Journal of Cellular Biochemistry 35:93–103 (1987)

Characterization of HMG CoA Synthase Activity of Rat Liver and CHO-K1 Cells

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This report describes the characterization and partial purification of rat liver 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) synthase activity. A preliminary characterization of Chinese hamster ovary (CHO) cell HMG CoA synthase activity is also presented. Ion-exchange chromatography of ammonium sulfate precipitates of rat liver cytosol indicate the existence of two isoenzymes of HMG CoA synthase. These isoenzymes are physically, catalytically, and immunologically distinct. One of these isoenzymes, peak 1, resembles mitochondrial HMG-CoA synthase activity as evidenced by similarities in elution upon ion-exchange chromatography, inhibition by $MgCl_2$, and cross reactivity with an antibody prepared against the mitochondrial enzyme. As peak 1 activity is unstable, further purification studies were performed on peak 2 activity. Peak 2 can be further resolved into two activities (peaks 2A and 2B) by gel filtration. In contrast, CHO-K1 cells (a permanent fibroblast line) possess only peak 2 type HMG CoA synthase activity.

Key words: HMG CoA synthase, cholesterol biosynthesis, protein purification, isoenzymes

The synthesis of HMG CoA, catalyzed by HMG CoA synthase, is a regulated step in both ketogenesis and mevalonate biosynthesis. Consistent with these two roles, liver HMG CoA synthase activity exhibits dual localization in the mitochondria and cytosol [1–3]. The mitochondrial and cytosolic forms of avian liver HMG CoA synthase are physically, catalytically, and immunologically distinct [4,5]. Moreover, the avian liver cytosolic activity is itself composed of four molecular species. Of these four, one is immunologically related to the mitochondrial species [5].

Other activities which participate in the generation of HMG CoA exhibit dual localization and/or a diversity of enzyme species. Two distinct reactions catalyze the formation of acetoacetyl CoA:acetoacetyl CoA synthase and acetoacetyl CoA thiolase. A third activity, acetoacetyl CoA:succinate CoA transferase, exists in several rat nonhepatic tissues [6]. Liver acetoacetyl CoA thiolase exhibits mitochondrial and

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Received February 11, 1987; accepted May 5, 1987.

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cytosolic localization [2] . Cytosolic rat thiolase, like avian cytosolic HMG CoA synthase activity, also consists of four molecular species [7].

In contrast, Clinkenbeard has reported the existence of only a single cytoplasmic molecular species of HMG CoA synthase activity in rat liver [5]. Considering the multiple forms of this activity in other species, we decided to further investigate the heterogeneity of rat liver cytosolic HMG CoA synthase. As we have previously described the isolation of a Chinese hamster ovary (CHO) cell mutant lacking detectable HMG CoA synthase activity [8], it was of interest to determine the multiplicity of cytosolic HMG CoA synthase in the parental cell line, CHO-K1. Therefore, a similar analysis of synthase heterogeneity was undertaken in the wild type, CHO-K1 fibroblast. We herein describe a partial purification and preliminary characterization of multiple forms of cytosolic rat liver HMG CoA synthase. We also present evidence which indicates that CHO fibroblasts possess a single form of synthase activity.

MATERIALS AND METHODS

Materials

Acetyl CoA, acetoacetyl CoA, oxaloacetic acid, DTNB, and Protein A-Sepharose were obtained from Sigma (St Louis, MO). Sephacryl S-300 and blue-Sepharose CL-6B were purchased from Pharmacia (Piscataway, NJ). DEAE-Trisacryl was obtained from LKB (Rockville, MD). Radioactively labelled acetyl-[1-C^{14}] CoA was purchased from New England Nuclear (Boston, MA). Normal rat serum was prepared by ammonium sulfate precipitation [9]. Antibody against avian mitochondrial HMG CoA synthase [10] was generously provided by H.M. Miziorko of the Medical College of Wisconsin (Milwaukee, WI). Sprague/Dawley male rats (100/150g) were fed rat chow ad libitum and housed in rooms with a controlled light/dark cycle (light 0700 to 1900) for at least 2 wk. Four days prior to death, they were fed rat chow containing 4% cholestyramine.

Cell Culture

The wild-type Chinese hamster fibroblast used in this study is the CHO-K1 cell [11]. CHO-K1 was grown in Hams F12 medium supplemented with 5% fetal calf serum. Delipidized serum was prepared from neonatal calf serum by organic solvent extraction [12].

Enzyme Assays

Citrate synthase activity was assayed in freshly prepared, sonicated mitochondrial and cytosolic extracts by the method of Srere [13] and contained in a final volume of 2.2 ml, 200 μmol Tris-HCl, pH 8.0, 0.2 μmol DTNB, 0.1 μmol acetyl CoA, and 10–60 μg protein. The reaction was initiated by the addition of 0.5 μm oxaloacetate that had been freshly prepared and neutralized with potassium hydroxide to pH 7.5 immediately prior to use. The absorption at 412 nm was monitored for 3 min at 25°C. HMG CoA synthase was assayed by the radiochemical procedure of Clinkenbeard et al as previously described [8]. Apparent Km values were determined by Lineweaver-Burke analysis at pH 8.0 and 400 μM acetyl CoA and 40 μM acetoacetyl CoA when acetoacetyl CoA and acetyl CoA concentrations, respectively, were varied.

CHO Cell Fractionation

CHO cells were harvested by trypsinization in 0.05% trypsin containing 0.02% EDTA. Cell pellets obtained after centrifugation at 500g for 5 min were resuspended in a small volume (1.0 ml per 10 × 150 mm culture dishes) of hypotonic buffer (20 mM Tris, pH 8.0). The cell pellets were allowed to swell on ice for 30 min. Cells were then lysed by homogenization with 20–30 strokes in a tight-fitting glass Dounce homogenizer. The cell extract was then diluted to normal tonicity with an equal volume of hypertonic buffer (0.5 M sucrose, 200 mM potassium chloride, 40 mM Tris, .02 unit ml aprotinin, 2×10^{-7} M pepstatin pH 7.2). Cell debris and nuclei were removed by centrifugation at 2,500g for 10 min. The resulting supernatant was centrifuged at 9,700g for 10 min. The mitochondrial pellet was washed two times and recentrifuged at 9,700g for 10 min. Cytosol was obtained by centrifuging the supernatant from the first 9,700g centrifugation at 100,000g for 1 hr. The mitochondrial and cytosolic fractions were assayed for HMG CoA synthase activity after brief sonication and dialysis overnight against 20 mM potassium phosphate, pH 7.0, 0.1 mM EDTA, 2.0 mM DTT.

Purification of Rat Liver Cytosolic HMG CoA Synthase

All steps were performed at 4°C and all buffers contained 0.1 mM EDTA, 2.0 mM DTT, 0.01 units/ml aprotinin, and 1×10^{-7} M pepstatin. Ammonium sulfate precipitates and concentrated fractions were stored at −70°C.

Steps 1 and 2: Cell-Free Extract and Cytoplasmic Fraction

Initial fractionations were performed via method A as follows: Approximately 121g of rat liver was excised and blended in a Waring blender for 3 × 15-sec bursts. The cell extract was then homogenized (three passes) in 210 ml of 0.25 sucrose, 2 mM Hepes, pH 7.2, in a Potter Elvehjem homogenizer. After dilution to 600 ml, PMSF was added to a final concentration of 0.5 mM. Cellular debris, nuclei, and mitochondria were removed by centrifugation at 20,000g for 20 min. Cytosol was obtained by centrifuging the supernatant at 100,000g for 1 hr. In method B, liver was excised, minced finely with scissors, and homogenized directly (three passes) in 9 × volume of rat liver of the buffer described above. PMSF was then added to 0.5 mM and the resulting extract was centrifuged at 2,500g for 10 min to remove cell debris and nuclei. The supernatant was then centrifuged at 9,700g for 10 min. The washed mitochondria were then assayed after sonication (3 × 15-sec bursts) for citrate synthase and HMG CoA synthase activities. The supernatant obtained from the first 9,700g centrifugation was then centrifuged at 100,000g for 1 hr in order to obtain the cytosolic fraction. As results indicate that method B yields cytosol free of mitochondrial matrix contaminants (Table I), subsequent fractionations were performed by method B.

Step 3: 38–55% Ammonium Sulfate Fractionation

The cytosolic fraction was brought to 38% saturation with solid ammonium sulfate (222.6 g/liter) and was stirred for 30 min. Precipitated protein obtained by centrifugation at 12,000g for 15 minutes was discarded. The supernatant was then adjusted to 55% saturation with solid ammonium sulfate (116.2 g/liter) and stirred for 30 min. The precipitated protein was recovered by centrifugation at 12,000g for 15 min.

TABLE I. Mitochondrial Contamination of Cytosolic HMG CoA Synthase Preparations*

	Enzyme activity		
		Cytosol A	Cytosol B
Activity	Mitochondria[a]	(nmol $min^{-1}mg^{-1}$)	
Citrate synthase	88	10	0[b]

*Mitochondrial and cytosolic fractions were prepared as described in Materials and Methods. Citrate synthase was determined on fresh fractions as described in Materials and Methods.
[a]Mitrochondrial results were obtained from fractions prepared by method B, as this procedure yields intact mitochondria.
[b]No detectable values were obtained.

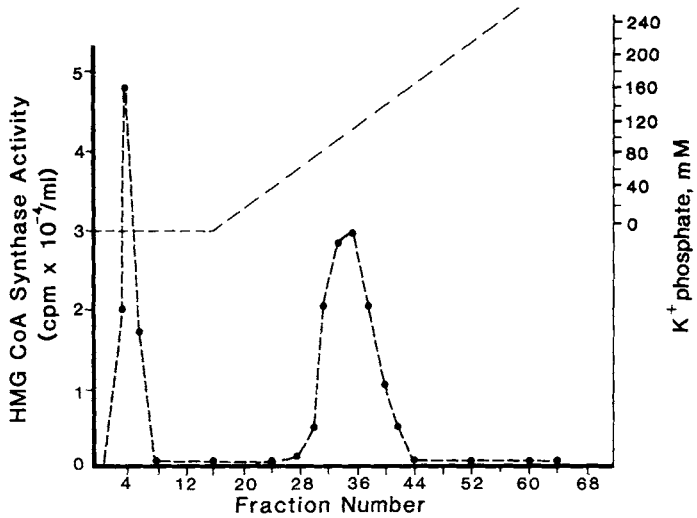

Fig. 1. DEAE-Trisacryl chromatography of rat liver cytosol. Approximately 537 mg of a 38–55% ammonium sulfate precipitate of rat liver cytosol prepared by method A was dialyzed overnight against 1 liter of 20 mM potassium phosphate buffer, pH 7.0, and applied to a column (1 × 76 cm) of DEAE-Trisacryl previously equilibrated with dialysis buffer. Elution and identification of HMG CoA synthase active fractions were as described in the Materials and Methods section. (●) HMG CoA synthase activity; (---), K^+ phosphate, mM.

Step 4: DEAE-Trisacryl Chromatography

The precipitated protein from step 3 was dissolved in 10 ml of 20 mM phosphate buffer, pH 7.0, and dialyzed overnight against the same buffer. The dialyzed enzyme was applied to a DEAE-Trisacryl (Pharmacia, Piscataway, NJ) column (1 × 76 cm) and was eluted with 2 column volumes of 20 mM potassium phosphate buffer, pH 7.0, followed by 800 ml of a linear 20–400 mM potassium phosphate gradient. Peak 1 typically elutes in 20 mM phosphate while peak 2 elutes at approximately 120 mM phosphate (Fig. 1). Both activity peaks were pooled separately and synthase activity was recovered from peak 1 by inward dialysis of 20 mM potassium phosphate buffer, pH 7.0, containing 65% ammonium sulfate. Peak 2 is applied directly to a blue-Sepharose CL-6B column.

Step 5: Blue-Sepharose CL-6B Chromatography

Peak 2 activity from step 4 was directly applied to a blue-Sepharose CL-6B column (3 × 20 cm) that had been equilibrated in 20 mM potassium phosphate buffer, pH 7.0. The column was washed with 4 column volumes of this buffer followed by 500 ml of a 0–0.6 M potassium chloride gradient in equilibration buffer. HMG CoA synthase activity eluted at approximately 200 mM potassium chloride. Active fractions were pooled, dialyzed overnight against 1 liter of 20 mM potassium phosphate buffer, pH 7.0, and concentrated to less than 1 ml by ultrafiltration through a Diaflo PM 10 membrane in a stirred Amicon cell.

Step 6: Sephacryl S-300 Chromatography

The enzyme from step 5 was applied to a Sephacryl S-300 (superfine grade) column (1 × 125 cm) equilibrated with 50 mM potassium phosphate, pH 7.0, at a flow rate of 0.3 ml/min. HMG CoA synthase activity elutes as two peaks with a Ve/Vo of 1.89 for peak 2A and 2.10 for peak 2B. Fractions containing activity were pooled separately and concentrated to less than 1 ml by ultrafiltration through a Diaflo PM 10 membrane in a stirred Amicon cell. Molecular weight determinations were made by comparing the elution volume of samples with that of standards of known molecular weight (thyroglobulin, 669,000; ferritin, 400,000; catalase, 232,000; aldolase, 158,000; ovalbumin, 45,000).

RESULTS

Localization of Multiple Forms of Rat Liver HMG CoA Synthase

Gradient elution ion-exchange chromatography of an ammonium sulfate precipitate of rat liver cytosol under the initial conditions described in Materials and Methods results in two peaks of HMG CoA synthase activity. One peak elutes in the flow-through fraction (peak 1) and the other elutes with 120 mM phosphate (peak 2; Fig. 1). Since avian liver mitochondrial HMG CoA synthase also elutes with the flow-through upon DEAE ion-exchange chromatography [4], an assessment of the subcellular localization of rat liver peak 1 synthase activity was undertaken.

Rat liver mitochondria and cytosol were prepared by two procedures: the initial procedure, A, and a modification of this procedure, B, utilizing gentle homogenization and several low-speed centrifugations in order to obtain a cytosolic preparation with minimal mitochondrial contamination. The mitochondrial and cytosolic fractions were then monitored for the presence of mitochondrial matrix components by examining the activity of citrate synthase—a mitochondrial matrix maker. Cytosol prepared by method A contains some mitochondrial contamination while cytosol prepared by method B appears free of mitochondrial matrix marker activity (Table I). When cytosol prepared by method B was chromatographed on DEAE-Trisacryl, two peaks of HMG CoA synthase were observed (Fig. 2). These two peaks exhibited an identical elution profile to peaks 1 and 2 from cytosol prepared via method A (Fig. 1). Moreover, the contribution of each peak to the total eluted HMG CoA synthase activity was similar in both fractionations, with peak 1 contributing 40% of the total eluted HMG CoA synthase in cytosol prepared via method A, and 38% in cytosol prepared by method B.

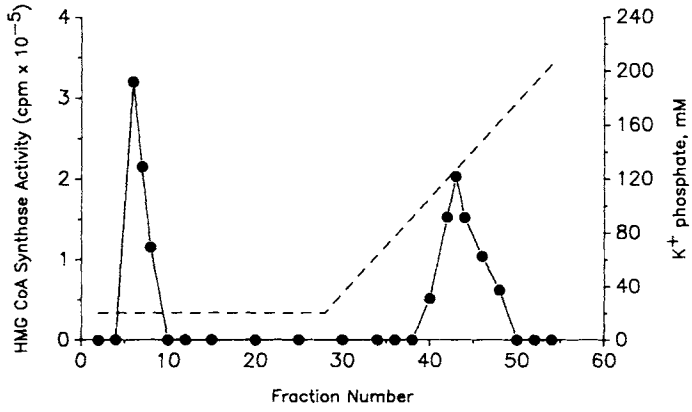

Fig. 2. DEAE-Trisacryl of rat liver cytosol with no detectable mitochondrial matrix activity. Approximately 600 mg of a 38–55% ammonium sulfate precipitate of rat liver cytosol prepared by method B was dialyzed overnight and chromatographed as described in the legend to Figure 1. (●) HMG CoA synthase activity; (---) K^+ phosphate, mM.

TABLE II. Comparison of Peak 1 and Peak 2 Activities

	Peak 1	Peak 2
Relative abundance in cytosolic extracts (% of total[a])	38 ± 6%	62 ± 6%
Stability (% of control activity[b])		
−24 hr at 4°C	23%	96%
−24 hr at −20°C	41%	88%
Relative activity when assayed in presence of 20 mM $MgCl_2$ (% of control)	0	72%
Apparent Km		
Acetoacetyl CoA	<0.1 μm	18 μm
Acetyl CoA	551 μm	127 μm

[a]Total activity represents total counts in all fractions such that recovery of HMG CoA synthase in peak 1 plus peak 2 equals 90% of the total counts recovered throughout the column.

[b]Control activity represents that activity monitored directly after chromatography on DEAE-Trisacryl at 4°C, and was, for peak 1, 1.65 nmol $min^{-1}mg^{-1}$, and for peak 2, 2.2 nmol $min^{-1}mg^{-1}$.

Properties of Peak 1 and Peak 2 Activities

Peak 2 activity is more abundant than peak 1 in rat liver cytosol (Table II). A preliminary characterization of peak 1 and peak 2 activities was undertaken in order to establish whether or not they represented two distinct molecular species of HMG CoA synthase activity. These two activities appear distinct catalytically with observed differences in the apparent Km for both substrates, acetyl CoA and acetoacetyl CoA (Table II). In addition, peak 1 activity is completely inhibited by 20 mM $MgCl_2$ while Peak 2 activity is only inhibited 28%. Peak 1 activity is also less stable than that of peak 2 to freezing or aging at 4°C. Rechromatography of peak 1 on DEAE-Trisacryl after 24 h at 4°C yielded an identical elution profile to that observed when chroma-

tographed fresh. Moreover, chromatography of rat liver ammonium sulfate precipitates prepared in the absence or presence of protease inhibitors yielded similar elution profiles (data not shown). Peak 1 and peak 2 activities also appear to be immunologically distinct. Treatment of peak 1 activity with antiserum prepared against the avian mitochondrial HMG CoA synthase [10] results in complete inhibition of activity (Fig. 3A), while treatment of peak 2 activity with this antiserum has no effect (Fig. 3B). It should be noted that normal rabbit serum is somewhat inhibitory to peak 1 while having no effect on peak 2 activity. This nonspecific inhibition could be distinguished from the effects of the specific antiserum by incubating a fixed amount of serum with increasing amounts of cytosolic protein (Fig. 3A).

Partial Purification of Peak 2 Activity

As Peak 2 activity is more abundant and more stable than that of peak 1, we have focused on the purification of the former. Progress in the purification of peak 2 activity is summarized in Table III. To date, a 650-fold purification of peak 2 activity has been achieved. However, peak 2 activity itself can be further resolved into two activities either by Sephacryl S-300 chromatography or by native polyacrylamide gel

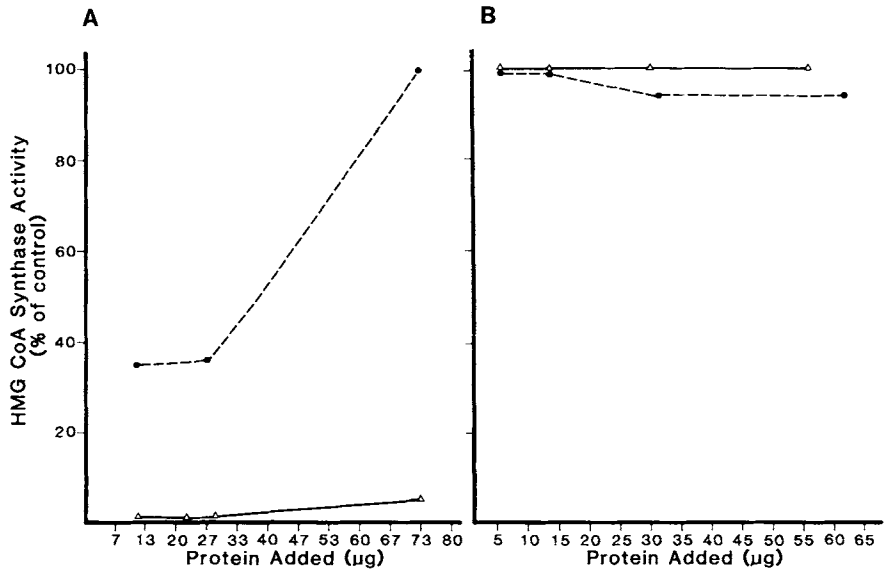

Fig. 3. Inhibition of peak 1 and 2 HMG CoA synthase activities with antiserum prepared against avian mitochondrial HMG CoA synthase. Inhibition of peak 1 and peak 2 synthase activities by antiserum prepared against the avian mitochondrial synthase [9] was assessed by incubating **(A)** 10–72 μg of peak 1 protein assayed directly following DEAE-Trisacryl chromatography (step 4 of purification procedure in Materials and Methods) or **(B)** 3–62 μg of peak 2 protein assayed directly following DEAE-Trisacryl chromatography with 50 μl of either antiavian mitochondrial synthase antiserum (△) or normal rabbit serum (●) and 1 μmol of potassium phosphate, pH 7.0, in a final volume of 0.1 ml for 1 hr at 22°C. Twenty-five microliters of a slurry of protein A Sepharose was added to precipitate immunecomplexes. After a 30-min incubation, precipitated protein was separated by centrifugation at 8,000g for 2 min. Aliquots of the supernatant were assayed for HMG CoA synthase activity as described in Materials and Methods. Control activity was measured in the absence of any added serum and was, for peak 1, 2.22 nmol/min^{-1}/mg^{-1} and for peak 2, 6.32 mnol/min^{-1}/mg^{-1}.

TABLE III. Purification Table

Step	Fold purification	% yield
Cell free extract	1.0	100
Cytosol	3.0	126
38-55% ammonium sulfate	9.6	62
DEAE-Trisacryl[a]	125	41
Blue Sepharose Cl-6B[a]	271	3.3
Sephacryl S-300[a]	650	2.0

[a]HMG CoA synthase activity derived from subsequent fractionation of peak 2 activity.

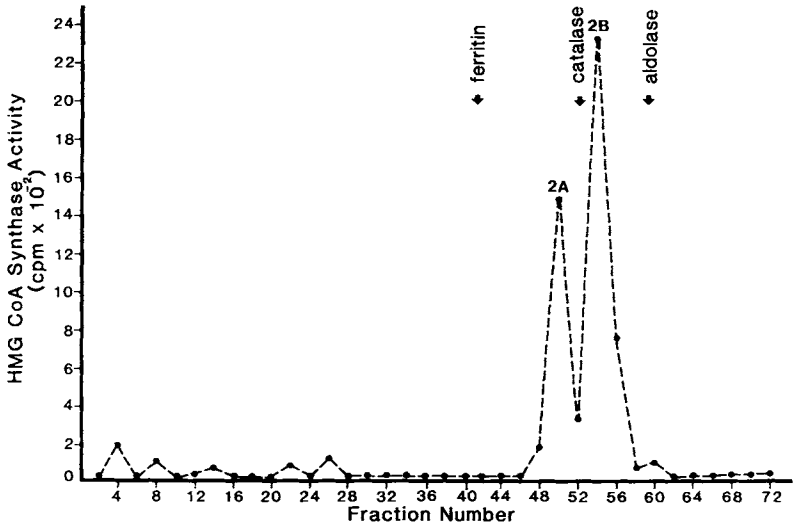

Fig. 4. Sephacryl S-300 chromatography of peak 2. Approximately 5 mg of peak 2 protein purified through step 5 of the purification protocol in Materials and Methods was applied to a column (1 × 125 cm) of Sephacryl S-300 (superfine grade) previously equilibrated with 50 mM potassium phosphate buffer, pH 7.0. Elution and identification of fractions containing HMG CoA synthase activity were as described in Materials and Methods: (●) HMG CoA synthase activity, (⬇) elution profile of molecular weight standards—ferritin, 440,000; catalase, 232,000; aldolase, 158,000.

electrophoresis. Peak 2 activity elutes as two peaks (2A and 2B) on Sephacryl S-300 with apparent molecular weights of 269,000 (2A) and 186,000 (2B) (Fig. 4). Native gradient polyacrylamide gel electrophoresis also resolves peak 2 into two assayable activities (data not shown). The apparent Km of peaks 2A and 2B isolated by Sephacryl chromatography for acetyl CoA were determined and identical values of 130 μM were obtained (Fig. 5). Vmax determinations revealed an apparent Vmax of 9.68 $\pm$ 0.95 nmol $min^{-1}mg^{-1}$ for peak 2A and 7.42 $\pm$ 0.93 nmol $min^{-1}mg^{-1}$ for peak 2B (Fig. 5).

Cytosolic HMG CoA Synthase Activity in CHO-K1 Cells

As multiple forms of HMG CoA synthase were observed in rat liver, an assessment of the possible existence of multiple forms of this enzyme was undertaken in CHO-K1 fibroblasts, which have been widely used in the in vitro analysis of

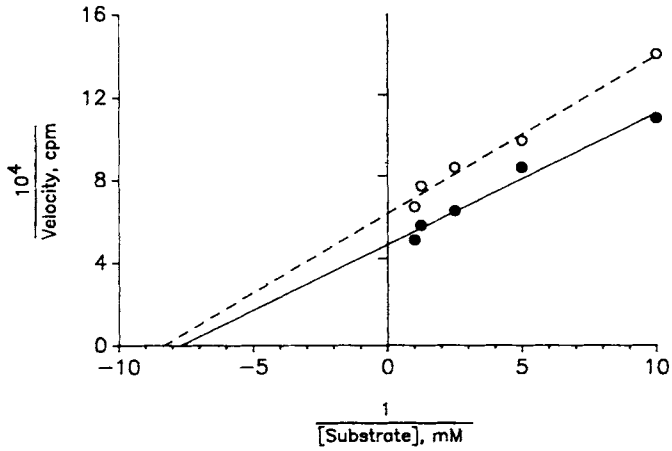

Fig. 5. Determination of the apparent Km for acetyl CoA for peak 2A and 2B HMG CoA synthase activities. Peaks 2A and 2B are isolated by Sephacryl S-300 chromatography as described in Materials and Methods. HMG CoA synthase assays were performed as described [8] utilizing 50 μg of protein and 40 μm acetoacetyl CoA. Acetyl CoA concentrations were varied from 100 to 1,000 μm. The data are plotted according to standard Lineweaver Burke analysis: (○) peak 2B; (●) peak 2A.

mevalonate biosynthesis [2]. Results from this analysis would also be useful in the further characterization of a previously described mevalonate auxotroph with no detectable HMG CoA synthase activity [8]. No measurable HMG CoA synthase activity was observed in CHO cell mitochondria. DEAE-Triacryl chromatography of freshly prepared CHO cell cytosol results in a single peak of activity, eluting with approximately 130 mM potassium phosphate (Fig. 6). Cytosolic HMG CoA synthase activity of CHO-K1 cells is not significantly inhibited by $MgCl_2$ up to concentrations of 40 mM [14]. This observation is also consistent with only a peak 2 type activity being present in CHO-K1 cytosolic extracts.

DISCUSSION

Based on electrofocusing studies, Clinkenbeard et al have reported that rat liver possesses a single cytoplasmic HMG CoA synthase species [5]. The more extensive studies presented in this report, in contrast, demonstrate the existence of at least three distinct forms of HMG CoA synthase activity in cytosolic preparations from rat liver. One of these forms (peak 1) appears similar to the mitochondrial activity with respect to several properties. These include elution upon ion-exchange chromatography [4], $MgCl_2$ inhibition (Table II) [4], and inhibition by specific antiserum (Fig. 3A). That peak 1 activity is indeed cytosolic and does not arise through leakage of mitochondrial components is demonstrated by identical elution profiles on ion-exchange chromatography of cytosolic fractions with variable degrees of mitochondrial marker enzyme contamination.

Peak 1 activity is distinct from the other two activities (peaks 2A and 2B) by several criteria. They differ catalytically as evidenced by differences in the apparent Km for the substrates acetyl CoA and acetoacetyl CoA and in $MgCl_2$ inhibition (Table II). They have distinct charge differences as evidenced by differences in elution on ion-exchange chromatography (Figs. 1, 2) and are not interconvertible upon aging.

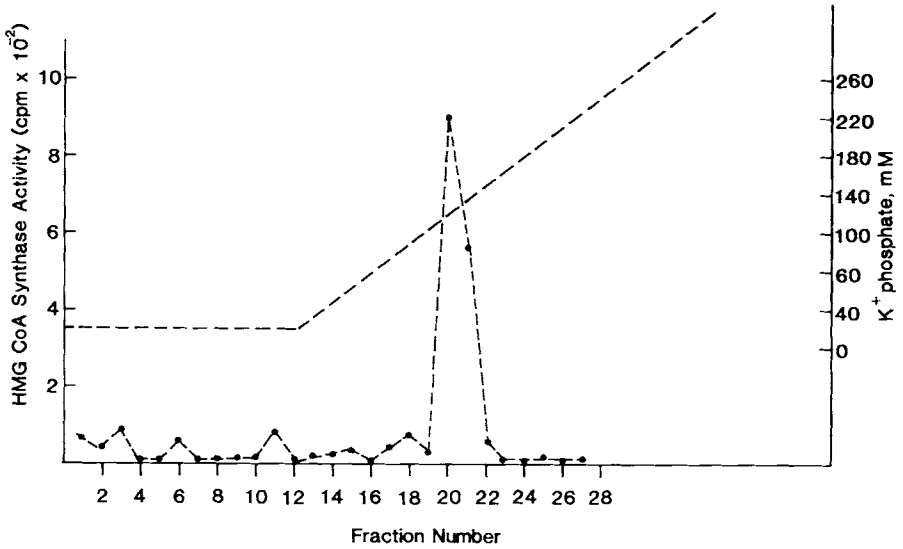

Fig. 6. DEAE-Trisacryl chromatography of CHO cell cytosol. CHO cells were inoculated at a density of 2×10^6 cells per 150-mm culture dish in F12 + 5% fetal calf serum into a total of 20 dishes and allowed to grow for 24 hr. The medium was then changed to F12 + 2% delipidized calf serum and cells were incubated for 48 hr. Cells were harvested and cytosol was prepared as described in Materials and Methods. The cytosolic fraction was concentrated to less than 1 ml in an Amicon microconcentrator and applied to a minicolumn (0.6 × 6 cm) of DEAE-Trisacryl equilibrated in 20 mM potassium phosphate, 0.1 mM EDTA, 2.0 mm DTT, pH 7.0. The column was washed with 5 ml of equilibrated buffer and HMG CoA synthase activity was eluted by addition of 5 ml of a 20–300 mM potassium phosphate, 0.1 mM EDTA, 2.0 mM DTT gradient, pH 7.0: (●) HMG CoA synthase activity; (---) K^+ phosphate, mM.

They also differ immunologically as antibody directed against the avian mitochondrial synthase cross-reacts specifically with peak 1 and not peak 2 (Fig. 3).

In contrast, kinetic determination reveal no significant differences in the apparent Km for acetyl CoA and Vmax of peaks 2A and 2B (Fig. 5). The distinction between peak 2A and peak 2B appears, therefore, largely one of molecular size. As this difference is only 80,000 daltons, it is unlikely that peak 2A is a dimeric form of peak 2B. The subunit molecular weight of cytosolic HMG CoA synthase in both avian and hamster liver is 53,000 daltons, [5,15]. Since the subunit structures of peaks 2A and 2B are presently unknown, it is possible that peak 2A is an oligomeric form of peak 2B composed of partially proteolyzed dimers.

It is presently unknown why there exist multiple forms of rat liver cytosolic HMG CoA synthase. Avian liver cystol also contains multiple synthases, although three of these forms (II, III, IV) are interconvertible [5]. Avian cytosolic synthase I, like rat cytosolic peak 1, bears close resemblance to the mitochondrial synthase. Clinkenbeard has speculated that synthase I is a cytoplasmic procursor of the mitochondrial enzyme [5].

In contrast, CHO fibroblasts contain only one form of HMG CoA synthase. The absence of a fibroblast mitochondrial synthase is not surprising since the avian mitochondrial activity is only present in those tissues capable of ketogenesis such as the liver and kidney [16]. A single fibroblast synthase activity is also consistent with the observation of Luskey et al, who have identified two amplified mRNAs in a

compactin-resistant, UT-I cell which expresses elevated levels of both HMG CoA synthase and reductase activity [17,18]. One of the amplied RNAs was identified as HMG CoA reductase mRNA while the other coded for the expression of a cDNA complementary to the 53,000-dalton protein believed to be a subunit of HMG CoA synthase. Additional evidence for this identification arises from a 90% homology between the amino acids coded by the 53,000-dalton mRNA and a peptide derived from the avian mitochondrial synthase [19]. This indicates that despite the physical and catalytic differences between the avian mitochondrial and fibroblast synthases, the active site structure has been conserved.

The fibroblast synthase appears similar to rat liver peak 2 with respect to elution on ion-exchange chromatography (Fig. 6) and lack of inhibition by $MgCl_2$ [14]. Recently, Gil et al have reported the isolation of a cDNA for cytoplasmic HMG CoA synthase from UT-1 cells [15]. A separation of hamster liver cytosolic HMG CoA synthase via DEAE cellulose chromatography by these same investigators yielded similar results, as we herein report with respect to rat liver peak 2 cytosolic synthase activity. Cross reactivity of the isolated hamster synthase with an antibody directed against part of the predicted amino acid sequence deduced from the UT-1 cell cDNA further indicates that the fibroblast synthase is most likely identical to the peak 2 HMG CoA synthase activity from rat liver.

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