Allosteric and non-allosteric forms of rat liver 3-hydroxy-3-methylglutaryl coenzyme A reductase: differential inhibition of activity by adenosine 2'-monophospho-5'-diphosphoribose

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Abstract Adenosine 2'-monophospho-5'-diphosphoribose (P-ADP-Rib) is a structural analog of NADPH which was reported to competitively inhibit (Ki_{app} = 21.7 μM) solubilized rat liver 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (Tanazawa, K., and A. Endo. 1979. *Eur. J. Biochem.* **98:** 195–201). However, microsomal HMG-CoA reductase, which at low thiol concentrations exhibits allosteric properties, is only poorly inhibited by P-ADP-Rib (Ki_{app} = 550 μM at 4.5 mM GSH). Gradual shift of the microsomal reductase towards a non-allosteric form by increasing glutathione (GSH) concentrations resulted in a higher inhibition by P-ADP-Rib. Under these conditions, Ki values for P-ADP-Rib were 165 μM and 53 μM at 9 mM and 27 mM GSH, respectively. The largest change in the degree of inhibition by P-ADP-Rib was observed within the 10 mM range of GSH. By contrast, freeze-thaw solubilized HMG-CoA reductase, which does not display allosteric properties, is readily inhibited by P-ADP-Rib, even when assayed at a low concentration of GSH (Ki_{app} = 50 μM at 4.5 mM GSH). Assaying the solubilized reductase in the presence of increased thiol concentration results in a minor decrease in the apparent Ki for P-ADP-Rib (22 μM at 27 mM GSH). Microsomal HMG-CoA reductase is allosterically activated by various nucleotides. When activated by NADH, the enzyme is effectively inhibited by P-ADP-Rib even at a 4.5-mM GSH concentration (Ki_{app} = 175 μM in the presence of 300 μM NADH). These results support a proposal that thiol-dependent reduction of enzyme disulfide(s) or binding of an allosteric activator to it cause conformational changes in the microsomal reductase protein that alter the properties of its catalytic NADPH-binding site(s). **—Roitelman, J., and I. Shechter.** Allosteric and non-allosteric forms of rat liver 3-hydroxy-3-methylglutaryl coenzyme A reductase: differential inhibition of activity by adenosine 2'-monophospho-5'-diphosphoribose. *J. Lipid Res.* 1986. **27:** 828–835.

Supplementary key words cholesterol synthesis • allosteric activation • glutathione • competitive inhibition

The major rate-limiting step in the biosynthetic pathway of sterols is considered to be the reduction of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) to mevalonate. This reaction is catalyzed by the microsomal enzyme HMG-CoA reductase (E.C. 1.1.1.34) (1–4).

Much attention has been focused on HMG-CoA reductase in attempts to elucidate the different modes of regulation of its activity. These modes have been proposed to involved processes of phosphorylation (inactivation) and dephosphorylation (activation) of the reductase protein (5–7), effects of the membrane lipids (8–10), alterations in the cellular content of specific mRNA coding for the reductase (11–13), and changes in the rates of enzyme synthesis and degradation (13–17).

There is a growing number of reports on the role of thiols and disulfides in determining the activity of HMG-CoA reductase in vitro (18–25). The activities of both microsomal and freeze-thaw solubilized HMG-CoA reductase preparations are expressed only in the presence of thiols (22–24). Thus, reduction of the enzyme by thiols is essential for the reaction (22, 23). In addition, the kinetic properties of HMG-CoA reductase are altered with thiol concentrations (23, 24). We have shown that, at low thiol concentrations, microsomal HMG-CoA reductase displays sigmoidal NADPH-dependent kinetics (24). These and similar observations (26) indicate positive cooperativity in binding NADPH to the enzyme's catalytic site(s). Under these conditions, NADH, which is neither a substrate nor an inhibitor of the reductase, greatly enhances the activity in a manner characteristic to an allosteric activator (25). As thiol concentrations increase, the sigmoidal NADPH-dependent kinetics gradually shift toward classical Michaelis-Menten hyperbola (24) and the NADH-enhancing effect is greatly diminished (25). All these phenomena were not observed with the freeze-thaw solu-

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; P-ADP-Rib, adenosine 2'-monophospho-5'-diphosphoribose; MVA, mevalonate; n_H, Hill coefficient.

bilized reductase, an active proteolyzed fragment of the microsomal enzyme (27, 28). The solubilized form of the enzyme displays classical Michaelis-type NADPH-dependent kinetics regardless of the thiol concentration used for its activation (24) and is not affected by NADH (25).

Based on this kinetic behavior, we proposed that microsomal HMG-CoA reductase is an allosteric enzyme, undergoing conformational changes in response to allosteric effectors (e.g., NADH) and alterations in thiol concentrations. In this report we present data suggesting that similar conformational changes are required for the inhibition of HMG-CoA reductase activity by a NADPH analog, adenosine 2'-monophospho-5'-diphosphoribose (P-ADP-Rib).

MATERIALS AND METHODS

Materials

GSH, NAD(P)H, P-ADP-Rib, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, leupeptin, and mevalonate (lactone form) were obtained from Sigma. Radiolabeled D,L-[3-^{14}C]HMG-CoA (55–57 mCi/mmol) was obtained from New England Nuclear and Amersham. All other reagents were of analytical grade and were purchased from local suppliers.

HMG-CoA reductase preparations

A microsomal fraction was obtained from livers of 200–250-g female rats (Wistar-Tel Aviv) that were fed ad libitum for 2 weeks with normal diet containing 10% (w/w) ground Amberlite XAD-2 resin (29). Animals were killed 4 hr after onset of the dark phase of a 12-hr light–dark cycle. Microsomes were prepared in buffer containing 0.3 M sucrose, 30 μM leupeptin, 10 mM K^+-HEPES, pH 7.5, and filtered through a 3 × 48 cm Sepharose 4B column, as described (24). Solubilized HMG-CoA reductase was prepared by the freeze-thawing procedure and was partially purified as previously described (24).

Assay of HMG-CoA reductase activity

Reductase activity was assayed by a slight modification of the procedure described by Shapiro et al. (30). Reactions were carried out in a total volume of 100 μl containing HEPES buffer (160 mM K^+-HEPES, 200 mM KCl, pH 7.5), 0.4 units of glucose-6-phosphate dehydrogenase, 10 mM glucose-6-phosphate, GSH, NAD(P)H, and P-ADP-Rib to concentrations as specified in the figure legends. After addition of the reductase preparations (7.2 μg of microsomal protein or 7.4 μg of soluble protein), the mixtures were preincubated at 37°C for 35–40 min in order to obtain maximal activation of the latent reductase. The reactions were initiated by addition of 30 μM [^{14}C]HMG-CoA and proceeded for 35–40 min. In NADPH-dependent kinetics, both NADPH and [^{14}C]HMG-CoA were omitted during preincubation and the reactions were initiated by simultaneous addition of both substrates. Measurements of the [^{14}C]MVA that was produced were as previously described (24). Under the above conditions, the formation of [^{14}C]MVA was linear with time and the amount of added protein.

Other assays

Protein was measured by a modification of the procedure of Lowry et al. (31). Concentrations of NAD(P)H and P-

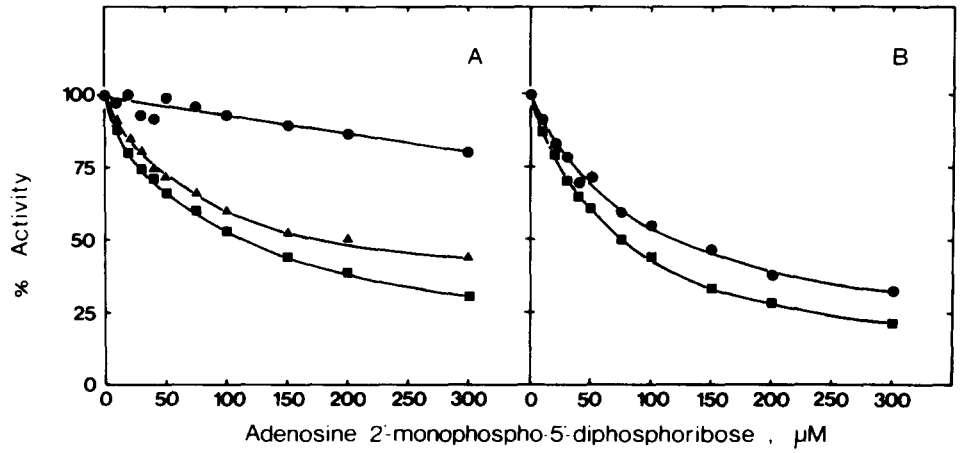

Fig. 1. The effect of P-ADP-Rib on the activity of HMG-CoA reductase. Microsomal (panel A) or solubilized (panel B) HMG-CoA reductase was preincubated in HEPES buffer with NADPH-generating system (10 mM glucose-6-phosphate, 0.4 I.U. of glucose-6-phosphate dehydrogenase), in the presence of 4.5 mM (●), 9.0 mM (▲, only the microsomal enzyme), or 27.0 mM (■) GSH, NADPH, and P-ADP-Rib to the indicated concentrations. The concentrations of NADPH used were in the range of $S_{0.5}$ for the microsomal enzyme (i.e., 60–75 μM NADPH) or at the K_m concentration at 4.5 mM GSH (i.e., 35 μM NADPH) for the solubilized reductase. Mixtures were preincubated for 35 min at 37°C in a volume of 90 μl. Reactions were initiated by addition of 10 μl (30 μM) of [^{14}C]HMG-CoA and were terminated after 35 min. Values represent the mean of two to six different experiments.

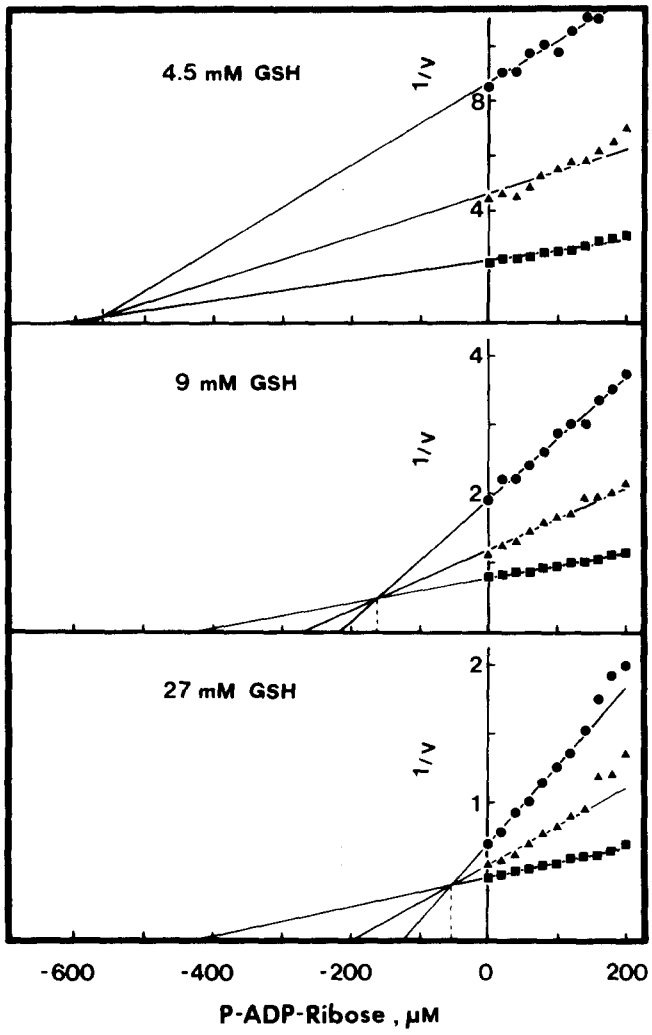

Fig. 2. Inhibition of microsomal HMG-CoA reductase by P-ADP-Rib. Column-filtered microsomes, suspended in HEPES buffer, were preincubated with 125 μM (●), 250 μM (▲) or 1000 μM (■) NADPH and an NADPH-generating system (10 mM glucose-6-phosphate, 0.4 I.U. of glucose-6-phosphate dehydrogenase), in the presence of the indicated concentrations of P-ADP-Rib and GSH. Preincubations, in a volume of 90 μl, werre for 35 min at 37°C after which reactions were initiated by addition of 30 μM [^{14}C]HMG-CoA in 10 μl. After 35 min at 37°C, the reactions were terminated and [^{14}C]MVA was measured. The results, plotted as 1/v versus [P-ADP-Rib], are the mean value of two different experiments. Enzyme activity, v, is in nmol MVA/mg protein per min.

ADP-Rib were measured spectrophotometrically at 259 nm. GSH was determined using Ellman's reagent (32).

Data analysis

V_{max} was determined from double reciprocal plots of rate versus the first and second powers of NADPH concentrations. Hill coefficients and $S_{0.5}$ values were calculated from Hill plots (log v/V_{max} – v vs. log [NADPH]). Ki values for P-ADP-Rib were determined from Dixon plots (1/v vs. [P-ADP-Rib]).

RESULTS

We have investigated the inhibition of HMG-CoA reductase by adenosine 2'-monophospho-5'-diphosphoribose (P-ADP-Rib), a structural analog of the substrate NADPH. Surprisingly, when microsomal HMG-CoA reductase was activated with a low concentration of GSH (4.5 mM) and assayed in the presence of $S_{0.5}$ concentrations of NADPH (60–75 μM) and increasing amounts of P-ADP-Rib, almost no inhibition of the reductase was observed (**Fig. 1A**). At 100 μM P-ADP-Rib there was only 7–10% inhibition of the activity, and even at the highest concentrations of P-ADP-Rib tested (up to 300 μM), the maximal decline in activity never exceeded 20% of that of the control (no P-ADP-Rib). At such low concentrations of NADPH, Dixon plots were nonlinear and the determination of Ki was practically impossible (data not shown). However, when similar experiments were performed at higher concentrations of NADPH (125–1000 μM), linear Dixon plots were obtained. These plots demonstrate competitive inhibition of the enzyme by P-ADP-Rib, with an apparent Ki of 550 μM (**Fig. 2**). When higher concentrations of GSH were used to activate microsomal HMG-CoA reductase, a drastic decrease in the Ki for P-ADP-Rib was observed. Under these conditions, Ki_{app} values of 165 μM and 53 μM were obtained at 9 mM and 27 mM GSH, respectively (Fig. 2).

The above results seemed inconsistent with those reported by Tanazawa and Endo (33). However, these investigators studied the freeze-thaw solubilized form of HMG-CoA reductase. When reductase solubilized by the same procedures is assayed, it is clearly shown that such enzyme is readily inhibited by P-ADP-Rib, even when activated with 4.5 mM GSH (Fig. 1B). Under these conditions, linear Dixon plots are obtained even at very low NADPH concentration, and the calculated Ki value is 50 μM (**Fig. 3**). When solubilized HMG-CoA reductase was activated with a higher concentration of GSH (27 mM), the apparent Ki for P-ADP-Rib decreased only slightly to 22 μM, in agreement with the value reported by Tanazawa and Endo (21.7 μM) (33).

The effect of P-ADP-Rib on the NADPH-dependent kinetics of microsomal reductase is shown in **Fig. 4.** At low GSH concentrations (Fig. 4, closed symbols), microsomal HMG-CoA reductase displayed sigmoidal kinetics (n_H = 1.8, **Table 1** and refs. 24, 25). Under these conditions, addition of 50 μM P-ADP-Rib had no apparent effect, and 150 μM of the nucleotide increased the $S_{0.5}$ value for NADPH by only 62% (Table 1). By contrast, at high GSH (Fig. 4, open symbols), HMG-CoA reductase approached classical Michaelis-Menten kinetics (n_H = 1.1, Table 1 and refs. 24, 25), and addition of 50 μM of P-ADP-Rib had already caused a 90% increase in the $S_{0.5}$ value for NADPH. P-ADP-Rib at a concentration of 150 μM increased $S_{0.5}$ by more than 260% (Table 1). Under these conditions, the estimated Ki value for

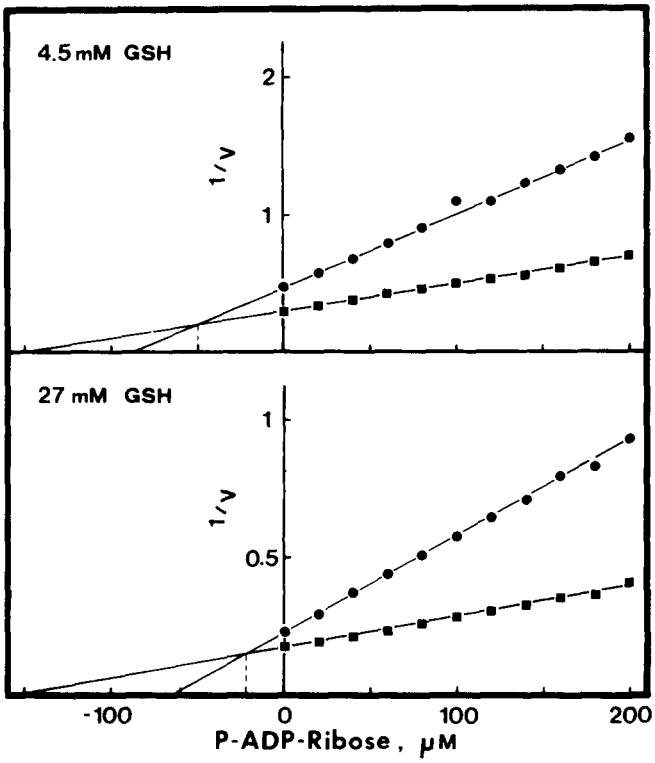

Fig. 3. Inhibition of solubilized HMG-CoA reductase by P-ADP-Rib. Freeze-thaw solubilized reductase was preincubated in HEPES buffer containing 35 μM (●) or 100 μM (■) NADPH and an NADPH-generating system (10 mM glucose-6-phosphate, 0.4 I.U. of glucose-6-phosphate dehydrogenase), in the presence of the indicated concentrations of P-ADP-Rib and GSH. Preincubations, in a volume of 90 μl, were for 35 min at 37°C after which reactions were initiated by addition of 30 μM [^{14}C]HMG-CoA in 10 μl. After 35 min at 37°C, the reactions were terminated and [^{14}C]MVA was measured. The results, plotted as 1/v versus [P-ADP-Rib], are the mean value of two different experiments. Enzyme activity, v, is in nmol MVA/mg protein per min.

P-ADP-Rib was 56.0 ± 0.4 μM, in accordance with the value determined by the Dixon plots (Fig. 2). At both thiol concentrations, P-ADP-Rib did not affect the V_{max} of the reaction (Fig. 4B), as expected for a competitive inhibitor (cf. Fig. 2).

The inhibition of microsomal HMG-CoA reductase by P-ADP-Rib depends on GSH concentration, as shown in **Fig. 5.** For any given concentration of P-ADP-Rib, the degree of inhibition of reductase activity increased as GSH concentrations increased (see inset). Note that the largest changes in the degree of inhibition occurred within the 10 mM range of GSH.

In a previous report from this laboratory we demonstrated that NADH, which is neither a substrate nor an inhibitor of hepatic HMG-CoA reductase, enhances the activity by increasing enzyme affinity toward NADPH (25). It was, therefore, of interest to study whether allosteric activation of microsomal HMG-CoA reductase by NADH affected the inhibition of the enzyme by P-ADP-Rib. **Fig. 6** shows that there exists an inverse relationship between the effects exerted by NADH and P-ADP-Rib. At 4.5 mM GSH, only 9% inhibition of microsomal HMG-CoA reductase activity was obtained by as much as 100 μM P-ADP-Rib, and 300 μM of the inhibitor decreased the activity by 20%. Addition of up to 100 μM NADH enhanced the activity 2.5-fold and, under these conditions, 100 μM and 300 μM P-ADP-Rib inhibited reductase activity by 25% and 50%, respectively (Fig. 6, inset). In order to distinguish whether NADH and P-ADP-Rib compete for a common site(s) on the reductase or act at different sites, we determined the Ki value for P-ADP-Rib in the presence of saturating concentration of NADH. As shown in **Fig. 7,** the apparent Ki value for P-ADP-Rib decreased to 175 μM in the presence of 300 μM NADH, as compared to Ki_{app} = 550 μM in its absence.

DISCUSSION

The mechanisms underlying the regulation of HMG-CoA reductase activity are not yet fully understood. We have recently shown that rat liver microsomal reductase displays sigmoidal NADPH-dependent kinetics, indicating cooperative binding of this substrate (24, 25). During our current studies on the allosteric properties of HMG-CoA reductase, we have investigated the effect of P-ADP-Rib on the enzyme activity. This nucleotide is a structural analog of NADPH (lacking the nicotinamide moiety) and was reported to competitively inhibit the reductase (33, 34). It can be predicted that when allosteric enzymes are assayed at low substrate concentration, low amounts of substrate analogs (competitive inhibitors) would enhance, rather than inhibit, the enzymatic activity. Such phenomena were observed with aspartate transcarbamylase (35) and threonine deaminase (36). However, the data presented in this report demonstrate that the activity of allosteric HMG-CoA reductase, assayed at low NADPH concentration, is not enhanced by low amounts of P-ADP-Rib. Moreover, higher concentrations of P-ADP-Rib do not significantly inhibit reductase activity. These findings indicate that, at low GSH concentrations, P-ADP-Rib is poorly recognized as an inhibitor by the allosteric form of the microsomal reductase. By contrast, both the microsomal reductase activated with high thiol concentrations and the freeze-thaw solubilized enzyme activated by any thiol concentration (the non-allosteric forms) are competitively inhibited by P-ADP-Rib (Figs. 1-4, Table 1 and ref. 33). Thus, the latter forms of the enzyme do recognize P-ADP-Rib as an analog of NADPH. Therefore, it can be concluded that the nicotinamide moiety is not crucial for the binding of the ligand to the catalytic site(s) of the nonallosteric forms of the enzyme. However, since P-ADP-Rib serves as a poor inhibitor of the allosteric form of the enzyme and also fails to activate it at low NADPH concentrations (Fig. 1A), P-

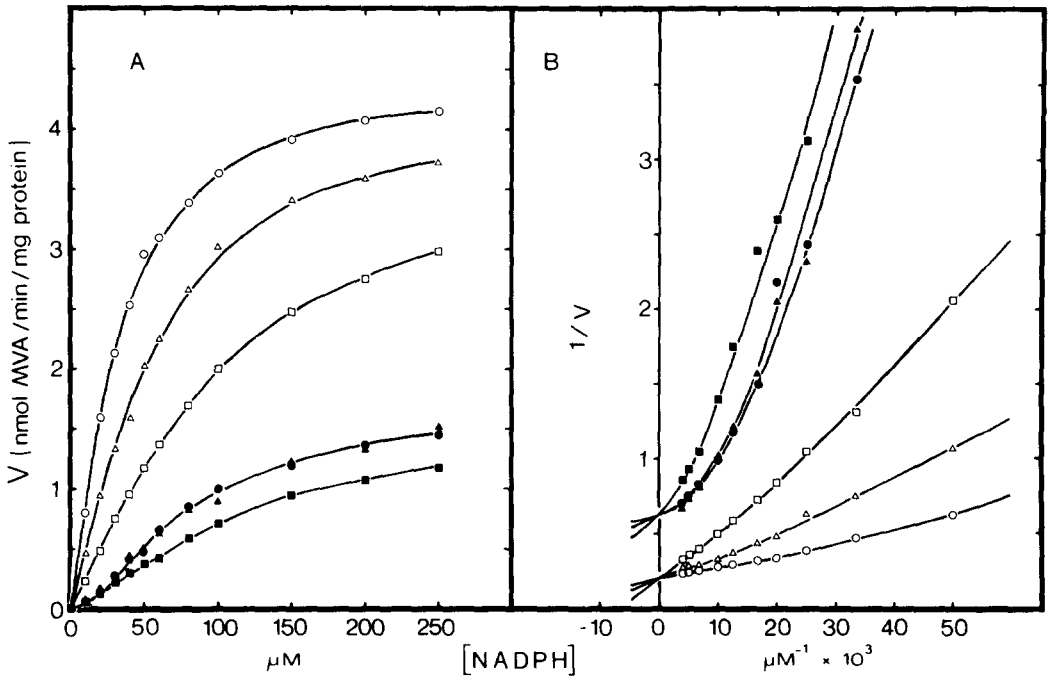

Fig. 4. The effect of P-ADP-Rib on the NADPH-dependent kinetics of microsomal HMG-CoA reductase. Column-filtered microsomes, suspended in HEPES buffer, were preincubated with an NADPH-generating system (10 mM glucose-6-phosphate, 0.4 I.U. of glucose-6-phosphate dehydrogenase), in the presence of 4.5 mM (closed symbols) or 27.0 mM (open symbols) of GSH and 50 μM (▲, △), 150 μM (■, □), or no (●, ○) P-ADP-Rib. Preincubation was in a total volume of 80 μl for 40 min at 37°C. Reactions were initiated by simultaneous addition of both substrates (30 μM [^{14}C]HMG-CoA and NADPH to the specified concentrations) in 20 μl. After 40 min at 37°C, reactions were terminated and the [^{14}C]MVA that was produced was measured.

ADP-Rib must have less structural fit than NADPH to the catalytic sites of allosteric reductase. It appears then, that the nicotinamide moiety is essential for cooperative interactions between these sites. Thus, P-ADP-Rib may be regarded as a "minimal inhibitor," analogous to the "minimal substrate" as discussed by Koshland (37).

The thiol-dependent alterations in the kinetic behavior of HMG-CoA reductase indicate that protein sulfhydryl/disulfide groups are involved in catalytic and regulatory properties of the enzyme. The nature and the spatial arrangement of the protein sulfhydryl groups is not yet known. It appears, however, that at least two distinct sets of sulfhydryl groups, located at different domains of the reductase molecule, are responsible for the observed phenomena. The first is a protein thiol(s)/dilsulfide(s) located at, or near, the HMG-CoA binding site(s). Reduction by thiols of this disulfide group(s) is essential for the binding of HMG-CoA, as demonstrated by binding studies to agarose/hexane/HMG-CoA affinity resin (22, 23) and by immunoinhibition studies (38). This set of protein sulfhydryl/disulfide group(s) is present in both the freeze-thaw solubilized and the microsomal forms of the reductase. The second is a postulated regulatory set of protein thiol/disulfide group(s) which is expressed only in the microsomal reductase. In the solubilized enzyme, this regulatory set has been either removed or rendered non-functional due to the proteolytic solubilization of the reductase. Reduction by thiols of this regulatory disulfide group(s) or its removal/inactivation by proteolysis changes the properties of the catalytic NADPH-binding site(s). These changes reduce the specificity for NADPH, allowing the competitive binding of P-ADP-Rib. Even under these conditions, this catalytic NADPH-binding site(s) still retains a high degree of specificity, excluding NADH, another structural analog of NADPH (Fig. 6 and ref. 25).

TABLE 1. The effect of P-ADP-Rib on the NADPH-dependent kinetics of microsomal HMG-CoA reductase

GSH	P-ADP-Rib	V_{max}	$S_{0.5}$	n_H
mM	*μM*	*nmol MVA per mg per min*	*%*	
	0		100	1.8[b]
4.5	50	1.56 ± 0.02[a]	105	1.6
	150		162	1.4
	0		100	1.1[b]
27.0	50	4.62 ± 0.02[a]	190	1.1
	150		366	1.0

V_{max} values were determined from double reciprocal plots of rate versus the first and second power of NADPH concentrations. Hill coefficients and $S_{0.5}$ values for NADPH were determined from the Hill plots (log $v/V_{max} - v$) versus log [NADPH]).

[a] Mean ± SD, n = 3.

[b] Values of n_H at 4.5 and 27.0 mM GSH are significantly different, $P < 0.05$.

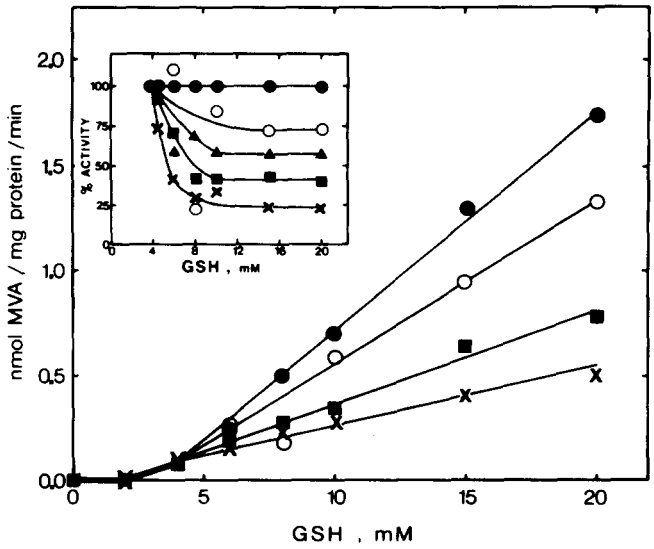

Fig. 5. The effect of GSH on the inhibition of microsomal HMG-CoA reductase by P-ADP-Rib. Column-filtered microsomes, suspended in HEPES buffer, were preincubated with 60 μM NADPH and an NADPH-generating system (10 mM glucose-6-phosphate, 0.4 I.U. of glucose-6-phosphate dehydrogenase), in the presence of 30 μM (○), 50 μM (▲, inset only), 100 μM (■), 300 μM (X) or no (●) P-ADP-Rib, and GSH to the specified concentrations. Preincubations, in a volume of 90 μl, were for 35 min at 37°C after which reactions were initiated by addition of 30 μM [^{14}C]HMG-CoA in 10 μl. After 35 min at 37°C, the reactions were terminated and the [^{14}C]MVA that was produced was measured.

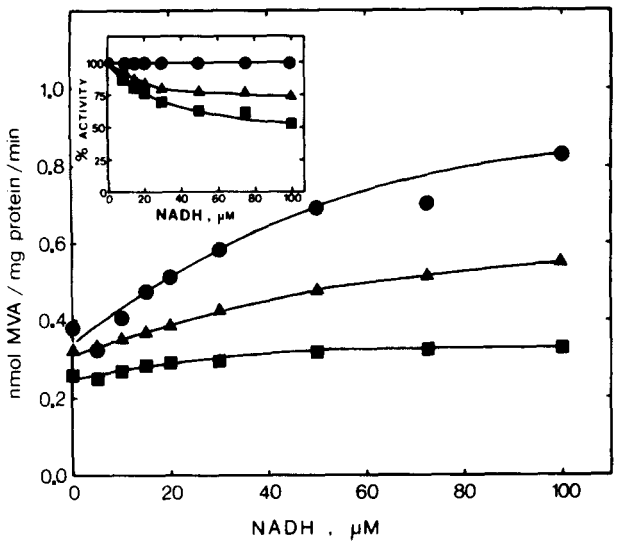

Fig. 6. The effect of NADH on the inhibition of microsomal HMG-CoA reductase by P-ADP-Rib. Column-filtered microsomes, suspended in HEPES buffer, were preincubated in a total volume of 90 μl containing 4.5 mM GSH, 60 μM NADPH, an NADPH-generating system (10 mM glucose-6-phosphate, 0.4 I.U. of glucose-6-phosphate dehydrogenase), 100 μM (▲), 300 μM (■), or no (●) P-ADP-Rib, in the presence of the specified concentrations of NADH. After 35 min at 37°C, 30 μM of [^{14}C]HMG-CoA in 10 μl was added and incubation was continued for 35 min at 37°C, after which reactions were terminated and the [^{14}C]MVA that was produced was measured.

Such thiol-induced changes in the enzyme properties are also expressed in the gradual shift from sigmoidal NADPH-dependent kinetics to Michaelis–Menten type hyperbolas (ref. 24 and Fig. 4). Further studies, employing differential labeling with specific sulfhydryl reagents and peptide-mapping techniques, should disclose the specific protein thiols involved in the changes of the kinetic properties of HMG-CoA reductase.

It is of interest to note that the largest variations in the degree of inhibition of microsomal MHG-CoA reductase activity by P-ADP-Rib lay in a rather narrow range (up to 10 mM) of GSH concentrations (Fig. 5, inset). Within this range of concentrations are also the largest changes in the Hill coefficient for NADPH (see Fig. 2 in ref. 24). This is also the reported range of variations in GSH levels in the rat liver (39, 40). These findings provide additional evidence for the possible role of the thiol-status (41) in the regulation of hepatic HMG-CoA reductase activity (20, 22–24).

We recently demonstrated that NADH, as well as other related nucleotides, enhances the activity of microsomal HMG-CoA reductase. We proposed that these compounds serve as allosteric activators of the reductase, increasing its affinity toward NADPH (25, 42). As shown above, addition of NADH also causes the allosteric form of the enzyme to become more susceptible to inhibition by P-ADP-Rib (Figs. 6, 7). Apparently, interaction of NADH with the allosteric site(s) induces protein conformational changes analogous to those caused by high thiol concentrations, allowing improved binding of P-ADP-Rib to the catalytic NADPH-binding site(s). The decreased Ki for P-ADP-Rib in the presence of NADH (Fig. 7) excludes the possibility that the two nucleotides compete for the same allosteric site(s) on the reductase. Therefore, it appears that P-ADP-Rib binds only to the

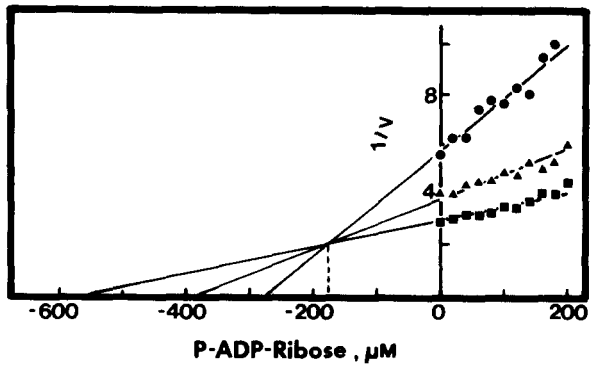

Fig. 7. The effect of NADH on the inhibition of microsomal HMG-CoA reductase by P-ADP-Rib. Column-filtered microsomes, suspended in HEPES buffer, were preincubated in a total volume of 90 μl containing 4.5 mM GSH, 125 (●), 250 (▲), or 1000 (■) μM NADPH, an NADPH-generating system (10 mM glucose-6-phosphate, 0.4 I.U. of glucose-6-phosphate dehydrogenase), 300 μM NADH and P-ADP-Rib to the specified concentrations. After 35 min at 37°C, 30 μM of [^{14}C]HMG-CoA in 10 μl was added and incubation was continued for 35 min at 37°C, after which reactions were terminated and the [^{14}C]MVA that was produced was measured.

catalytic site(s), and that this binding is improved when NADH interacts with the allosteric site(s). This conclusion is further supported by our previous observation that adenosine-derived nucleotides, phosphorylated at *both* the 2' and the 5' positions of the adenosyl moiety, fail to allosterically activate microsomal HMG-CoA reductase (42).

Studies with catalytic site-directed monospecific antibodies have suggested that the binding of substrates or their analogs to HMG-CoA reductase is accompanied by marked conformational changes, as demonstrated by altered antigenicity of the enzyme (36). The data presented in this report indicate that HMG-CoA reductase undergoes conformational changes that alter its kinetic properties, and further support the role of allosteric transitions in the regulation of HMG-CoA reductase activity.

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