Regulation of rat liver microsomal cholesterol 7α-hydroxylase: reversible inactivation by ATP + Mg^{2+} and a cytosolic activator

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Abstract Modulation of cholesterol 7α-hydroxylase catalytic activity by adenine nucleotides was studied in rat liver microsomal preparations. Inactivation of cholesterol 7α-hydroxylase showed specific requirements of ATP and ADP. AMP and cyclic AMP were stimulatory and cyclic AMP had no effect in the ATP inactivation. The inactivation reactions by ATP were dependent on Mg^{2+} ions, a cytosolic factor, and time. Ca^{2+} ions were less effective whereas Mn^{2+} ions were highly inhibitory to hydroxylase activity. The inactivation could be reversed in a time-dependent reaction requiring a cytosolic activator that was precipitable by ammonium sulphate of saturation up to 65%. The current data suggest that cholesterol 7α-hydroxylase can exist in two catalytic forms that are reversible.—**Kwok, C. T., W. Burnett, and I. R. Hardie.** Regulation of rat liver microsomal cholesterol 7α-hydroxylase: reversible inactivation by ATP + Mg^{2+} and a cytosolic activator. *J. Lipid Res.* 1981. **22:** 580–589.

Supplementary key words adenine nucleotides · bile acids · cholesterol · gallstones

Cholesterol 7α-hydroxylase (EC 1.14.13.7) catalyzes the committed step in the biosynthesis of bile acids from cholesterol (1, 2), its catalytic activity having a diurnal variation with maximum enzyme activity at midnight and minimum enzyme activity at noon. The increased catalytic activity at the onset of the dark period is associated with an increased enzyme synthesis and an increase in supply of substrate (3, 4). The catalytic activity of cholesterol 7α-hydroxylase can also be enhanced by cholesterol feeding and by interruption of the return of bile salts to the liver by bile fistula and cholestyramine treatment, without affecting its diurnal rhythm (4, 5). Regulation of the catalytic activity under these conditions is not fully understood, although it is apparent that these experimental conditions do not modify the activating properties of 100,000 *g* supernatant on cholesterol 7α-hydroxylase (1, 3, 6).

Our preceding paper (6) demonstrated the involvement of a cytosolic factor in the modulation of cholesterol 7α-hydroxylase activity. The stimulatory effect of this heat-stable, non-dialyzable, and non-catalytic activator can be counteracted by ATP and 3-hydroxy-3-methylglutaryl-coenzyme A. These inactivating effects are concentration-dependent.

This study reports further findings on the effects of adenine nucleotides and also provides evidence for a short-term regulation of cholesterol 7α-hydroxylase activity by a reversible inactivation mechanism requiring ATP + Mg^{2+} and factors in the cytosol.

MATERIALS AND METHODS

Chemicals

ADP, AMP, and cyclic AMP were products of Sigma Chemical Co., St. Louis, MO.

General experimental details were fully described in the preceding paper (6). The following additional experimental methods were employed in the present studies.

Cholesterol 7α-hydroxylase assay

Cholesterol 7α-hydroxylase was assayed as previously described (6) using washed microsomes in all experiments. The standard incubation medium contained potassium phosphate buffer, pH 7.4 (80 mM), nicotinamide (3 mM), with or without EDTA (1 mM), dithiothreitol (5 mM), [4-^{14}C]cholesterol (0.05 μCi, 0.1 mM), microsomal fraction (0.5–1.0 mg protein), and a NADPH-regenerating system containing glucose-6-phosphate (2.5 mM), $NADP^+$ (1.25 mM), and glucose-6-phosphate dehydrogenase in 0.2 M potassium phosphate buffer, pH 7.4. The final volume was 0.5 ml.

ATP inactivation assay

Preincubation was carried out with ATP (3 mM) + $MgCl_2$ (4.5 mM) for various time intervals as

specified in the individual experiments. Then hydroxylase activity was assayed by a 20-min incubation after adding the NADPH-regenerating system.

Reactivation assay

Cholesterol 7α-hydroxylase was inactivated by preincubation with ATP (3 mM) and $MgCl_2$ (4.5 mM) for 1 hr at 37°C, and the enzyme activity was then assayed by a 20-min incubation after adding the NADPH-regenerating system. At the end of the reaction, crude cytosol or the ammonium sulfate-fractionated cytosolic protein was added as specified to initiate the reactivation reaction.

The reactions were terminated after various time intervals from 5 to 60 min and extracted by chloroform–methanol 2:1 (v/v) containing 0.005% butylated hydroxytoluene, as described in the preceding paper (6).

RESULTS

Effect of adenine nucleotides

ATP was previously found to inactivate cholesterol 7α-hydroxylase activity (6). In the present studies, a limited number of related adenine nucleotides was also tested (**Fig. 1**). ADP was found to inactivate cholesterol 7α-hydroxylase also and to counteract the activating effect of the crude cytosol. These effects of ADP were dependent on concentration, with a maximum inhibitory effect at 5 mM. AMP was slightly stimulatory with a maximal effect at 1 mM and this stimulatory effect, though small, persisted in the presence of the crude cytosol. Cyclic AMP was also found to stimulate hydroxylase activity, an effect that was maximal at 1 mM cyclic AMP, and then diminished with increasing cyclic AMP concentrations. When the hydroxylase activity was activated by the crude cytosol, the stimulatory effect of cyclic AMP increased with increasing concentrations.

Requirements for ATP inactivation

The ATP inactivation of cholesterol 7α-hydroxylase demonstrated in the initial experiments (6), was comparatively small in the absence of crude cytosol, and the present studies provided further information about the mechanism of these reactions. **Fig. 2** shows that the inactivation of cholesterol 7α-hydroxylase by ATP depended on the preincubation time and the

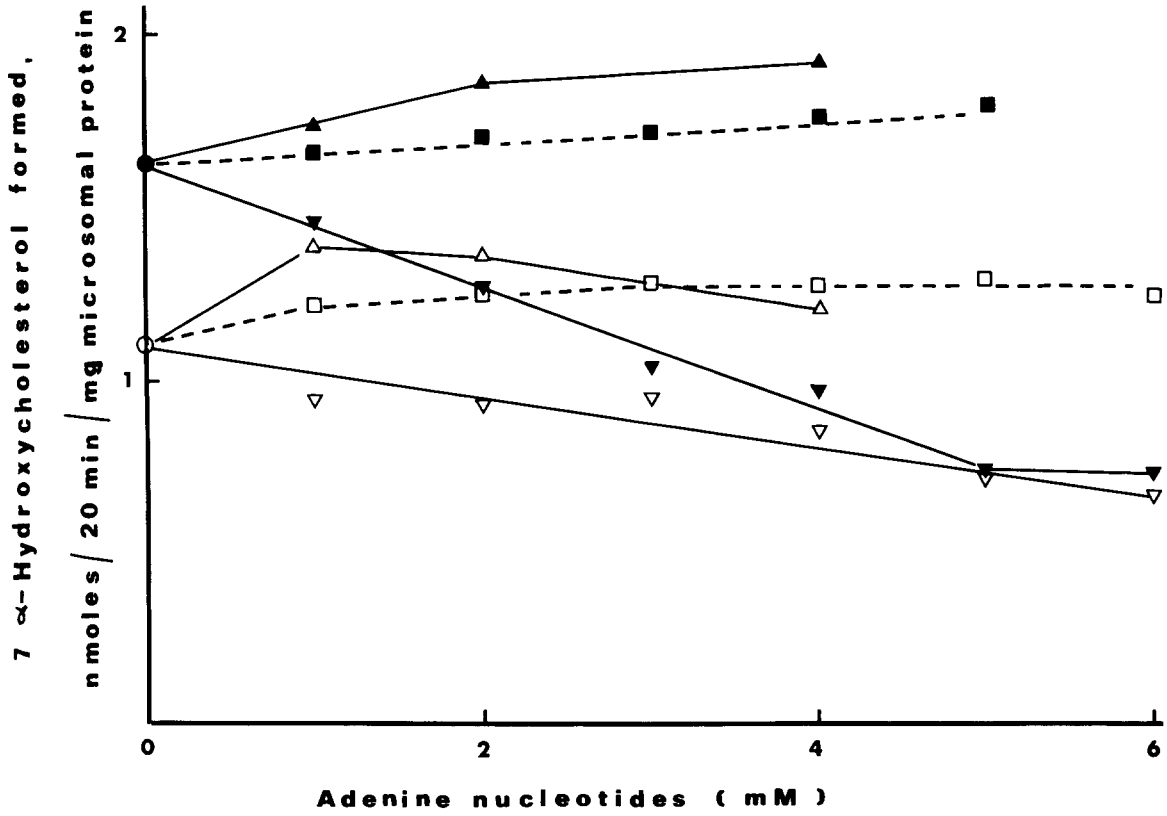

Fig. 1. Effects of adenine nucleotides on cholesterol 7α-hydroxylase activity. Adenine nucleotides: ADP (▽,▼), AMP (□,■), and cyclic AMP (△,▲) in increasing concentrations were preincubated for 10 min in the presence or absence of the crude cytosol (300 μg protein) as indicated by the closed or open symbols, respectively. The enzyme activities were assayed as described in the Experimental section.

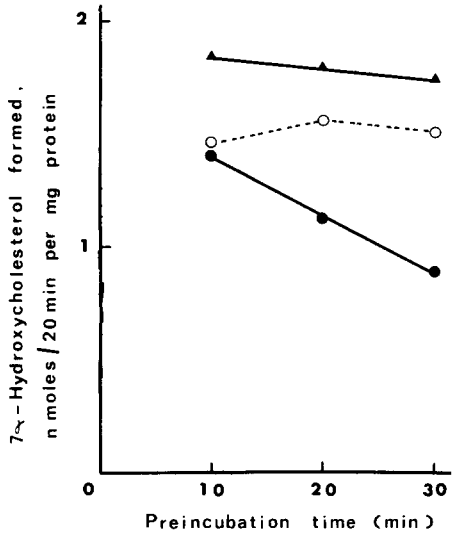

Fig. 2. Inactivation assay of cholesterol 7α-hydroxylase. The inactivation of cholesterol 7α-hydroxylase was carried out by preincubation with 3 mM ATP + 4.5 mM Mg^{2+} for the indicated preincubation times. Enzyme activity was assayed as described in the Experimental section following the addition of the NADPH-regenerating system and crude cytosol (●). Comparative studies were also made having the crude cytosol omitted (○), and included initially in the preincubation as denoted by (▲).

crude cytosol. It is interesting to note that the same cytosol that was shown to contain an activator (6) was required in the inactivation. This requirement was essential for maximal inactivation and it was also apparent that the inactivating effect of this cytosol could be differentially measured from that of the activator. To assay the inactivating activity, the cytosol must be added after preincubation, otherwise the activating activity would be preferentially assayed. Thus the observed effects of ATP or ADP added in the latter reaction represent some form of competitive reaction with the cytosol for the hydroxylase and this was supported by the diminishing effect of the cytosol with increasing nucleotide concentrations (Fig. 1 and Ref. 6). These findings thus explained the small effect of ATP in the absence of the cytosol.

The inactivation of cholesterol 7α-hydroxylase activity by ATP required bivalent cations. **Table 1-A** shows that Mn^{2+} was clearly more effective than Mg^{2+} or Ca^{2+} ions, but subsequent studies indicated that the Mn^{2+} effect was independent of ATP (**Table 2**). Mg^{2+} ions also caused some inhibition per se of the catalytic activity of cholesterol 7α-hydroxylase but were considerably less active in this regard than Mn^{2+}. Ca^{2+} substitution, however, had no apparent effect on hydroxylase activity. The results in Table 1-B also show that EDTA had little effect on either the hydroxylase activity or on ATP + Mg^{2+} inactivation.

TABLE 1. Some requirements for ATP inactivation of cholesterol 7α-hydroxylase

Addition	7α-Hydroxycholesterol Formed	
A. Standard incubation[a]	1.45 ± 0.28 (6)	1[d]
ATP	1.41 ± 0.26 (4)	0.93 ± 0.09
ATP + Mg^{2+}	0.89 ± 0.10 (6)	0.61 ± 0.12
ATP + Ca^{2+}	1.47 ± 0.03 (3)	0.78 ± 0.05
ATP + Mn^{2+}	0.82 ± 0.02 (3)	0.43 ± 0.02
B. Standard incubation[b]	1.37 ± 0.28 (4)	1
EDTA + Mg^{2+}	1.28 ± 0.26 (4)	0.92 ± 0.11
EDTA + ATP	0.89 (2)	0.90
EDTA + ATP + Mg^{2+}	0.65 (2)	0.66
C. None[c]	0.91	
ATP	0.55	
ATP + 1 mM cyclic AMP	0.51	
ATP + 2 MM cyclic AMP	0.53	
ATP + 4 mM cyclic AMP	0.53	

[a] Preincubation was carried out with ATP (3 mM), Mg^{2+} (4.5 mM), Mn^{2+} (4.5 mM), or Ca^{2+} (4.5 mM) without EDTA in the standard incubation mixture for 10 min.

[b] Effect of EDTA in 3 mM ATP + Mg^{2+} inactivation was studied.

[c] Effect of cyclic AMP on 3 mM ATP + Mg^{2+} inactivation was determined.

[d] Specific activity relative to control incubations which served as the internal controls for each experiment.

Enzyme activities were assayed by adding the NADPH-regenerating system plus crude cytosol (300 μg protein) as described in the Experimental section. Enzyme activity is expressed as nmol 7α-hydroxycholesterol formed/20 min per mg protein. The results are mean specific activity ± S.D. Number of independent experiments is shown in the parentheses.

Having shown that ATP inactivation of hydroxylase is bivalent cation-dependent, **Fig. 3 a** and **b** further show that these reactions were cation concentration-dependent. Fig. 3 b also provides further support for the requirement for the cytosol in the inactivation. In the absence of cytosol the inactivation of hydroxylase by ATP ranged from 10 to 25% with Mg^{2+} or Ca^{2+} at concentrations increasing from 1.8 to 7.2 mM. Mn^{2+}, as indicated earlier, proved to be highly inhibitory with inhibition ranging from 70 to 90% at a similar

TABLE 2. Effects of bivalent cations on cholesterol 7α-hydroxylase

Cations	7α-Hydroxycholesterol Formed	
None	1.83 ± 0.09	1[a]
Mg^{2+}	1.59 ± 0.06	0.87 ± 0.05
Mn^{2+}	0.59 ± 0.03	0.33 ± 0.11
Ca^{2+}	1.78 ± 0.11	0.89 ± 0.05

[a] Specific activity relative to controls without added bivalent cations.

Preincubation was carried out with chloride salts of Mg^{2+}, Mn^{2+}, or Ca^{2+} at concentrations of 4.5 mM as described in the standard incubation mixture without EDTA. The NADPH-regenerating system was added to commence the assay after 10 min preincubation. Enzyme activity is expressed as nmol 7α-hydroxycholesterol formed/20 min per mg microsomal protein. The results are mean (± S.D.) of four independent experiments.

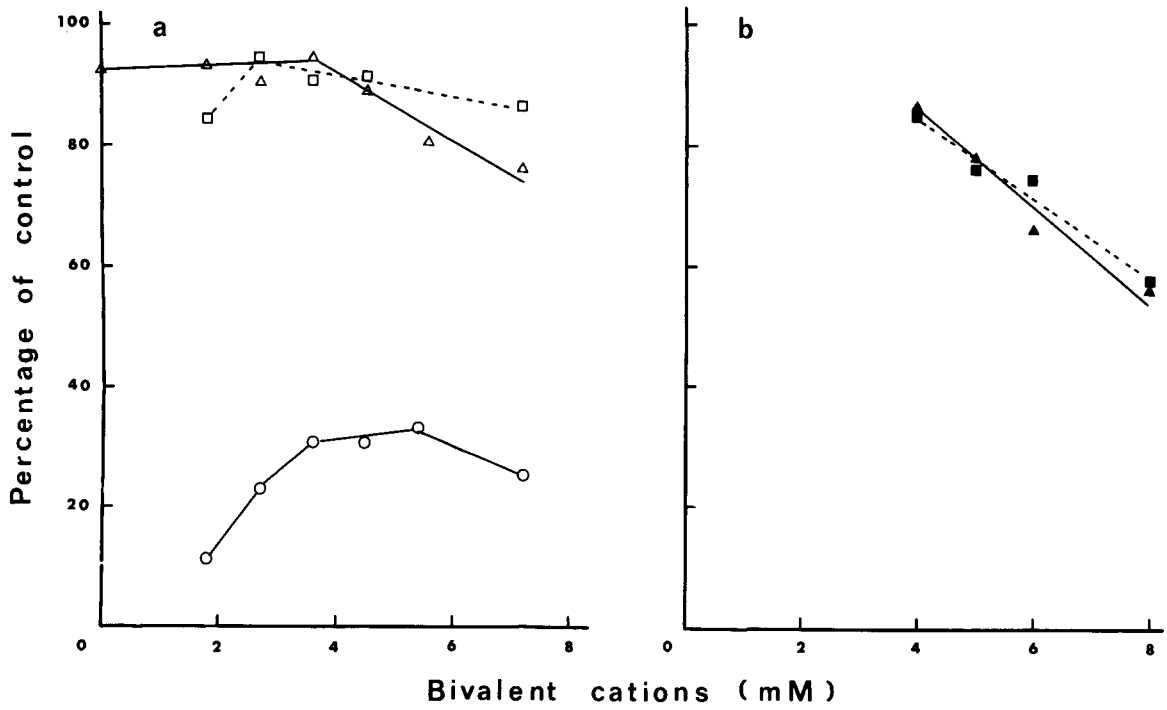

Fig. 3. Effects of bivalent cations on ATP inactivation of cholesterol 7α-hydroxylase. Chloride salt of bivalent cations Mg^{2+} (△), Mn^{2+} (○), or Ca^{2+} (□) was preincubated with 3 mM ATP in the absence of EDTA. Hydroxylase activity was assayed after a 30-min preincubation (a). The effect of the crude cytosol on ATP inactivation with Mg^{2+} (▲) and Ca^{2+} (■) was also studied as a function of relative enzyme activity against the cationic concentrations (b). In (b) hydroxylase activity was assayed after a 20-min preincubation by adding the NADPH-regenerating system plus the crude cytosol. The controls were that of standard incubation without ATP as described in the Experimental section.

range of ionic concentrations (Fig. 3 a). The presence of cytosol greatly enhanced the inactivating effects of Ca^{2+} and Mg^{2+} with inhibition ranging from 15 to 45% with increasing ionic concentrations (Fig. 3 b).

Combined effects of ATP and ADP

Although ATP and ADP were shown to be specific inhibitors of hydroxylase activity it was not clear whether one of these nucleotides by itself was adequate for the maximal inactivation, and the mechanism of reaction was not defined. The data in **Fig. 4** provide some answers to these questions. Fig. 4 a shows the combined effect of ATP and ADP on hydroxylase inactivation, hydroxylase activity being measured as a function of ADP concentration from 0 to 3 mM at two ATP concentrations, 1 and 3 mM, as indicated. The results clearly indicate that the ATP inactivation was concentration-dependent; as indicated, greater inactivation was observed with 3 mM ATP (39% of control value as compared to 75% at 1 mM ATP). The presence of ADP further accentuated the ATP effect, and this effect was also concentration-dependent. At both ATP concentrations the inactivation increased more or less in parallel with increasing ADP concentrations. It was significant that the inactivation was almost complete at an equimolar concentration of ATP and ADP at 3 mM.

Fig. 4 b shows the effects of equimolar ADP and AMP on 3 mM ATP inactivation of hydroxylase. Unlike the preceding figure, the preincubation time was reduced from 20 to 10 min as occurred with ADP. The ATP inactivation of hydroxylase increased with increasing equimolar nucleotide concentrations of AMP and ADP. However, the addition of AMP further increased the inactivation, the effect being about two-fold at ADP concentrations above 1.5 mM in comparison to Fig. 4 a. The same figure also shows the requirement of cytosolic factor(s) for maximal inactivation of hydroxylase as was shown earlier in this study. Similarly, the results confirm that the correct sequence of adding the cytosol to the assay system was crucial for maximal inactivation.

Fig. 4 c shows the effects of molar ratio of ATP/ADP at the indicated concentrations from 0 to 3 mM on the inactivation of hydroxylase, in addition to the effect of 50 mM NaF on these reactions. ATP or ADP, as previously observed, inactivated hydroxylase activity and the individual effect was comparable at the respective concentrations of 3 mM, though ATP appeared to be more inhibitory. The inactivating effect was minimal when the molar ratio of ATP/ADP was at 1:2. NaF was clearly antagonistic in effect to ATP, causing significant reduction of inactivation from 12 to 41% at increasing molar ratio of ATP/ADP from 0:3

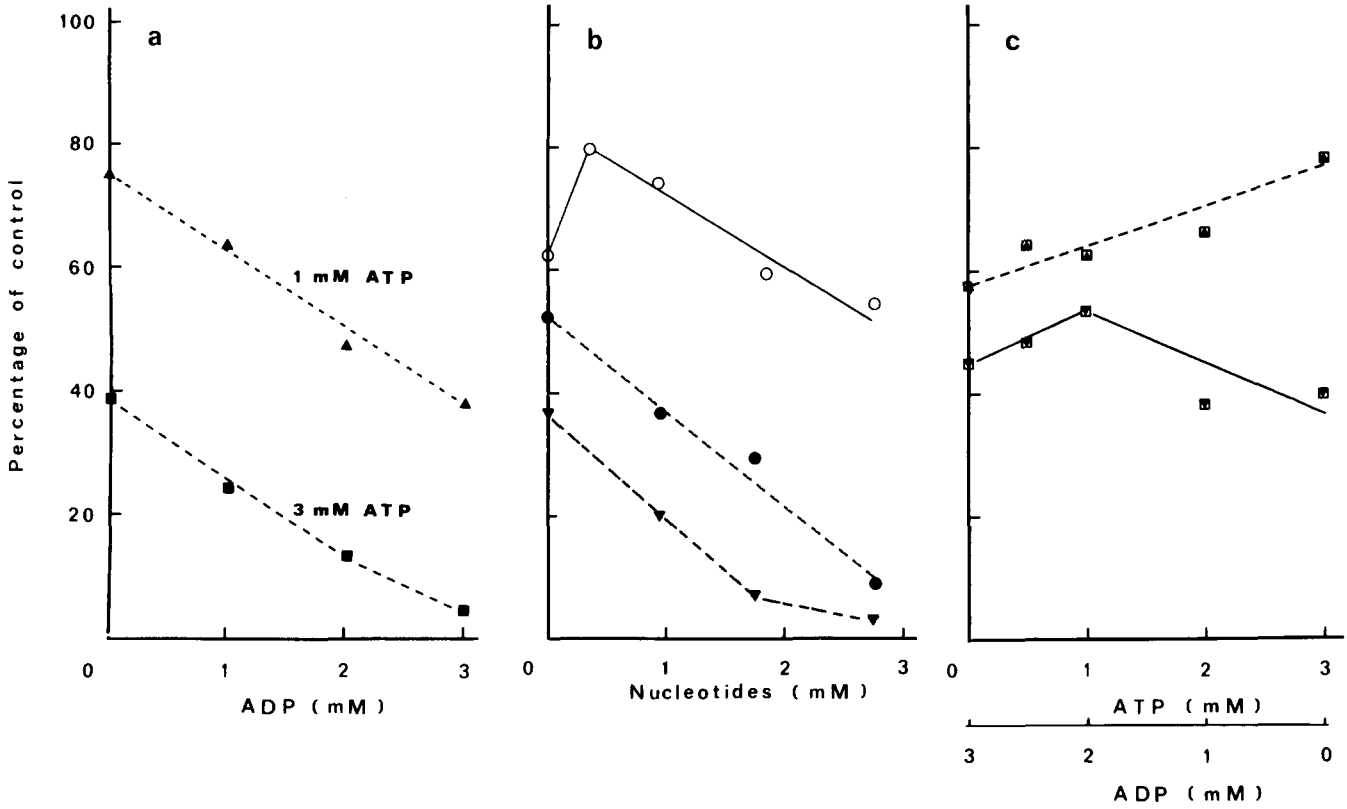

Fig. 4. Combined effects of adenine nucleotides on cholesterol 7α-hydroxylase. The effects of ADP, AMP, NaF, and crude cytosol on ATP inactivation of hydroxylase activity were studied. In (a), hydroxylase activity was measured as a function against graded concentrations of ADP with 1 mM (▲) and 3mM (■) ATP, respectively, in the presence of 8 mM Mg^{2+}. In both (a) and (c), a 20-min preincubation was allowed for. In (b), 3 mM ATP + 8 mM Mg^{2+} was preincubated with combined nucleotides represented by ADP and AMP (at equal amounts of 0.91 mM, 1.75 mM, and 2.80 mM) for 10 min. Comparative studies on the effect of the crude cytosol in this reaction were determined as indicated by its deletion (○), addition during the preincubation (●), and addition during the assay (▼). In (c), the effect of various molar ratios of ATP/ADP on hydroxylase inactivation was measured at the indicated concentration of ATP and ADP (▼). The effect of NaF (50 mM) on this inactivation was also determined (▲).

to 3:0. This is consistent with our previous observations (6).

Cyclic AMP effect on ATP inactivation

Cholesterol 7α-hydroxylase was inactivated by preincubation with ATP and enzyme activity was assayed by adding crude cytosol and a NADPH-regenerating system. The presence of cyclic AMP at concentrations ranging from 1 to 4 mM had little or no effect on ATP inactivation (Table 1-C).

Reversible inactivation of cholesterol 7α-hydroxylase

The results so far had suggested that the cytosol contained factors that aided in the activation as well as in the inactivation of cholesterol 7α-hydroxylase. It was of interest to determine whether the activator was capable of reversing the inactivation by ATP. **Fig. 5 a** shows that, in the absence of crude cytosol, ATP was capable of inactivating cholesterol 7α-hydroxylase activity, albeit to a much smaller extent (Fig. 5 a, open circles). The inactivation was reversed by adding the crude cytosol. However, the reactivation reaction was dependent on both reaction and preincubation times. When the crude cytosol was added following an assay carried out after the 10-min preincubation with ATP, the hydroxylase activity declined during the first 5 min and then increased with increasing incubation times with the added crude cytosol (Fig. 5 a, solid circles). The hydroxylase catalytic activity was restored to normal after a 20-min challenge by the crude cytosol and this activity continued to increase with increasing incubation times.

When the inactivation of hydroxylase activity was challenged after 60 min of preincubation with ATP by introducing the crude cytosol, hydroxylase activity was restored only to the level of the 20-min inactivation, even after a further 60-min incubation (Fig. 5 b, solid circles), showing partial reactivation. In contrast, introduction of the crude cytosol to the control without ATP in the preincubation normalized hydroxylase catalytic activity as was observed in the challenge after

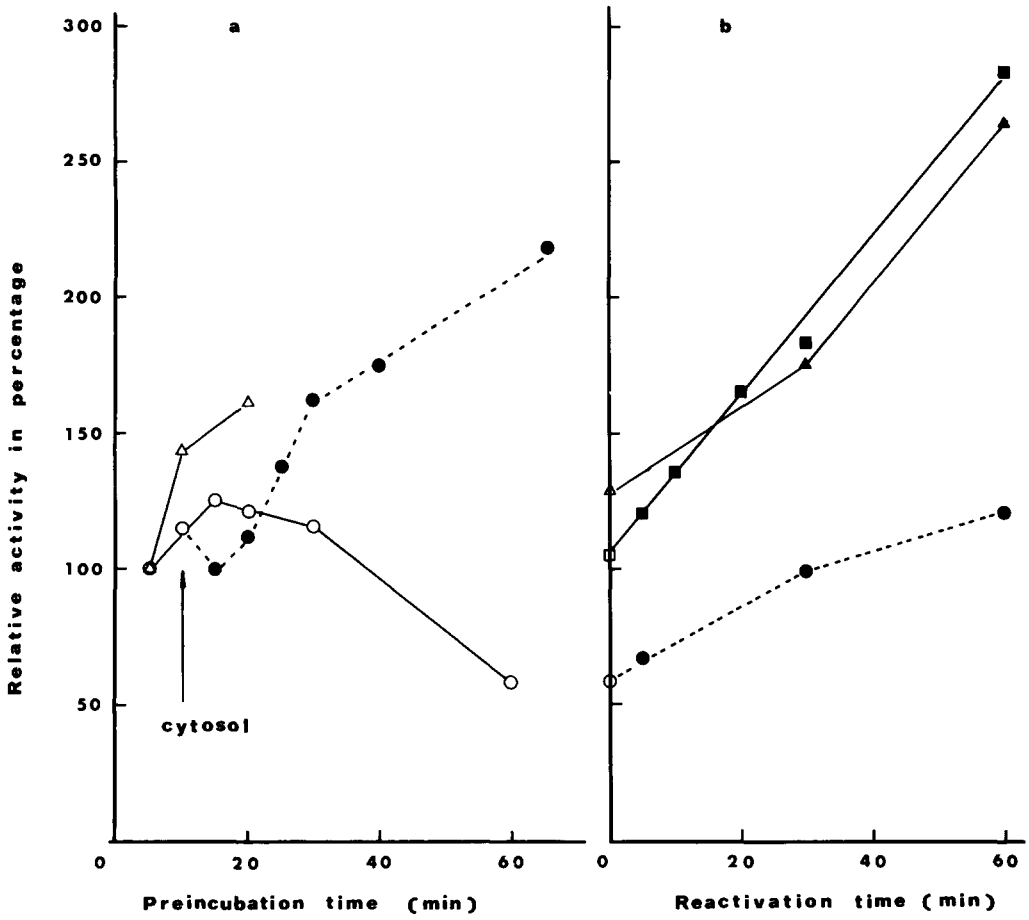

Fig. 5. Inactivation and reactivation of cholesterol 7α-hydroxylase. The inactivation and reactivation of hydroxylase activity was studied as a function of time. Preincubation was carried out with (○,●) or without (△,▲) ATP for the indicated time intervals and hydroxylase activity was assayed as described in the Experimental section. In (a), hydroxylase activity was mesured at 5, 10, 15, 20, 30, and 60-min intervals following the addition of crude cytosol. The ordinate represents the percentage of hydroxylase activity relative to that of the 5-min preincubation. The open symbols in (a) represent the hydroxylase activity remaining at the end of the indicated time of preincubation, while in (b) they represent the activities remaining at the end of a 60-min preincubation. Crude cytosol (300 μg) was added to the assays after a 10- and 60-min preincubation as shown in (a) and (b), respectively. The hydroxylase activity was determined at the indicated time of reactivation following the introduction of the crude cytosol as represented by the close symbols. The effect of 50 mM NaF on these reactions was also compared (□,■).

the 10-min preincubation (Fig. 5 b, triangles). The effect of NaF on reactivation was also studied. The presence of NaF again reduced the inhibitory effect of ATP considerably (Fig. 5 b, open square) and the reactivation reaction by the crude cytosol resulted in a full return to normal activity (Fig. 5 b, solid squares).

It was of interest to determine whether the ammonium sulfate subfractions, as previously reported (6), contained inactivator activity in addition to the activator activity already demonstrated in these fractions. Inactivation assay of cholesterol 7α-hydroxylase was carried out with ammonium sulfate fractions of 40% and 40–65% saturation. The presence of these fractions did not completely prevent the inactivation, but reduced the magnitude and the onset of inactivation considerably (**Fig. 6**, solid triangles and squares) as compared with that with crude cytosol (open squares). Subjecting the crude cytosol to heat and ammonium sulfate-fractionation treatments appeared to have eliminated the inactivator activity sensitive to NaF from the 40% and 40–65% ammonium sulphate subfractions. This was supported by the reactivation activities of these fractions without requiring NaF for maximal reactivation to the level of activity of the standard assay (**Fig. 7 a** and **b**). The reactivation reaction was dependent on the amount of activator present as indicated by the assay with the 65% ammonium sulphate fraction (Fig. 7 a). The subfractions of 40% and 40–65% ammonium sulfate saturation were as active in the reactivation

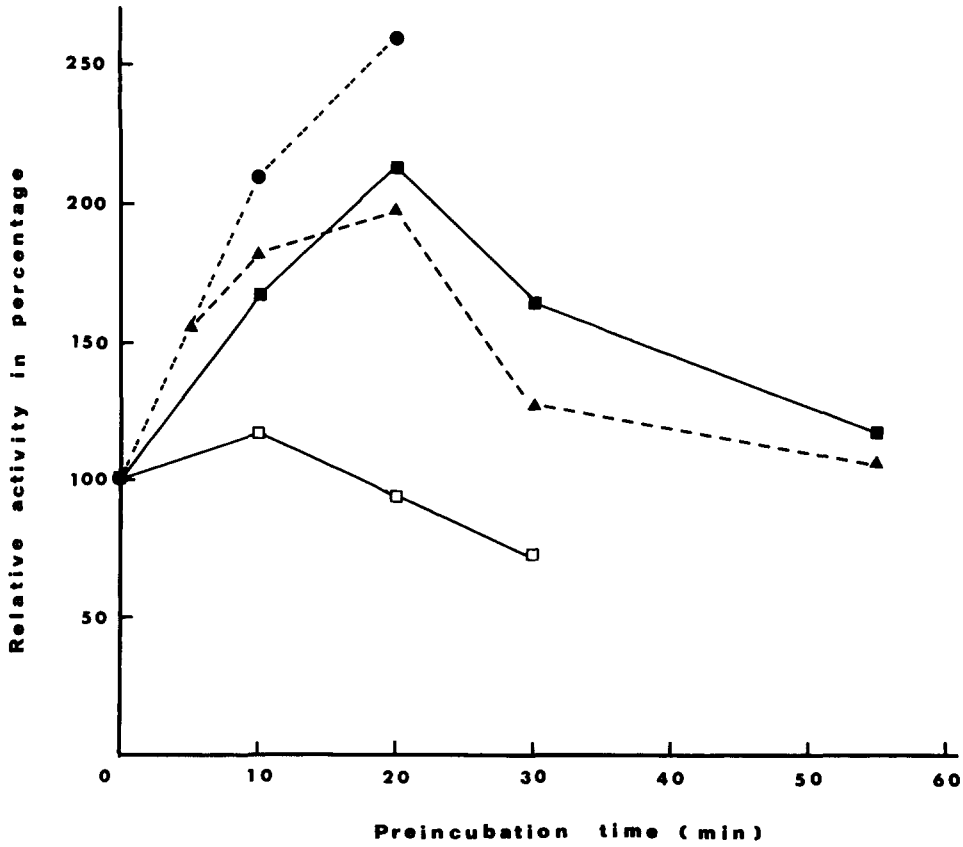

Fig. 6. Effects of the ammonium sulfate fractionated cytosol on ATP inactivation of cholesterol 7α-hydroxylase. Inactivation of cholesterol 7α-hydroxylase was carried out with 3 mM ATP + 4.5 mM Mg^{2+} at various preincubation intervals up to 55 min. Ammonium sulfate fractionations of the heat-treated cytosol were prepared as previously described (6). Crude cytosol (□), 40% (▲) (78 μg protein), and 40–65% (■) (75 μg protein), saturated ammonium sulfate fractions of the cytosol were added during the assay. Control (●) assays without added ATP were similarly carried out. Enzyme activities were expressed relative to the zero time preincubation.

(Fig. 7 b) though twice as much of the 40–65% fraction was present.

DISCUSSION

Studies on molecular modulations of cholesterol 7α-hydroxylase and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase are of great importance in understanding the regulation of cholesterol metabolism, since they are the rate-limiting enzymes. Knowledge of the regulation of the activities of these enzymes will provide insight into the pathogenesis, and perhaps give a lead towards the prevention, of those metabolic diseases related to the enterohepatic and systemic circulation of cholesterol. Several studies have shown that gallstone patients have altered activities of these hepatic enzymes. Thus, abnormal regulation of these two rate-limiting enzymes appears to be an important factor in the pathogenesis of gallstones (7–9).

There is good evidence that these enzymes are subject to similar regulatory mechanisms and this has been reviewed by Myant and Mitropoulos (10). In the rat liver, these enzymes are found in a common location endoplasmic reticulum with low RNA density (11). The enzymic activities vary similarly in a circadian rhythm with maximum activities at midnight and minimum activities at noon (12, 13). The enzymic activities are altered in a parallel fashion by various treatments such as cholestyramine and cholic acid feeding, ethanol and phenobarbital administration (14, 15).

At molecular levels, these enzymes are activated by a cytosolic factor and inactivated by ATP + Mg^{2+} (6, 16–18). It is interesting to note that both of these enzymes are maximally activated, 2- to 3-fold, by a 15–20-min preincubation (6, 19). These activations are probably associated with desensitization of ATP + Mg^{2+} inactivation (20). Washing the microsomes enhances hydroxylase activity and this may be related to loss of a microsomal inhibitor as shown by Beg,

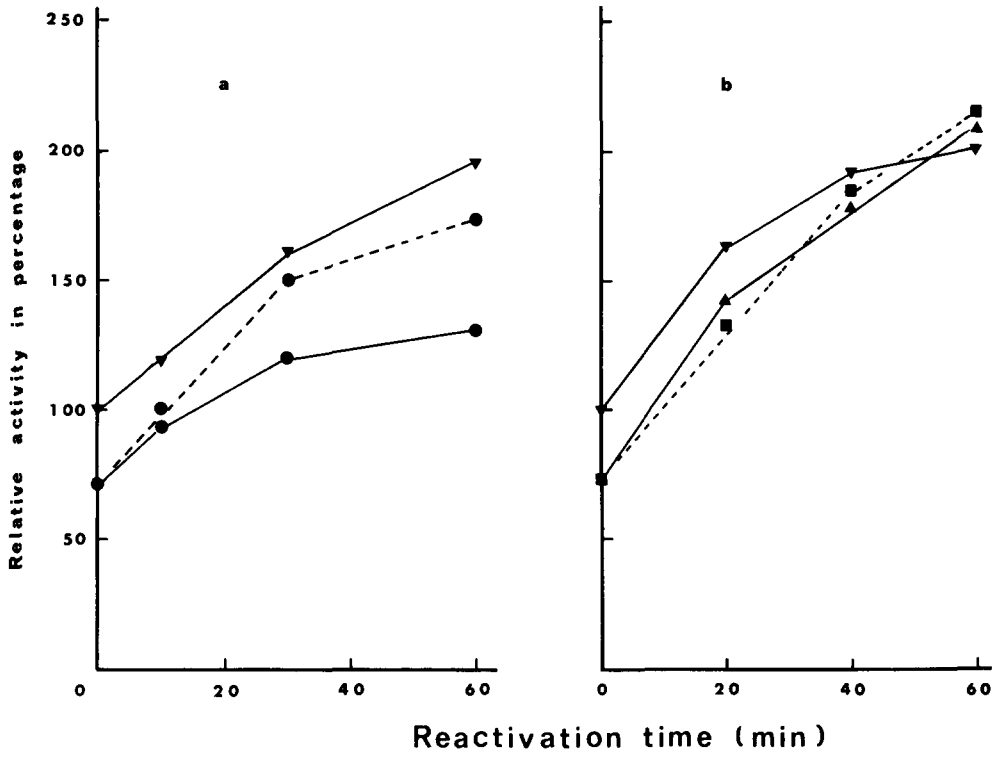

Fig. 7. Reactivation of cholesterol 7α-hydroxylase by ammonium sulfate fractionated cytosol. Cholesterol 7α-hydroxylase was inactivated by preincubation with ATP + Mg^{2+} as described in the Experimental section. (a), Reactivation by 65% saturation ammonium sulfate fractions, 49 μg cytosolic protein (● —— ●) and 98 μg cytosolic protein (● – – – ●), and control (▼). (b), Reactivation by 40% (▲) and 40–65% (■) saturation ammonium sulfate fractions with respective protein concentrations of 78 μg and 150 μg, and control (▼) with 40% saturation ammonium sulfate fraction added. Enzyme activities were expressed as percentage relative to control without ATP added.

Allmann, and Gibson (16), or to sequential loss of ATP + Mg^{2+} inactivation of HMG-CoA reductase when the microsomes were repeatedly extracted with buffer solution.

However, our initial data suggested that the molecular modulations of cholesterol 7α-hydroxylase differ in many respects from those of HMG-CoA reductase (6) and the present findings provide further support to those observations. The inactivation of cholesterol 7α-hydroxylase by adenine nucleotides shows specificity for ATP and ADP. AMP and cyclic AMP appear to be stimulatory. ADP appears comparable to ATP as an inactivating agent in the modulation of cholesterol 7α-hydroxylase activity. It has been demonstrated recently that modulations of HMG-CoA reductase and associated protein kinase are mediated via a reversible reaction of phosphorylation-dephosphorylation (21, 22). However, we have no data yet to support a similar proposition for cholesterol 7α-hydroxylase, nor are we clear as to whether the inactivation is directly linked to the hydroxylase, to the inhibitor, or both. Inactivation of cholesterol 7α-hydroxylase by ATP is dependent on time and a cytosolic factor. Cyclic AMP has no effect in the inactivation reaction though preincubation with cyclic AMP was shown to diminish HMG-CoA reductase activity (16, 19). The NaF effect on hydroxylase activity is consistent in counteracting the effect of ATP. Although the present findings do not provide a clear indication of this reaction mechanism, it appears that NaF is acting specifically on the inhibition of ATP interaction with hydroxylase and, in doing so, it relieves the inactivation. In contrast, NaF has considerably less effect on ADP inactivation of 7α-hydroxylase activity, suggesting that the hydrolysis of ATP is a crucial step in mediation of the inactivation reaction. This is a reaction quite unlike its inhibitory effect in the activation of HMG-CoA reductase (18, 19). The specificity of these reactions suggest that covalent modification by ATP + Mg^{2+} may be involved in the regulation of cholesterol 7α-hydroxylase activity.

Support for the two catalytic forms of hydroxylase is based on the reversible inactivation of the enzyme mediated by a cytosolic factor or factors. The present findings suggest that the cytosol contains both the activator and inactivator activities, a property in com-

mon with that of HMG-CoA reductase (16, 18). The activities of these factors in the crude cytosol can be differentially assayed by manipulating the preincubation procedure. The inclusion of the crude cytosol in preincubation stimulates 7α-hydroxylase activity by 30–45% and this activation can be counteracted by ATP (6). Similarly, Nordstrom, Rodwell, and Mitschelen (18) have demonstrated that the cytosolic activator can activate 5–25% of HMG-CoA reductase. These findings suggest that considerable amounts of both hydroxylase and HMG-CoA reductase are present in catalytically latent forms. The inactivation of cholesterol 7α-hydroxylase by ATP can be reversed by a cytosolic activator in a time-dependent reaction. The following observations suggest that the crude cytosol also contains the inactivator. Addition of the crude cytosol in the inactivation assay enhanced the rate of reaction. This effect, however, was eliminated from the 40% or 40–65% saturation ammonium sulfate fractions. Furthermore, the lack of requirement of NaF for complete reactivation mediated by these fractions lends further support for its presence. In contrast, NaF is required for complete reactivation by the crude cytosol. At this stage it is not clear whether the inactivator has been eliminated from the cytosol by such treatment, and this awaits further investigation.

The inactivation of hydroxylase activity by ATP requires Mg^{2+}, and the magnitude of this inactivation is comparable to that of HMG-CoA reductase (16, 18). It is interesting to note that Mn^{2+} ions are highly inhibitory to hydroxylase activity. Other bivalent cations such as Hg^{2+}, Pb^{2+}, Cu^{2+}, and Zn^{2+} are also reported to be highly inhibitory to hydroxylase activity (23). Mg^{2+} ions have been routinely included in the assay of cholesterol 7α-hydroxylase (1, 23, 25, 26). It is interesting to note that its inhibitory effect on 7α-hydroxylase activity has not been reported. These elements have been reported in gallstones (24), but any significance of these associations awaits further investigation.

The present findings demonstrate that cholesterol 7α-hydroxylase can be reversibly inactivated by cytosolic factors and ATP. The mechanism of modulation reactions appears to be dissimilar to the 'phosphorylation-dephosphorylation' model proposed for HMG-CoA reductase (21, 22). Studies are being undertaken to isolate the activator and the inactivator, and to investigate further the nature of the reversible inactivation reaction by ATP.

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