Dietary Modification of the Activation State of Intestinal 3-Hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) Reductase

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Received May 21, 1984

To ascertain whether the phosphorylation-dephosphorylation reaction is actually involved in the in vivo regulation of intestinal 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, dietary modulation of the activation state of the enzyme was studied in isolated epithelial cells of rats. Substitution of a sucrose-enriched semipurified diet for the commercial non-purified diet caused a significant increase in jejunal activity with a concomitant decrease in ileal activity. Jejunal activity increased without influencing the activation state whereas at the early stage of dietary manipulation, there was a rapid decrease in apparent activity compared to total activity in the ileum, hence the reduction of the activation state. These observations favor the view that the phosphorylation (inactivation) reaction is responsible for the regulation of intestinal HMG-CoA reductase in vivo. In contrast, dietary fat-dependent stimulation of jejunal reductase activity was mainly attributable to an increase in enzyme protein rather than in the level of the activation. The results suggest a complex controlling feature of the cholesterol synthesis in the intestine.

The liver and intestine are perhaps the only tissue which can synthesize cholesterol for circulation. 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase is the rate limiting enzyme of the cholesterol biosynthetic pathway in these tissues. The activity of hepatic HMG-CoA reductase is subjected to regulation by the level of catalytic efficiency as well as the amount of enzyme protein. Since the report of Beg et al. concerning the Mg2+-ATP dependent reversible inactivation of rat liver microsomal HMG-CoA reductase, it is now evident that the phosphorylation-dephosphorylation mechanism is responsible for the intra-cellular short term regulation of hepatic HMG-CoA reductase. With the intestinal enzyme, available information is quite meager in this respect, in particular on the in vivo regulation.

Panini et al. recently demonstrated a bicarbonate dependent reversible modulation of the reductase in isolated rat intestinal epithelial cells and suggested the existence of an interconvertible regulatory mechanism for short term regulation of intestinal cholesterogenesis. Field et al. also reported the in vitro regulation of intestinal microsomal HMG-CoA reductase in which they observed activation of reductase prepared in the presence of NaF. In addition, our in vitro studies evidenced inactivation by Mg2+-ATP of the intestinal reductase and reversible reactivation by a partially purified activator protein prepared from the liver and intestine. Considered together, the catalytic activity of intestinal HMG-CoA reductase is acknowledged to be modulated by the phosphorylation-dephosphorylation reaction in vitro. However, it remains unclear whether this type of regulatory mechanism also operates in vivo.

To ascertain the participation of this type of the mechanism in the regulation of the intestinal reductase in vivo, nutritional manipulations demonstrated to modify the reductase activity were adopted. Thus the present study asked whether the activation state of the intestinal reductase could be rapidly modulated in response to dietary manipulations.
Modulation of the hepatic enzyme was also studied for comparison.

MATERIALS AND METHODS

Materials. [3-14C]-3-Hydroxy-3-methylglutaric acid (Amersham International plc, Buckinghamshire, England) was converted to the anhydride and reacted with coenzyme A (Kyowa Hakko Kogyo Ltd., Tokyo) to yield [3-14C]-HMG-CoA.11,12 The specific activity was adjusted to 5000 dpm/nmol. The other chemicals used were all reagent grade.

Animals and diets. Male Wistar rats (Kyudo Co., Kumamoto) were housed individually in stainless steel cages in a room with controlled temperature (22 to 25°C) and illumination (lighting on 0800 to 2000 hr). Animals were allowed access to experimental diets and water ad libitum. A commercial stock diet (Type NMF, Oriental Yeast Co., Tokyo) and a semipurified diet were employed. The basal composition of the latter was, in weight percent: casein (vitamin-free, ICN Pharmaceutical Inc., Cleveland OH), 20; mineral mixture, 4 and water soluble-vitamin mixture, 1 (both mixtures according to Harper13) were purchased from Oriental Yeast Co., Tokyo); choline chloride, 0.15; cellulose powder (Type E, Toyo Kagaku Sango Co., Tokyo), 2; and sucrose to 100. As a fat source, corn oil, 1 to 10% was added at the expense of sucrose. The diet contained 400 IU vitamin A palmitate, 200 IU vitamin D₃, and 10 mg dl-α-tocopheryl acetate per 100 g. The stated composition of the commercial pellets was, in percentages; crude protein, 27.5; crude fat, 5.3; crude ash, 7.9; crude fiber, 3.4; moisture, 7; carbohydrate, 48.9; and a reasonable amount of vitamins. To investigate whether the changes in HMG-CoA reductase activities induced by dietary manipulations affect the ratio of the active to inactive form, animals were killed periodically after replacing experimental diets and the alteration of the activation state of intestinal or hepatic reductase was determined.

Isolation of intestinal epithelial cells. The animals were killed at 0900 hr by decapitation and the small intestine was immediately removed and flushed with ice-cold saline. Intestinal epithelial cells were prepared by the method of Weiser14 with a following minor modification.10) The gut loops were tied up at one end and were distended with buffer A (1.5 mM KCl, 96 mM NaCl, 27 mM sodium citrate, 8 mM KH₂PO₄, and 5.6 mM Na₂HPO₄, pH 7.3). After the other end was tied up, the loops were immersed in 0.9% NaCl solution and kept for 15 min at 37°C in a water bath with gentle shaking (90/min oscillation frequency). The buffer A was discarded and the loop was redistended with buffer B (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na₂HPO₄, 1.5 mM KH₂PO₄, 1.5 mM EDTA, and 0.5 mM dithiothreitol (DTT), pH 7.4). After 60 min incubation at 37°C, the intestine was emptied and released cells were sedimented by centrifugation (1500 x g, 5 min) and homogenized in 100 mM phosphate buffer containing 20 mM EDTA and 10 mM DTT. During the isolation of epithelial cells, dephosphorylation (activation) of HMG-CoA reductase occurs and hence enzyme assays performed on these cells represent an index of the total quantity of enzyme present in the tissue. When the reductase activity was assayed on epithelial cells isolated in the presence of fluoride ion, a potent inhibitor of dephosphorylation, the results indicate the apparent enzyme activity. To determine the apparent activity in isolated intestinal cells, NaF, 50 mM was added to both buffer A and B and also to the homogenizing and activity assay media. For this purpose, the gut loop was cut open and divided longitudinally into two equal portions, and epithelial cells were isolated by the same buffer system as described above in the presence or absence of NaF. The activation state, namely the ratio of apparent to total reductase activity, was estimated by the ratio of the activity in the cells isolated in the presence and absence of NaF.

Assay of HMG-CoA reductase. HMG-CoA reductase activity was determined in hepatic microsomes15) and whole homogenate of intestinal epithelial cells10,16) as described previously. Enzyme protein was determined by the method of Lowry et al.17) using bovine serum albumin (Sigma Chemical Co., St. Louis, MO) as a standard.

Statistical analysis. The data were analyzed by Student’s t-test18) or one way analysis of variance where appropriate.19)

RESULTS

Diet-type-dependent alteration of the distribution of HMG-CoA reductase activity

We have previously demonstrated using scraped cells, that the jejunoileal-gradient for the intestinal microsomal reductase, characteristic for the rats maintained on a commercial nonpurified diet, was reversed in a few days by replacing the diet with a sucrose-enriched semipurified diet, thus resulting in a new distribution pattern, the ileojejunal gradient.20,21) Consistent with these observations, HMG-CoA reductase activity in epithelial cells isolated by the dual buffer method14) also exhibited the jejunoileal gradient when rats were given a non-purified diet while the ileojejunal gradient was achieved on feeding a semipurified diet containing 5% fat (Fig. 1). The
Regulation of Intestinal HMG-CoA Reductase Activity in Rats Fed either a Commercial Non-purified Diet or a Semipurified Diet Containing 5% Corn Oil.

Rats, approximately 160g, were fed these diet for 7 days and killed at 0900hr. Segment 0 corresponds to the duodenum and the remaining portion of the small intestine was divided into four segments of equal length. Reductase activities were determined on isolated epithelial cells. Each point represents the mean ± S.E. for 6 rats. * Significantly different from the semipurified diet group at p<0.05 by Student’s t-test. Symbols: ○, semipurified diet; ●, commercial non-purified diet.

Fig. 1. Intestinal Distribution of HMG-CoA Reductase Activity in Rats Fed either a Commercial Non-purified Diet or a Semipurified Diet Containing 5% Corn Oil.

reversal in the distribution of reductase activities was found to be reproducible when the activities were calculated in terms of total activities (nmol/hr/segment) instead of specific activities (pmol/min/mg protein). Thus, the observed changes in the enzyme activities could not be solely ascribed to the probable morphological changes of the intestine induced by the different types of diet.

The effect of diet replacement on the activation state of the reductase was then studied with the upper jejunum and lower ileum because of substantial alterations of the activities in these two tissues in response to dietary manipulations. The change in the activation state of reductase activity was also evaluated on hepatic microsomes isolated in the presence or absence of NaF. As shown in Fig. 2, the activity of upper jejunal cells separated without NaF increased progressively while that of the corresponding lower ileal cells decreased with the lapse of feeding on the sucrose-enriched semipurified diet. The jeunoileal gradient was completely diminished in 5 days and the ileojejunal gradient was being established. The course of changes in the apparent reductase activity also depicted a pattern similar to the total activity.

Hepatic microsomal reductase activities, both NaF-treated and non-treated, were significantly depressed in one day after feeding a semipurified diet and were virtually maintained at the low levels thereafter (Fig. 3).

Figure 4 summarizes the alteration in the existing state of jejunal and ileal HMG-CoA reductase together with that of the hepatic microsomal enzyme following ingestion of the sucrose-enriched semipurified diet. The activation state of the upper jejunum and liver remained approximately constant throughout the feeding period of up to 7 days (p = 0.51 and p = 0.89, respectively, by one way analysis of variance), though the value for the jejunal cells was obviously higher (about 80%) than that of liver (about 20%). On the contrary, though there were some degrees of fluctuations, the activation state of the ileum was initially

Fig. 2. Effects of Replacement of a Non-purified Diet with a Semipurified Diet on Intestinal HMG-CoA Reductase Activity.

Rats, approximately 140g, were fed a commercial non-purified diet for 4 days followed by a semipurified diet containing 5% corn oil and were killed at 0900hr as indicated at day-intervals. Intestinal epithelial cells were isolated from the upper jejunum and lower ileum in the presence or absence of NaF, and HMG-CoA reductase activities were determined. Each point represents the mean for 2~5 rats. Symbols: ○, upper jejunum (-NaF); ●, upper jejunum (+NaF); □, lower ileum (-NaF); ■, lower ileum (+NaF).
Fig. 3. Effects of Replacement of a Non-purified Diet with a Semipurified Diet on Hepatic Microsomal HMG-CoA Reductase.

Microsomes were isolated in the presence or absence of NaF, and reductase activities were determined. Each point represents the mean ± S.E. for 3 rats. Symbols: △, −NaF; ▲, +NaF.

Fig. 4. Alteration in the Activation State of Intestinal or Liver Microsomal HMG-CoA Reductase after Replacing a Commercial Non-purified Diet with a Semipurified Diet Containing 5% Fat.

The data were calculated from the results shown in Figs. 2 and 3. Each point represents the mean ± S.E. for 3~5 rats except for the mean of 2 rats on day 1 of intestinal reductase. Symbols: ○, upper jejunum; □, lower ileum; △, liver microsomes.

lowered (p<0.05) by the semipurified diet while it was restored to the initial level after 7 days.

Effect of dietary fat on HMG-CoA reductase activity

Consistent with the previous observation with microsomes from mucosal cells separated by scraping, dietary fat at the 10% level compared to the 1% level, significantly stimulated the reductase activity of isolated epithelial cells (Fig. 5). Based on this observation, an experiment similar to the preceding one on the course of changes in activity was carried out. The reductase activities, both in the jejunum and ileum, reached a peak 2 days after feeding the 10% fat diet, and 3 days and thereafter they remained virtually constant at levels approximately two times that observed on feeding 1% fat (Fig. 6). As shown in Fig. 7, though there were large fluctuations, the ratio of the active to total reductase activity appeared to be maintained at the fixed range in both tissues (p=0.11 in the jejunum and p = 0.82 in the ileum).

DISCUSSION

Consistent with previous studies, substi-
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Fig. 6. Effects of Replacement of the 1% Fat Diet with the 10% Fat Diet on Intestinal HMG-CoA Reductase Activity.

Rats, approximately 165 g, were fed the semipurified diet containing 1% fat for 6 days followed by the 10% fat diet for the periods indicated. Reductase activities were determined as described in legend to Fig. 2. Each point represents the mean for 3 rats except for the mean for 6 rats on day 0. Symbols: O, upper jejunum (-NaF); ●, upper jejunum (+NaF); □, lower ileum (-NaF); ■, lower ileum (+NaF).

Fig. 7. Alteration in the Activation State of Intestinal HMG-CoA Reductase after Replacing the 1% Fat Diet with 10% Fat Diet.

The data were calculated from the results shown in Fig. 6. Each point represents the mean ± S.E. for 3~6 rats. Symbols: O, upper jejunum; ■, lower ileum.

tution of the semipurified diet for the commercial non-purified diet caused a significant increase in the jejunal activity with a concomitant decrease in the ileal activity (Fig. 1). Although the dietary ingredients responsible for causing the changes in the distribution pattern of the intestinal reductase were not fully understood at present, the difference in the type and amount of dietary fiber and/or carbohydrate appears to be a determining factor presumably through the modification of intestinal uptake of cholesterol and bile acid and/or intestinal lipoprotein production. The alteration of epithelial cell dynamics should also be taken into account, since cholesterol is a membrane constituent of the cells which proliferate at the crypt site and migrate along the length of the villi sloughing into the intestinal lumen.

The modification of the activation state of HMG-CoA reductase in isolated intestinal epithelial cells was chased on substituting the non-purified diet for the semipurified diet. Despite these diet-dependent alterations, the ratios of the active to total reductase activities in the jejunum remained unaltered throughout the feeding periods of 7 days (Fig. 4), indicating that the increase in the synthesis of enzyme protein is mainly responsible for the observed enhancement of the jejunal reductase activity. In contrast, the ratios were considerably lowered in the ileum on feeding up to 5 days of the semipurified diet, suggesting a possible participation of the phosphorylation reaction (inactivation) in the reduction of the ileal reductase activity. After 7 days on the same diet, however, the activation state was restored to the initial level. Since the extent of the reduction of total activity was moderate compared to that of apparent activity, it is reasonable that the reduction of the activation state due to short term regulation is expected to be restored to the initial level as a consequence of the decrease in enzyme protein due to long term regulation.

Hepatic HMG-CoA reductase activities both in NaF-treated and -non-treated microsomal fractions were significantly depressed as early as one day after feeding the semipurified diet (Fig. 3). According to Beg et al., the hepatic reductase is under regulation of the bicyclic cascade system. Thus in the present study, liver microsomal HMG-CoA reductase
appears to be rapidly converted to a phosphorylated inactive form in a few hours, or otherwise at least in a day, following ingestion of the sucrose-enriched semipurified diet, with a possible subsequent decrease in the enzyme protein, hence the total activity (Fig. 3). Under these situations, the ratio of NaF treated to NaF-non-treated microsomal reductase activity (the activation state) should remain constant throughout the feeding period. The rapid decrease in hepatic total reductase activity may be effected by a relatively short half-life (about 4 hr) of the hepatic enzyme in vivo. Alternatively, the gradual decrease in total ileal activity may be relevant to a rather longer half-life of the intestinal enzyme compared to the liver enzyme.

The activation state of the hepatic enzyme was relatively low in comparison with that of the intestine (Fig. 4), suggesting an intrinsic difference in the extent of the activation state between these two enzymes; considerable portions existed in an active form in the intestine and the opposite was the case for the liver.

On the basis of our previous observations on dietary fat-dependent stimulation of the reductase activity of jejunal villi,(15) a similar type of experiment was introduced. The dietary fat markedly stimulated the reductase activity in epithelial cells separated from the upper jejunum (Fig. 5). It is plausible that the jejunal cholesterogenesis is increased by fat feeding for assembly and transport of intestinal lipoprotein to process absorbed fat.(15,29) After substitution of the 10% fat diet for the 1% fat diet, both jejunal and ileal reductase activities were increased without greatly influencing the activation state (Fig. 7). Thus the increase in enzyme protein, not the catalytic efficiency, should be the major cause for dietary fat-induced enhancement of reductase activity.

In the present study, the activation state of HMG-CoA reductase in the ileum was rapidly changed in response to dietary manipulations. The magnitude of the activation state of the intestinal reductase was evidently higher than that of the hepatic enzyme. Thus, in the intestine, the inactivation reaction appears, in a relative sense, to be physiologically more significant than the activation reaction. The observed results indicate the involvement of the phosphorylation-dephosphorylation reaction in the in vivo regulation of intestinal HMG-CoA reductase activity.

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