

Does microsomal glycerophosphate acyltransferase also catalyze the acylation of dihydroxyacetone phosphate?

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Rat liver microsomal dihydroxyacetone phosphate acyltransferase, in contrast to the glycerophosphate acyltransferase, was found to be active at low pH (5.5), stable towards heat (55°C, 15 min) and trypsin (in the absence of detergents) and was not inhibited by high concentrations of *N*-ethyl maleimide. Dihydroxyacetone phosphate acyltransferase is only slightly and non-competitively inhibited by *sn*-glycerol-3-phosphate whereas glycerophosphate acyltransferase is strongly inhibited by dihydroxyacetone phosphate in a competitive manner. Kinetic analysis indicates that this competitive inhibition is not due to the competition of two common substrates for the same active center of one enzyme. These results demonstrate that microsomal glycerophosphate acyltransferase and dihydroxyacetone phosphate acyltransferase are two distinct and separate enzymes.

Liver microsome Glycerophosphate acyltransferase Dihydroxyacetone phosphate acyltransferase

1. INTRODUCTION

Phosphatidic acid, the precursor of all glycerides and phosphoglycerides, is biosynthesized either via the acylation of *sn*-glycerol-3-phosphate (GP) or via the acyl dihydroxyacetone phosphate (acyl DHAP) pathway. Though a significant proportion of glycerolipids has been shown to be biosynthesized via the acyl DHAP pathway, the relative importance of this pathway in different tissues compared to that of the GP pathway has not been firmly established [1-3]. DHAPAT, the key enzyme of the acyl DHAP pathway, has been shown to be mainly localized in liver peroxisomes [4,5] but a portion of this enzyme is also present in the microsomal fraction [4,5]. Conflicting results have been obtained from different laboratories regard-

ing the specificity of this microsomal acyltransferase. Authors in [6,7] and recently authors in [8,9], using different rat tissues, have presented evidence that there is no specific microsomal DHAPAT and the acylation of DHAP in microsomes is due to the dual catalytic activity of GPAT (reviewed in [10]). On the other hand, we have shown [11] that the properties of microsomal DHAPAT are different from that of GPAT in the same subcellular fraction indicating that these enzymes are not the same. Results from other laboratories also show that properties of these two acyltransferases in the microsomal fraction of lung [12], liver [13], harderian gland [14] and 3T3 preadipocytes [15] are quite different. However, in a recent publication [16] it was concluded that these enzymes in rat liver microsomes may be the same because microsomal DHAPAT was partially inhibited by a very high concentration of GP. To resolve these controversies we extensively studied the properties of these two long chain acyltransferases in rat liver microsomes and the results are presented here.

Abbreviations: DHAP, dihydroxyacetone phosphate; GP, *sn*-glycerol-3-phosphate; AT, acyltransferase

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2. EXPERIMENTAL

Male albino rats of Sprague-Dawley strain (Harlan Sprague-Dawley, Indianapolis, IN), 150–200 g each, were used for this study. [1,3-¹⁴C]-GP, [³²P]DHAP, and [³²P]GP were prepared in this laboratory as in [11]. All other materials were the same as in [4,11].

Subcellular fractionation of rat liver was performed as in [17] and the fraction sedimenting between 2.5×10^5 g · min and 6×10^6 g · min (P fraction of DeDuve et al.) was used as the microsomal fraction, as in [4]. The microsomal fraction was stored at -20°C and thawed only once before use. Acyltransferase activities were determined by measuring the formation of radioactive lipids from the water-soluble labeled substrates ([³²P]DHAP or [³²P]GP) resulting from the acylation by palmitoyl CoA [4,11]. The incubation mixture contained 75 mM Tris-HCl buffer at pH 7.5 (unless otherwise stated), palmitoyl CoA (60 μM), fatty acid-poor bovine serum albumin (1 mg), NaF (8.3 mM), MgCl₂ (8.3 mM), [³²P]DHAP or [³²P]GP (0.42 mM, unless otherwise stated, 7×10^3 cpm/nmol) and enzyme preparation (50–100 μg protein) in a total volume of 0.6 ml. The radioactive lipids were extracted using an acidic Bligh and Dyer [11,18] extraction method.

3. RESULTS

3.1. Comparison of the properties of rat liver microsomal DHAPAT and GPAT

3.1.1. pH activity profile of the acyltransferases

Fig.1A shows the influence of pH of the incubation mixture on the activities of DHAPAT and GPAT. The activities were found to be different over the entire pH range investigated. It should be noted that the maximum activity for both the acyltransferases were observed at pH 7.4 whereas at lower pH (pH 5.5) only DHAPAT was active (60% of maximum activity). At this low pH GPAT activity was not detectable.

3.1.2. Sensitivity to thiol inhibitor

Thiol agents such as *N*-ethyl maleimide have been employed to differentiate between various acyltransferases [19]. This reagent has been shown

to inhibit microsomal GPAT but not mitochondrial GPAT [19] or peroxisomal DHAPAT [18]. Therefore, the effect of *N*-ethyl maleimide on these two microsomal acyltransferases was studied and the results are shown in fig.1B. In agreement with previous reports it was observed that even at very low concentrations (20 μM), *N*-ethyl maleimide strongly inhibited GPAT (80% decrease) with little inhibition (2%) of DHAPAT (fig.1B).

3.1.3. Effect of heat treatment

The susceptibility of these enzymes to thermal inactivation at 50°C was examined by heating the microsomes for different time periods and then assaying the enzyme at 37°C . The results are shown in fig.1C. It is evident that in contrast to DHAPAT, GPAT is very heat labile. For example, all of the GPAT activity was destroyed by heating the microsomes for 15 min, whereas very little reduction of activity of DHAPAT is seen under such conditions (fig.1C).

3.1.4. Effect of detergents

Detergents at low concentrations have been shown to stimulate (and solubilize) membrane-bound DHAPAT [18]. This was also found to be true for the microsomal enzyme. Fig.1D shows that Triton X-100 stimulated DHAPAT at low concentrations (up to 0.2 mg/ml) and the activity is only inhibited by 15% at higher Triton X-100 concentrations (0.5–1 mg/ml). In contrast, GPAT activity is strongly inhibited by Triton X-100 (85% at 0.5 mg/ml., fig.1D). Similar differential inhibition of these two microsomal acyltransferases has been observed when deoxycholate was used (not shown).

3.2. Effects of GP on DHAPAT and DHAP on GPAT activities

GP only slightly inhibits DHAPAT when it is added to the enzyme assay mixture. When kinetic analysis was done by reciprocal plots of velocity vs increasing DHAP concentrations in the presence or absence of GP, a slight non-competitive type of inhibition was observed (fig.2A). On the other hand, DHAP inhibited GPAT strongly and competitively. To determine whether this inhibition is due to DHAP acting as an inhibitor or due to the competition of common substrates (GP and DHAP) for the same active site of an enzyme, the

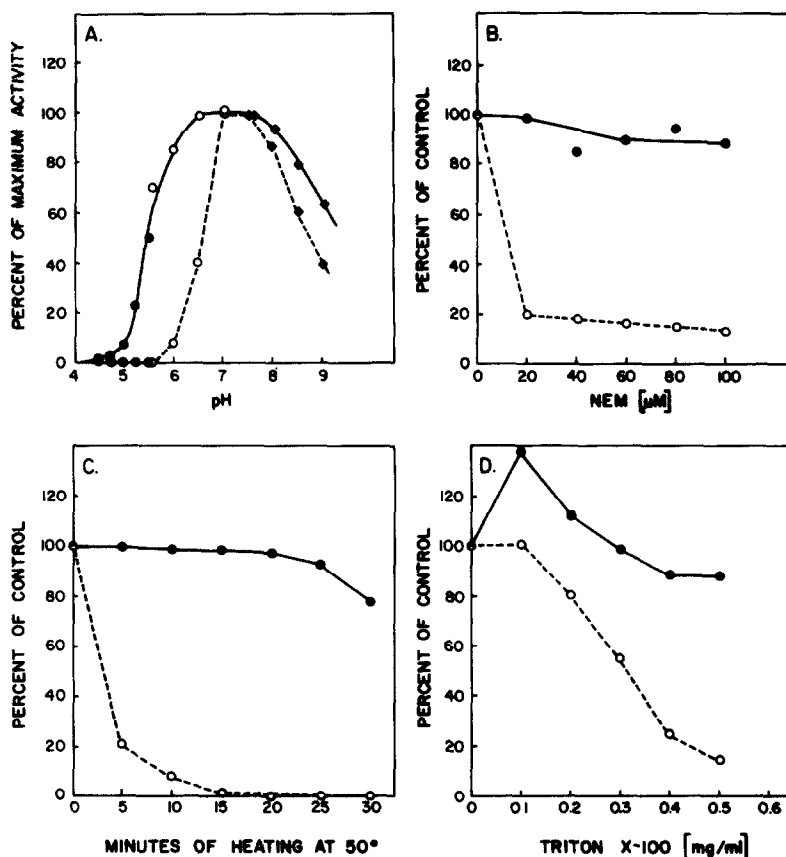


Fig.1. Effect of various treatments on rat liver microsomal DHAPAT (solid line) and GPAT (dashed line). (A) pH dependence. Different buffers were used for different pH values; acetate (●—●), 2-(*N*-morpholino)ethanesulfonic acid (○—○), and Tris-HCl (◆—◆). The assay and the incubation conditions are described in the text. Results have been expressed as the percentage of maximum activity; for DHAPAT 0.61 nmol/min per mg protein and for GPAT 3.15 nmol/min per mg protein. (B) Effect of *N*-ethylmaleimide (Nem). Microsomes in 0.25 M sucrose, 10 mM Tris-HCl (pH 7.4) were first preincubated with different concentrations of Nem (as indicated) at 4°C. Aliquots of these mixtures were used to assay the enzyme activities at 37°C. The maximal activities of the acyltransferases are similar to those described in A. (C) Effect of heat treatment. Microsomes were heated at 50°C up to 30 min before determination of DHAP or GPAT and aliquots were taken out at different time periods (as indicated) for the assay of the enzymes at 37°C. Results have been expressed as percentages of activity remaining after heat treatment for the period. The activities before heat treatment are 0.58 nmol/min per mg protein for DHAPAT and 2.98 nmol/min per mg protein for GPAT. (D) Effect of preincubation of rat liver microsomes with increasing amounts of Triton X-100. Microsomes were first preincubated with different amounts of Triton X-100 (as indicated) for 10 min and aliquots of these preincubated enzymes were used to determine DHAP and GPAT activities. Results have been expressed as percentages of control activities which are the same as in 1C.

following experiment was performed. Constant amounts of [^{32}P]DHAP were added to the assay mixtures which contained variable amounts of [^{14}C]GP and the amounts of ^{32}P - and ^{14}C -labeled lipids formed were determined. In this way, the products of both the reactions formed in the same incubation mixture are measured simultaneously.

If the competitive inhibition is due to the competition between common substrates for the same enzyme, then it is expected that the amount of acyl DHA ^{32}P formed will be progressively decreased with the increasing concentration of GP. The results of the experiment presented in fig.2B showed that DHAP competitively inhibited ($K_i =$

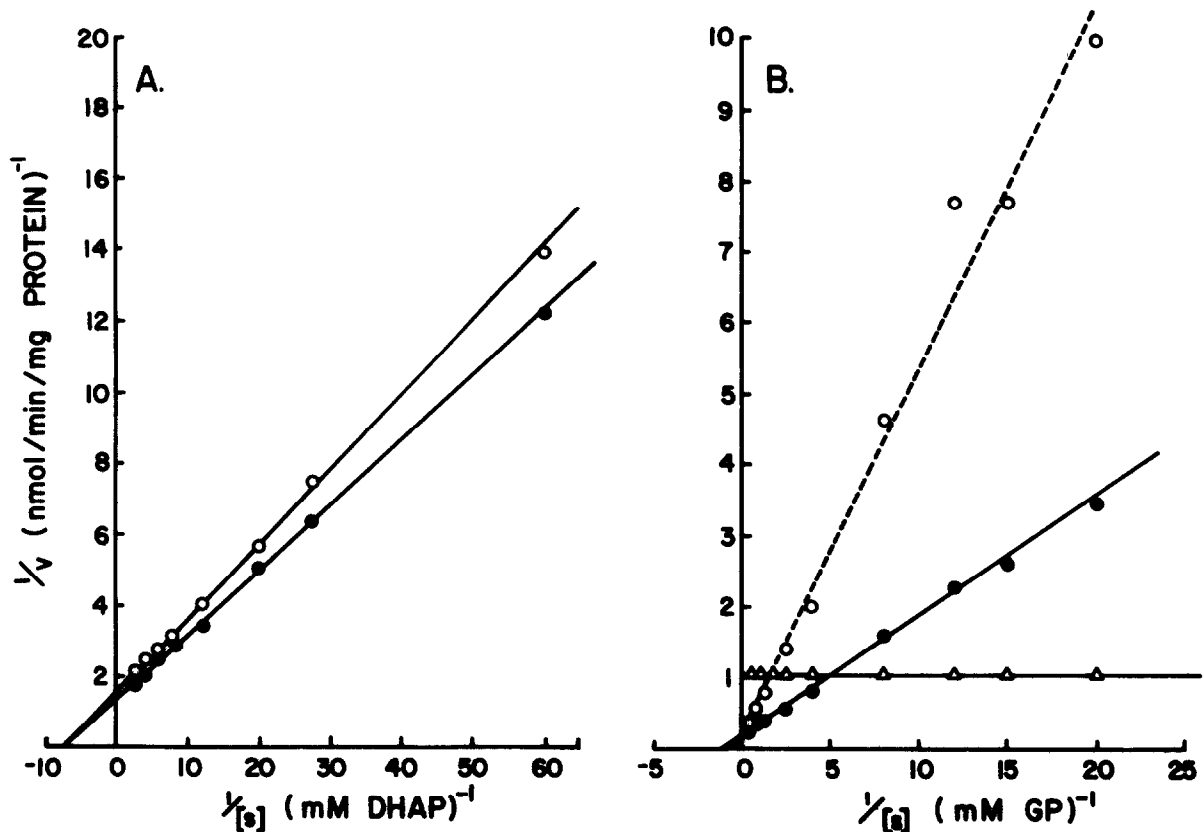


Fig.2. Activities of rat liver microsomal acyltransferase at different substrate concentrations. Results are presented as a double reciprocal plot. The assays were done as described in the text. (A) DHAPAT activity with (○—○) and without (●—●) a constant amount (2.5 mM) of GP. K_m for DHAP is 0.14 mM; V_{max} is 0.77 nmol/min per mg protein; V_{max} inhibited 0.67 nmol/min per mg protein. (B) GPAT activity with (○—○) and without (●—●) a constant amount (2.5 mM) of DHAP. The activity of DHAPAT with varying GP concentrations was also measured (△—△) in the same experiment by using [³²P]DHAP and [¹⁴C]GP in the incubation mixtures as described in the text and the results are plotted in the same graph. V_{max} for GP acyltransferase, 5 nmol/min per mg protein, K_m for GP 0.83 mM and K_i DHAP 2.5 mM.

2.5 mM) GPAT, but increasing GP up to 3.0 mM had little effect (5–10% inhibition) on the amount of acyl DHAP formed in the same incubation mixture.

4. DISCUSSION

The data presented above show the striking differences in properties of these two microsomal long-chain acyltransferases with respect to the pH vs activity, sensitivity to *N*-ethyl maleimide, heat stability and sensitivity to detergents. We also found, like others [16], that trypsin (20 μg/ml) in the absence of detergents completely destroyed

microsomal GPAT with little inactivation of DHAPAT. Most of the results presented here are completely different from those obtained in similar studies, [6,7] or [8,9]. The only results we obtained which were in agreement with those of Bell and coworkers (which were also originally reported from this laboratory, see [20] are the inhibition of one acyltransferase by the other substrate. However, the inhibition of DHAPAT by GP is not significant and the inhibition as seen in fig.2A (slight reduction in V_{max} with K_m remaining the same) is non-competitive which was only apparent at a relatively high concentration of GP. On the other hand, a strong inhibition of GPAT by

DHAP, in which K_m increased without any change in V_{max} (fig.2B), was observed which was similar to that obtained in [7] and in [8]. This inhibition could be either due to the competitive inhibition of the enzyme by DHAP or due to the utilization of both DHAP and GP as substrates by the same enzyme. The data presented in fig.2B prove that the inhibition is not due to the competition between common substrates for the same active center of the enzyme. This interpretation is also supported by the fact that the K_i (2.5 mM) for DHAP in the GPAT reaction is not equal to the K_m (0.14 mM) of DHAP when the formation of acyl DHAP is measured. That the enzymes are not the same is strengthened by our recent finding that in the fibroblasts and leukocytes from patients suffering from Zellweger syndrome, DHAPAT activity at both pH 5.5 and 7.4 is practically negligible, whereas GPAT activity is almost normal [21,22]. Similar results, as presented above, are also obtained with the microsomal fractions of all other rat organs such as kidney, brain, adipose tissue, testis, heart and lung. From these results and from the results obtained by other workers [12-16] we conclude that the same microsomal enzyme does not catalyze both the acylation of DHAP and GP.

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