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Characterization of triacylglycerol hydrolase activities in isolated myocardial cells from rat heart

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Triacylglycerol (TG) hydrolase activities were characterized in myocytes isolated from rat hearts. Acid hydrolase activity with a pH optimum of 5 could be measured in myocyte homogenates, and the subcellular distribution suggested that this activity originated in lysosomes. Lipoprotein lipase (LPL) was also present in myocyte homogenates, as evidenced by TG hydrolase activity that was stimulated by serum and apolipoprotein CII, and inhibited by apolipoprotein $CIII_2$, high ionic strength (NaCl and $MgCl_2$, $I = 1$ M) and antibodies to LPL. Serum-independent neutral (pH 7.5) TG hydrolase activity was less sensitive to inhibition by 1 M-NaCl, by antibodies to LPL and by preincubation at 40 °C than was serum-stimulated hydrolase activity. Furthermore, there were modest but significant differences in the subcellular distribution of the serum-independent and serum-stimulated hydrolase activities. Hydrolase activities in myocyte homogenates could be solubilized by 7.2 mM-deoxycholate. Acid hydrolase activity was recovered in the unbound fraction after heparin–Sepharose chromatography, whereas LPL was bound to the affinity column and was eluted by 0.9–1.2 M-NaCl. Approximately one-third of the serum-independent TG hydrolase activity was not bound to the heparin–Sepharose affinity column. This unbound TG hydrolase activity had a pH optimum of 7 and was stimulated by 50 mM-$MgCl_2$, but not by serum and was resistant to inhibition by high ionic strength (1 M-NaCl), to preincubation at 40 °C for 2 h, and by antibodies to LPL. It is concluded that, in addition to an acid lysosomal TG hydrolase and LPL, myocytes from rat heart contain a serum-independent TG hydrolase with unique characteristics.

INTRODUCTION

Investigations of the regulation of cardiac metabolism can be complicated by the cellular heterogeneity of the heart, and so it is important to determine the cellular localization of an enzyme before proposing that it has a regulatory role in the metabolism of the myocardial cells. LPL (EC 3.1.1.34) has been located at the capillary endothelium of the heart by immunocytochemical techniques (Pedersen *et al.*, 1983). Displacement of this endothelium-bound enzyme by perfusion with heparin (Borensztajn & Robinson, 1970) or inhibition of enzyme activity by antibodies to LPL (Schotz *et al.*, 1977) results in a marked decrease in the ability of the heart to metabolize chylomicrons. Therefore LPL at the capillary endothelium of the heart has a functional role in the degradation of the TG component of circulating lipoproteins. The heparin-non-releasable or residual LPL may represent the precursor of this functional LPL (Ben-Zeev *et al.*, 1981). This residual LPL in cardiac tissue after heparin perfusion may exist in several cellular compartments; enzyme activity has been detected in the interstitium (Jansen *et al.*, 1980; Hulsmann *et al.*, 1982), mesenchymal cells (Chajek *et al.*, 1977) and isolated myocardial cells (Bagby *et al.*, 1977; Chohan & Cryer, 1978; Vahouny *et al.*, 1980). In addition to LPL, cardiac tissue also contains TG lipases with acid (pH 4.5–5) and neutral (pH 7–7.5) pH optima when assayed in the absence of serum (Severson, 1979*a*; Rosen *et al.*, 1981; Severson *et al.*, 1981; Hulsmann *et al.*, 1981). More recently, Stam & Hulsmann (1983) have proposed that the neutral serum-independent TG lipase activity in post-nuclear supernatant fractions from heparin-perfused hearts is identical with LPL.

Under certain circumstances, the mobilization of endogenous TG (lipolysis) can support the energy requirement of the heart, and rates of myocardial lipolysis are regulated by a variety of hormonal and metabolic factors (Crass, 1977; Lech *et al.*, 1977; Severson, 1979*b*). Catecholamine-stimulated lipolysis has also been observed in isolated myocardial cells (myocytes) from rat hearts (Palmer & Kane, 1983*b*; Kryski *et al.*, 1985). It is unclear, however, which TG lipase is responsible for hormone-stimulated rates of myocardial lipolysis. Acid lipase activity has been observed in dog heart myocytes (Knauer & Weglicki, 1983), and LPL activity has been measured in myocytes from adult rat ventricular tissue (Bagby *et al.*, 1977; Chohan & Cryer, 1978; Vahouny *et al.*, 1980). The objective of the present investigation was to characterize the TG lipase(s) in myocytes from rat hearts. The results indicate that rat heart myocytes contain a serum-independent neutral TG hydrolase (lipase) with unique characteristics, in addition to the acid lysosomal TG hydrolase and LPL.

MATERIALS AND METHODS

Isolation of myocytes, homogenization and subcellular fractionation

Ca^{2+}-tolerant myocytes were isolated from the hearts of male Sprague–Dawley rats (250–300 g) as described by Kryski *et al.* (1985). The viability, determined as the

Abbreviations used: LPL, lipoprotein lipase; TG, triacylglycerol.

percentage of cells that were rod-shaped and excluded Trypan Blue, was 73±1% ($n = 10$). After isolation, the myocytes were collected by centrifugation and the cell pellet was frozen and stored at −70 °C until use, usually within 1 week.

Myocytes were homogenized by suspending the cell pellet in 5 ml of buffer A (0.25 M-sucrose/1 mM-EDTA/1 mM-dithiothreitol/10 mM-Hepes, pH 7.4), and sonicating (Braun-Sonic 1510; 75 W) for 4 × 30 s at 4 °C. The homogenate was then diluted with buffer to give approx. 10^6 cells/ml. Myocyte homogenates could be stored at −70 °C for up to 1 month with no loss of enzyme activity. When myocyte homogenates were to be subjected to differential centrifugation, the isolated myocytes were first washed twice with Buffer A before homogenization by sonication in order to remove the albumin present in the isolation solutions. The subcellular fractions were obtained by differential centrifugation of a post-nuclear (700 *g*) supernatant as described by Wang *et al.* (1977).

Triacylglycerol hydrolase assays

In preliminary experiments, TG hydrolase activity at pH 7.5 was measured with an ethanolic triolein substrate preparation (Severson, 1979*a*). Subsequently, a sonicated (liposomal) substrate preparation was used. A portion of glycerol tri[1-^{14}C]oleate or glycerol tri[9,10(n)-^{3}H]oleate solution in toluene (0.69 μmol; 1 μCi/μmol) was added to a glass tube and dried under a stream of N_2. Buffer (0.143 ml of 0.2 M-Pipes/0.4% albumin, pH 7.5), 0.043 ml of 1.33 M-$MgCl_2$, and (when indicated) 0.034 ml of heat-inactivated (60 min at 50 °C) rat serum were added to the tube, and the volume was adjusted to 1 ml with water. The triolein was dispersed by sonication (75 W) for 3 or 4 × 30 s at 4 °C, and the substrate emulsion was used within 1 h. Assays were initiated by adding 50 μl of the enzyme preparation to 350 μl of substrate. The final concentration of assay components were : 0.6 mM-triolein, 25 mM-Pipes, pH 7.5, 0.05% (7.6 μM) albumin, 50 mM-$MgCl_2$, and (when present) 3% (v/v) serum. Assay incubations were terminated after 30 min at 30 °C by the addition of 3 ml of a fatty-acid-extraction solution (Belfrage & Vaughan, 1969) and 0.1 ml of 1 M-NaOH. After vortex-mixing and centrifugation, the radioactivity (sodium [1-^{14}C]oleate or sodium [^{3}H]oleate) in a sample of the upper phase (total volume 1.61 ml) was determined. Under these conditions, the recovery of [^{14}C]oleate added to assay tubes was 97–98%. Assays were linear with time for 60 min and with protein up to 250 μg. Assay blanks contained the appropriate buffer in place of the enzyme protein and averaged 0.01% of the total substrate radioactivity. All assays were performed in duplicate.

Acid lipase activity was determined with sonicated [^{14}C]- or [^{3}H]-triolein emulsions prepared as described above, except that the buffer was 25 mM-acetate at pH 5, and $MgCl_2$ and serum were omitted. In preliminary experiments, acid hydrolase activity at pH 5 was measured with a glycerol-dispersed triolein substrate preparation (Severson *et al.*, 1981).

Immunotitration experiments

Antibodies to purified rat heart LPL were isolated as described by Schotz *et al.* (1977). Control IgG and the anti-LPL IgG fractions were dialysed before use against 5 mM-sodium phosphate/15 mM-NaCl, pH 7.5, and then centrifuged to remove any insoluble material. Enzyme preparations were preincubated with different amounts of control or anti-LPL IgG either for 60 min at 4 °C or for 10 min at 30 °C in a total volume of 100 μl. Then assays were initiated with the addition of 300 μl of a sonicated substrate emulsion. To increase the sensitivity of assays performed in the absence of serum, the specific radioactivity of the triolein substrate was increased to 5 μCi/μmol.

Solubilization of hydrolase activities and heparin–Sepharose affinity chromatography

Myocytes were suspended in Buffer A containing 7.2 mM-sodium deoxycholate and sonicated as described above. After incubation for 45 min at 4 °C, the homogenate was centrifuged for 60 min at 100000 *g*. The supernatant was removed and the pellet was resuspended in the buffer containing deoxycholate, re-sonicated, incubated and re-centrifuged. The supernatants from both centrifugations were combined, adjusted to 0.15 M-NaCl, and applied to a heparin–Sepharose CL-6B column (1.4 cm × 7.5 cm) equilibrated with 5 mM-barbital/30% (v/v) glycerol/0.15 M-NaCl, pH 7.5 (flow rate 15 ml/h). The column was then washed with 10 bed vol. of the equilibrating buffer. The NaCl concentration in the elution buffer was increased to 0.75 M, and 100 ml was passed through the column. The LPL was then eluted by increasing the NaCl concentration to 1.2 M. Column fractions (5 ml) were collected and the protein content was determined (Robrish *et al.*, 1978). Albumin was added to fractions containing LPL activity to a final concentration of 1 mg/ml; these fractions could then be stored at −70 °C for 1 month without loss of activity. The unbound fraction eluted from the column at 0.15 M-NaCl was concentrated by ultrafiltration on an Amicon 8MC apparatus with a YM-10 membrane.

Materials

Hepes, Pipes, dithiothreitol, bovine albumin (essentially fatty-acid-free), goat IgG, and triolein were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Radiochemicals (glycerol tri[1-^{14}C]oleate and glycerol tri[9,10(n)-^{3}H]oleate) were obtained from either NEN Canada Ltd., Lachine, Que., Canada, or from Amersham Canada, Missisauga, Ont., Canada, and were purified by Florosil chromatography before use (Carroll, 1961), Heparin–Sepharose CL-6B was purchased from Pharmacia Fine Chemicals, Dorval, Que., Canada. Human apolipoproteins CII and $CIII_2$ were generously given by Dr. M. Huff, University of Western Ontario.

Statistics

Results are presented as means±S.E.M.; *P* values were calculated by a paired Student's *t* test.

RESULTS AND DISCUSSION

Acid TG hydrolase activity in myocytes

The specific activity for TG hydrolase in homogenates from whole hearts and from myocytes, measured at pH 5 with a glycerol-dispersed triolein substrate preparation, was 7.2±0.4 ($n = 8$) and 9.6±1.0 ($n = 16$) nmol/h per mg of protein respectively. Subsequent experiments to characterize the acid lipase activity in myocytes used a sonicated triolein substrate preparation. The pH optimum

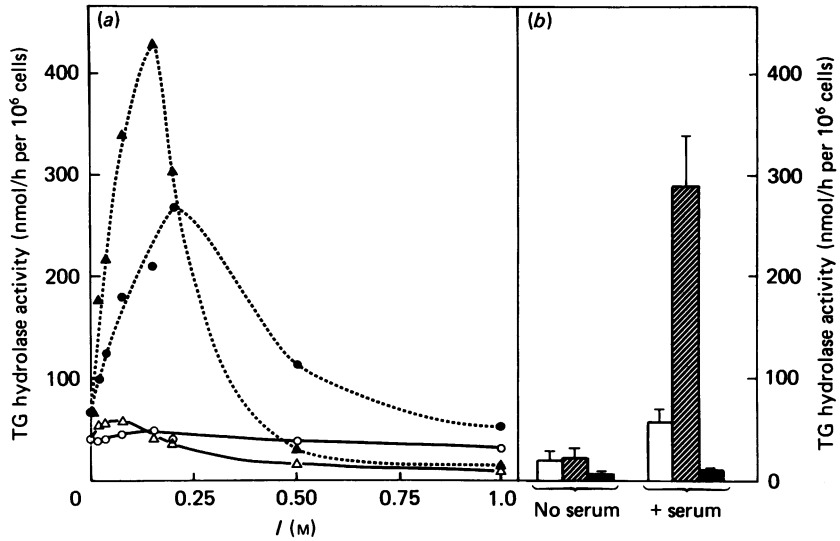

Fig. 1. Effect of ionic strength on neutral TG hydrolase activity in myocyte homogenates

(*a*) Hydrolase activity at pH 7.5 was determined in the presence (●, ▲) and absence (○, △) of 3% serum at the indicated ionic strengths, obtained by adding various concentrations of NaCl (○, ●) or $CaCl_2$ (△, ▲) to the assay. (*b*) TG hydrolase activity at pH 7.5 in the absence and in the presence of 3% serum was measured in the absence of $MgCl_2$ (□) and in its presence: ▨, $I = 0.15$ M; ■, $I = 1.0$ M). Results are means ± S.E.M. for three experiments.

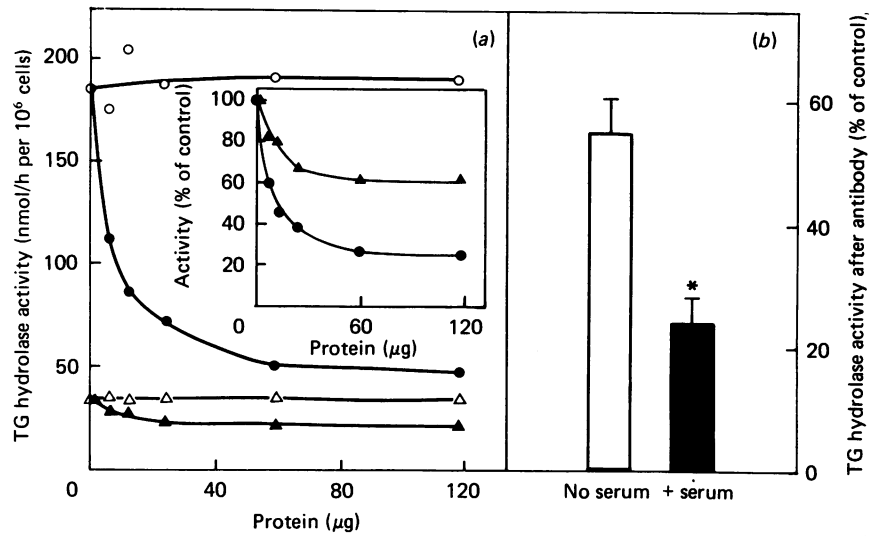

Fig. 2. Effect of antibodies to LPL on neutral TG hydrolase activities in myocyte homogenates

(*a*) Serum-independent (△, ▲) and 3%-serum-stimulated (○, ●) TG hydrolase activity was measured in the presence of the indicated amounts of control IgG (○, △) or anti-LPL IgG (●, ▲). Insert shows the activity calculated as a percentage of the initial activity (100%). (*b*) Hydrolase activity was measured in the absence and in the presence of 3% serum in assays with saturating amounts (4 μg of IgG/unit of serum-dependent activity, defined as the amount of enzyme that catalyses the releases of 1 nmol of oleate/h) of anti-LPL IgG or control IgG. Results, calculated as the percentage of control activity, are means ± S.E.M. for four experiments. $*P < 0.01$.

for acid hydrolase activity in myocyte homogenates was 5 (results not shown); an acid pH optimum of 4.5–5 has previously been observed for TG lipase activity in a variety of preparations from cardiac tissue (Severson, 1979*a*; Rosen *et al.*, 1981; Hulsmann *et al.*, 1981). Over 80% of the acid hydrolase activity was particulate after differential centrifugation. The relative specific activity (% of total recovered activity/% of total recovered protein) was 4.6 ± 0.3, 3.2 ± 0.5 and 1.6 ± 0.3 ($n = 4$) in lysosomal, mitochondrial and microsomal subcellular fractions respectively, consistent with a lysosomal localization within the intact cell.

Neutral TG hydrolase activity in myocyte homogenates

The specific activity for TG hydrolase activity measured at pH 7 in the absence of serum by using an

Table 1. Effect of sodium deoxycholate on TG hydrolase activities in myocyte homogenates

Enzyme activities in myocyte homogenates were determined after a 45 min preincubation in the absence and in the presence of 7.2 mM-sodium deoxycholate (DOC). The final concentration of deoxycholate in the hydrolase assays, when present, was 150 μM. The percentage of hydrolase activity recovered in the supernatant after centrifugation at 100000 *g* relative to the sum of activities in the supernatant plus pellet was also determined. Results are from a single representative experiment.

Enzyme	DOC	Enzyme activity (nmol/h per 10^6 cells)	Percentage soluble
Acid hydrolase	−	78	19
	+	96	83
Serum-independent neutral hydrolase	−	42	21
	+	102	87
Serum-stimulated neutral hydrolase	−	212	31
	+	678	93

ethanolic triolein substrate preparation with homogenates from myocytes (10.5±1.0 nmol/h per mg of protein; $n = 18$) was comparable with that in whole heart homogenate (11.4±1.2 nmol/h per mg; $n = 16$). The pH optimum for serum-independent TG hydrolase activity measured with a sonicated substrate preparation was 7.5 (results not shown), similar to values reported for cardiac tissue preparations (Severson, 1979*a*; Rosen *et al.*, 1981; Hulsmann *et al.*, 1981). The further characterization of TG hydrolase activity is shown in Fig. 1. In contrast with results with enzyme preparations from whole heart, where NaCl and $MgCl_2$ produced a 2–3-fold increase in serum-independent hydrolase activity at pH 7.5 (Severson, 1979*a*), increasing the ionic strength to 0.2 M had only a small effect on serum-independent TG hydrolase activity in myocyte homogenates (Fig. 1*a*). The addition of serum to hydrolase assays resulted in a small increase in activity, but this serum activation was markedly enhanced when the ionic strength of the assay was increased; optimal serum stimulation of TG hydrolase activity occurred at ionic strengths of 0.15 M and 0.2 M for $CaCl_2$ and NaCl respectively. Similar results were obtained with $MgCl_2$ (Fig. 1*b*); the serum stimulation of hydrolase activity was increased from 2.5-fold to 12-fold in the presence of 50 mM-$MgCl_2$. The stimulation by bivalent metal ions (Ca^{2+}, Mg^{2+}) was consistently greater than that produced by univalent (Na^+) cations. When the ionic strength of the assay was increased further to 1 M, the inhibition of hydrolase activity was greatest with the bivalent metal ions (Fig. 1).

Apolipoprotein CII also produced a concentration-dependent stimulation of TG hydrolase activity, with maximal effects observed at a concentration of 10 μg/ml. Both serum- and apolipoprotein-CII-stimulated activities were inhibited to 23% of control by 100 μg of apolipoprotein $CIII_2$/ml. Serum- and apolipoprotein-CII-stimulated TG hydrolase activities had an optimal pH of about 7.5; serum-stimulated lipase activity in cardiac tissue preparations was also reported to have a pH optimum of 7.4 (Stam & Hulsmann, 1983).

Thermal inactivation and inhibition of neutral TG hydrolase activities in myocyte homogenates by antibodies to LPL

Further experiments were designed to determine if serum-independent TG hydrolase activity in myocytes was, at least in part, due to a lipase that was separate and distinct from LPL. Preincubation of a myocyte homogenate at 40 °C before assay resulted in a greater loss of serum-stimulated than of serum-independent TG hydrolase activity; after 120 min, serum-stimulation was decreased from 5.3- to 1.5-fold. Rates of thermal inactivation were increased if the preincubation temperature was raised to 50 °C; a 10 min preincubation resulted in a 66±13% loss of serum-stimulated TG hydrolase activity but only a 27±14% loss of serum-independent activity ($n = 5$; $P < 0.05$).

The effect of antibodies to LPL on neutral TG hydrolase activities in myocyte homogenates is shown in Fig. 2. Various concentrations of control IgG had no effect on either serum-independent or serum-stimulated activities, but the addition of antibodies to LPL produced a concentration-dependent inhibition of both TG hydrolase activities (Fig. 2*a*). However, when activities were normalized by expressing them as a percentage of control (100%), it was clear that the antibodies to LPL produced a greater inhibition of serum-stimulated than of serum-independent TG hydrolase activity. In the presence of saturating concentrations of antibody to LPL, serum-independent hydrolase activity was decreased to 55±6% of control, but the serum-stimulated activity was decreased to 24±5% of control (Fig. 2*b*).

Subcellular distribution of neutral TG hydrolase activities in myocytes

Myocyte homogenates were subjected to differential centrifugation in order to determine the subcellular distribution of the neutral TG hydrolase activities. For hydrolase activity measured in the absence of serum, most of the total activity in the various subcellular fractions was recovered in the particulate fractions (82% of the sum of total recovered activities), with the highest relative specific activity (2.3±0.03; $n = 4$) in the microsomal fraction. Less serum-stimulated activity was present in the microsomal (59% of the total, $P < 0.05$) and lysosomal (8%; $P < 0.01$) fractions and more was present in the soluble fraction (27%; $P < 0.01$) compared with the serum-independent TG hydrolase activity. This distribution of TG hydrolase activity is markedly different from that observed with subcellular fractions from whole hearts, where both LPL and serum-independent neutral TG hydrolase activities were predominantly soluble (Severson, 1979*a*), but the distribution of serum-stimulated TG hydrolase is similar to results obtained previously with LPL in cardiac myocytes (Chohan & Cryer, 1979).

Solubilization and heparin–Sepharose chromatography of TG hydrolases

Further attempts to purify and characterize the TG hydrolases in myocytes required solubilization of the predominantly particulate activities. As shown in Table 1, treatment of myocyte homogenates with deoxycholate resulted in the recovery of 80–90% of acid and neutral

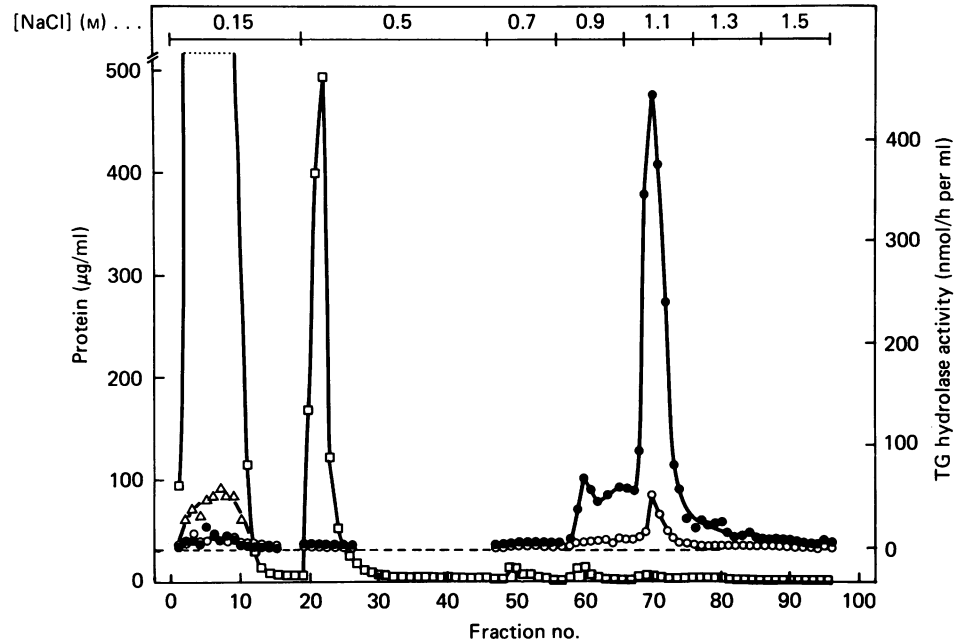

Fig. 3. Heparin–Sepharose affinity chromatography of solubilized hydrolase activities from myocytes

The high-speed supernatant fraction after deoxycholate treatment of myocytes was loaded on to the heparin–Sepharose column in buffer containing 0.15 M-NaCl, as described in the Materials and methods section. Fractions were obtained after the stepwise increase in NaCl concentration as indicated at the top. Acid hydrolase activity (△) could not be detected in fractions eluted at 0.5–1.5 M-NaCl, and so, for the sake of clarity, activity measurements are only indicated for the unbound fractions (0.15 M-NaCl). Neutral TG hydrolase activity was measured in the absence (○) and in the presence (●) of 3% serum. For assays with the unbound fraction, 50 mM-$MgCl_2$ was present in the assay. For fractions eluted at higher salt concentrations, the assays were adjusted so that a final concentration of 0.2 M-NaCl was obtained. The protein content of all fractions was also measured (□). Results are from a single experiment.

Table 2. Recovery and purification of TG hydrolase activities after heparin–Sepharose chromatography

Results are calculated from the data presented in Fig. 3. A unit of hydrolase activity was defined as the amount of enzyme that catalysed the release of 1 nmol of oleate/h. Percentage recoveries are given in parentheses, and purification (fold) in square brackets.

Fraction	Protein (mg)	Acid hydrolase activity (units)	Acid hydrolase activity (units/mg)	Neutral hydrolase activity, no serum (units)	Neutral hydrolase activity, no serum (units/mg)	Neutral hydrolase activity + serum (units)	Neutral hydrolase activity + serum (units/mg)
Applied to column	242	1.64	0.007	1.67	0.007	8.01	0.033
0.15 M-NaCl eluate	170	2.10 (128%)	0.012 [1.8]	0.48 (29%)	0.003 [0.4]	0.53 (6%)	0.003 [0.0]
0.9 M-NaCl eluate	0.26	0	0	0.27 (16%)	1.03 [149]	2.34 (29%)	9.01 [271]
1.1 M-NaCl eluate	0.14	0	0	0.80 (48%)	5.71 [828]	9.20 (119%)	66.4 [2010]

serum-independent and serum-stimulated TG hydrolase activities in the high-speed (100000 *g*) supernatant after centrifugation. The total activity of the neutral TG hydrolase measured in the absence and in the presence of serum was increased by the detergent treatment; similar results have been reported with deoxycholate treatment of heart homogenates (Borensztajn *et al.*, 1970) and microsomal fractions from cardiac myocytes (Chohan & Cryer, 1979). Deoxycholate was very effective in solubilizing hydrolase activities in myocytes. The percentage of activity recovered in the 100000 *g* supernatant for the acid hydrolase, serum-independent and serum-stimulated neutral lipase was 92 ± 2, 90 ± 2 and 93 ± 1% respectively ($n = 6$). Chromatography of the solubilized myocyte preparation on heparin–Sepharose columns is shown in Fig. 3. Large quantities of protein were excluded from the column, and acid hydrolase activity was detected in this unbound fraction with a recovery of 128% (Table 2). A small amount of serum-stimulated neutral TG hydrolase activity (LPL) was eluted at 0.9 M-NaCl, but the majority was recovered at 1.1 M-NaCl (Fig. 3). The total recovery of serum-stimulated TG hydrolase activity was greater than 100%, with a 2010-fold increase in specific activity in the fraction eluted at 1.1 M-NaCl (Table 2). With respect to serum-independent TG hydrolase activity, the fractions eluted at 0.9 M-and 1.1 M-NaCl contained 64%

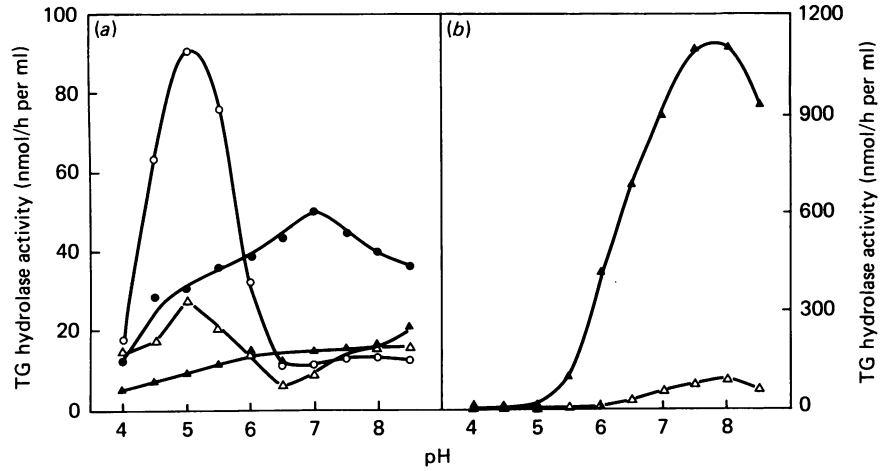

Fig. 4. Effect of pH on hydrolase activity in fractions after heparin–Sepharose chromatography

(*a*) Hydrolase activity in the unbound (0.15 M-NaCl eluate) fraction was measured at the indicated pH values (25 mM-acetate, pH 4–6; 25 mM-phosphate, pH 6.5–8.6) with the following additions: ○, none; ●, 50 mM-$MgCl_2$; △, 3% serum; ▲, 50 mM-$MgCl_2$ plus 3% serum. Results are means from two experiments. (*b*) Hydrolase activity in the 1.2 M-NaCl eluate was measured at the indicated pH values in the absence (△) and in the presence (▲) of 3% serum; the final NaCl concentration in the assay was 150 mM.

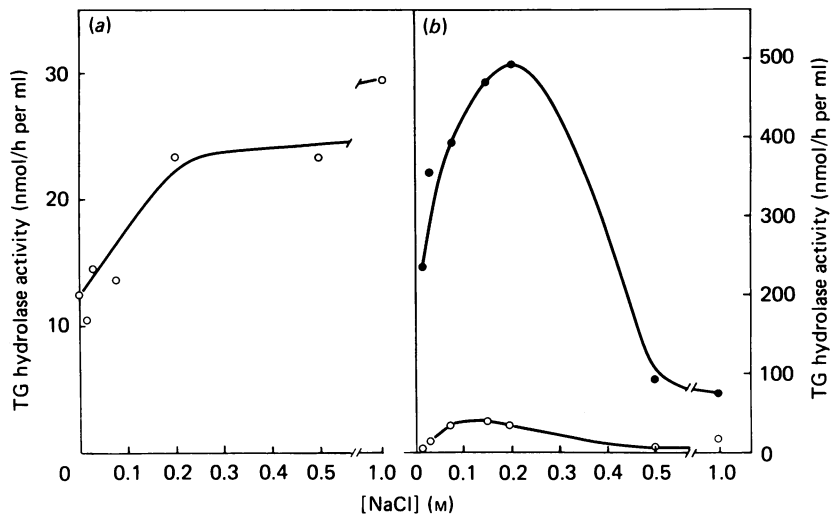

Fig. 5. Effect of NaCl on hydrolase activity in fractions after heparin–Sepharose chromatography

(*a*) Neutral hydrolase in the unbound fraction was assayed at pH 7 in the presence of various concentrations of NaCl (○) to give the indicated ionic strengths. (*b*) Serum-independent (○) and 3% serum-stimulated (●) TG hydrolase (LPL) activity in the 1.2 M-NaCl eluate was assayed at pH 7.5 with the indicated concentrations of NaCl.

of the initial activity (Table 2). However, the unbound fraction (0.15 M-NaCl) also contained low but detectable amounts of serum-independent TG hydrolase activity (Fig. 3), representing 29% of the initial activity (Table 2), and this activity was not increased by the addition of serum to the assay.

Characterization of TG hydrolase activities after heparin–Sepharose chromatography

Unbound fractions from the heparin–Sepharose column were concentrated by ultrafiltration, and the activity of TG hydrolase in this fraction was compared with the serum-stimulated TG hydrolase (LPL) eluted from the column at high salt concentrations. The effect of pH on hydrolase activities is shown in Fig. 4. For the unbound fraction (Fig. 4*a*), hydrolase activity had a distinct pH optimum of 5, and the addition of serum, $MgCl_2$ or serum + $MgCl_2$ resulted in an inhibition of acid hydrolase activity. Serum has previously been reported to inhibit acid hydrolase activity in whole heart preparations (Stam & Hulsmann, 1983). At pH 7, it is apparent that TG hydrolase activity can be enhanced by 50 mM-$MgCl_2$, but not by serum, either in the presence or in the absence of $MgCl_2$ (Fig. 4*a*). In five experiments, the recovery of $MgCl_2$-stimulated neutral TG hydrolase in the unbound fraction (0.15 M-NaCl eluate) after heparin–Sepharose chromatography was 32.6±6.0%. For the LPL fraction eluted at 1.2 M-NaCl, activity in the absence and in the presence of serum had a pH optimum of 7.5–8, and serum produced a 12-fold increase in activity (Fig. 4*b*).

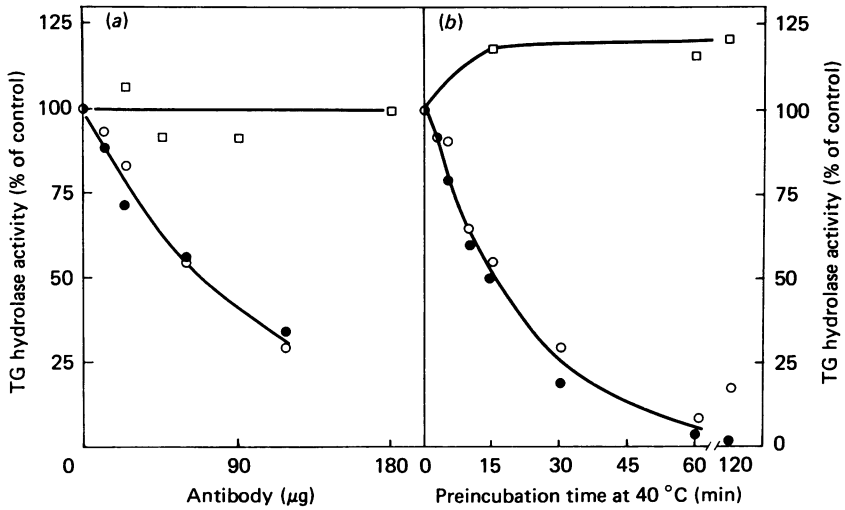

Fig. 6. Effect of anti-LPL antibodies on, and rate of thermal inactivation of, hydrolase activities in fractions from heparin–Sepharose chromatography

(*a*) The 1.2 M-NaCl eluate (LPL) fraction was preincubated for 10 min at 30 °C with either control IgG or anti-LPL IgG, and then TG hydrolase activity was determined in the absence (○) and in the presence (●) of 3% serum. The immunotitration experiment with the unbound fraction was performed with a different antibody preparation from that used with the LPL fraction. Initial activities in the absence and in the presence of serum were 38 and 472 nmol/h per ml. Neutral TG hydrolase activity in the unbound fraction was measured at pH 7 in the presence of 50 mM-$MgCl_2$ (□); initial activity was 13 nmol/h per ml. Results are calculated as a percentage of the corresponding activity measured with control IgG. (*b*) The 1.2 M-NaCl eluate was preincubated at 40 °C for the indicated times before measurement of TG hydrolase activity in the absence (○) and in the presence (●) of 3% serum. Results are presented as a percentage of the initial activity measured with (100% = 320 nmol/h per ml) and without (100% = 31 nmol/h per ml) serum. TG hydrolase activity in the unbound fraction was also measured at pH 7.5 in the presence of 50 mM-$MgCl_2$ after preincubation at 40 °C for the indicated times (□); zero time (100%) corresponded to 21 nmol/h per ml.

The effect of NaCl on the neutral TG hydrolase activity in the unbound fraction is presented in Fig. 5(*a*). Increasing concentrations of NaCl resulted in a modest stimulation of hydrolase activity that was still evident at a concentration of 1.0 M. In four experiments, the neutral TG hydrolase activity in the unbound fraction was increased from 12.7 ± 0.8 to 31.3 ± 1.4 nmol/h per ml by the presence of 1.0 M-NaCl in the assay; the activity in the presence of 50 mM-$MgCl_2$ was 71.8 ± 1.5 nmol/h per ml. For LPL, optimal activity in the absence and in the presence of serum was obtained at an ionic strength of 0.15–0.2 M-NaCl, and activity was inhibited by 1.0 M-NaCl (Fig. 5*b*).

The LPL activity eluted at 1.2 M-NaCl could be inhibited by the addition of antibodies to LPL (Fig. 6*a*). In contrast with the immunotitration experiments performed with myocyte homogenates (Fig. 2), activity determined in the absence and in the presence of serum showed the same sensitivity to inhibition by the antibody (Fig. 6*a*). The Mg^{2+}-stimulated neutral TG hydrolase activity in the unbound fraction was not decreased by preincubation with antibodies to LPL (Fig. 6*a*).

Hydrolase activity eluted at 1.2 M-NaCl and measured with and without serum showed the same rate of thermal inactivation (Fig. 6*b*), in contrast with the previous results with myocyte homogenates. The $MgCl_2$-stimulated neutral TG hydrolase in the unbound fraction was not decreased by a 2 h preincubation at 40 °C (Fig. 6*b*).

General discussion

The objective of this investigation was to characterize the TG hydrolase activities in isolated myocardial cells (myocytes) from rat heart. An acid TG hydrolase activity with a pH optimum of 5 was observed in myocyte homogenates, and the subcellular distribution was consistent with a lysosomal location within the myocyte. Acid lipase activity has also been observed in dog myocytes (Knauer & Weglicki, 1983). LPL activity was also identified in myocytes by the following criteria: stimulation by serum and apolipoprotein CII of neutral TG hydrolase activity in myocyte homogenates; inhibition of serum- and apolipoprotein-CII-stimulated hydrolase activity by apolipoprotein $CIII_2$ and high ionic strength; pH optimum for serum- and apolipoprotein-CII-stimulated TG hydrolase of 7.5; binding of solubilized serum-stimulated activity to heparin–Sepharose columns and elution with high salt (0.9–1.2 M-NaCl) concentrations; and inhibition of serum-stimulated activity by antibodies to LPL. These results are consistent with the characteristics of purified LPL from rat (Twu *et al.*, 1975; Chung & Scanu, 1977), human (Twu *et al.*, 1976) and pig heart (Enholm *et al.*, 1975), and from previous publications with myocytes from rat heart (Bagby *et al.*, 1977; Chohan & Cryer, 1978, 1979; Vahouny *et al.*, 1980).

On the basis of experiments with post-nuclear supernatants from heparin-perfused rat hearts, it has been proposed that neutral TG hydrolase activity measured in the absence of serum is identical with LPL (Stam & Hulsmann, 1983). This proposal was based on: (i) complete inhibition of serum-independent TG hydrolase activity by antibodies to LPL (Hulsmann *et al.*, 1982), although the serum-stimulated activity was more sensitive to inhibition by the antibody (Stam &

Hulsmann, 1983); (ii) similar effects of high salt concentrations, protamine sulphate and heparin on hydrolase activity determined in the presence and in the absence of serum (Stam & Hulsmann, 1983); (iii) substantial (83%) binding of the serum-independent TG hydrolase to heparin–Sepharose columns and elution at about 1.0 M-NaCl (Stam & Hulsmann, 1984). In contrast with these results, our experiments with myocyte homogenates indicated that the serum-stimulated TG hydrolase was inhibited to a greater extent than the serum-independent hydrolase activity by preincubation at 40 °C, by 1.0 M-NaCl, and by the addition of antibodies to LPL. In addition, there were modest but statistically significant differences in the subcellular distributions between particulate and soluble subcellular fractions for these two hydrolase activities. After treatment of the myocyte homogenates with deoxycholate, a neutral TG hydrolase activity could be detected in the unbound fraction from heparin–Sepharose columns that had unique characteristics. This neutral TG hydrolase had a pH optimum of 7 and could be stimulated by 50 mM-$MgCl_2$ and 1.0 M-NaCl. Furthermore, this TG hydrolase activity in the unbound fraction was not stimulated by serum, was not inhibited by antibodies to LPL, and was resistant to any inactivation by pre-incubation at 40 °C for up to 2 h. By comparison, the inhibition by antibodies to LPL and the rate of thermal inactivation at 40 °C was identical for LPL activity eluted from the heparin–Sepharose column at 1.2 M-NaCl when assayed in the presence and in the absence of serum. Therefore, it seems reasonable to conclude that a significant portion of the serum-independent TG hydrolase activity measured in myocyte homogenates is due to an enzyme that is separate and distinct from LPL. The difference between this conclusion and the results of Stam & Hulsmann (1983) may be due to their use of a post-nuclear supernatant fraction from heparin-perfused hearts, since this enzyme preparation will still contain large quantities of LPL localized in the interstitial spaces (Jansen *et al.*, 1980; Hulsmann *et al.*, 1982). Consequently, it would be much more difficult to detect a neutral TG hydrolase activity that is separate from LPL by using this preparation.

Rates of lipolysis can be stimulated by the addition of catecholamines to myocyte incubations (Palmer & Kane, 1983*b*; Kryski *et al.*, 1985). The lysosomotropic agent methylamine (de Duve *et al.*, 1974) has been reported to inhibit basal and glucagon-stimulated rates of lipolysis (glycerol output) in isolated perfused hearts (Hulsmann *et al.*, 1981), and so the potential involvement of the acid lysosomal TG hydrolase in the mobilization of endogenous TG in myocytes will have to be assessed. In addition, the activation of LPL in heart homogenates (Palmer *et al.*, 1981), defatted heart powders (Palmer & Kane, 1983*a*) and in acetone–ether-dried powders from cardiac myocytes (Palmer & Kane, 1983*b*) by adrenaline has been reported. Future investigations will have to determine if the serum-independent TG hydrolase present in myocytes can be regulated by hormones.

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