THE STEREOCHEMISTRY OF THE REDUCTION OF MEVALDIC ACID-COENZYME A HEMITHIOACETAL BY RAT LIVER 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE

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1. Introduction

The enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase is now established as the primary site of control of mammalian hepatic cholesterol biosynthesis. This enzyme catalyses a two step reduction of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) to mevalonic acid (II) using two molecules of NADPH. It has been shown for the corresponding enzyme from yeast that the hemithioacetal addition compound of mevaldic acid and coenzyme A (I) was a good substrate, and a possible intermediate, for the reaction [1]. We have recently demonstrated [2] that both reductive steps catalysed by the rat liver enzyme occur by direct hydrogen transfer from the 4A (4R) position of NADPH and this is the same stereochemistry as has been previously reported for the yeast enzyme [3, 4].

We now wish to report that the mevaldic acid-coenzyme A hemithioacetal addition compound (I) is a good substrate for the rat liver enzyme and that the hydrogen atom transferred in this step appears at the 5-pro-S position of the resulting mevalonic acid (scheme 1). A similar result has been reported for the yeast enzyme [4].

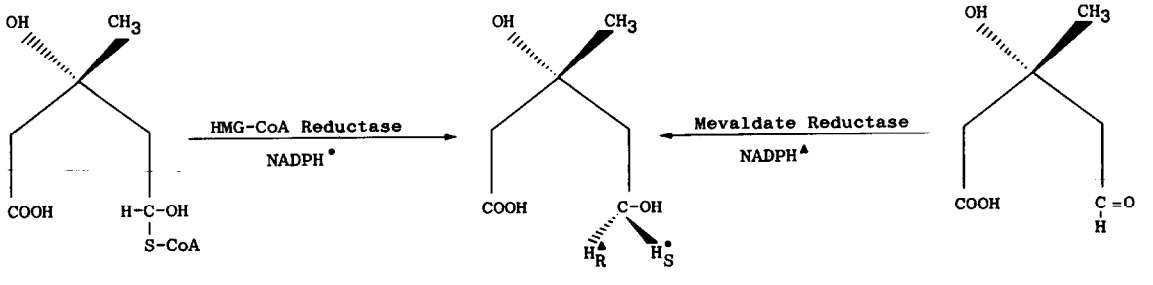
2. Methods

The mevaldic acid-coenzyme A hemithioacetal was prepared by the method of Retey et al. [1]. The preparation of partially purified rat liver HMG-CoA reductase by the method of Kawachi and Rudney [5] and the method of isolation and stereospecific analysis of 5-tritiated mevalonic acid have been previously described [2].

Incubations contained RS-mevalic acid coenzyme A hemithioacetal (approx. 3 μmole); [4-$^3H_2$]NADPH, (specific activity 6.0 dpm × pmole^{-1}) (3 μmole); potassium RS mevalonic acid (3 μmole) and partially

Scheme 1

The stereochemistry of mevalonic acid biosynthesis from mevaldic acid.



3S-Mevaldic acid coenzyme A hemithioacetal (I)

3R-Mevalonic acid (II)

3R-Mevaldic acid (III)

purified HMG-CoA reductase (4 mg of protein, specific activity 50 nmole mevalonate formed from HMG-CoA × mg protein^{-1} × hr^{-1}) in a total volume of 3 ml of 10 mM potassium phosphate buffer pH 6.9 containing 1 mM EDTA and 5 mM 2-mercaptoethanol. Incubations at 38° for 2 hr were terminated by the addition of 10 M KOH (0.3 ml) and 10^5 dpm of RS [2-^{14}C]mevalonic acid lactone.

3. Results

Mevaldic acid-coenzyme A hemithioacetal and [4-$^3H_2$]NADPH were incubated with partially purified rat liver HMG-CoA reductase. The resulting tritiated mevalonic acid was mixed with [2-^{14}C]-mevalonic acid and the tritium at the C-5 position of this mevalonic acid was analysed by the classical method [6] which we have previously described [2]. The double labelled mevalonic acid was converted to farnesyl pyrophosphate by enzymes from the soluble fraction of rat liver and dephosphorylated by the addition of alkaline phosphatase. The position of tritium at the C-1 of farnesol was determined by oxidation with liver alcohol dehydrogenase. The $^3H/^{14}C$ ratios of the compounds were measured and are shown in table 1.

Incorporation of tritium into the mevalonic acid from [4-$^3H_2$]NADPH indicates that the hemithioacetal addition compound was reduced by the rat liver enzyme. From the amount of tritium incorporated it was calculated that the hemithioacetal was reduced at about one-third the rate of the normal substrate. When the mevalonic acid was converted to farnesol and then oxidised to farnesal with liver alcohol dehydrogenase no tritium was lost from the farnesol, indicating that all of the tritium at the C-1 position of farnesol was located at the 1-pro-S position and hence must have occupied the 5-pro-S position of the original mevalonic acid. Liver alcohol dehydrogenase removes the 1-pro-R hydrogen atom from farnesol.

Table 1
Degradation of mevalonic acid arising from incubation of mevaldic acid co-enzyme A hemithioacetal with [4-^{3}H]-NADPH and partially purified rat liver 3-hydroxy-3-methylglutaryl coenzyme A reductase.

Compound	$^3H/^{14}C$ ratio	Loss of tritium (%)
1. Mevalonic acid	0.51	
2. Farnesol biosynthesised from (1.)	1.00	
3. Farnesal prepared from (2.) by oxidation with liver alcohol dehydrogenase and NAD	1.01	0

The enzyme mevalonic acid kinase is absolutely specific for the 3R isomer of mevalonic acid and only half the racemic [2-^{14}C]mevalonic acid will be converted to farnesol. Therefore the doubling of the $^3H/^{14}C$ ratio between mevalonic acid and farnesol means that all of the [5-^{3}H]mevalonic acid is metabolised and hence must have had the 3R configuration. This establishes that the reduction of the mevalonic acid-coenzyme A hemithioacetal produces only the 3R isomer of mevalonic acid.

4. Discussion

We have shown that the hemithioacetal addition compound of 3R mevaldic acid and coenzyme A is a substrate for the rat liver HMG-CoA reductase and that its reduction involves hydride transfer to the 5-pro-S position of mevalonic acid. This information, together with that previously reported [2–4] establishes that the stereochemistry of hydride transfer is identical for the reactions catalysed by either rat liver or yeast HMG-CoA reductase. These observations suggest that the two enzymes have a common evolutionary origin.

The stereochemistry of the reduction catalysed by rat liver HMG-CoA reductase is opposite to that found for the reduction of 3R mevaldic acid (III) by the mevaldate reductase from the cytosol of liver, since with the latter enzyme hydride transfer occurs to the 5-pro-S position of mevalonic acid [6] (scheme 1). It is interesting to note that both these enzymes from rat liver catalyse the reduction of mevaldic acid, or its derivative, to mevalonic acid (scheme 1) in a process that can involve the direct transfer of hydrogen from NADPH. The precise role

of the soluble mevaldate reductase in metabolism has yet to be elucidated, however, the present results are not consistent with the possibility of HMG-CoA reductase and mevaldate reductase being structurally or genetically related since the two enzymes must bind their substrates in opposite manners.

References

[1] J. Retey, E. von Stetten, U. Coy and F. Lynen, European J. Biochem. 15 (1970) 72.
[2] A.S. Beedle, K.A. Munday and D.C. Wilton, European J. Biochem. 28 (1972) 155.
[3] R.E. Dugan and J.W. Porter, J. Biol. Chem. 246 (1971) 5361.
[4] P. Blattmann and J. Retey, Hoppe-Seylers Z. Physiol. Chem. 352 (1971) 367.
[5] T. Kawachi and H. Rudney, Biochemistry 9 (1970) 1701.
[6] C. Donninger and G. Popjak, Proc. Roy. Soc. (London) B 163 (1966) 465.