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Partial Purification and Properties of Mevalonate Kinase from Neonatal Chick Liver

J. García-Martínez, A. Linares, M. D. Suárez and E. García-Peregrín

Departamento de Bioquímica
Universidad de Granada (Spain)

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J. GARCIA-MARTINEZ, A. LINARES, M. D. SUAREZ and E. GARCIA-PEREGRIN. *Partial Purification and Properties of Mevalonate Kinase from Neonatal Chick Liver.* Rev. esp. Fisiol., 38, 261-266. 1982.

Mevalonate kinase from neonatal chick liver has been partially purified by ammonium sulphate precipitation and Sephadex G100 and DEAE-cellulose fractionation. The kinetic characteristics agreed with the sequential mechanism suggested for the enzyme and provided apparent K_m values of 0.01 mM for mevalonic acid and 0.25 mM for ATP. Partially purified mevalonate kinase from neonatal chick liver showed an absolute specificity for ATP. Mn^{2+} was a better activator than Mg^{2+} at low concentrations (0.1-1.0 mM). Higher Mn^{2+} concentrations produced a clear inhibition of mevalonate kinase. Likewise, addition of EDTA, with or without metal ions, clearly inhibited the enzymatic reaction.

Mevalonate kinase (ATP: mevalonate-5-phosphotransferase, EC 2.7.1.36) catalyzes the phosphorylation of mevalonic acid (MVA) to mevalonate-5-phosphate (MVAP), as the first stage in the conversion of MVA into isopentenyl pyrophosphate and then into numerous isoprenoids in animals, plants and microorganisms. Although this enzyme has been detected in several sources, little is known about its properties in avian tissues. There is general agreement that 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (EC 1.1.1.34) is the rate limiting enzyme in cholesterol synthesis (19). However, MVA kinase may play an important role in the regulation of this process. So, DORSEY and PORTER (5) have shown that geranyl pyrophosphate and farnesyl pyrophosphate are powerful inhibitors of pig liver mevalonate kinase, suggesting that both terpenyl pyrophosphates act as physiological control after MVA. More recently, the existence of secondary regulatory sites for cholesterol synthesis between MVA and isopentenyl pyrophosphate has been postulated, which would agree with the rhythmic activity observed in the utilization of MVA for biogenesis of cholesterol by rat liver (2). Recent results of JABALQUINTO and CARDEMIL (12) indicated that MVAPP decarboxylase does not present diurnal variation in activity, so that the rhythm observed by BHAT and RAMASARMA (2) should be due to variations in one or both kinases.

In a previous paper, we have showed a clear increase in the amount of MVAP formed by chick liver at day 7 after hatching (7), while no significant variations have been observed in the amount of MVAPP formed at the same age. Likewise, changes in mevalonate kinase activity have been reported in neonatal chick brain and kidney (15) so that an important role for this enzyme during myelination can be suggested. The high mevalonate kinase concentration found in 6-day *Sarcophaga bullata* larvae also suggested an important metabolic role for this enzyme during this developmental time (11).

In this communication we report the partial purification of neonatal chick liver mevalonate kinase as well as some of its characteristics.

Materials and Methods

Newborn male chick *(Gallus domesticus)* were obtained from a commercial hatchery and maintained fed *ad libitum* on a commercial diet. (2-^{14}C)MVA was supplied as the lactone by the Radiochemical Centre, Amersham. The potassium salt was prepared as previously described (20). Livers were excised from 10 day-old chicks, pooled and homogenized in a Potter-Elvehjem homogenizer with 0.05 M Tris-maleate buffer pH 7.5, so that the final liver/buffer ratio was 1/5, w/v. Assuming that MVA-activating enzymes from neonatal chick liver are essentially located in the soluble fraction (7), 105,000 × *g* supernatant was used as enzyme preparation.

Protein from 105,000 × *g* supernatant was fractionated with solid $(NH_4)_2SO_4$. The fraction obtained between 0-60 % saturation was dissolved in the buffer above mentioned and applied to a Sephadex G100 column (2.5 × 70 cm), previously equilibrated with the same buffer. Protein was eluted at a flow rate of 30 ml/h. Fractions of 10 ml were collected. The active fractions eluted from Sephadex G100 column were applied to a DEAE-cellulose column (1.5 × 15 cm), previously equilibrated with 0.05 M Tris-maleate buffer pH 7.5 containing 0.1 M KCl; they were eluted with the same buffer using a continuous gradient of 0.1-0.5 M KCl and a flow rate of 30 ml/h. Fractions of 5 ml were collected. Fractions with mevalonate kinase activity were filtered upward through a Sephadex G100 column in the same way as above.

Protein content in enzyme preparations was determined by the method of LOWRY *et al.* (17) using bovine albumins as standard. Protein concentration of column eluates was determined by absorption at 280 nm. Unless otherwise specified, the reaction system contained 6 μmol of $MgCl_2$, 12 μmol of ATP, 75 nmol of (2-^{14}C)MVA, 150 μmol of Tris-maleate buffer pH 7.5 and 0.01-0.02 mg of protein in a final volume of 1.5 ml. Reaction and chromatographic identification of MVAP were carried out as previously described (8, 9).

Results

Table I shows the results of partial purification of MVA kinase from neonatal chick liver. According to the developmental pattern of this enzyme (7), 10 day-old chicks were used. About 80 % of the enzyme was precipitated between 0-60 % ammonium sulphate saturation with about 1.2-fold increase in specific activity. Further purification was carried out by Sephadex G100 gel filtration. One fraction with MVA kinase activity was eluted and, then, applied to a DEAE-cellulose column. Fraction eluted at 0.25 M KCl contained the mevalonate kinase activity with about 11.5-fold increase in specific activity. This fraction was filtered again through a Sephadex G100 column. One active fraction with about 33.5-fold purification was obtained. This fraction

Table I. *Purification of mevalonate kinase from neonatal chick liver.*

	Protein (mg)	Activity (dpm × 10^{-5})	Specific activity (dpm/mg × 10^{-5})	Purification	Yield (%)
105,000 × *g* supernatant	714	2,706	3.79	1	100
$(NH_4)_2SO_4$ (0-60 %)	440	1,987	4.52	1.19	73.4
Sephadex G100	126	1,642	13.03	3.44	60.7
DEAE-cellulose	35	1,535	43.86	11.57	56.7
Sephadex G100	9	1,103	126.56	33.33	40.7

was free of phosphomevalonate kinase and phosphatases as contaminating activities.

MVAP formation by this active fraction was investigated as a function of incubation time. The reaction occurred linearly between 0-120 min. MVA phosphorylation was also studied as a function of purified protein added to the reaction mixture. MVAP formation was proportional to the amount of protein added over the range 0-0.02 mg.

The rate of MVA phosphorylation by partially purified MVA kinase was studied at variable MVA concentrations and different, fixed concentrations of ATP. Lineweaver-Burk plots showed a family of intersecting lines (fig. 1). If the vertical-axis intercepts of each of these lines were represented as a function of the corresponding value of 1/[ATP], a straight line was observed, providing an apparent K_m value of 0.25 mM for ATP. Similar results were obtained working with variable ATP concentrations and different, fixed MVA concentrations (fig. 2), giving an apparent K_m value of 0.01 mM for MVA.

Partially purified mevalonate kinase from neonatal chick liver showed an absolute specificity for ATP. Only 5.0-10 mM ITP could replace in part ATP, but the MVAP formed was only about 10%

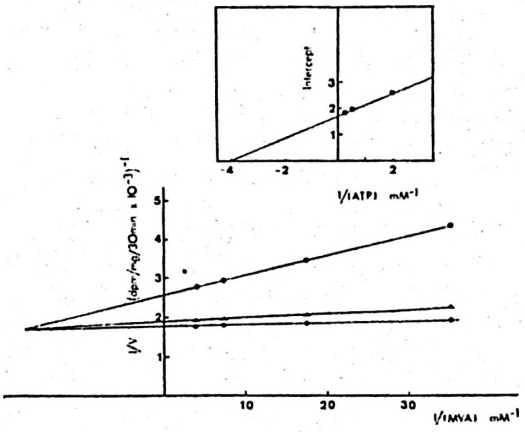

Fig. 1. *Double reciprocal plots of 1/V against 1/S (MVA).*
Incubations contained the standard radiochemical assay mixture except for variable concentrations of MVA and different, fixed concentrations of ATP: (✲), 4.0 mM; (▲), 2.0 mM; (●), 0.5 mM.

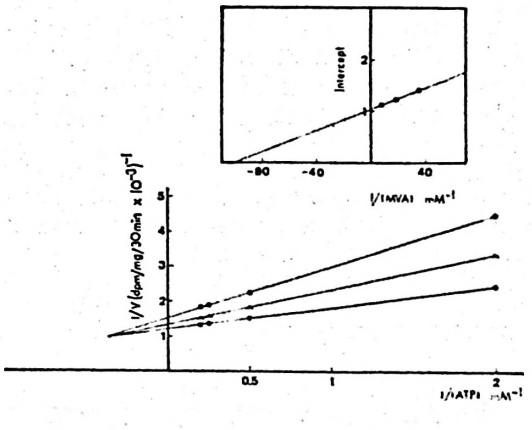

Fig. 2. *Double reciprocal plots of 1/V against 1/S (ATP).*
Incubations contained the standard radiochemical assay mixture except for variable concentrations of ATP and different, fixed concentrations of MVA: (✲), 113.32 μM; (▲), 56.66 μM; (●), 28.33 μM.

Table II. *Nucleotide dependence of partially purified mevalonate kinase from neonatal chick liver.*
Incubations contained the standard radiochemical assay mixture, except for the nucleotide.

Conc. (mM)	Specific activity (dpm/mg × 10^{-5})	
	Nucleotide	
	ATP	ITP
—	0.00	0.00
0.1	30.23	0.00
0.5	45.34	0.00
1.0	54.41	0.00
5.0	96.72	6.04
10.0	60.45	8.46

of that obtained with ATP at the same concentrations (table II). At the concentrations assayed (0.1-10 mM) neither GTP nor UTP were effective in replacing ATP. It is important to notice that the maximum amount of MVAP was obtained when ATP was added at 5 mM concentration.

The effect of metal ions on partially purified mevalonate kinase was also studied. A little MVA phosphorylation was observed when enzymatic reactions were carried out without addition of metal ions. Supplementation of Ca^{2+} (0.1-10 mM) had no effect on the reaction. Results in table III show that MVA kinase

Table III. *Effect of metal ion on partially purified mevalonate kinase from neonatal chick liver.*
Incubations contained the standard radiochemical assay mixture, except for metal ion.

Conc. (mM)	Specific activity (dpm/mg × 10^{-5})		
	Metal ion		
	Mg^{2+}	Mn^{2+}	Ca^{2+}
—	9.07	9.07	9.07
0.1	12.09	18.13	8.47
0.5	20.33	108.81	6.04
1.0	30.22	102.77	6.04
5.0	96.72	60.45	9.07
10.0	90.69	30.22	12.09

Table IV. *Effect of EDTA on partially purified mevalonate kinase from neonatal chick liver.*
Incubations contained the standard radiochemical assay mixture, except for the metal ion and EDTA.

Additions (mM)	Specific activity (dpm/mg × 10^{-5})
None	9.07
EDTA (1.0)	0.00
EDTA (10.0)	0.00
M^{2+} (5.0)	96.72
Mg^{2+} (5.0) + EDTA (1.0)	36.27
Mg^{2+} (5.0) + EDTA (10.0)	10.03
Mn^{2+} (0.5)	114.86
Mn^{2+} (0.5) + EDTA (1.0)	6.04
Mn^{2+} (0.5) + EDTA (10.0)	0.00

reached its maximal activity in the presence of 0.5-1 mM Mn^{2+} or 5-10 mM Mg^{2+}. Higher concentrations of Mn^{2+} were clearly inhibitory.

As would be expected, the enzymatic reaction was inhibited by EDTA (table IV). Radiochemical assays carried out with incubation mixtures containing the optimum Mg^{2+} and Mn^{2+} concentrations showed that 1.0 and 10 mM EDTA clearly inhibited MVAP formation in the presence of Mg^{2+}, inhibition that was more pronounced in reactions carried out in the presence of Mn^{2+}.

Discussion

In previous papers (7, 8, 14, 16) we have reported some properties of chick mevalonate kinase from crude extracts, some of them clearly different to those reported in other sources. In view of these differences, it appeared to be of interest to purify the chick liver mevalonate kinase and to study the characteristics of the purified preparation. Some of these characteristics have been reported in this paper and some others, especially important because of the differences with those found in other sources, will be reported in a next paper (6).

Although Sephadex G200 has been successfully used as a purification technique for both *Hevea* latex (22) and *Sarcophaga bullata* larvae (11), we had obtained a good recovery of enzyme activity from Sephadex G100 columns working with cell-free extracts from *Pinus pinaster* (21) and *Agave americana* (20). Furthermore, in both sources two fractions with mevalonate kinase activity were eluted from Sephadex G100 (10), both fractions being equally active at the same pH 7.9 and with about 20-fold increase in specific activity over the original acetone powder extracts. In the chick liver enzyme, only one active fraction was eluted from Sephadex G100 column. On the other hand, DEAE-cellulose chromatography has been successfully used as a purification procedure for chick liver mevalonate kinase, as well as the hog liver enzyme (1).

The kinetic characteristics of partially purified MVA kinase from chick liver agreed with the results of BEYTIA *et al.* (1) for the hog liver enzyme, giving a pattern which is typical for a sequential reaction according to Cleland's terminology (3). Several authors have reported K_m values of mevalonate kinase from different preparations, obtaining a wide variety of results. Differences could be related to the extent of purification besides the origin, so that comparison with other results is difficult.

Regarding nucleotide requirements, the partially purified mevalonate kinase from chick liver was very specific for ATP, whereas pig liver (13) and *Hevea* latex (22) enzymes could utilize ATP and ITP equal effectively at similar concentrations. The specific requirement for ATP was more pronounced in the purified enzyme than that in the 15,000 × *g* supernatant enzyme from chick liver, in which about 30 % activity observed in the presence of ATP was found in the presence of ITP at the same concentrations (8). On the other hand, the inhibition of mevalonate kinase by high concentrations of ATP has been reported in crude preparations from chick liver (8) and in plant sources (4, 18) but not in other crude preparations from chick kidney (14) and brain (16).

Metal ion requirements of partially purified mevalonate kinase from chick liver showed that Mn^{2+} was a better activator than Mg^{2+} at low concentrations. Maximal enzyme activity was reached at 0.5-1 mM Mn^{2+}; at higher concentrations Mn^{2+} was clearly inhibitory. We have also found an inhibition by Mn^{2+} in mevalonate kinase partially purified from *Agave americana* (20), similar to that reported for the enzyme from hog liver (1). As in this origin, Ca^{2+} was ineffective while LEVY and POPJAK (13) had reported that Ca^{2+} was effective in replacing Mg^{2+} as metal cofactor.

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Resumen

Se ha purificado parcialmente la mevalonato quinasa de hígado de pollo recién nacido mediante precipitación con sulfato amónico y fraccionamiento por Sephadex G100 y DEAE-celulosa. Las características cinéticas concuerdan con el mecanismo secuencial sugerido para la enzima y dan K_m aparentes de 0,01 mM para el ácido mevalónico y 0,25 mM para el ATP. La enzima parcialmente purificada muestra una especificidad absoluta frente al ATP. El ion Mn^{2+} es mejor activador que el Mg^{2+} a bajas concentraciones (0,1-1,0 mM). Concentraciones superiores de Mn^{2+} producen una clara inhibición de la mevalonato quinasa. Asimismo, la adición de EDTA, con o sin iones metálicos, inhibe claramente la reacción enzimática.

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