IRREVERSIBLE INHIBITION OF 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE BY PHENICIN (PHOENICINE)

KEIJI HASUMI, MASAOMI ARAHIRA, KAORU SAKAI and AKIRA ENDO*

Department of Agricultural and Biological Chemistry, Tokyo Noko University, 3-5-8 Saiwaicho, Fuchu-shi, Tokyo 183, Japan

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Phenicin (phoenicine) was first isolated from cultures of *Penicillium phoeniceum* and *Penicillium rubrum* as a red pigment in 1933[1]. Since then its production[2], chemical synthesis[3,4] and biosynthesis[5] have been reported by several groups. However, biological activities of phenicin have not yet been fully studied.

In our search for new microbial metabolites having inhibitory activity against cholesterol synthesis, phenicin was found to be an active compound. This communication describes studies of the mechanism of action of phenicin. They have demonstrated that it inhibits 3-hydroxy-3-methylglutaryl(HMG)-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis specifically. The enzyme is inactivated irreversibly.

Phenicin was obtained by growing *Penicillium phoeniceum* IFO 5801 as described previously[2]. The enzymatic synthesis of cholesterol was assayed by measuring the radioactive digitonin-precipitable sterols derived from [^{14}C]acetate, [^{14}C]HMG-CoA or [^{14}C]mevalonate in a rat liver enzyme system as described by KURODA and ENDO[6]. Phenicin inhibited 50% sterol synthesis from [^{14}C]acetate at a concentration of 30 μM. Sterol synthesis from [^{14}C]HMG-CoA was similarly inhibited by phenicin, while that from [^{14}C]mevalonate was not sensitive to phenicin at concentrations up to 150 μM. These results indicated that phenicin inhibited specifically the conversion of HMG-CoA to mevalonate catalyzed by HMG-CoA reductase.

When rat liver microsomes were isolated and assayed for HMG-CoA reductase by the method described previously[6,7], it was found that the activity was inhibited to the extent of approximately 50% by phenicin at a concentration of 200 μM (Fig. 1).

Fig. 1. Inhibition of rat liver microsomal HMG-CoA reductase by phenicin.

Reductase was assayed with no preincubation.

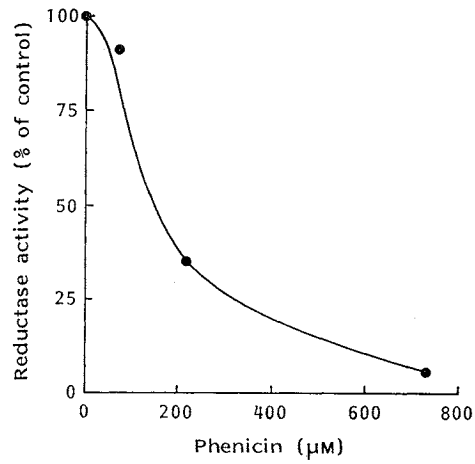
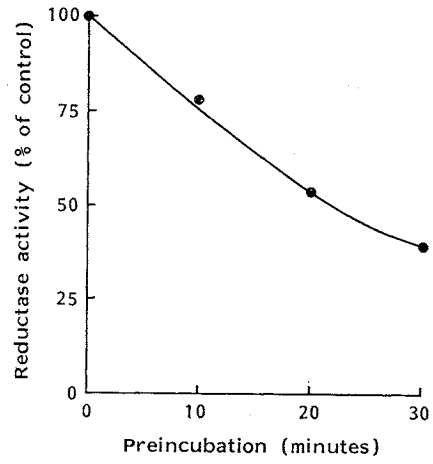


Fig. 2. Effects of preincubation on the phenicin inhibition of rat liver HMG-CoA reductase.

Partially purified rat liver reductase (5 μg protein) was mixed with 125 mM potassium phosphate buffer, pH 7.4 containing 12.5 mM EDTA, 6.25 mM NADPH, 2.5 mM dithiothreitol and 4.6 μM phenicin (total volume 40 μl) and incubated at 37°C as indicated. The mixture was then mixed with 10 μl of 500 μM [^{14}C]HMG-CoA and incubated at 37°C for 10 minutes.



Preincubation of phenicin with rat liver HMG-CoA reductase, which had been solubilized and partially purified as described previously[8], caused increasingly greater inhibition as the time of exposure to the enzyme increased (Fig. 2). Table 1 shows the results of experiments in which the concentrations of phenicin in the

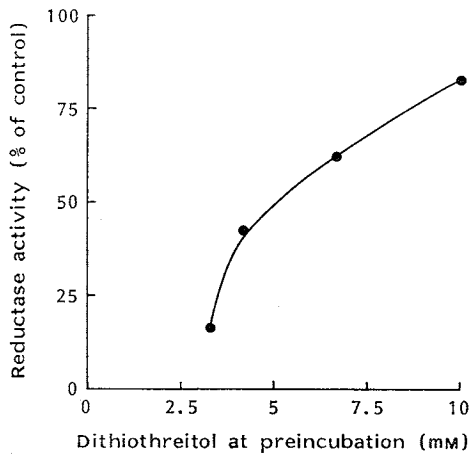
Table 1. Irreversible inhibition of rat liver HMG-CoA reductase by phenicin.

Preincubation mixture (40 μl) contained 0.25 mg (protein) of partially purified enzyme, 9.1 μM phenicin, 160 mM potassium phosphate buffer, pH 7.4, 2.5 mM dithiothreitol, 12.5 mM KCl, 31.3 mM sucrose, and 20 mM EDTA. After incubation at 37°C for 20 minutes, mixture was diluted 1- to 4-fold by mixing with cold 160 mM potassium phosphate buffer, pH 7.4 containing 2.5 mM dithiothreitol, 12.5 mM KCl, 31.3 mM sucrose and 20 mM EDTA. Mixtures for enzyme assay (50 μl) contained 40 μl of the diluted mixtures and 10 μl of a solution containing 500 μM [^{14}C]HMG-CoA and 25 mM NADPH, which were incubated at 37°C for 10 minutes.

Phenicin (μM)		Reductase inhibition (%)
Preincubation	Enzyme assay	
9.1	7.3	37.3
9.1	5.5	46.4
9.1	3.7	47.6
9.1	1.8	43.6

Fig. 3. Effects of dithiothreitol on phenicin inhibition of rat liver HMG-CoA reductase.

Partially purified rat liver HMG-CoA reductase (5 μg) was preincubated for 20 minutes as described in Fig. 2, except that concentrations of dithiothreitol were varied as indicated, and the remaining reductase activities were determined.



enzyme assay were reduced to varying extents, as compared with those at preincubation. Inhibition depended on the concentration of phenicin at preincubation. The activity of the inactivated enzyme could not be restored by decreasing the phenicin concentration in the enzyme assay. These results demonstrated that phenicin inhibition of HMG-CoA reductase was time dependent and irreversible.

The inhibitory activity of phenicin was reduced in the presence of the reducing agent dithiothreitol at preincubation. Thus, when solubilized HMG-CoA reductase was preincubated with 4.6 μM phenicin at 37°C for 20 minutes in the presence of 3.3 mM dithiothreitol, inhibition was 84%, while inhibition was reduced to approximately 17% in the presence of 10 mM dithiothreitol at preincubation (Fig. 3). These results suggest that phenicin inhibition is caused by interaction with the sulfhydryl group(s) of the HMG-CoA reductase molecule. This interpretation is supported by the observation that thiols have a regulatory role in the catalytic action of HMG-CoA reductase[9].

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