DECARBOXYLATION OF MEVALONATE PYROPHOSPHATE IS ONE RATE-LIMITING STEP IN HEPATIC CHOLESTEROL SYNTHESIS IN SUCKLING AND WEANED RATS[1]

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SUMMARY

The phosphorylation and decarboxylation of mevalonate by 105,000g supernatant fractions from livers of suckling and weaned rats was studied. The rate of mevalonate phosphate formation by S_{105} fraction from suckling rats was not significantly different from that observed in rats weaned on control (Purina Chow) diet. The decarboxylation of mevalonate by preparations from weaned rats, however, was 5 - 20 times higher than the decarboxylation by preparations from suckling rats. In spite of the fact that the amount of mevalonate pyrophosphate (MVAPP) is slightly lower in preparations from suckling rats, the ratio of MVAPP/CO_2 formed was considerably higher. In preparations from rats weaned on diet supplemented with 1% cholesterol the decarboxylation of mevalonate was reduced by 50 - 80%, while the rate of MVAP formation and the ratio of MVAPP/CO_2 produced were similar to those observed in the preparations from suckling rats. These results hence indicate that decarboxylation of MVAPP is a key rate-limiting step in the conversion of mevalonate to squalene.

INTRODUCTION

We recently reported that the incorporation of mevalonate (MVA) into non-saponifiable lipids (squalene) is low in cell-free preparations of livers from suckling rats as compared with the incorporation in preparations from weaned rats (1). It was further suggested that the hepatic activities

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of one or more enzymes catalyzing the conversion of MVA into squalene increase after weaning. In the present report we have examined the 105,000g supernatant fractions from livers of suckling and weaned rats for the activities of the enzymes which catalyze the initial three steps in the conversion of mevalonate to squalene. It is shown that the phosphorylation of MVA and mevalonate phosphate (MVAP) is not significantly different in preparations from suckling and weaned rats, while the decarboxylation of MVA is considerably higher in preparations from rats weaned on Purina Chow diet as compared to that in suckling rats. These results provide evidence that decarboxylation of MVAPP is one key regulatory step in hepatic cholesterol synthesis.

MATERIALS AND METHODS

DL-mevalonic acid lactone [1-^{14}C] was obtained from Amersham/Searle, Arlington Heights, Ill. ATP was purchased from Sigma Chemical Company, St. Louis, Mo. Unlabeled mevalonic acid lactone was a product of Calbiochem, Los Angeles, Ca. The lactone form of mevalonic acid was converted to potassium salt prior to its use for incubations. Rats of Sprague-Dawley strain, bred in this laboratory, were used in the present study. Pups were weaned from their mother at 20 days of age. Animals were fed laboratory chow obtained from Ralston Purina Co., St. Louis, Mo.

Livers of rats (sacrificed between 8 and 10 a.m.) were homogenized with 2.5 volumes of 0.1M potassium phosphate buffer (pH 7.4) containing 0.004M $MgCl_2$, 0.001M EDTA and 0.01M 2-mercaptoethanol. The homogenates were centrifuged at 105,000g for one hour and the supernatant (S_{105}) was used as the enzyme source. The protein concentration was determined by the biuret procedure of Gornall *et al.* (2). Incubations were carried out in rubber-stoppered, 10ml conical flasks fitted with center wells. The incubation mixture contained 5.4μmoles of ATP, 10μmoles of $MgCl_2$, 100μmoles of potassium phosphate buffer (pH 7.4), 0.275μmoles (225 x 10^3cpm) of mevalonate [1-^{14}C], and 1mg of S_{105} protein, all in a final volume of 1ml. For decarboxylation assay, 0.2ml of 30% NaOH was placed in the center wells,

and the reactions were terminated by injecting 0.2ml of 5 N H_2SO_4 into the main compartments. The flasks were shaken for an additional 60 min, for the complete absorption of the liberated [$^{14}CO_2$]. The contents of the center wells were transferred quantitatively to liquid scintillation vials for radioactivity measurements. To measure phosphorylation of MVA and MVAP, the reactions were terminated by immersing the flasks in boiling water for 3 min. The contents of the flasks were transferred to centrifuge tubes and the precipitated proteins were sedimented. MVA, MVAP and mevalonate pyrophosphate (MVAPP) present in the supernatant fractions were separated by descending paper chromatography in a solvent system consisting of tert-butyl alcohol:formic acid:H_2O (20:5:8, v/v/v) as described by Tchen (3). Discrete areas containing [^{14}C], located by scanning in Nuclear Chicago Actigraph III, were transferred to scintillation vials containing aquasol and assayed for radioactivity.

RESULTS AND DISCUSSION

The conversion of MVA to MVAPP is accomplished by two successive phosphorylations by the enzymes mevalonate kinase (EC 2.4.1.36) and phosphomevalonate kinase (EC 2.7.4.2). MVAPP is then enzymatically decarboxylated to form isopentenyl pyrophosphate. The data presented in Fig. 1 show decarboxylation of MVA as a function of time and of protein concentration. As can be seen, the rate of decarboxylation by hepatic S_{105} from suckling rats was very low as compared with the rate of decarboxylation by preparations from weaned rats.

To determine whether the low rate of decarboxylation of MVA was associated with reduced activities of mevalonate kinase or phosphomevalonate kinase or of both, we examined the formation of MVAP and MVAPP. The results in Table I show that the rate of formation of MVAP by S_{105} fractions from suckling rats was not significantly different from the rates observed in animals weaned on control (Purina Chow) diet. In contrast, there was a significant and progressive decrease in MVA decarboxylase activity during

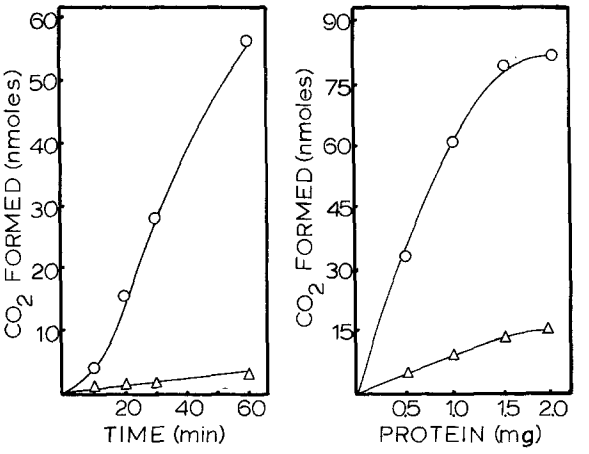

FIGURE 1. Decarboxylation of mevalonate as a function of time and protein concentration.

S_{105} fractions from livers of suckling and weaned rats were incubated with 275nmoles of mevalonate-1[^{14}C] under conditions described in the text. Each point in the Figure is the average of closely agreeing duplicate determinations. In the time course studies 1mg of S_{105} protein was used. O---O weaned rats (23 day); Δ---Δ suckling rats (18 day). The incubation period of 1 hr was used for studying the effect of protein concentration. O---O weaned rats (30 day); Δ---Δ suckling rats (14 day).

the suckling period followed by a surge in the weaned animals. This change in the decarboxylase activity in suckling and weaned animals is strikingly similar to the pattern observed in the incorporation of MVA into non-saponifiable lipids and digitonin precipitable sterols (1). The data presented in Table I further show that the ratio of MVAPP to CO_2 produced was considerably higher in suckling rats as compared to the ratio in weaned rats. These results thus suggest that decarboxylation of MVAPP is one of the key regulatory steps in the conversion of mevalonate to squalene.

The data in Table I also show that when the rats were weaned on diet supplemented with 1% cholesterol, the post-weaning increase in the decarboxylase activity was partially prevented. Again, as in suckling rats, the formation of MVAP and the ratio of MVAPP/CO_2 formed remained high in preparations from rats weaned on 1% cholesterol diet. These results, taken

TABLE I

DECARBOXYLATION AND PHOSPHORYLATION OF MEVALONATE BY S105 PREPARATIONS OF LIVERS FROM SUCKLING AND WEANED RATS

Group	Age In Days	Total MVAP Formed* nmoles/mg protein/hr	Total MVAPP Formed** nmoles/mg protein/hr	MVA Decarboxylated nmoles/mg protein/hr	MVAPP/CO_2***
Suckling	8	64.45	35.79	14.96	1.97
	12	66.12	39.78	10.00	3.33
	19	67.87	24.91	2.70	8.33
Weaned on Purina Chow Diet	23	65.95	64.26	60.35	0.06
	27	85.63	84.69	83.41	0.01
Weaned on Diet Containing 1% Cholesterol+	23	70.90	69.98	29.38	1.38
	27	89.21	54.59	11.27	3.84

S_{105} fractions (1mg protein) prepared from livers of rats were incubated with 275nmoles of mevalonate-1[^{14}C]. See text for details of the assay procedure.

*nmoles of total MVAP formed was estimated by adding the amount of CO_2 produced to the amount of MVAP and MVAPP present.

**nmoles of total MVAPP formed was calculated by adding the amount of CO_2 produced and the MVAPP present.

***The ratio was calculated by dividing the nmoles of MVAPP present by the nmoles of CO_2 produced.

+Cholesterol was added to Purina Chow diet as described earlier (4).

in conjunction with our earlier findings (4) that the post-weaning increase in the conversion of mevalonate to squalene was partially prevented by weaning animals on diet supplemented with cholesterol, further suggest that decarboxylation of MVAPP is a regulatory step in weaned animals as well. It is likely that the low levels of hepatic decarboxylase activity in suckling rats are associated with the cholesterol in maternal milk.

Further examination of the data in Table I reveals that the formation of MVAPP is approximately 50% lower in suckling rats than in the rats weaned on Purina Chow diet. This observation raises the possibility that the activity of MVAP kinase is also rate-limiting. It should, however, be borne in mind that since the reaction catalyzed by MVAP kinase is reversible (5), the amount of MVAPP formed or accumulated is regulated by the equilibrium constant (K_{eq}) of the kinase reaction, particularly when the rate of forward reaction, namely MVAPP decarboxylation, is low. Hence, the reduced amounts of MVAPP formed in the preparations from the suckling rats do not necessarily reflect the lower activity of MVAP kinase.

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