Pig Liver Phosphomevalonate Kinase. 1. Purification and Properties†

Sergio Bazaes, Enrique Beytía,‡ Ana María Jabalquinto,§ Francisco Solís de Ovando, Isabel Gómez, and Jaime Eyzaguirre*

ABSTRACT: Pig liver phosphomevalonate kinase (EC 2.7.4.2) has been purified to homogeneity as shown by polyacrylamide gel electrophoresis. The molecular weight estimates range from 21 000 to 22 500. Each molecule is composed of one polypeptide chain. The presence of SH-containing reagents is essential for the preservation of enzyme activity at all steps in the purification. The enzyme shows absolute specificity for ATP and requires for activity a divalent metal cation, Mg^{2+} being most effective. The optimum pH for the enzyme ranges from 7.5 to over 9.5. Kinetics are hyperbolic for both substrates, showing a sequential mechanism; true K_m values of 0.075 mM and 0.46 mM have been obtained for phosphomevalonate and ATP, respectively. Amino acid composition shows a high content of acid amino acids, one cysteine residue per molecule of enzyme, and the absence of methionine. The results obtained suggest that the enzyme plays no regulatory function in cholesterol biosynthesis in pig liver, although a variable enzyme content was detected in different livers.

Phosphomevalonate kinase (ATP:5-phosphomevalonate phosphotransferase, EC 2.7.4.2) is one of the enzymes in the biosynthetic pathway of the polyisoprenoid precursor isopentenyl pyrophosphate. The enzyme catalyzes the reaction

(3*R*)-phosphomevalonate + ATP $\overset{Mg^{2+}}{\rightleftharpoons}$ (3*R*)-pyrophosphomevalonate + ADP

Phosphomevalonate kinase has been detected in several organisms and tissues: yeast (Bloch et al., 1959; Henning et al., 1959), latex of *Hevea brasiliensis* (Skilleter & Kekwick, 1971), rat liver (Levy & Popják, 1960), pig liver (Hellig & Popják, 1961), and developing rat brain (Ramachandran & Shah, 1977).

Little is known about the properties of the enzyme (Beytía & Porter, 1976). All phosphomevalonate kinases studied are specific for ATP, except the *H. brasiliensis* enzyme which can utilize ITP and UTP at a lower rate (Skilleter & Kekwick, 1971). All enzymes are activated by divalent metal cations, Mg^{2+} being most effective. The apparent K_m for MVAP,[1] estimated for the pig liver enzyme, is 3×10^{-4} M. This rather high value has led to the suggestion that the enzyme may be a rate-limiting step in isoprenoid biosynthesis (Helling & Popják, 1961). The *H. brasiliensis* enzyme, however, shows a K_m for MVAP of 4.2×10^{-5} M (Skilleter & Kekwick, 1971). The studies so far reported on phosphomevalonate kinase are of a preliminary nature and have been performed with crude preparations. No information is available on the molecular size and quaternary structure of the enzyme.

In this communication we report the purification to homogeneity of the pig liver enzyme as well as some of its characteristics. In the following paper (Bazaes et al., 1980), the participation of lysyl and cysteinyl residues in the enzyme active site is described.

Experimental Procedure

Chemicals. Nucleotides, pyruvate kinase, lactic dehydrogenase, Tris, and (*RS*)-mevalonic lactone were purchased from Sigma Chemical Co. 2-Mercaptoethanol was obtained from Calbiochem. DEAE-cellulose DE-52 was supplied by Whatman, Ltd. Bio-Gel P-60, Bio-Gel P-150, and hydroxylapatite were from Bio-Rad Laboratories, and DL-[5-^{3}H]-mevalonic acid was from New England Nuclear. All other reagents employed were of analytical grade.

Preparation of Mevalonate 5-Phosphate. This substrate was prepared by enzymatic phosphorylation of mevalonate (Levy & Popják, 1960) in 200 mM potassium phosphate buffer, pH 7.4, and 10 mM 2-mercaptoethanol. The lactone of mevalonic acid was converted previously to the potassium salt with KOH. Mevalonate kinase was partially purified according to the method of Beytía et al. (1970) up to the DEAE-cellulose step, obtaining a preparation with a specific activity of 0.35 unit/mg, free of phosphatases and phosphomevalonate kinase. In later experiments, a mevalonate kinase preparation, obtained as the byproduct in the purification of phosphomevalonate kinase at the Bio-Gel P-150 step (see below), was employed. This preparation, with a specific activity of 3.2 units/mg, was also free of the contaminating activities mentioned above. For identification of the reaction products during the purification steps, [^{3}H]mevalonate was added to the reaction mixture, at a final specific activity of at least 0.03 μCi/μmol.

The synthesis reaction was stopped by immersing the reaction mixture in boiling water for 4 min, and the precipitated protein was eliminated by centrifugation. For the initial purification steps, the procedure of Levy & Popják (1960) was used, up to the first elimination of barium as $BaSO_4$. Further purification was achieved by DEAE-cellulose chromatography according to Dugan et al. (1968), using sequential elution with $(NH_4)_2CO_3$. The product [free of $(NH_4)_2CO_3$] was kept at −20 °C, either as a lyophilized powder or in solution at pH 7.5. The product was identified by Dowex 1–formate chromatography, according to Bloch et al. (1959), and by paper chromatography using 1-propanol–ammonia–water (60:20:20) (Shah et al., 1965; Dugan et al., 1968) or 1-butanol–formic acid–water (77:10:13) (Tchen, 1958). Its phosphorus content [determined according to King (1932)] indicated the presence of 1 g-atom/mol of MVAP. The concentration of MVAP was

† From the Laboratorio de Bioquímica, Universidad Católica, Casilla 114-D, Santiago, Chile. *Received December 14, 1978; revised manuscript received December 27, 1979.* This work was supported by Grant No. 64/72 from the Dirección de Investigación, Universidad Católica, by the Regional Program of UNDP–UNESCO RLA 76/006, and by funds from the Organization of American States.

‡ Deceased March 18, 1979.

§ Present address: Departamento de Medicina Experimental, Facultad de Medicina Oriente, Universidad de Chile, Casilla 16038, Santiago, Chile.

[1] Abbreviations used: MVAP, phosphomevalonate; MVAPP, pyrophosphomevalonate; MVA, mevalonic acid; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; DTNB, 5,5′-dithiobis(2-nitrobenzoate); Mes, 2-(*N*-morpholino)ethanesulfonic acid; Tes, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid; Bicine, *N*,*N*-bis(2-hydroxyethyl)glycine.

0006-2960/80/0419-2300$01.00/0

determined with phosphomevalonate kinase by using the standard spectrophotometric enzyme assay. The product was found to be free of mevalonate and ATP by using the mevalonate kinase assay and ADP, as shown with pyruvate kinase.

Enzyme Purification. All steps were performed at 0–4 °C. All buffers used contained 10 mM 2-mercaptoethanol.

Crude Extract. Pig liver was obtained immediately after slaughter and brought to the laboratory on ice. One kilogram was cut in small pieces and homogenized in a Waring Blendor in 2 L of 100 mM potassium phosphate buffer, pH 7.5. After filtration through cheesecloth, the extract was centrifuged successively at 8000*g* for 10 min, 43500*g* for 20 min, and 100000*g* for 45 min. The supernatant was filtered through glass wool.

Ammonium Sulfate Fraction. Solid enzyme-grade ammonium sulfate (Schwarz/Mann) was added to 30% saturation, and after 30 min the preparation was centrifuged for 10 min at 27000*g*. The supernatant was brought to 60% saturation with solid ammonium sulfate and after 30 min was centrifuged as before. The precipitate was resuspended in 400 mL of 5 mM potassium phosphate buffer, pH 7.5, and dialyzed for 18 h against two 5-L changes of the same buffer.

DEAE-cellulose Fraction. The dialysate was diluted with 1 mM phosphte buffer, pH 7.5, to a conductivity of 3–4 mS and applied to a DEAE-cellulose column (6.4 × 30 cm) equilibrated with 10 mM phosphate buffer, pH 7.5. The column was washed with 7 L of 50 mM phosphate buffer, and the enzyme was eluted with a linear gradient of 1.6 L each of 60 and 250 mM phosphate buffer, pH 7.5; eluates with a specific activity over 0.15 unit/mg were pooled, and the enzyme was precipitated by adding solid ammonium sulfate to 80% saturation. After centrifugation for 10 min at 27000*g*, the precipitate was resuspended in 50–60 mL of 10 mM phosphate buffer, pH 7.5. At this stage the enzyme is free of phosphatase and NADH-oxidase activities.

Bio-Gel P-150 Fraction. The DEAE-cellulose fraction was applied to a Bio-Gel P-150 column (5 × 75 cm) equilibrated with 10 mM phosphate buffer, pH 7.5, and then washed with the same buffer. Fractions of 10 mL were collected at a flow rate of 20 mL/h (upward flow). The active fractions were pooled and concentrated to 10–15 mL in an Amicon ultrafiltration apparatus with a UM 10 membrane. This fraction could be kept for several months at −20 °C in 45% glycerol without loss of activity. At this step, phosphomevalonate kinase is completely separated from mevalonate kinase. This last enzyme, obtained in earlier fractions, was used for MVAP synthesis.

Hydroxylapatite Fraction. The P-150 fraction (diluted 1:3 in 10 mM phosphate buffer, pH 7.5, if containing glycerol) was applied to a 1.6 × 10 cm column containing hydroxylapatite, equilibrated with 10 mM phosphte buffer, pH 7.5. The column was washed with 70 mL of the same buffer, and 2-mL fractions were collected at a flow rate of 30 mL/h. The enzyme is obtained in this washing. After concentration by ultrafiltration, the active fraction was kept in 50% glycerol at −20 °C.

Blue Dextran–Sepharose Fraction. The enzyme was applied to a column (2.4 × 7.5 cm) containing Blue Dextran–Sepharose prepared according to Ryan & Vestling (1974), equilibrated with 10 mM Tris-HCl (pH 7.5)–10% glycerol. After being washed with 40 mL of the equilibration buffer, the enzyme was eluted with 60 mL of the same buffer containing 1 mM ATP. After being pooled and concentrated by ultrafiltration, the enzyme was stored in 50% glycerol at −20 °C.

Enzyme Assays. Mevalonate kinase was assayed spectrophotometrically according to Levy & Popjâk (1960). The standard assay for phosphomevalonate kinase was a modification of that proposed by Tchen (1962). The reaction mixture (1-mL final volume) contained 100 mM potassium phosphate buffer, pH 7.5, 10 mM 2-mercaptoethanol, 0.24 mM NADH, 2.5 mM ATP, 2.5 mM phosphoenolpyruvate, 5 mM $MgCl_2$, 8 units each of pyruvate kinase and lactate dehydrogenase, enzyme, and 0.75 mM MVAP added to start the reaction. The spectrophotometric assays were performed at 30 °C in a Cary 118C spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme required to phosphorylate 1 μmol of MVAP per min under the assay conditions.

Protein Determination. For crude extracts the biuret method was used. Otherwise, the micromethod of Bensadoun & Weinstein (1976) was employed.

Molecular Weight Determination. Three methods were used. (1) The first was gel filtration in a Bio-Gel P-60 column, according to Andrews (1965). A column of 1.6 × 82 cm was equilibrated with 10 mM Tris-HCl buffer (pH 8.0)–100 mM KCl–1 mM EDTA–10 mM 2-mercaptoethanol. Separate samples of insulin (M_r 5750), cytochrome *c* (M_r 12400), chymotrypsinogen A (M_r 25000), pepsin (M_r 35000), or phosphomevalonate kinase were applied to the column. Cytochrome *c* was determined spectrophotometrically at 412 nm, the other standards were determined at 280 nm, and phosphomevalonate kinase was determined by using the usual assay. (2) The second was sucrose density gradient centrifugation according to Beytia et al. (1970). Gradients were prepared from 5 to 20% sucrose in 10 mM phosphate buffer, pH 7.5, containing 10 mM 2-mercaptoethanol. Centrifugtion was performed at 160000*g* for 17 h in a Beckman Model L 5 40 centrifuge. Cytochrome *c*, bovine serum albumin (M_r 67000), and lactate dehydrogenase (M_r 140000) were used as standards; their concentration was estimated as in the gel filtration method, except for lactate dehydrogenase which was determined spectrophotometrically according to Kornberg (1955). (3) The third was sodium dodecyl sulfate–polyacrylamide slab gel electrophoresis. Electrophoresis was performed as described below, using cytochrome *c*, chymotrypsinogen A, and ovalbumin (M_r 43000) as standards.

Amino Acid Analysis. Purified phosphomevalonate kinase was dialyzed for 15 h against distilled water, lyophilized, and resuspended in 0.5 mL of 6 N HCl. The sample was divided into two equal aliquots and the tubes were sealed under nitrogen. Hydrolysis was carried out at 110 °C for 24 and 48 h, respectively. Analysis was performed in a Glenco Custom Modular amino acid analyzer (Glenco Scientific, Houston, TX). Tryptophan was determined according to the method of Edelhoch (1967), and cysteine was determined by the procedure of Ellman (1959), in the presence of 6 M guanidinium chloride.

Polyacrylamide Gel Electrophoresis. Electrophoresis and staining of native proteins were performed in cylindrical gels (0.6 × 10 cm) as described by Gabriel (1971). Separating gels contained 10–12% acrylamide, and stacking gels contained 3.7% acrylamide. The electrode buffer (pH 8.3) contained 25 mM Tris base and 190 mM glycine. Gels were run at 4 °C at a constant current of 1 mA/gel until the dye band entered the separating gel and at 2.5 mA/gel until this band reached near the bottom.

Phosphomevalonate kinase activity in the gels was measured by the standard procedure, after homogenizing 2-mm slices in 0.2 mL of 0.2 M phosphate buffer, pH 7.5, containing 10 mM 2-mercaptoethanol.

Table I: Purification of Phosphomevalonate Kinase from Pig Liver

step	vol (mL)	protein (mg)	units	sp act. (units/mg)	purifn (x-fold)	cumulative recovery (%)
crude extract	1530	85 680	752	0.0088	1	100
30–60% ammonium sulfate	521	49 495	590	0.0119	1.35	78.4
DEAE-cellulose	660	1 716	420	0.24	27.3	55.8
Bio-Gel P-150	340	80.9	290	3.57	406	38.6
hydroxylapatite	14	13.6	123	9.0	1022	16.3
Blue Dextran-Sepharose	4.6	1.02	70	69.1	7852	9.3

Sodium dodecyl sulfate gel electrophoresis was performed in slab gels according to Laemmli (1970). Separating gels of 11% acrylamide and stacking gels of 3% acrylamide were used. Gels were run at room temperature at 70 V until the dye entered the separating gel and continued at 110 V. The electrode buffer was the same as that used for the native gels, containing, in addition, 0.1% sodium dodecyl sulfate.

Results

Enzyme Purification. Table I summarizes the results of the purification of the enzyme starting with 1 kg of liver. The DEAE-cellulose step gives a high increase in specific activity and a phosphatase-free preparation. The Bio-Gel P-150 column separates mevalonate kinase, because of its higher molecular weight [98 000 (Beytía et al., 1970)]. This preparation was used in many experiments not requiring a homogeneous enzyme. The elution of the enzyme from the Blue Dextran–Sepharose column is achieved with 1 mM ATP; this elution effect seems to be specific since the enzyme is not eluted with KCl solutions of similar ionic strength.

Purity of the Enzyme. The purity was established by polyacrylamide gel electrophoresis. Figure I shows that a homogeneous preparation is obtained after Blue Dextran–Sepharose chromatography. The figure also shows that the protein band corresponds to the position of the enzyme activity in the gel.

Stability of the Enzyme. The enzyme requires the presence of sulfhydryl reagents in order to protect its activity. Preparations with a specific activity of 3–4 units/mg of protein lose 99% of their activity in 20 days at 4 °C in the absence of thiol reagents, but no loss is observed in 3 months if 10 mM 2-mercaptoethanol is added. The homogeneous preparations can be maintained without loss of activity for 60 days at −20 °C in 10 mM Tris or phosphate buffer, pH 7.5, containing 50% glycerol and 10 mM 2-mercaptoethanol.

Molecular Weight. Gel filtration in Bio-Gel P-60 gives a value of 22 500. With sucrose density gradient centrifugation, a value of 22 000 is interpolated. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis gives a value of 21 000, showing also that the enzyme is composed of only one polypeptide chain.

Amino Acid Analysis. The amino acid composition of the enzyme is summarized in Table II; no methionine could be detected under the conditions of the analysis.

Substrate and Cofactor Requirements. The enzyme has an absolute specificity for ATP, which cannot be replaced by other nucleotide triphosphates. Mg^{2+} is the best activator among several divalent metal cations assayed, as shown in Table III, in agreement with previous results of Hellig & Popják (1961). Optimal reaction rates measured in the presence of 2.5 mM ATP are obtained at a Mg^{2+} concentration of 5 mM; no inhibitory effect is observed at a Mg^{2+} concentration of 13 mM.

Effect of pH. Figure 2 shows the effect of pH on the enzyme activity by using several bufers (Good et al., 1966)

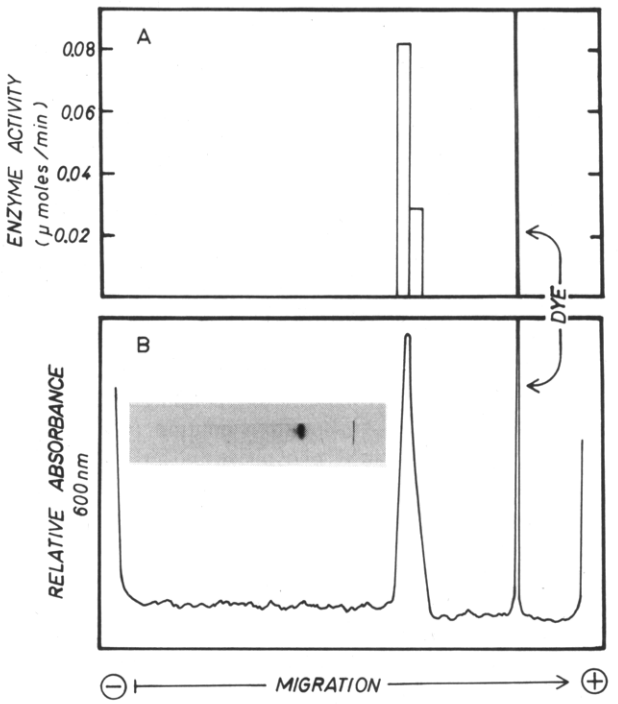

FIGURE 1: Polyacrylamide gel electrophoresis of purified pig liver phosphomevalonate kinase. Electrophoresis was performed in 10% acrylamide gels. The gel was sliced longitudinally; half was used for activity assay (A) and the other half was stained for protein. (B) represents the photograph and the scanning of the protein-stained section. Scanning was performed at 600 nm in a Varian-Techtron 635 spectrophotometer. 23 μg of protein of the Blue Dextran–Sepharose chromatography step was applied to the gel. All procedures used were as described under Experimental Procedure.

Table II: Amino Acid Analysis of Pig Liver Phosphomevalonate Kinase

amino acid	residues/21 900 M_r 24-h hydrolysis	48-h hydrolysis
Asp plus Asn	19	20
Thr	9	9
Ser	14	12
Glu plus Gln	33	32
Pro	9	10
Gly	23	22
Ala	18	18
Val	8	8
Ile	8	7
Leu	19	18
Tyr	2	2
Phe	7	7
Lys	7	8
His	5	6
Arg	11	11
Met	*c*	*c*
Cys[a]	1	1
Trp[b]	4	4

[a] Determined by the method of Ellman (1959). [b] Determined by the method of Edelhoch (1967). [c] Undetected.

Table III: Effect of Several Divalent Cations on the Activity of Pig Liver Phosphomevalonate Kinase[a]

metal ion	rel act. (%)
$MgCl_2$	100.0
$MnCl_2$	65.2
$ZnSO_4$	56.1
$CoSO_4$	63.6

[a] In a volume of 0.99 mL were added the following at 30 °C: 1 mM MVAP, 2.5 mM ATP, 10 mM 2-mercaptoethanol, and the corresponding metal ion at 5 mM concentration, in 100 mM Tris-HCl buffer, pH 7.5. The reaction was started by the addition of 0.01 mL of phosphomevalonate kinase (15 milliunits). After 10 min, the reaction was stopped with 1 mL of 3% $HClO_4$ and neutralized with KOH. The ADP produced was measured by the spectrophotometric assay of activity. The results are expressed in percent of the activity obtained with Mg^{2+}. The enzyme assay was linear with time under the conditions used.

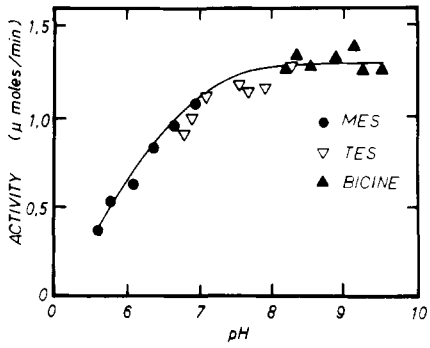

FIGURE 2: Effect of pH on the activity of phosphomevalonate kinase. The reaction mixtures contained phosphomevalonate kinase (12 milliunits; sp act. 2.3 units/mg), 0.24 mM NADH, 2.5 mM ATP, 2.5 mM phosphoenolpyruvate, 5 mM $MgCl_2$, 1 mM MVAP, 10 mM 2-mercaptoethanol, and pyruvate kinase and lactate dehydrogenase, 8 units of each, in different 100 mM buffers. The enzyme activity is expressed as micromoles of MVAP phosphorylated per minute per milliliter of the solution of the enzyme from which the samples for enzyme assay were taken.

in the range from pH 5.5–9.5. An optimum activity plateau is observed between pH 7.5 and pH 9.5.

Initial Velocity Studies. Phosphomevalonate kinase shows hyperbolic kinetics with both Mg-ATP and MVAP when assayed over a 60-fold concentration range starting at $0.2K_m$. Lineweaver–Burk plots at variable concentrations of MVAP and at different, fixed concentrations of Mg-ATP (Figure 3) show a family of intersecting lines. Similar results (not shown) are obtained with a plot of $1/v$ vs. $1/[ATP]$ at different, fixed concentrations of MVAP. These findings agree with a sequential mechanism for the enzyme (Cleland, 1963). Secondary plots of the slopes and intercepts, according to Florini & Vestling (1957), give true K_m values of 0.075 mM for MVAP and 0.46 mM for ATP (inset, Figure 3).

Discussion

This paper describes a procedure for the purification of pig liver phosphomevalonate kinase to apparent homogeneity. The enzyme is purified about 7800-fold, showing that its relative content in the liver (on a soluble protein basis) is very small. The amount of enzyme in the extract varied among different preparations, fluctuating from 250 to 750 units/kg of liver; its content may depend on genetic or nutritional factors.

The last step in the purification of phosphomevalonate kinase utilizes a Blue Dextran–Sepharose column. This gel has been successfully used in the purification of several dehydrogenases and kinases possessing the "dinucleotide fold", due to a structural similarity between the chromophore (Cibacron Blue F3 GA) and NAD^+ (Thompson et al., 1975; Wilson, 1976). The fact that phosphomevalonate kinase was eluted from this column by using 1 mM ATP, but not KCl of similar ionic strength, suggests that the binding of the enzyme may be specific and due perhaps to the presence of this dinucleotide fold in its tertiary structure. More direct evidence of a specific interaction between enzyme and dye may be obtained by studying the competitive effect between dye and ATP and the spectral changes brought about in the dye by its interaction with the protein (Thompson & Stellwagen, 1976; Beissner & Rudolph, 1978).

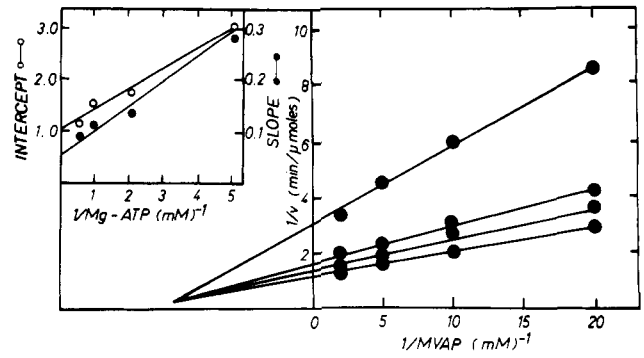

FIGURE 3: Effect of ATP on the initial velocity of the reaction with phosphomevalonate as the varied substrate. The reaction mixture contained 10 milliunits of phosphomevalonate kinase (sp act. 2.3 units/mg), the required concentration of MVAP and ATP, and the rest of the components of the assay of the enzyme, as described under Experimental Procedure. In the inset, the intercepts and slopes obtained are plotted against the ATP concentration.

Pig liver phosphomevalonate kinase shows a molecular weight similar to that found for the smallest phosphotransferase known to date, adenylate kinase [22 000 (Schulz et al., 1974)], and as shown by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, it is composed of only one polypeptide chain. Adenylate kinase is known to present, also, one polypeptide chain, with the dinucleotide fold in its structure (Schulz et al., 1974; Schulz & Schirmer, 1974; Thompson & Stellwagen, 1976).

Purified pig liver phosphomevalonate kinase is very unstable in the absence of SH-containing reagents like 2-mercaptoethanol or dithiothreitol. The enzyme contains only one cysteine residue which may be essential for activity [this aspect is studied further in the following paper (Bazaes et al., 1980)].

The pig liver phosphomevalonate kinase has an absolute requirement for bivalent metal cations, as shown by all kinases, Mg^{2+} being most effective. It also shows absolute specificity for ATP. This suggests that the true substrate is Mg-ATP. The enzyme shows hyperbolic kinetics for both substrates, and the data support a sequential mechanism, but product inhibition studies are required to specify the type of sequential mechanism involved.

The pH–activity profile reported in this work does not agree with the results of Hellig & Popják (1961), obtained with a 14-fold purified preparation from pig liver, or those obtained with the partially purified enzyme from *H. brasiliensis* (Skilleter & Kekwick, 1971), which show a distinct pH optimum at 7–7.3. However, Bloch et al. (1959), with a crude preparation of the yeast enzyme, observe an optimum pH range from 5.7 to 10. Several authors have reported pH–activity curves for the phosphorylation of MVA to MVAPP in different tissues, obtaining a wide variety of results (Garcia-Martinez et al., 1976; Linares et al., 1977; Ramachandran & Shah, 1977). Since these experiments were carried out with crude extracts containing phosphatases and other interfering enzymes, they are not comparable to those reported in this work.

Secondary regulatory sites in the biosynthesis of cholesterol from mevalonic acid have been proposed (Dempsey, 1974). Recently, Shama Bhat & Ramasarma (1977) have reported rhythmic activity in the utilization of mevalonate, and the step responsible is likely to be between mevalonate and isopentenyl pyrophosphate involving the activities of mevalonate kinase, phosphomevalonate kinase, and pyrophosphomevalonate decarboxylase. Several observations described in this work (low molecular weight, monomeric structure, and hyperbolic kinetics) argue against an allosteric character for phosphomevalonate kinase.

Although variable amounts of phosphomevalonate kinase have been found in different liver samples, as pointed out above, this does not seem to be caused by changes in the nutritional state of the animal, since Slakey et al. (1972) have demonstrated that the levels of activity of mevalonate and phosphomevalonate kinases do not change on fasting and refeeding. All these findings, together with the evidence presented by Ramachandran & Shah (1977), make it unlikely that phosphomevalonate kinase is a regulatory site in cholesterol biosynthesis.

Acknowledgments

We thank Drs. Frank Marcus and Leo J. Saidel (University of Health Sciences, Chicago, IL) for kindly performing the amino acid analysis and Drs. Emilio Cardemil and Catherine C. Allende for helpful suggestions in the preparation of the manuscript.

References

Andrews, P. (1965) *Biochem. J. 96*, 595.
Bazaes, S., Beytía, E., Jabalquinto, A. M., Solis de Ovando, F., Gómez, I., & Eyzaguirre, J. (1980) *Biochemistry* (following paper in this issue).
Beissner, R. S., & Rudolph, F. B. (1978) *Arch. Biochem. Biophys. 189*, 76.
Bensadoun, A., & Weinstein, D. (1976) *Anal. Biochem. 70*, 241.
Beytía, E., & Porter, J. W. (1976) *Annu. Rev. Biochem. 45*, 113.
Beytía, E., Dorsey, J. K., Marr, J., Cleland, W. W., & Porter, J. W. (1970) *J. Biol. Chem. 245*, 5450.
Bloch, K., Chaykin, S., Phillips, A. H., & de Waard, A. (1959) *J. Biol. Chem. 234*, 2595.
Cleland, W. W. (1963) *Biochim. Biophys. Acta 67*, 104.
Dempsey, M. E. (1974) *Annu. Rev. Biochem. 43*, 967.
Dugan, R. E., Rasson, E., & Porter, J. W. (1968) *Anal. Biochem. 22*, 249.
Edelhoch, H. (1967) *Biochemistry 6*, 1948.
Ellman, G. L. (1959) *Arch. Biochem. Biophys. 82*, 70.
Florini, J. R., & Vestling, C. S. (1957) *Biochim. Biophys. Acta 25*, 575.
Gabriel, O. (1971) *Methods Enzymol. 22*, 565.
García-Martínez, J., Segovia, J. L., Suárez, M. D., & García-Peregrin, E. (1976) *Biochem. Biophys. Res. Commun. 72*, 202.
Good, N. E., Winget, G. D., Winter, W., Connolly, T. N., Izawa, S., & Singh, R. M. N. (1966) *Biochemistry 5*, 467.
Hellig, H., & Popják, G. (1961) *J. Lipid Res. 2*, 235.
Henning, U., Möslein, E. M., & Lynen, F. (1959) *Arch. Biochem. Biophys. 83*, 259.
King, E. J. (1932) *Biochem. J. 20*, 292.
Kornberg, A. (1955) *Methods Enzymol. 1*, 441.
Laemmli, U. K. (1970) *Nature (London) 227*, 680.
Levy, H. R., & Popják, G. (1960) *Biochem. J. 75*, 417.
Linares, A., Suárez, M. D., & García-Peregrin, E. (1977) *Biochem. Biophys. Res. Commun. 77*, 974.
Ramachandran, C. K., & Shah, S. N. (1977) *J. Neurochem. 28*, 751.
Ryan, L. D., & Vestling, C. S. (1974) *Arch. Biochem. Biophys. 160*, 279.
Schulz, G. E. & Schirmer, R. H. (1974) *Nature (London) 250*, 142.
Schulz, G. E., Elzinga, M., Marx, F., & Schirmer, R. H. (1974) *Nature (London) 250*, 120.
Shah, D. H., Cleland, W. W., & Porter, J. W. (1965) *J. Biol. Chem. 240*, 1946.
Shama Bhat, C., & Ramasarma, T. (1977) *Biochem. Biophys. Res. Commun. 74*, 785.
Skilleter, D. N., & Kekwick, R. G. O. (1971) *Biochem. J. 124*, 407.
Slakey, L. L., Craig, M. C., Beytia, E., Briedis, A., Feldbruegge, D. H., Dugan, R. E., Quershi, A. A., Subbarayan, C., & Porter, J. W. (1972) *J. Biol. Chem. 247*, 3014.
Tchen, T. T. (1958) *J. Biol. Chem. 233*, 1100.
Tchen, T. T. (1962) *Methods Enzymol. 5*, 489.
Thompson, S. T., & Stellwagen, E. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 361.
Thompson, S. T., Cass, K. H., & Stellwagen, E. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 669.
Wilson, J. E. (1976) *Biochem. Biophys. Res. Commun. 72*, 816.