Reversible inactivation-reactivation of 3-hydroxy-3-methylglutaryl coenzyme A reductase of rat intestine

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Abstract 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase in the ileum of rats was inactivated by Mg^{2+}-ATP and reversibly reactivated by cytoplasmic activator from the liver. The mevalonate kinase reaction was presumably not involved in this inactivation. Studies of nucleotide specificity for the inactivation revealed that ATP was most effective in the reaction among the nucleotides tested. In contrast to the hepatic microsomal HMG-CoA reductase, more than one-half of intestinal reductase existed in an active form. These observations indicated the presence of phosphorylation-dephosphorylation mechanism for modulation of intestinal HMG-CoA reductase.—**Oku, H., T. Ide, and M. Sugano.** Reversible inactivation-reactivation of 3-hydroxy-3-methylglutaryl coenzyme A reductase of rat intestine. *J. Lipid Res.* 1984. **25:** 254–261.

Supplementary key words HMG-CoA reductase • phosphorylation-dephosphorylation • intestine

In recent years, the concept of reversible covalent modification of hepatic HMG-CoA reductase (EC 1.1.1.34) has been proposed as a mechanism of short-term regulation of hepatic cholesterogenesis: inactivation by reductase kinase in the presence of Mg^{2+}-ATP and reversible reactivation by phosphoprotein phosphatase both in vitro (1, 2) and in vivo (3, 4). The intestine actively synthesizes cholesterol, perhaps next to or equal to the liver, and contributes cholesterol to circulation (5, 6). However, little is known about modification of intestinal reductase.

Field et al. (7, 8) showed that intestinal microsomal HMG-CoA reductase prepared in the presence of NaF, a potent inhibitor of phosphoprotein phosphatase activity, was activated by partially purified hepatic or intestinal phosphatase. However, they were unable to demonstrate the reductase kinase-mediated reduction of the reductase activity. Consequently, Field et al. (8) suggested that the intestinal reductase might be less sensitive to regulation by the phosphorylation-dephosphorylation mechanism. Also Panini and Rudney (9) indicated that HMG-CoA reductase activity of isolated intestinal cell undergoes rapid reversible modulation in response to bicarbonate, and suggested the existence of short-term regulation of intestinal HMG-CoA reductase activity.

Since intestinal cholesterogenesis, like the liver, is under the control of various factors (10–14), demonstration of short-term regulation of intestinal HMG-CoA reductase activity should be important for full comprehension of the role of the intestine in cholesterol dynamics. Accordingly, we studied the modulation of intestinal HMG-CoA reductase activity by Mg^{2+}-ATP and by the liver cytoplasmic activator and determined the ratio of the expressed to total reductase activity in this tissue.

MATERIALS AND METHODS

Chemicals

[3-^{14}C]-R,S-3-hydroxy-3-methylglutaric acid (HMG) was from New England Nuclear, Boston, MA. [3-^{14}C]-R,S-HMG-CoA was prepared by the method of Suzuki, Oba, and Uritani (15) and its specific activity was adjusted to 5000 dpm/nmol. Other chemicals were all reagent grade and were purchased from the following sources: ATP and coenzyme A (Kyowa Hakko Co., Tokyo); cAMP, dibutyryl-cAMP, dibutyryl-cGMP, and GTP (Yamasa Shoyu Co., Tokyo); AMP, ADP, and hexokinase (Oriental Yeast Co., Tokyo); cGMP, GDP, and UTP (Sigma Chemical Co., St. Louis, MO); GMP (Kohjin Co., Tokyo); dithiothreitol and creatine kinase (Boehringer Mannheim GmbH, Germany); phosphocreatine disodium salt (Nakarai Chemical Co., Kyoto); and trypsin inhibitor (BDH Biochemicals Ltd., Poole, England).

Animals

Male Wistar rats (Kyudo Co., Kumamoto) weighing about 150 g were maintained on a commercial stock diet (Type NMF, Oriental Yeast Co., Tokyo) fed ad libitum under controlled lighting conditions (illuminated from 0800 to 2000 hours).

Abbreviation: HMG, 3-hydroxy-3-methylglutaryl

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Isolation of intestinal epithelial cells

Rats were killed at 1000 hours (10 AM) and the small intestine was immediately excised. The lower ileum (about 25 cm length) was washed with 0.9% NaCl solution and intestinal epithelial cells were prepared by the method of Weiser (16) with the following minor modification. The gut loop was distended by filling it with buffer A at 37°C. Buffer A contained 1.5 mM KCl, 96 mM NaCl, 27 mM sodium citrate, 8 mM KH_2PO_4, 5.6 mM Na_2HPO_4, pH 7.3. The loop was placed in 0.9% NaCl solution at 37°C and incubated in a metabolic shaker for 15 min with gentle shaking. The buffer was discarded. The intestine was then distended with buffer B at 37°C which contained 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.47 mM KH_2PO_4, 1.5 mM EDTA, 0.5 mM dithiothreitol (DTT), pH 7.4. Weiser (16) showed that short-term incubation in buffer B consecutively released cells starting from villus to crypt cells. Merchant and Heller (17) modified the Weiser method and harvested villus cells by an initial 40-min incubation in buffer B; the crypt cell fraction was obtained by further incubation for 20 min in the same buffer. We utilized a 60-min incubation in buffer B to obtain whole epithelial cells of the lower ileum. Epithelial cells were collected by centrifugation at 1500 *g* for 5 min and homogenized in a loose-fitting Teflon homogenizer with 2 ml of 100 mM potassium phosphate buffer (pH 7.2) containing 15 mM DTT. To prevent the activation or inactivation during homogenization, NaF or EDTA was added to the homogenizing medium when needed (concentrations of NaF and EDTA were 50 mM and 20 mM, respectively).

Assay of HMG-CoA reductase

HMG-CoA reductase activity in epithelial cell homogenate was measured as previously described (18, 19). Despite uncertainty as to the cellular localization of intestinal HMG-CoA reductase, the microsomal fraction has so far been used mainly for the determination of reductase activity. The highly purified mitochondrial fraction does not contain reductase activity and more than 70% of total activity exists in the low speed pellet fraction which contains intact cells, nuclei, and amorphous materials (8). To optimize the yield of enzyme, whole cell homogenates for physiological studies were recommended for intestinal reductase (8). Consequently, the total epithelial cell homogenate was used as an enzyme source in the present study. Briefly, 50 μl of substrate solution in 100 mM potassium phosphate containing 50 nmol [^{14}C]HMG-CoA and 2 μmol NADPH (pH 7.2) was added to the cell homogenate (0.08–0.30 mg protein) to start the enzyme reaction. Incubation was for 60 min at 37°C. The final volume was 150 or 155 μl. The reaction was terminated by the addition of 50 μl of 6 N HCl. An aliquot of the reaction mixture was applied to a silica gel G plate and the radioactivity in [^{14}C]mevalonolactone was determined. The reaction was linear for 60 min and was proportional to the amount of protein up to 0.3 mg. The variation in the enzyme activities from the same source never exceeded ±5% by this method.

Preparation of reductase activator

The reductase activator was prepared from liver cytosol essentially according to the method of Nordstrom, Rodwell, and Mitschelen (20). Cytosolic protein precipitating in 35–60% acetone solution was further purified at 40% saturation of ammonium sulfate. Activator thus obtained was dissolved in 50 mM phosphate buffer containing 1 mM EDTA, 5 mM DTT, 250 mM sucrose, pH 7.2. This activator preparation did not contain detectable reductase activity. The activity of the preparation was found to be fully retained at least for a week when stored at −20°C and hence it was used within 1 week after preparation.

Inactivation and reactivation procedures

Inactivation was started by the addition of 5 μl of concentrated Mg^{2+}-ATP solution to 100 μl of cell homogenate (concentrations as stated in the figure and table legends). After 5–45 min incubation at 37°C, 50 μl of the substrate solution containing EDTA, [^{14}C]HMG-CoA, and NADPH in 100 mM potassium phosphate (pH 7.2) was added to terminate the inactivation reaction and simultaneously to start the reductase reaction. The substrate solution contained NaF to prevent activation of the enzyme during the assay. In the inactivation-reactivation study, inactivation was started by adding 5 μl of Mg^{2+}-ATP solution to 50 μl of cell homogenate; the reaction was terminated by the addition of activator solution containing EDTA, and the reactivation reaction was started. After 10–30 min incubation at 37°C, 50 μl of the substrate solution containing NaF, [^{14}C]HMG-CoA, and NADPH was added. The concentration of EDTA and NaF in inactivation, reactivation and enzyme assay was adjusted to be 20 mM and 50 mM, respectively.

Statistical analysis

Statistical evaluation of the results was carried out by one-way analysis of variance followed by inspection of all differences between pairs of means, or analysis of variance of two-way layout without repetition where appropriate (21).

RESULTS

Validation of the reaction product

Young et al. (22, 23) observed the presence of HMG-CoA cleavage enzyme in intestinal homogenates. The

NADPH-independent metabolite produced by the cleavage enzyme behaves like mevalonolactone on thin-layer chromatography. However, in our study, thin-layer chromatography of the reaction mixture revealed that only one metabolite with a mobility corresponding to that of mevalonolactone was formed in the presence of NADPH. No NADPH-independent metabolite was detected (data not shown). Cleavage enzyme activity, thus, is not expressed in our epithelial cells at detectable levels.

Inactivation by Mg^{2+}-ATP

It has been shown that incubation of rat liver microsomes with Mg^{2+}-ATP decreases the activity of HMG-CoA reductase and this can be fully restored to initial levels by incubating microsomes with cytosol, indicating that the reductase activity can be regulated by a phosphorylation-dephosphorylation mechanism (24). To ascertain whether similar regulation exists in the intestine, the effect of Mg^{2+}-ATP on intestinal enzyme was investigated. Mg^{2+}-ATP was added to the cellular homogenate and preincubation was carried out at 37°C. Addition of Mg^{2+}-ATP caused a significant reduction of activity (**Fig. 1**). Maximum inactivation was achieved even in the relatively low concentration of ATP and in the relatively short-term of preincubation.

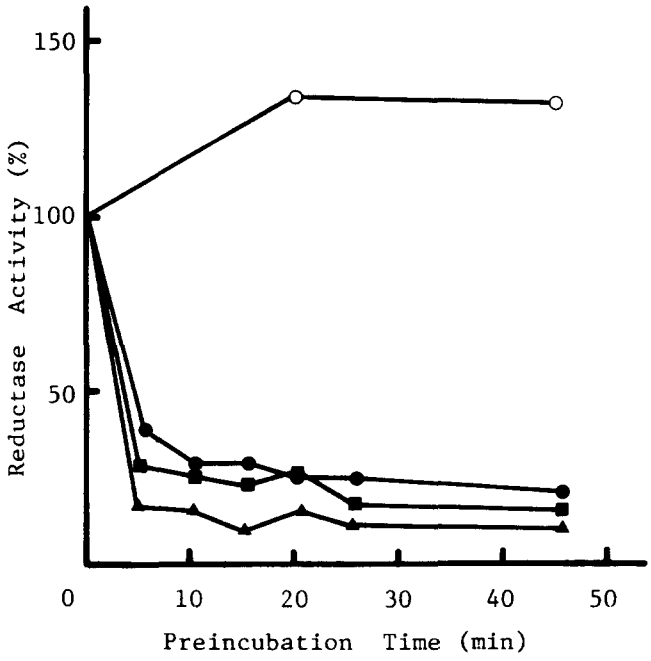

Fig. 1. Inactivation of intestinal HMG-CoA reductase by Mg^{2+}-ATP. Total homogenates of intestinal epithelial cells (0.30 mg of protein) were preincubated for 5–45 min without (control) or with various amounts of Mg^{2+}-ATP. At appropriate intervals, the enzyme reaction was started by the addition of the substrate solution containing EDTA and NaF (final concentrations were 20 mM and 50 mM, respectively). The control experiment contained the same concentrations of EDTA and NaF as Mg^{2+}-ATP-inactivated preparations during the enzyme assay. The initial enzyme activity was 80 pmol/min per mg protein. Symbols: ○, control; ●, 2 mM ATP + 4 mM $MgCl_2$; ■, 4 mM ATP + 8 mM $MgCl_2$; ▲, 8 mM ATP + 16 mM $MgCl_2$.

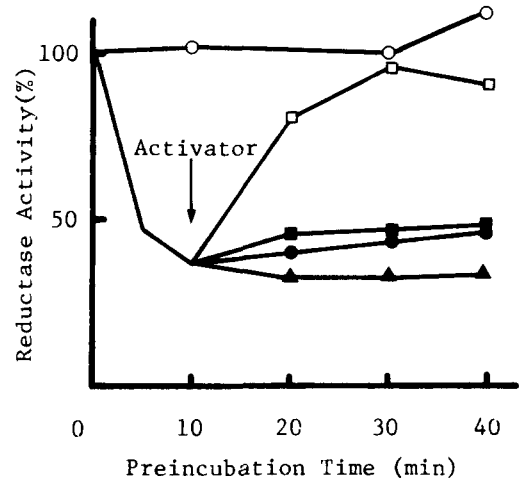

Fig. 2. Inactivation and reactivation of intestinal HMG-CoA reductase. Inactivation was started at zero time in the presence of 4 mM ATP + 8 mM $MgCl_2$. After 10 min, an activator solution (0.61 mg of protein) or control buffer, both containing excess EDTA (final concentration was 20 mM), was added to terminate the inactivation reaction, and reactivation was started in the presence or absence of 50 mM NaF. At 10-min intervals, reactivation was terminated by the addition of the substrate solution containing NaF, and reductase activity was determined. The initial activity was 164 pmol/min per mg protein (enzyme protein, 0.29 mg). Symbols: ○, control; □, activator; ■, activator + NaF; ●, control buffer; ▲, control buffer + NaF.

The possibility that Mg^{2+}-ATP promotes the conversion of mevalonate to phosphomevalonate, thereby decreasing apparent reductase activity (25, 26) was examined by adding a 45-nmol trapping pool of mevalonate. This did not decrease the apparent inactivation of reductase, indicating that the decrease in the reductase activity was real and was not due to the conversion of mevalonate to phosphomevalonate (data not shown).

Reactivation by cytosolic activator

Reactivation of Mg^{2+}-ATP-inactivated reductase was studied using liver cytosolic activator. The reductase was initially inactivated by incubation with Mg^{2+}-ATP for 10 min and the reactivation was examined. Reductase inactivated by Mg^{2+}-ATP was fully reactivated to initial levels in the absence of NaF, whereas the reactivation was completely blocked when NaF was added together with the activator (**Fig. 2**). When similar types of experiments were performed using an enzyme preparation from four individual animals, 10-min Mg^{2+}-ATP treatment decreased the enzyme activities to 49.6 ± 5.6% of the control value. Following 30 min treatment with cytosolic activator, the inactivated enzymes restored their activities to 92.5 ± 9.5% of the control value ($P < 0.05$ vs. Mg^{2+}-ATP-inactivated enzyme). The activities did not change (51.9 ± 7.9% of the control value) when NaF coexisted during the activation reaction.

Specificity of the nucleotide requirement for inactivation

The effect of several nucleotides on inactivation of intestinal HMG-CoA reductase was studied. A significant decrease in enzyme activity was obtained with ATP and ADP (**Table 1**). The decrease in the enzyme activities with ATP and ADP was also confirmed in other sets of experiments (Fig. 1 to Fig. 4).

According to Nordstrom et al. (20), the presence of equimolar ATP and ADP was required for maximal inactivation of solubilized hepatic reductase by the partially purified inactivator preparation from the liver. Brown, Brunschede, and Goldstein (27) also reported the requirement of both nucleotides for inactivation of liver microsomal reductase by a factor extracted from human fibroblasts. Since the two nucleotides are interconvertible in cellular homogenate, experiments were undertaken to determine which nucleotide is required for inactivation. For this purpose, incubation conditions that prevent the conversion of one nucleotide to the other were adopted. The ATP-regenerating system consisting of creatine phosphate and creatine kinase did not prevent intestinal HMG-CoA reductase from Mg^{2+}-ATP-induced inactivation (**Fig. 3**). In the same manner, an ADP-regenerating system (glucose plus hexokinase), contrary to the ATP-regenerating system, blocked the inactivation of the reductase by ADP ($P < 0.05$, **Fig. 4**). With three different enzyme sources, the presence of the ATP-regenerating system failed to prevent the enzyme from inactivation by ATP (61.0 ± 4.4% of the control value at 4 mM ATP, $P < 0.05$ vs. the control value) while that of the ADP-regenerating system completely blocked the ADP-induced inactivation (102.0 ± 2.7% of control value at 4 mM ADP).

TABLE 1. Effect of several nucleotides on intestinal HMG-CoA reductase activity

Additions (no. of animals)	HMG-CoA Reductase Activity	
	pmol/min per mg	%
None (4)	197 ± 15	(100)
ATP (4)	129 ± 26[a]	63.9 ± 11.6[b]
ADP (4)	133 ± 21[a]	66.5 ± 6.9[b]
AMP (3)	208 ± 12	99.8 ± 11.0
GTP (4)	186 ± 24	93.7 ± 8.6
GDP (4)	199 ± 8	102.2 ± 5.5
GMP (3)	206 ± 9	97.9 ± 2.2
UTP (4)	157 ± 28	78.6 ± 11.0
UDP (4)	195 ± 15	98.8 ± 1.6

Inactivation was carried out for 10 min in the presence of 4 mM nucleotide + 8 mM $MgCl_2$ as described in Experimental Procedures. Data are mean ± SE.

[a] $P < 0.05$ vs. none.

[b] $P < 0.01$ vs. none.

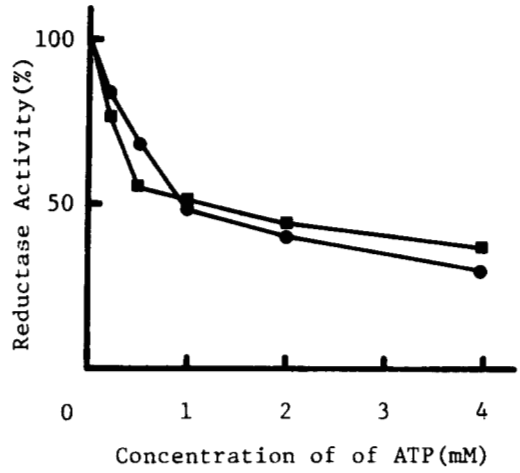

Fig. 3. Effect of ATP-regenerating system on the inactivation reaction of intestinal HMG-CoA reductase achieved by Mg^{2+}-ATP. Regenerating system consisted of creatine kinase and creatine phosphate. ATP was added at various concentrations together with regenerants and the inactivation reaction was continued for 10 min. The substrate solution containing EDTA and NaF was then added and enzyme activity was determined. Mg^{2+} was present at the concentration twofold that of nucleotides. The control activity was 96 pmol/min per mg protein (enzyme protein, 0.30 mg). Symbols: ●, ATP + 2 U creatine kinase; ■, ATP + 2 U creatine kinase + creatine phosphate (10 mM).

Apparent and total reductase activity

It has been reported that more than 80% of liver microsomal HMG-CoA reductase exists in an inactive form (28). To estimate apparent reductase activity, NaF has been most commonly used to inhibit activation of the reductase during the preparation of the enzyme source. Thus, the lower ileum was cut open and divided longi-

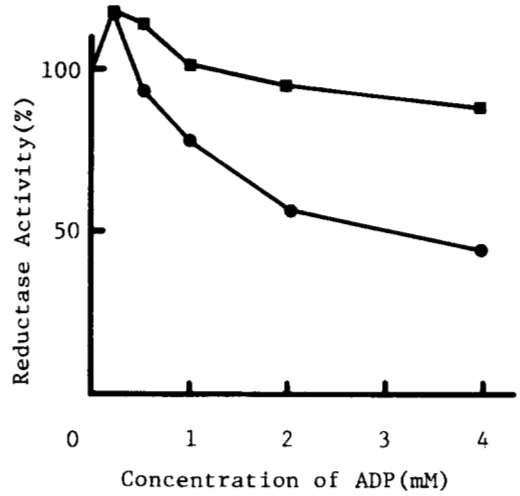

Fig. 4. Effect of ADP-regenerating system on inactivation of intestinal HMG-CoA reductase achieved by Mg^{2+}-ADP. ADP-regenerating system consisted of hexokinase and glucose. Experimental procedure was the same as described in the legend to Fig. 3. The control activity was 96 pmol/min per mg protein (enzyme protein, 0.30 mg). Symbols: ●, ADP + 2 U hexokinase; ■, ADP + 2 U hexokinase + glucose (10 mM).

tudinally into two equal portions. Cells were then isolated either in the presence or absence of NaF. Two experiments were performed. In one experiment, 50 mM NaCl in buffer for cell preparation was replaced with an equimolar solution of NaF. In the other, 50 mM NaF was added to the buffer. However, since substitution of NaF for NaCl resulted in the low recovery of mucosal protein (data not shown), addition of NaF to the buffer was adopted in later experiments. A considerable portion of the intestinal reductase was shown to exist in an active form (**Table 2**). However, it was not clear whether activation during cell isolation could be completely prevented by NaF, since the possibility remains that there is a time-lag for fluoride to enter the cells. For reconfirming the existing state of the intestinal reductase, the scraping technique was adopted to obtain a total homogenate of the lower ileum (19, 29). Although the reductase activity in scraped cells was considerably lower than that in cells isolated by the method of Weiser (16), more than one-half of intestinal HMG-CoA reductase existed in an active form (**Table 3**).

Activation of intestinal reductase prepared in the presence of NaF

In the intestinal homogenate prepared in the presence of NaF, reductase activity was fully activated by the hepatic activator when activation was carried out in the absence of fluoride. As **Table 4** shows, the reductase in the homogenate prepared in the absence of NaF was also activated, but the degree of activation was relatively low. Consequently, it was necessary to determine the extent of activation and hence total reductase activity. Maximal activation was achieved in 10 min with 0.42 mg of activator protein (0.08 mg of enzyme protein, **Fig. 5**). Under these conditions, it was again confirmed that at least 50% of intestinal HMG-CoA reductase existed in an active form (Table 4).

TABLE 2. Apparent HMG-CoA reductase activity of intestinal cells separated by the Weiser's method (16)

	Animal	Reductase Activity −NaF	Reductase Activity +NaF	+NaF/−NaF
		pmol/min per mg		
Exp. A	1	110	88	0.80
	2	142	58	0.41
	3	101	94	0.93
Exp. B	4	303	142	0.47
	5	179	126	0.71
	6	87	71	0.82
	7	139	97	0.70
Mean ± SE	(1 to 7)	152 ± 28	97 ± 11	0.69 ± 0.07

The lower ileum was cut open and divided longitudinally into two equal portions. Epithelial cells were then isolated in the presence or absence of NaF. In Exp. A, 50 mM NaCl of buffer for cell preparation was replaced with equimolar NaF, and in Exp. B, 50 mM NaF was added to the buffer. NaF was included throughout the homogenization and reductase activity measurement when cells were isolated in the presence of NaF.

TABLE 3. Apparent HMG-CoA reductase activity of intestinal cells separated by scraping

Animals	Reductase Activity −NaF	Reductase Activity +NaF	+NaF/−NaF
	pmol/min per mg		
8	33	26	0.78
9	24	14	0.57
10	38	26	0.68
11	18	9	0.50
12	14	10	0.71
13	14	9	0.64
14	21	12	0.57
Mean ± SE	23 ± 4	15 ± 3	0.64 ± 0.04

Scraped intestinal mucosa was homogenized in 100 mM phosphate buffer containing 15 mM DTT and 2 mg trypsin inhibitor/15 ml in the presence or absence of 50 mM NaF. Whole homogenate was used for the determination of HMG-CoA reductase.

DISCUSSION

The regulation of hepatic HMG-CoA reductase by phosphorylation-dephosphorylation has been well established. The intestine also has a potent capability to synthesize cholesterol and plays an important role in cho-

TABLE 4. Apparent and total HMG-CoA reductase activity of intestinal cells separated by Weiser's method

No.	Preparation Conditions	Activation Conditions	Activator	Reductase Activity
				pmol/min per mg
1	+NaF	−NaF	+	332 ± 70[a]
2	+NaF	−NaF	−	185 ± 44
3	+NaF	+NaF	+	171 ± 7[b]
4	+NaF	+NaF	−	140 ± 7[c]
5	−NaF	−NaF	+	284 ± 29
6	−NaF	−NaF	−	238 ± 27
7	−NaF	+NaF	+	228 ± 29
8	−NaF	+NaF	−	196 ± 25

The lower ileum was cut open and divided longitudinally into two equal portions. Epithelial cells were then isolated in the presence or absence of NaF. Activation was carried out for 20 min in the presence or absence of 50mM NaF and/or 0.42 mg of activator protein. The activator preparation was different from that used in the experiment described in Fig. 2. Each value represents the mean ± SE of three rats.

[a] $P < 0.05$ vs. 2, 3, 4, 7, and 8.
[b] $P < 0.05$ vs. 5.
[c] $P < 0.05$ vs. 5 and 6.

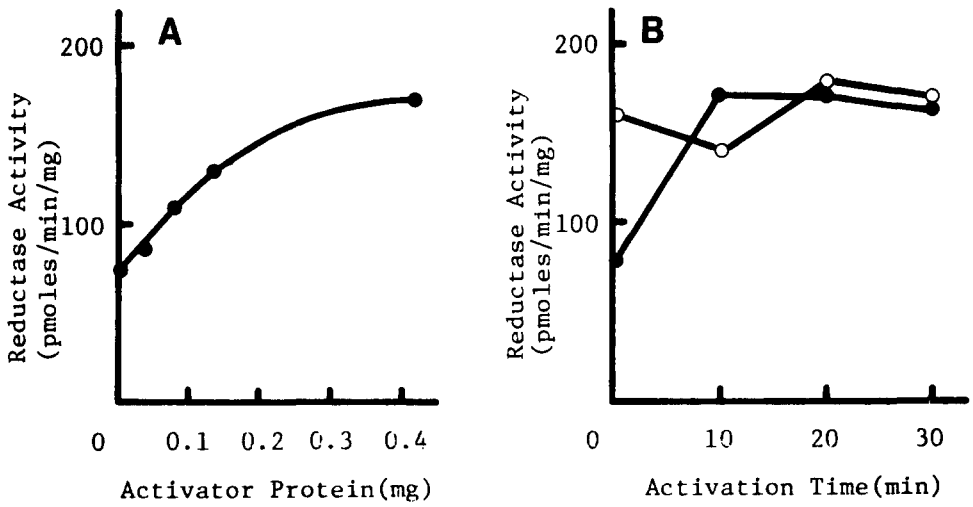

Fig. 5. Kinetic studies on the activation reaction by liver cytoplasmic activator. Intestinal epithelial cells were prepared as described in the footnote to Table 2. A, Activator was added at various concentrations to total cell homogenate (0.08 mg protein) prepared in the presence of NaF and incubated for 10 min; B, 0.42 mg of activator protein was added to cell homogenate prepared in the presence or absence of NaF. The activator was the same preparation as that used in the experiment shown in Table 4. Symbols: ●, prepared in the presence of NaF; ○, prepared in the absence of NaF.

lesterol and lipoprotein metabolism. Nevertheless, a phosphorylation-dephosphorylation mechanism for intestinal HMG-CoA reductase has not yet been fully clarified. The aim was to ascertain whether this type of regulatory mechanism exists in the intestine.

A considerable decrease in intestinal HMG-CoA reductase activity was observed when Mg^{2+}-ATP was added to the intestinal cell homogenate (Fig. 1). On the other hand, in the absence of Mg^{2+}-ATP, HMG-CoA reductase activity was elevated during the course of preincubations. This elevation could not be prevented by the addition of 100 mM NaF (data not shown), suggesting the existence of a regulatory mechanism independent of phosphorylation-dephosphorylation. Heller and Gould (30) reported reversible heat activation-cold inactivation of solubilized microsomal HMG-CoA reductase from rat liver. They suggested that this was an intrinsic property of solubilized reductase and postulated the reversible dissociation of enzyme protein into subunits with an accompanying loss of activity. The elevation of the activity during preincubation is presumably attributed to a similar property of intestinal HMG-CoA reductase.

Our results are inconsistent with those of Field et al. (8), in which they failed to inactivate the intestinal microsomal HMG-CoA reductase by Mg^{2+}-ATP. Though the reason for the discrepancy remains to be clarified, differences in the enzyme preparations, microsomes, or homogenate may be involved. The degree of inactivation varied from preparation to preparation (compare Table 1, Fig. 1, and Fig. 2). These variations might be due to the preparation-dependent difference in the activity of reductase kinase. The Mg^{2+}-ATP-dependent inactivation was fully restored to initial levels by liver cytoplasmic activator, whereas the reactivation was completely blocked by NaF. These results agreed with those of Panini and Rudney (9), and favor the view that the intestinal reductase is subjected to the regulation by reversible phosphorylation. Studies of nucleotide specificity for inactivation reaction revealed that only ATP, but not ADP, could participate in the inactivation reaction. These observations may suggest a different substrate specificity between intestinal and hepatic HMG-CoA reductase kinase.

Beg et al. (31, 32) reported the bicyclic cascade system of hepatic reductase, consisting of a HMG-CoA reductase cycle and a HMG-CoA reductase kinase cycle. According to their proposal, cAMP participates in the regulation of HMG-CoA reductase activity through the allosteric activation of reductase kinase. No evidence that indicates the involvement of cyclic nucleotides in the regulation of intestinal HMG-CoA reductase is available at present. In our study, cAMP and cGMP added to intestinal cell homogenate or their dibutyryl derivatives added to isolated cells failed to modify the activity of HMG-CoA reductase (data not shown). In addition, Shakir, Sundaram, and Margolis (33) showed that theophylline had no effect on intestinal cholesterogenesis. Thus, it is plausible that cyclic nucleotides are not involved in the regulation of intestinal cholesterogenesis. Apparently, more detailed study is needed before the definite conclusion is made.

In the present study, the hepatic cytosolic activator was used for reactivation for convenience. Since Field et al. (8) have shown that hepatic and intestinal phosphatase were both effective for reactivation, our observation with

the hepatic activator does not invalidate the existence of phosphatase-mediated reactivation. In fact, the preliminary experiment with the intestinal activator showed a similar type of reactivation of both liver and intestinal reductase.

Panini and Rudney (9) reported that the majority of HMG-CoA reductase in microsomes from scraped intestinal cells was indeed in an inactive form, whereas the opposite was the case with the epithelial cells prepared by the procedure of Weiser (16). Nevertheless, total activity was comparable in the two preparations. Hence, it is plausible that the conversion of HMG-CoA reductase into an active form proceeds during cell isolation by Weiser's method. From our experiments with the addition of the fluoride to the buffer for cell preparation, it seems that the considerable portion of intestinal reductase exists in an active form (Tables 2 and 3).

In conclusion, our observations have provided evidence that intestinal HMG-CoA reductase can be regulated by phosphorylation-dephosphorylation. However, it seems that intestinal reductase is less sensitive to such a regulatory mechanism than the liver enzyme. The degree of inactivation by Mg^{2+}-ATP was relatively low. In addition, a considerable portion of intestinal reductase existed in an active form.

Supported by a Grant-in-Aid for Scientific Research B (No. 547104) from the Ministry of Education, Science, and Culture.

Manuscript received 6 January 1983.

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