Comparison of long-chain fatty acyl-CoA synthetases from rabbit heart and liver: substrate preferences and effects of Mg^{2+}

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Rabbit heart has a single, non-specific, fatty acyl-CoA synthetase (HP1) which is dependent on Mg^{2+} , apart from the requirement for $MgATP^{2-}$. Two long-chain fatty acyl-CoA synthetase activities (LP1 and LP2) can be resolved by hydroxyapatite chromatography of liver preparations; the Mg^{2+} requirement for these enzymes is undefined. These experiments were done to define the Mg^{2+} requirements of the liver enzymes and to compare them with the heart enzyme. For all three sources of enzyme and for arachidonic, oleic and palmitic acid substrates, the overall velocity of the reaction increased as $[Mg^{2+}]$ increased. Depending on the substrate and the source of enzyme, the increase in overall velocity could be attributed to changes in affinity or maximal

velocity or both. The substrate preference of the HP1 enzyme for arachidonic acid (AA) was fifth or sixth of eight substrates regardless of the concentration of Mg^{2+} . In contrast, increasing $[Mg^{2+}]$ shifted the relative substrate preference of both liver enzymes for AA. At low $[Mg^{2+}]$, AA was ranked seventh or eighth (least preferred) of eight substrates, whereas at high $[Mg^{2+}]$, AA was ranked as fifth or sixth. Hill plots of competition studies were consistent with Mg^{2+} -induced positive co-operativity in LP1, but not in HP1 or LP2. Although enzymes from the three sources exhibit substantial kinetic differences, it is uncertain whether they are three different enzymes.

INTRODUCTION

The free arachidonic acid (AA) levels in any tissue are closely regulated, and are rate-limiting in the synthesis of prostaglandins and other eicosanoids [1]. Agonist-induced prostaglandin synthesis seems to be dependent on the activation of phospholipases, which increase free AA availability [2–5]. In contrast, the availability of arachidonic acid for basal prostaglandin synthesis might be limited not by the activity of phospholipase but by the activity of the enzymes that incorporate AA into tissue lipids [6,7]. Thus, whereas stimulus-induced prostaglandin synthesis might be an index of phospholipase activity, basal prostaglandin synthesis might be an index of AA re-acylation. Although stimulus-induced prostaglandin synthesis has been extensively studied, much less is known about the mechanisms that control AA re-incorporation into phospholipid.

Two peaks of long-chain fatty acyl-CoA synthetase activity can be resolved by hydroxyapatite chromatography of solubilized preparations of several tissue types, including platelets, liver and brain [8,9]. The early peak is relatively non-selective as to fatty acid substrate, whereas the later peak contains arachidonoyl-CoA synthetase, the AA-specific activity. Tissues whose membranes contain a high proportion of AA might be expected to contain high levels of arachidonoyl-CoA synthetase. Experimental evidence has not supported this hypothesis. Kinetic studies suggest that there is only one long-chain fatty acyl-CoA synthetase in rat testis [10]. Only a single, non-specific, peak of activity can be resolved from solubilized heart preparations [11], yet both testicular and cardiac phospholipids are rich in AA [10,12].

The heart enzyme too is activated by Mg^{2+} ; apart from the requirement for $MgATP^{2-}$ it is also non-selective as to substrate, and it has hydroxyapatite elution characteristics similar to the early (non-specific) enzyme from other tissues. This similarity has led us to ask whether the heart enzyme is similar in other ways to the early peak. The work presented here was done to compare and contrast the single activity peak from heart with both peaks from

liver. The parameters examined were their affinities for AA, their requirements for free Mg^{2+} and their substrate preference ratios.

MATERIALS AND METHODS

Materials

$[^3H]$ AA (220–240 Ci/mmol), $[^3H]$ oleic acid (OA; 10–15 Ci/mmol) and $[^3H]$ palmitic acid (PA; 50–55 Ci/mmol) were purchased from New England Nuclear (Boston, MA, U.S.A.), and diluted to the appropriate specific radioactivity with unlabelled fatty acid. Unlabelled AA was purchased from NuChek Preps (Elysian, MN, U.S.A.). Other unlabelled fatty acids, CoA (lithium salt) and ATP (disodium salt) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other chemicals were of the highest grade available.

Enzyme preparation

Male New Zealand White rabbits (1.5–2 kg) were killed with an intravenous overdose of sodium pentobarbital (60–65 mg/kg). The hearts and a 4–5 g portion of the livers were removed. Hearts were trimmed of atria and large blood vessels. The enzyme was prepared as described [8]. Briefly, a single heart or liver was homogenized in 10 vol. of buffer composed of 320 mM sucrose, 10 mM Hepes, pH adjusted to 7.4 with 1 M Tris, supplemented with 10 mM 2-mercaptoethanol, 20 μ g/ml PMSF, 1 mM benzamidine and 0.01% soybean trypsin inhibitor. The homogenate was centrifuged at 1000 *g* for 10 min. The pellet was discarded and the supernatant was centrifuged at 35000 *g* for 1 h. This pellet was resuspended in 20 mM potassium phosphate buffer, pH 7.4, containing 1% Nonidet-P40, 10 mM 2-mercaptoethanol and 10 mM EDTA, stirred at 4 °C for 2 h, then centrifuged at 35000 *g* for 1 h. The supernatant, which contained the solubilized enzyme, was loaded on a hydroxyapatite column (void volume 12 ml) equilibrated with 20 mM potassium phosphate buffer, pH 7.4, containing 1% Nonidet-P40 and 10 mM 2-mercaptoethanol. All elution buffers contained 10 mM 2-mer-

Abbreviations used: AA, arachidonic acid; OA, oleic acid; PA, palmitic acid.

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captoethanol and 1 % Nonidet-P40, and were adjusted to pH 7.4. The column was eluted with 100 ml of 80 mM potassium phosphate, followed by a 200 ml linear gradient of potassium phosphate (80–300 mM). The eluate was collected in 6 ml fractions. Each fraction was assayed for enzyme activity as described below. Protein content was measured by the method of Lowry et al. [13]. The prepared enzyme was stored at -20°C .

Enzyme assay

Fatty acyl-CoA synthetase activity was measured as described [8,9]. Briefly, 50 μl of enzyme preparation (5–15 μg of protein) was added to 100 μl of an assay cocktail containing (final concentrations) 100 mM Tris/HCl, pH 8.0, 2 mM Triton X-100, 0.66 mM CoA, 6.6 mM ATP, radiolabelled (^{14}C or ^3H) fatty acid, and the appropriate concentrations of unlabelled fatty acid and bivalent cations. The reaction was allowed to proceed for 2–3 min at 37°C and was terminated by the addition of 2.25 ml of stopping solution (propan-2-ol/heptane/2 M H_2SO_4 , 40:10:1 by vol.). Unreacted substrate was extracted by adding 1.5 ml of heptane and 1 ml of water, vortex-mixing vigorously and removing the upper (organic) layer. The aqueous layer was re-extracted twice with 2 ml of heptane containing 4 mg/ml PA. The radioactivity in a 1 ml portion of the final aqueous layer was determined by liquid-scintillation spectroscopy. Product formation was calculated as mol of product formed per min per mg of protein.

The dissociation constant of MgATP^{2-} was assumed to be 50 μM , on the basis of previous measurements [11]. Under these conditions, total Mg^{2+} concentrations of 3, 7 and 80 mM yielded calculated free Mg^{2+} concentrations of 40.6 μM , 792 μM and 73.4 mM respectively.

Analysis of results

Results are expressed as means $\pm$ S.E.M. The GB Stat (version 5.0.4) computer program, run on a Macintosh Quadra 840AV, was used to analyse the data by one-, two- or three-way analysis of variance, followed by Fisher's LSD test for significance. Differences were judged to be statistically significant at $P < 0.05$.

RESULTS

A single peak of activity (HP1) was consistently isolated from heart. Two peaks, one eluted with 80 mM phosphate (LP1) and one with the phosphate gradient (LP2), were consistently isolated

from liver. The average elution volume of the heart enzyme was 60 ± 6 ml. The average elution volumes of LP1 and LP2 were 60 ± 6 and 150 ± 6 ml respectively. The phosphate concentration at peak LP2 elution was approx. 145–155 mM.

Initial velocities

Initial velocities were determined for all enzyme sources at 7 mM total Mg^{2+} , with AA, OA or PA as substrate. Substrate concentrations ranged from 1 to 256 μM , and incubation times from 10 s to 5 min. The initial velocity (slope of the linear portion of the product-time curve) was graphed as a function of the substrate concentration. The results are shown in Figure 1. The relative velocities of HP1 and LP1 were $\text{PA} > \text{OA} > \text{AA}$. The relative velocities of LP2 were not markedly different among the three substrates. For all enzyme sources and all substrates, the velocity curves remained linear for at least 2 min. In subsequent experiments, the incubation time was 2 min.

Apparent K_m and $V_{\max}$

Velocity was measured for enzyme from the three sources at 18 substrate concentrations (0.75–256 μM) and three concentrations of Mg^{2+} . The data were graphed as Lineweaver–Burk plots. Figure 2 shows the plots for AA. The plots of the heart enzyme intersect in the third quadrant, whereas those of LP1 and LP2 intersect at or near the y-axis.

The apparent K_m and $V_{\max}$ values were calculated from the Lineweaver–Burk plots and graphed as a function of the calculated free Mg^{2+} concentration. For each substrate, the $K_m(\text{app})$ of the heart enzyme increased (affinity decreased) as a function of free Mg^{2+} (Figure 3A). At all concentrations of Mg^{2+} the HP1 K_m is lowest for OA and highest for PA. At 80 mM Mg^{2+} the $K_m(\text{app})$ is significantly greater than at 3 mM total Mg^{2+} , 2.12 ± 0.12 -fold for AA, 1.7 ± 0.148 -fold for OA and 2.16 ± 0.657 -fold for PA. In contrast, Mg^{2+} decreases the $K_m(\text{app})$ (increases the affinity) of LP1 and LP2 for AA (Figures 3B and 3C) by 1.71 ± 0.43 -fold and 1.73 ± 0.41 -fold respectively. The affinity of LP1 and LP2 for OA does not seem to be changed by free Mg^{2+} . Finally, the affinity of LP1 for PA is decreased in the presence of increasing Mg^{2+} , whereas the affinity of LP2 for PA is not changed by Mg^{2+} .

For the heart enzyme, $V_{\max}(\text{app})$ was significantly greater for OA and PA than for AA. For all substrates, $V_{\max}(\text{app})$ increased significantly as the free Mg^{2+} concentration increased from

Figure 1 Velocity plotted against substrate concentration ($[S]$) for fatty acyl-CoA synthetase from heart and liver

Enzyme was isolated and activity measured as described in the text. The total Mg^{2+} concentration was 7 mM (approx. 792 μM free Mg^{2+}). Each point represents the mean for two different enzyme preparations. (A) HP1; (B) LP1; (C) LP2.

Figure 2 Representative Lineweaver-Burk plots of fatty acyl-CoA synthetase from heart and liver, with AA as substrate

(A) HP1; (B) LP1; (C) LP2. Symbols: $\square$, 3 mM Mg^{2+} ; $\blacksquare$, 7 mM Mg^{2+} ; $\blacktriangledown$, 80 mM Mg^{2+} .

40.6 μM to 73.4 mM (Figure 4A). The magnitude of the increase was 3.26 ± 0.197 -fold for AA, 3.22 ± 0.305 -fold for OA and 4.37 ± 0.354 -fold for PA. The relative increase for PA was significantly greater than for OA and AA, which were not different from each other. Free Mg^{2+} had no effect on the apparent V_{max} of either liver enzyme for the AA substrate. However, as free Mg^{2+} concentration increased from 40.6 μM to

Figure 4 Apparent maximal velocity of fatty acyl-CoA synthetase isolated from heart and liver, expressed as a function of free Mg^{2+} concentration

Note the change of scale in (C). Each point represents the mean $\pm$ S.E.M. for six different preparations. * $P < 0.05$ compared with 46 μM Mg^{2+} . (A) HP1; (B) LP1; (C) LP2.

73.4 mM, the apparent V_{max} for OA substrate increased 1.96 ± 0.761 -fold for LP1 and 2.38 ± 0.552 -fold for LP2. By the same token, V_{max} (app) for PA substrate increased 4.07 ± 0.601 -fold for LP1 and 2.08 ± 0.246 -fold for LP2.

Figure 3 Apparent K_m of fatty acyl-CoA synthetase isolated from heart and liver, expressed as a function of free Mg^{2+} concentration

Each point represents the mean $\pm$ S.E.M. for six different preparations. * $P < 0.05$ compared with 46 μM Mg^{2+} . (A) HP1; (B) LP1; (C) LP2.

Table 1 Ratio of velocity at 80 mM Mg²⁺ to velocity at 3 mM Mg²⁺

The values shown represent the mean $\pm$ S.E.M. Statistical significance: *value significantly different from HP1 within a substrate group; †value significantly different from arachidonic acid within an enzyme source group; *n* is the number of preparations.

Enzyme source	[³ H]AA (<i>n</i> = 4)	[³ H]OA (<i>n</i> = 4)	[³ H]PA (<i>n</i> = 3)
HP1	1.98 $\pm$ 0.128	2.31 $\pm$ 0.160	2.17 $\pm$ 0.0418
LP1	1.67 $\pm$ 0.0388*	1.61 $\pm$ 0.0695*	1.60 $\pm$ 0.0756*
LP2	1.47 $\pm$ 0.073*	1.70 $\pm$ 0.0852*†	1.94 $\pm$ 0.0454*†

Substrate preference

The substrate preference of enzyme from each source was determined in the presence of 3 or 80 mM total Mg²⁺. Product formation at 35 μ M substrate ([³H]AA, [³H]OA or [³H]PA) was measured in the presence or absence of increasing concentrations of unlabelled fatty acids. In some cases (see below), the unlabelled fatty acid competed for labelled substrate only at very high concentrations. Thus the substrate preference ratios were constructed by ranking the percentage competition at the highest unlabelled fatty acid concentration used (256 μ M). The rank order of unlabelled substrate preference was not

different between the three labelled fatty acids for any of the enzyme sources. The unlabelled substrates were assigned integer values from 1 to 9 for each labelled fatty acid substrate. An integer value of 1 indicated the most potent competitor and 9 the least potent. The mean and S.E.M. of these ranks were analysed by ANOVA to determine whether Mg²⁺ changed the rank order of substrate preference for any of the enzyme sources.

Table 1 shows the ratio of velocity at 80 mM Mg²⁺ to that at 3 mM Mg²⁺ at 35 μ M labelled substrate. In all cases the velocity was significantly greater at the higher Mg²⁺ concentration. However, the magnitude of the Mg²⁺ effect was greatest for HP1, regardless of labelled substrate. For HP1, the Mg²⁺ effect was slightly, although significantly, greater for [³H]OA than for [³H]AA. For LP2, the magnitude of the Mg²⁺ effect was significantly greater for the [³H]OA and [³H]PA substrates than for [³H]AA. There were no differences between the substrates for the LP1 enzyme.

Table 2 shows the substrate preference ratio for enzyme from each source at 3 and 80 mM Mg²⁺. The Mg²⁺ concentration had no effect on the substrate preference of the HP1 enzyme. The relative affinities of LP1 and LP2 for AA are significantly greater in the presence of 80 mM Mg²⁺ than in the presence of 3 mM Mg²⁺. It is interesting to note that in the HP1 enzyme, AA ranks fifth or sixth among the competing substrates, regardless of the Mg²⁺ concentration. In both liver enzymes, AA ranks eighth or ninth at low [Mg²⁺] (significantly lower than with HP1) and fifth or sixth at high [Mg²⁺] (no different from HP1). The relative

Table 2 Relative substrate preference of HP1, LP2 and LP2 in the presence of 3 or 80 mM Mg²⁺

Statistical significance: *significant difference in rank position compared with 3 mM Mg²⁺; †significant difference in rank position compared with HP1. Abbreviations: DA, decanoic acid; LA, lauric acid; MA, myristic acid; LN, linolenic acid; LI, linoleic acid; OC, octanoic acid.

[Mg ²⁺] (mM) ...	HP1		LP1		LP2	
	3	80	3	80	3	80
DA	2.00 $\pm$ 0	LA 1.67 $\pm$ 0.667	LA 1.00 $\pm$ 0	LA 1.00 $\pm$ 0	LA 1.33 $\pm$ 0.333	LA 1.33 $\pm$ 0.667
LA	2.33 $\pm$ 1.33	DA 2.00 $\pm$ 0	DA 2.33 $\pm$ 0.33	DA 3.00 $\pm$ 0.577	DA 2.33 $\pm$ 0.667	MA 2.00 $\pm$ 0.577
OA	2.33 $\pm$ 0.667	OA 2.33 $\pm$ 0.667	MA 3.67 $\pm$ 0.333	MA 3.33 $\pm$ 0.882	MA 2.67 $\pm$ 0.667	DA 3.00 $\pm$ 0.577
MA	4.00 $\pm$ 0	MA 4.00 $\pm$ 0	PA 4.00 $\pm$ 0.577	OA 3.67 $\pm$ 0.882	OA 5.33 $\pm$ 0.667	OA 3.67 $\pm$ 0.333
PA	5.33 $\pm$ 1.45	AA 5.33 $\pm$ 0.333	OA 4.33 $\pm$ 1.20†	PA 5.00 $\pm$ 0.577†	PA 5.33 $\pm$ 0.882	AA 5.67 $\pm$ 0.667*
AA	5.67 $\pm$ 0.333	LI 6.33 $\pm$ 0.333	LN 7.00 $\pm$ 0	AA 5.67 $\pm$ 1.45*	LN 5.67 $\pm$ 1.45†	PA 6.33 $\pm$ 0.882
LN	7.33 $\pm$ 0.667	PA 6.67 $\pm$ 0.882	OC 7.33 $\pm$ 1.20	LI 7.67 $\pm$ 0.333	OC 5.67 $\pm$ 0.667†	OC 7.00 $\pm$ 0.577
LI	7.67 $\pm$ 0.667	LN 7.67 $\pm$ 0.333	AA 7.67 $\pm$ 0.882†	LN 7.67 $\pm$ 0.667	AA 8.00 $\pm$ 0.577†	LN 7.33 $\pm$ 0.882*
OC	8.33 $\pm$ 0.667	OC 9.00 $\pm$ 0	LI 7.67 $\pm$ 0.882	OC 8.0 $\pm$ 1.00	LI 8.67 $\pm$ 0.333	LI 8.67 $\pm$ 0.333

Table 3 Hill-plot slopes in the presence of 3 or 80 mM Mg²⁺

Statistical significance: * value significantly different from 3 mM Mg²⁺; †value significantly different from -1. Each point represents the mean $\pm$ S.E.M. for three or four preparations.

Substrate	HP1		LP1		LP2	
	[Mg ²⁺] (mM) ... 3	80	3	80	3	80
[³ H]AA	-1.13 $\pm$ 0.0905	-1.14 $\pm$ 0.128	-1.11 $\pm$ 0.0350	-1.84 $\pm$ 0.184*†	-1.21 $\pm$ 0.0988	-1.26 $\pm$ 0.0978
[³ H]OA	-0.934 $\pm$ 0.117 ^{b,c}	-1.12 $\pm$ 0.125 ^{b,c}	-1.03 $\pm$ 0.156 ^{a,b,c}	-1.78 $\pm$ 0.249*† ^{a,b,c}	-1.20 $\pm$ 0.183 ^{a,b,c}	-1.35 $\pm$ 0.0717 ^{a,b,c}
[³ H]PA	-0.771 $\pm$ 0.0642†	-1.16 $\pm$ 0.110*	-0.752 $\pm$ 0.0477† ^{a,b,c,d}	-1.25 $\pm$ 0.163 ^{a,b,c,d}	-0.945 $\pm$ 0.101 ^{b,c}	-1.17 $\pm$ 0.0699 ^{b,c}

^a Insufficient data for competition by arachidonic acid.

^b Insufficient data for competition by linoleic acid.

^c Insufficient data for competition by linolenic acid.

^d Insufficient data for competition by oleic acid.

affinity of both liver enzymes for several other competing fatty acids was significantly different from that of the heart enzyme.

In most (but not all) cases, there were sufficient results to construct a pseudo-Hill plot. For each enzyme source, the pseudo-Hill coefficients of the competing fatty acids in the presence of 3 mM Mg^{2+} were analysed by a one-way ANOVA, as were the pseudo-Hill coefficients of the competing fatty acids in the presence of 80 mM Mg^{2+} . As there were no significant differences between the pseudo-Hill coefficients for the various competing fatty acids at 3 or 80 mM Mg^{2+} , the data were pooled for each enzyme source and each labelled substrate at each Mg^{2+} concentration, as shown in Table 3. For the HP1 enzyme, the average pseudo-Hill coefficients at 3 mM Mg^{2+} were not different from those at 80 mM Mg^{2+} for the [3H]AA or [3H]OA substrates. However, for the [3H]PA substrate, the pseudo-Hill coefficient at 3 mM Mg^{2+} was significantly greater than at 80 mM Mg^{2+} . For the LP1 enzyme, the pseudo-Hill coefficient at 3 mM Mg^{2+} was significantly greater than at 80 mM Mg^{2+} for all three of the labelled substrates. For the LP2 enzyme there were no significant differences in the pseudo-Hill coefficients for any of the three labelled substrates.

DISCUSSION

The kinetic properties of HP1, LP1 and LP2 are sufficiently different from each other to suggest that these are three distinct enzymes. The affinity of HP1 for all substrates decreases as the concentration of Mg^{2+} increases. In contrast, the affinity of LP1 and LP2 for AA increases as $[Mg^{2+}]$ increases. The affinity of LP1 for OA is unchanged and is decreased for PA. LP2 differs from LP1 in that the affinity for both OA and PA is unchanged as $[Mg^{2+}]$ increases. The maximal velocity of the HP1 preparation is increased 3–4-fold, regardless of substrate, as $[Mg^{2+}]$ increases from 3 to 80 mM. In contrast, the maximal velocities of LP1 and LP2 are unchanged for the AA substrate, and are only approximately doubled for the OA or PA substrates. Finally, the substrate preference of the HP1 preparation is not changed as $[Mg^{2+}]$ increases, whereas the relative preference for AA increases as $[Mg^{2+}]$ increases in both liver enzymes. Taken together, these results strongly suggest that the liver enzymes are kinetically different from the heart enzyme and from each other.

The overall velocities of HP1, LP1 and LP2 increase as $[Mg^{2+}]$ increases. However, the mechanism by which this is accomplished seems to be different for the heart and liver enzymes. The results presented here confirm an earlier observation that fatty acyl-CoA synthetase activity isolated from heart increases as the free Mg^{2+} concentration increases [11], and extend this observation to the fatty acyl-CoA synthetases isolated from liver, suggesting that in general Mg^{2+} increases fatty acyl-CoA synthetase activity.

The major mechanism of increased overall velocity of the HP1 enzyme seems to be an increase in the apparent maximal velocity. This might be accomplished by Mg^{2+} activation of otherwise inactive enzyme molecules. (That is, in the absence of Mg^{2+} the enzyme is inactive, whereas in the presence of Mg^{2+} the enzyme is active.) This is supported by the Hill-plot findings. For both [3H]OA and [3H]AA, the pseudo-Hill coefficients are not changed by increasing Mg^{2+} concentrations and are not significantly different from -1 for HP1. This is consistent with a single enzyme activated by Mg^{2+} in an all-or-nothing fashion. Although the PA data, with a pseudo-Hill coefficient of less than -1 , suggest the possibility of negative co-operativity at low $[Mg^{2+}]$, it is also consistent with the possibility of failure to achieve rapid equilibrium. Interestingly, the pseudo-Hill coefficients are not different from -1 for labelled PA at 7 mM Mg^{2+} (792 μM free

Mg^{2+}) [11], a Mg^{2+} concentration that is similar to that seen in intact cells [14].

The results suggest that the mechanism of Mg^{2+} activation of LP1 is different from that of HP1. For the [3H]OA and [3H]AA substrates, Mg^{2+} seems to shift LP1 enzyme from a non-co-operative state to one exhibiting positive co-operativity (absolute value of the pseudo-Hill coefficient greater than 1), as well as shifting the relative substrate preference to favour the formation of arachidonoyl-CoA. For the PA substrate the pseudo-Hill coefficient decreases significantly as $[Mg^{2+}]$ increases. Although the coefficient never becomes less than -1 , the results are nevertheless consistent with Mg^{2+} -induced positive co-operativity in the LP1 enzyme.

The LP2 fatty acyl-CoA synthetase shares characteristics of both LP1 and HP1. As in LP1, Mg^{2+} shifts the relative substrate preference of LP2 for AA. As in HP1, Mg^{2+} seems to have no effect on the co-operativity of LP2, regardless of substrate. For no substrate was the pseudo-Hill coefficient of LP2 significantly different from -1 .

Although the catalytic and kinetic characteristics of HP1, LP1 and LP2 seem to be distinct, it is unclear whether these are three different enzymes or a single enzyme in three different conformational/aggregation states. Results from other laboratories would suggest the latter. Western blots of long-chain fatty acyl-CoA synthetase isolated from microsomes, peroxisomes and mitochondria of rat liver show a single band of immunoreactivity, corresponding to purified enzyme [15]. Although rat testicular phospholipids contain high concentrations of AA, as in the heart, kinetic evidence indicates that there is only one long-chain fatty acyl-CoA synthetase [10]. The substrate specificity of fatty acyl-CoA synthetase seems to be a function of its aggregation state [16,17]. Detergents seem to shift the aggregate molecular size, stability and substrate specificity [16], suggesting that the membrane microenvironment of the enzyme influences its catalytic properties. Other studies have indicated that immobilization of the enzyme on various matrices alters the substrate specificity and stability [17], again indicating that the conformation and degree of rotational freedom of the enzyme are critical to both the absolute catalytic activity and substrate specificity. Long-chain fatty acyl-CoA synthetase seems to be highly conserved [18], consistent with the notion that there is a single enzyme with several conformational states.

In the liver enzymes, the substrate preference is changed by Mg^{2+} . The heart enzyme substrate preference is not influenced by Mg^{2+} , but a previous study showed that formation of oleoyl-CoA by HP1 is supported by low concentrations of Ca^{2+} , whereas arachidonoyl-CoA formation is not [11]. In HP1, LP1 and LP2, substrate preference can be influenced to some extent by the ionic composition of the reaction mixture. Enzymes that require ATP as a co-substrate usually also require Mg^{2+} for activity. It was thought that this requirement indicated that the $MgATP^{2-}$ complex is the true co-substrate for ATP-requiring reactions. However, recent examination of several ATP-requiring enzymes has forced a re-examination of this assumption. Protein kinase C has been found to have a Mg^{2+} -binding pocket [19–21]. When this site is occupied by Mg^{2+} , the conformation of the protein changes, as does the activity of the protein [20]. In a similar fashion, calmodulin-dependent protein kinase [22] and insulin-dependent tyrosine kinase [23] also have a requirement for Mg^{2+} , which seems to be separate from the requirement for $MgATP^{2-}$. Thus, fatty acyl-CoA synthetase(s) might be additional ATP-requiring enzyme(s) regulated by Mg^{2+} ions. Although enzymes isolated from different sources exhibit different kinetic properties, it remains to be seen whether they are a single enzyme or a family of enzymes.

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