Rat Adipose Tissue Phosphatidic Acid Phosphatase: Lack of Effect of Nucleotides on Cytosolic Enzyme Activity

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Al-Shurbaji, A. and Berglund, L., 1989. Rat Adipose Tissue Phosphatidic Acid Phosphatase: Lack of Effect of Nucleotides on Cytosolic Enzyme Activity. – Acta Chem. Scand. 43: 680–683.

The soluble phosphatidic acid phosphatase from rat adipose tissue was partially purified using ammonium sulfate fractionation and hydroxyapatite chromatography. Administration of ethanol has been found to increase phosphatidic acid phosphatase activity. The enzyme activity has been found to be dependent on magnesium ions with maximal activity at 2–5 mM magnesium. The enzyme displays an apparent pH optimum of 7.0. The activity of the enzyme is not affected by addition of ATP or ADP, in contrast with the results for hepatic phosphatidic acid phosphatase. The results suggest that these two enzymes may be regulated by different mechanisms and that they may thus represent two different types of isoenzyme.

The activity of phosphatidic acid phosphatase (E.C. 3.1.3.4) varies considerably when subjected to physiological stimuli that affect the rate of triglyceride biosynthesis.[1] The activity of this enzyme in liver, as measured *in vitro* seems to be the lowest among all enzymes involved in triglyceride biosynthesis.[1-3] Also, the rate of triglyceride biosynthesis varies in parallel with phosphatidic acid phosphatase activity.[1,2] It has therefore been suggested that the activity of this enzyme may be of importance in the regulation of triglyceride biosynthesis in the liver.[1,2] The enzyme present in adipose tissue is also affected by various agents. Adipose tissue phosphatidic acid phosphatase activity is increased by lipolytic agents *in vitro* as well as *in vivo*.[4-6] Long-term regulation of the enzyme activity has also been described, and phosphatidic acid phosphatase activity was found to decrease during starvation and with aging, and increase in obesity and with increased adipocyte size.[7-10] In both hepatic and adipose tissue, phosphatidic acid phosphatase activity is present in the cytosol as well as in the microsomes.[11] In a previous study, evidence was presented that the cytosolic phosphatidic acid phosphatase activity from rat liver changes under the effect of nucleotides.[12] Thus, ATP was found to inhibit the enzyme activity whereas ADP acted as a stimulant, indicating that hepatic phosphatidic acid phosphatase might be under regulatory control. Little is known, however, about the regulation of the enzyme present in adipose tissue. The present study was undertaken in order to compare the phosphatidic acid phosphatase from adipose tissue and liver with respect to the effect of nucleotides on enzyme activity.

Experimental

Materials. (1-^{14}C)palmitic acid with a specific activity of 56 Ci/mol was obtained from the Radiochemical Centre, Amersham, Great Britain. Unlabelled phosphatidic acid, ATP, ADP and dithiothreitol were products of Sigma Chemical Co., St. Louis, MO. Thin-layer chromatography plates, precoated with silica gel, were purchased from Merck AG, Darmstadt, West Germany. Hydroxyapatite was a product of Bio-Rad. In order to prepare labelled soluble phosphatidic acid, microsomal-bound phosphatidic acid was prepared using undiluted (1-^{14}C)palmitic acid as previously described.[13] The labelled phosphatidic acid obtained was extracted and isolated using thin-layer chromatography and diluted with dipalmitoyl phosphatidic acid to give a final specific activity of 2.5×10^3 dpm/nmol. Polyacrylamide gradient gels (4/30) were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden.

Purification of rat adipose tissue phosphatidic acid phosphatase. In order to induce phosphatidic acid phosphatase activity, rats were treated with ethanol as described.[12] The rats were killed and the epididymal fat pads were excised. The fat pads were cut into pieces and homogenized with two volumes of 50 mM Tris/HCl buffer, pH 7.4, containing 0.225 M sucrose and 0.1 mM dithiothreitol. The homogenate was centrifuged at $10.000\times g$ for 20 min at 4 °C and the pellets discarded. The supernatant was recentrifuged at $100.000\times g$ for 60 min at 4 °C and ammonium sulfate (0.23 g/ml) was added to the resulting supernatant. After being stirred for 30 min at 4 °C, the mixture was centrifuged at $10,000\times g$ for 20 min. The pellets were gently dissolved in 0.25 volumes of the homogenization buffer and dialyzed overnight against 10 mM Tris/HCl buffer, pH 7.4, containing 0.3 M sucrose and 0.1 mM dithiothreitol. The mixture was thereafter transferred to a column of hydroxyapatite (17×1.5 cm), equilibrated with 10 mM potassium phosphate buffer, pH 7.5, containing 30% glycerol and 0.1 mM dithiothreitol. The column was washed with 50 ml of the

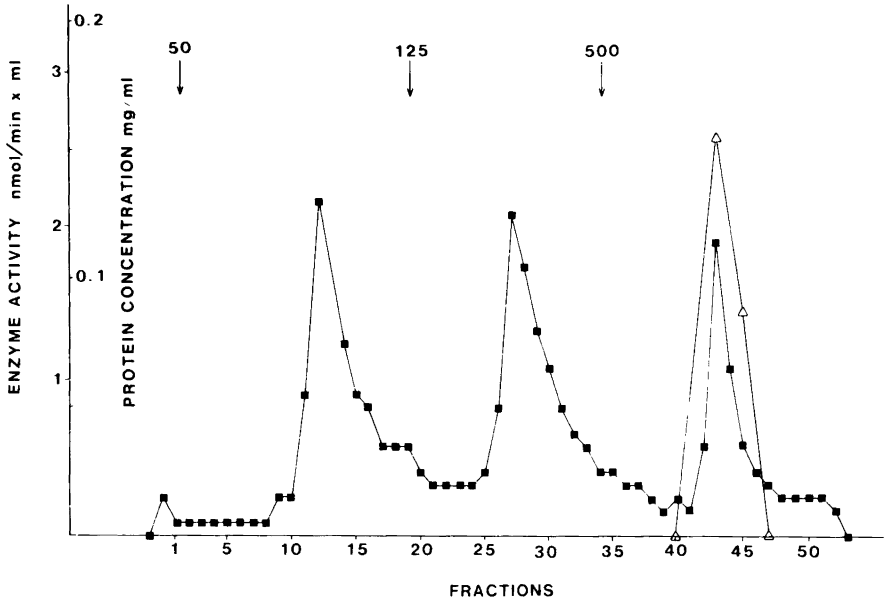

Fig. 1. Hydroxyapatite chromatography of rat adipose tissue cytosolic fraction after treatment with ammonium sulfate: phosphatidic acid phosphatase activity (Δ), protein concentration (■). Elution was performed using increasing concentrations of potassium phosphate, as indicated by the arrows. The conditions were as described in the text.

same buffer after application of the sample and thereafter developed by stepwise elution with increasing concentrations of potassium phosphate (50, 125 and 500 mM). Several protein peaks were eluted by this procedure (Fig. 1). Phosphatidic acid phosphatase activity was eluted with 500 mM potassium phosphate and the enzyme-containing fractions were pooled. Analysis of the partly purified enzyme with SDS gel electrophoresis demonstrated the presence of several protein bands (data not shown). The enzyme activity was found to be linear with time and with enzyme concentration.

Phosphatidic acid phosphatase (25–50 μg protein/assay) activity was assayed for 15 min at 37 °C. The standard assay mixture contained 60 μmol Tris-maleate, pH 6.9, 1.6 μmol $MgCl_2$ and free micellar phosphatidic acid, 500 nmol, to give a final volume of 0.8 ml. The extraction procedure, thin-layer chromatography and radioscanning of the chromatoplates were performed as described previously.[13] Protein concentration was determined as described by Lowry[14] or by measuring the ultraviolet absorption at 280 nm assuming that A^{280} for 1% was 10. Polyacrylamide gel electrophoresis in detergent was performed as described by Shapiro *et al.*[15]

Results

Effect of ethanol on adipose tissue phosphatidic acid phosphatase. It is well known that liver phosphatidic acid phosphatase activity is stimulated by the administration of ethanol.[12,16,17] In order to evaluate the effect of ethanol on adipose tissue phosphatidic acid phosphatase, rats were injected intraperitoneally with ethanol (2.5 mg/kg body weight) 16 h prior to being sacrificed. The adipose tissue enzyme was thereafter prepared up to the ammonium sulfate precipitation step, as outlined below, from the treated rats and from a group of control rats. As seen in Table 1, the adipose tissue phosphatidic acid phosphatase activity was clearly stimulated by ethanol.

Dependence on magnesium and pH. Magnesium was found to be an absolute requirement for cytosolic phosphatidic acid phosphatase from rat adipose tissue (Fig. 2). The optimal activity was seen at low magnesium concentrations, whereas levels above 5 mM were found to be inhibitory. The dependence on magnesium was similar to that of the hepatic enzyme, when assayed under similar conditions.[12] The dependence on pH is shown in Fig. 3. The maximal activity was found at pH 7.0, the enzyme activity decreased at alkaline pH. In contrast with the finding for the liver enzyme, acid pH levels were found to be inhibitory.[13]

Table 1. Effect of ethanol on adipose tissue phosphatidic acid phosphatase.

Condition	Phosphatidic acid phosphatase activity (nmol/min×mg protein)
Controls	1.42±0.05
Ethanol-treated	2.85±0.23

The enzyme activity was assayed after the preparation procedure had been performed up to the ammonium sulfate precipitation step. Both groups consisted of four rats and the results are expressed as the mean ± S.E.M.

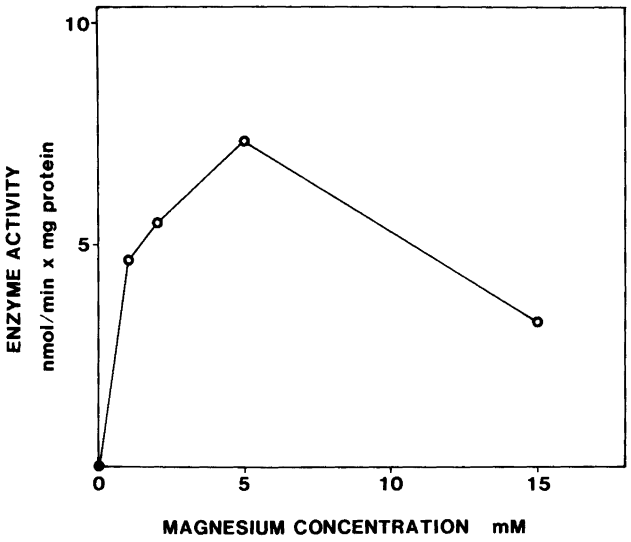

Fig. 2. Magnesium dependence of rat adipose tissue phosphatidic acid phosphatase.

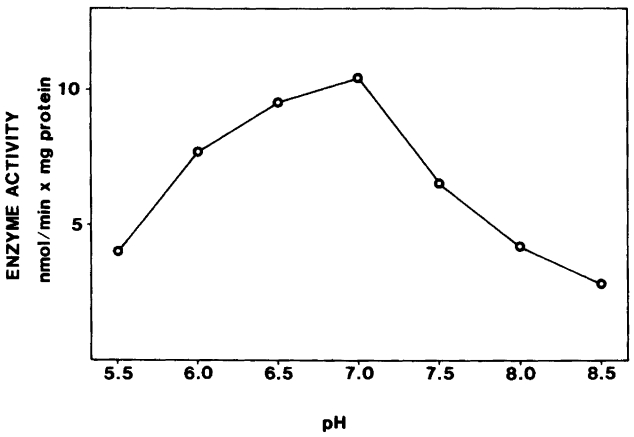

Fig. 3. Dependence upon pH. The assay for rat adipose tissue phosphatidic acid phosphatase activity was performed as described in the text, except that the Tris–maleate buffers used were of the pH levels indicated in the figures.

Table 2. Effect of nucleotides on phosphatidic acid phosphatase.

Additions	Phosphatidic acid phosphatase activity (%)	
	Adipose tissue	Liver
None (control)	100	100
2 mM ATP	92±3.1	60± 5
2 mM ADP	96±9.2	177±14

The results represent mean values from six experiments with the variation given as S.E.M. The enzyme activity was assayed as described above except that the final magnesium chloride concentration was 4 mM in the presence of nucleotides. The data for the liver enzyme are from Ref. 12.

Effect of nucleotides. The activity of adipose tissue phosphatidic acid phosphatase was assayed in the presence of either 2 mM ATP or 2 mM ADP.

As outlined in Table 2, no effect of either nucleotide on the enzyme activity was found. In contrast, the rat liver phosphatidic acid phosphatase activity was readily affected when assayed under these conditions.

Discussion

Several similarities between the cytosolic phosphatidic acid phosphatase in rat liver and adipose tissue were found. The adipose tissue enzyme was stimulated by administration of ethanol. It seems likely that ethanol treatment also increases the substrate availability for the adipose tissue phosphatidic acid phosphatase.[16] In addition, both enzyme activities showed an absolute requirement of magnesium and displayed a similar dependence on this ion. It was also possible to utilize similar techniques in the purification procedure employed for both enzymes. These properties may reflect some basic similarities of the two enzymes. However, the dependence on pH was different, as the adipose tissue enzyme showed a pH optimum of 7.0, whereas the liver enzyme was more active at acidic pH levels.[12] Furthermore, no effect of either ADP or ATP on adipose tissue phosphatidic acid phosphatase was seen. This suggests that these nucleotides, which greatly influence the activity of cytosolic hepatic phosphatidic acid phosphatase, do not play any regulatory role for the adipose tissue enzyme. Thus, the phosphatidic acid phosphatase present in adipose tissue may not be under allosteric control. This might reflect a lesser need for metabolic control of triglyceride biosynthesis in adipose tissue, where this pathway is mainly used for storage of fatty acids liberated by the action of lipoprotein lipase on triglyceride-rich lipoproteins. The findings indicate that the cytosolic phosphatidic acid phosphatase activities in adipose tissue and in liver represent different types of isoenzyme. Furthermore, these isoenzymes may be under different types of control reflecting the different regulatory requirements for triglyceride biosynthesis in the two organs. Further studies are needed to characterize the molecular basis of these differences.

Acknowledgements. This work was supported by a personal grant (L.B.) from the Swedish Medical Research Council (Project No. B84-19P-6742-O1A) and by grants from the Tore Nilsson Foundation, the Harald and Greta Jeansson Foundation and the Loo and Hans Osterman Foundation.

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Received February 14, 1989.