinolin/cholestyramine-treated rats were given dietary cholesterol, reductase activity and immunodetectable protein declined to control levels and the reductase mRNA was reduced to barely detectable levels (Liscum *et al.*, 1983). These findings suggested that the treatment of rats with cholestyramine and mevinolin increases the concentration of reductase protein by increasing the rate of mRNA synthesis, and that cholesterol administration to such induced rats lowers the concentration of hepatic reductase by decreasing the level of mRNA. Cholestyramine and mevinolin could act either by depleting cholesterol and/or a nonsterol metabolite of mevalonate, which act as repressors of transcription, or could trigger the appearance of a positive effector that increases the transcription rate.

B. Posttranslational Control

A number of studies have recently suggested that the relationship between HMG-CoA reductase activity and enzyme concentration is different in the microsomal fraction from animals in various experimental conditions. Such observations imply that direct modulation of preexisting enzyme can contribute to changes in the activity that are associated with transition from one experimental condition to another. However, there can be little doubt that changes in enzyme concentration also contribute to changes in enzyme activity.

Thus, it has been observed that when dietary cholesterol is given to rats at the beginning of the dark period of the light cycle, enzyme activity in the