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# Peroxisomal localization of glucose-6-phosphate dehydrogenase and pyrophosphate-stimulated dihydroxyacetone-phosphate acyltransferase in mouse kidney

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1. The subcellular localization of dihydroxyacetone-phosphate acyltransferase (DHAPAT) (assayed in the presence of pyrophosphate) and glucose-6-phosphate dehydrogenase ($NADP^+$-dependent) activity in mouse kidney was investigated by density-gradient centrifugation. 2. DHAPAT has a predominantly peroxisomal distribution, and the activity in purified peroxisomes is stimulated by various organic and inorganic phosphate-containing compounds. The pH optimum is acid. 3. Approx. 10% of the cellular $NADP^+$-dependent glucose-6-phosphate dehydrogenase activity is associated with peroxisomal fractions and may provide a source of NADPH for the peroxisomal reduction of acyl-dihydroxyacetone phosphate formed by DHAPAT activity.

## INTRODUCTION

The acylation of dihydroxyacetone phosphate (DHAP) is thought to be obligatory for the synthesis of ether-linked glycerolipids and is catalysed by dihydroxyacetone-phosphate acyltransferase (DHAPAT), which, in the liver, is predominantly peroxisomal [1–3]. In the rare congenital disease Zellweger Syndrome there is a severe deficiency of plasmalogens [4], an absence of morphologically identifiable peroxisomes [5] and less than 10% of normal tissue activity of DHAPAT [6,7].

After its formation, acyl-DHAP is reduced in an NADPH-dependent step which, in the liver, is also predominantly peroxisomal [8]. A source of NADPH might be provided by peroxisomal $NADP^+$-dependent isocitrate dehydrogenase [9] and by $NADP^+$-dependent glucose-6-phosphate dehydrogenase, which has been reported to be partly localized in rat liver peroxisomes [10].

Relatively little attention has been directed at DHAPAT of mammalian kidney, which like liver is a rich source of peroxisomes. We reported that in mouse kidney DHAPAT is mainly peroxisomal [11], as has also been found to be the case for rat kidney [12]. We have noted [13] that in mouse liver and kidney DHAPAT activity is enhanced when assays include $PP_i$. Here we extend this work to examine the subcellular distribution of DHAPAT assayed in the presence of $PP_i$, and to investigate some of the properties of the enzyme by using purified kidney peroxisomes. Since isocitrate dehydrogenase is apparently absent from kidney peroxisomes, we also investigated the subcellular site of glucose-6-phosphate dehydrogenase ($NADP^+$-dependent) from the viewpoint that it might provide a peroxisomal source of NADPH for reduction of acyl-DHAP.

## MATERIALS AND METHODS

### Materials

The materials used in this study were as previously described [11]. In addition, fructose-bisphosphate aldolase and triose-phosphate isomerase were obtained from Boehringer–Mannheim.

### Dual-centrifugation procedure

Preparation of kidney post-nuclear supernatant (PNS) from the kidneys of 15–20 adult MFI-strain mice of mixed sex, the treatment of PNS with $PP_i$ (final concn. 6–7 mM) and rate- and density-dependent banding in sucrose gradients in a BXIV zonal rotor were performed as previously described [14,15]. Treatment of PNS with $PP_i$ specifically lowers the density of endoplasmic reticulum by removing loosely bound proteins and ribosomes [16].

This dual-centrifugation procedure was used both for the study of the subcellular localization of glucose-6-phosphate dehydrogenase and for the preparation of purified peroxisomes required to investigate the properties of peroxisomal DHAPAT.

### Single-centrifugation procedure

Subcellular fractionation of PNS was also carried out in a single-step procedure. The gradient used in the BXIV zonal rotor was as described previously [11]. Centrifugation was at 21000 rev./min for 127 min. The gradient was unloaded with fluorochemical FC-43 [11,15]; the 34–60%-sucrose portion of the gradient was fractionated into 4 ml fractions, and the lower-density portion of the gradient into larger fractions.

Abbreviations used: DHAP, dihydroxyacetone phosphate; DHAPAT, dihydroxyacetone-phosphate acyltransferase (EC 2.3.1.42); PNS, post-nuclear supernatant

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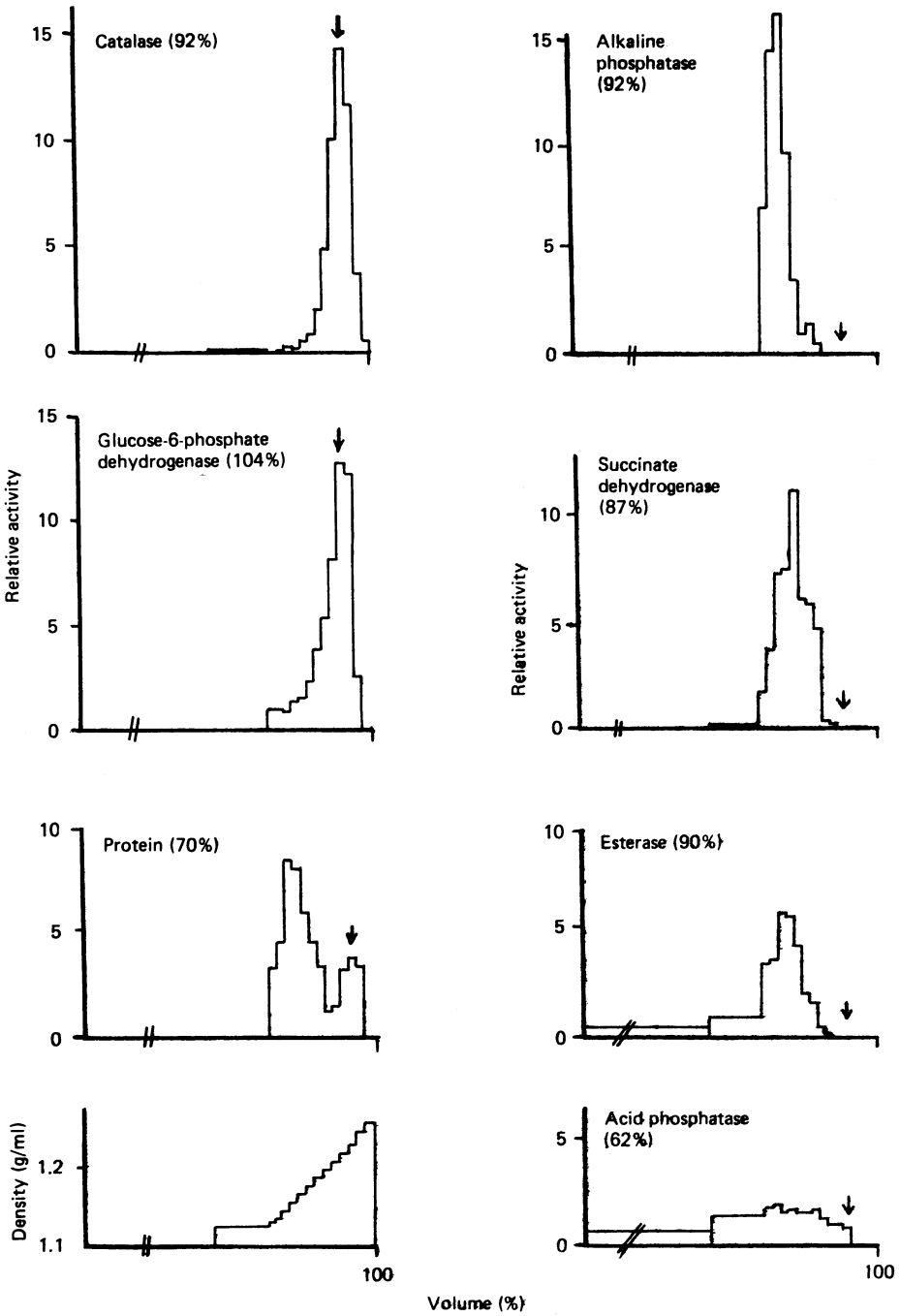


**Fig. 1. Density-dependent separation of a peroxisome-enriched sample obtained from rate-dependent banding of kidney PNS**

Note the correspondence of catalase and glucose-6-phosphate dehydrogenase distributions. The number accompanying the name of each enzyme represents the percentage recovery of activity after fractionation. The arrow represents the peak peroxisomal (catalase) fraction. Experimental procedure is described in the Materials and methods section.

## Assays

Marker enzymes for subcellular organelles were assayed as previously described [17]. Glucose-6-phosphate dehydrogenase was assayed as described by Lohr & Waller [18]. DHAPAT was measured essentially as described by Bates & Saggerson [19], routinely at pH 7.4 with DHAP substrate at 0.1 mM and in the presence of 10 mM-$PP_i$ with a molar ratio of bovine serum albumin to palmitoyl-CoA (60 $\mu$M) of 1. Substrate (DHAP) is generated from [U-$^{14}$C]fructose 1,6-bisphosphate by the action of aldolase and triose-phosphate isomerase. In calculating enzyme activities, the specific radioactivity of DHAP was taken to be half that of the parent fructose 1,6-bisphosphate. Protein was measured by the dye-binding method [20].

## Presentation of fractionation results

The distribution of enzymes between subcellular fractions is given in the form of frequency histograms, following the practice of de Duve [21].

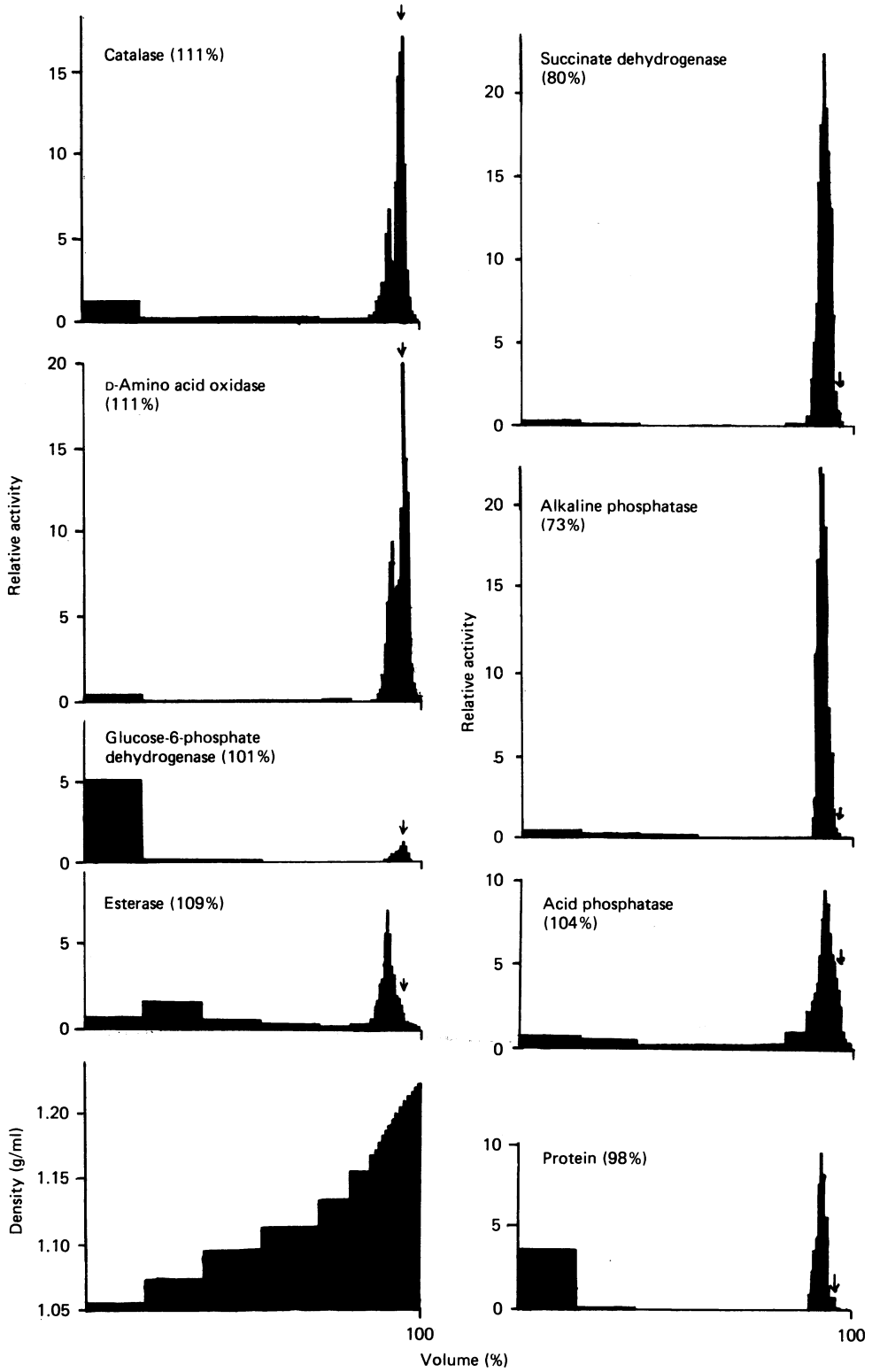


**Fig. 2. Subcellular localization of glucose-6-phosphate dehydrogenase analysed by a single-step centrifugation of kidney PNS**

The number accompanying the name of each enzyme represents the percentage recovery of activity after fractionation. The arrow represents the peak peroxisomal (catalase) fraction. Experimental procedure is described in the Materials and methods section.

## RESULTS

The subcellular markers used in this study were: catalase and D-amino acid oxidase for peroxisomes, esterase for endoplasmic reticulum, alkaline phosphatase for brush borders and plasma membrane, acid phosphatase for lysosomes, and succinate dehydrogenase for mitochondria. When a peroxisome-enriched sample (prepared by rate-dependent banding) from mouse kidney was further analysed by density-dependent

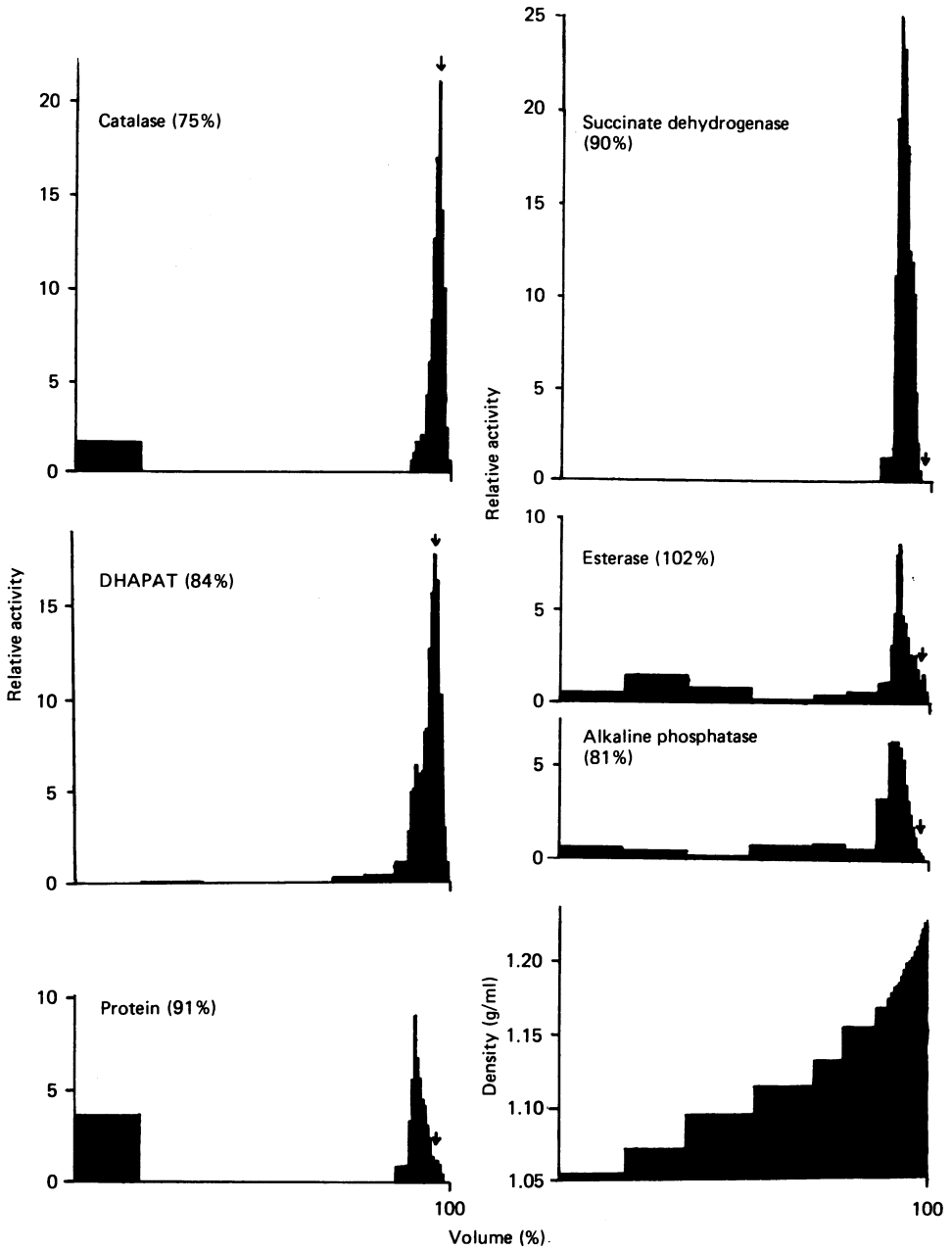


**Fig. 3. Subcellular localization of DHAPAT analysed by a single-step centrifugation of kidney PNS**

The number accompanying the name of each enzyme represents the percentage recovery of activity after fractionation. The arrow represents the peak peroxisomal (catalase) fraction. Experimental procedure is described in the Materials and methods section.

banding (Fig. 1), virtually all the glucose-6-phosphate dehydrogenase was found to be associated with the peroxisomes. To compare peroxisome-associated activity with the total glucose-6-phosphate dehydrogenase in kidney, whole PNS was analysed in a single-gradient system, which allows some separation of the major subcellular organelles from peroxisomes. Nearly all the glucose-6-phosphate dehydrogenase remains in the sample band (Fig. 2), and is presumably cytosolic, whereas particulate activity ($\sim$ 10% of total) is associated with the peroxisomes. The distribution of this enzyme is very similar to that which we reported for mouse kidney $NAD^+$-dependent $\alpha$-glycerophosphate dehydrogenase [11].

Previously [11] we found that mouse kidney DHAPAT (assayed without $PP_i$) was predominantly peroxisomal, but poor recoveries of the enzyme were obtained from the gradient, and activity in PNS was relatively low ($\sim$ 0.007 nmol of acyl-DHAP formed/min per mg of protein). We have also noted that $PP_i$ caused an apparent activation of DHAPAT activity in mouse kidney and liver [13]. Therefore, in the present study the subcellular localization of renal DHAPAT assayed in the presence of 10 mM-$PP_i$ was examined. Fig. 3 shows the distribution of markers and DHAPAT in whole PNS of mouse kidney. DHAPAT is predominantly peroxisomal, but some activity is present in the region of the gradient enriched in mitochondria and endoplasmic reticulum.

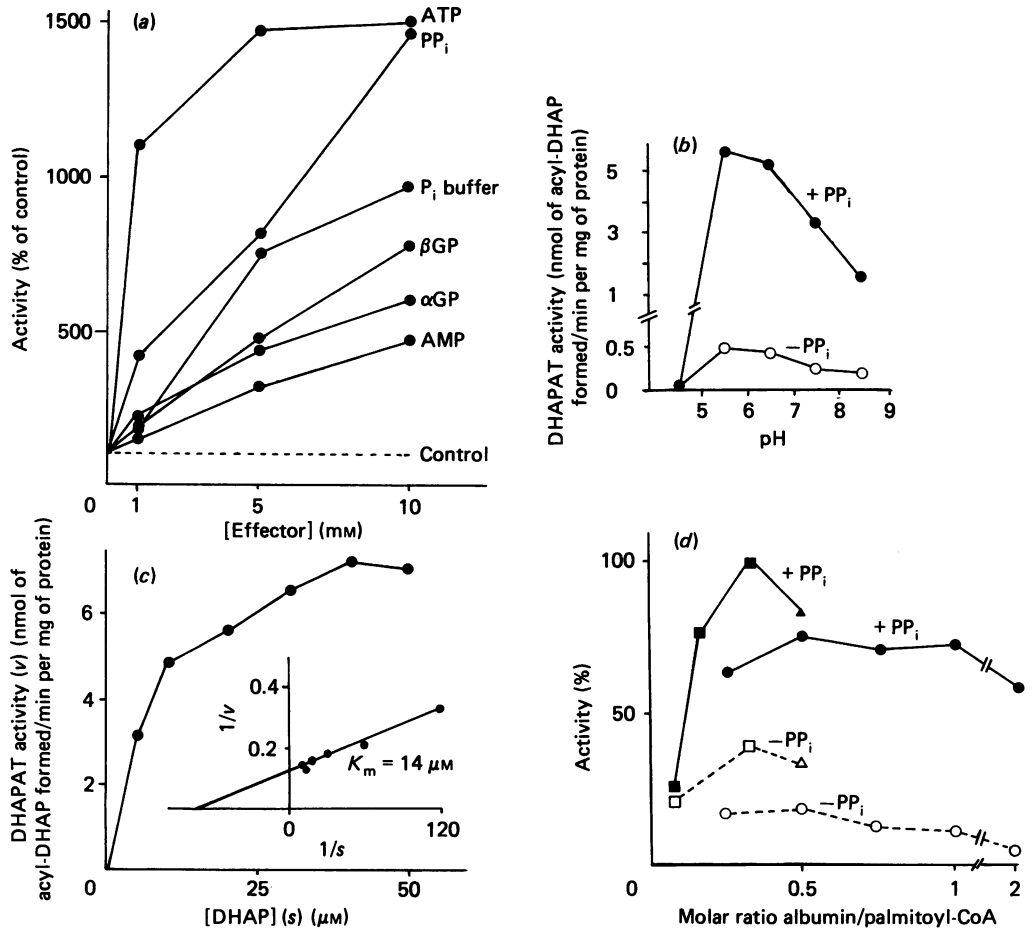


**Fig. 4. Properties of DHAPAT of kidney peroxisomes**

Sources of enzyme were fractions with peak catalase activity prepared as described for Fig. 1. Peroxisomes were purified 30–40-fold relative to PNS. (*a*) Effects of various phosphate-containing compounds on activity. Abbreviations: αGP, α-glycerophosphate; βGP, β-glycerophosphate. (*b*) pH-dependence of DHAPAT in the presence and absence of 10 mM-pyrophosphate. (*c*) Effect of fructose 1,6-bisphosphate concentration on activity in the presence of 10 mM-$PP_i$. The DHAP concentration generated at each of the tested fructose 1,6-bisphosphate concentrations was measured by the enzymic method of Michal & Beutler [23] and used to calculate the $K_m$ of the enzyme (inset). (*d*) Effect of the ratio of bovine serum albumin to palmitoyl-CoA on DHAPAT. [Palmitoyl-CoA]: ■, □, 180 μM; ▲, △, 120 μM; ●, ○, 60 μM.

This could be attributable to one or both of these organelles or to free peroxisomal membranes released during homogenization [3].

Some properties of $PP_i$-activated peroxisomal DHAPAT were investigated by using preparations of purified peroxisomes from mouse kidney. These results are presented in Fig. 4. Both organic pyrophosphate (ATP) and $PP_i$ caused an activation. A smaller activation was observed with several organic and inorganic monophosphates (Fig. 4*a*). The degree of activation by $PP_i$ at 10 mM varied from experiment to experiment (30 to 5 times activation), but was usually in the range 10–15-fold. In the presence of 10 mM-$PP_i$, maximal activity was observed at pH 5.5 (Fig. 4*b*). Measured at pH 7.4, the enzyme was nearly saturated at only 0.05 mM-DHAP in the presence of $PP_i$ (Fig. 4*c*), which is a much lower concentration than reported for the rat or guinea-pig enzyme [2,3]. The effect of $PP_i$ is unlikely to be attributable to displacement of palmitoyl-CoA from bovine serum albumin, since activation was observed over a wide range of molar ratios of albumin to palmitoyl-CoA (Fig. 4*d*).

## DISCUSSION

In mouse kidney, DHAPAT (assayed in the presence of 10 mM-$PP_i$) is predominantly peroxisomal. Some or all of the non-peroxisomal activity may be attributable to free peroxisomal membranes [3]. The pH optimum for activity is acid. These results are in good agreement with those of others for rat and guinea-pig liver [1–3]. With the assay used here, mouse kidney has a low DHAPAT activity in the absence of $PP_i$; however, with $PP_i$ (10 mM) and 0.1 mM-DHAP, the activity for purified peroxisomes of mouse tissues (2–7 nmol/min per mg of protein) was of the same order as that reported by others for purified rat liver peroxisomes (~ 3 nmol/min per mg [3], 1–2 [22], or 17 [2]). However, the mouse enzyme has greater apparent affinity for DHAP than has been reported for the hepatic enzyme of other species. $PP_i$ activation of DHAPAT could be due to protection of substrates, or products, or to an effect on DHAPAT, or to a combination of these. At present the mechanism is unknown; however, it is not due to an activation of the DHAP-generating system. Results of preliminary experi-

ments with rat liver samples indicate that the mouse system may behave differently with respect to $PP_i$.

Our results indicate that in mouse kidney, as reported for rat liver [10], a small proportion of glucose-6-phosphate dehydrogenase is peroxisomal. The peroxisomal enzyme is probably not loosely associated with organelles, since it was not displaced by $PP_i$ treatment of PNS. As far as we are aware, there is no report of peroxisomal isocitrate dehydrogenase in kidney; thus it is possible that in this tissue NADPH for reduction of acyl-DHAP may be provided by peroxisomal glucose-6-phosphate dehydrogenase.

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