Distinct immunoreactivities suggest the existence of potential tissue variants in rat lipoprotein lipase

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Lipoprotein lipases (LPL) isolated from rat cardiac muscle and bovine milk were each used as immunogens to produce polyclonal anti-LPL sera and two anti-LPL monoclonal antibodies. The immunological reactivities of these antibody sources with LPL purified from rat cardiac muscle, lung, adipose tissue, mammary gland and skeletal muscle were compared by an e.l.i.s.a. and by Western blotting. Differences between the immunoreactivities of LPL from the distinct tissue sources were revealed in both systems. A synthetic peptide with a sequence corresponding to the heparin-binding site of LPL (Ser-Arg-Thr-Asn-Thr-Lys-Val-Ser-Arg-Ile-Thr-Gly-Leu) was produced and used as an immunogen. The antiserum produced against the synthetic

peptide was found to bind specifically to the region of the heparin-binding site, as determined by use of a competition e.l.i.s.a. In use against the five tissue LPL preparations, this antiserum revealed only minor variations between the tissue sources, compared with the hierarchy of reactivity observed when antibodies raised against the whole molecule were used. In combination with the outcome of previous studies on some of the physical properties of these preparations [Soteriou and Cryer (1993) *Int. J. Biochem.* **25**, 1483–1490], the observations reported here on the distinct immunoreactivities exhibited by LPL prepared from the different tissue sources of a single species indicate the necessity to characterize fully the nature of these differences.

INTRODUCTION

Lipoprotein lipase (LPL) is the key enzyme in the metabolism of plasma lipoprotein triacylglycerol (Kotze and Spiers, 1976; Cryer and Jones, 1979; Carnheim et al., 1984; Cheng et al., 1985). The action of LPL at the endothelial cell surface of extrahepatic tissues releases chylomicron and very-low-density lipoprotein triacylglycerol-derived fatty acids that are then available for uptake (see Cryer, 1981, for review).

LPL is under tissue-specific control with respect to its synthesis and translocation to the endothelial site of action, and it has been suggested previously that tissue-specific variations of the enzyme may exist (Fielding, 1976; Ben-Zeev et al., 1983). Indeed, when LPL was purified from various tissues taken from a single species, variations in the specific activity, isoelectric point and in the patterns observed after two-dimensional electrophoresis were observed between the individual tissue LPL preparations (Soteriou and Cryer, 1993). These preliminary indications of putative tissue-specific LPL variants must, however, be contrasted with the existence of a single, highly conserved gene for LPL (Zechner et al., 1988; Enerback et al., 1988; Edwards et al., 1993), which makes it probable that, if variations do exist, they arise because of distinct post-transcriptional changes, as discussed by Soteriou and Cryer (1993).

With the aim of investigating the possible existence of tissue-specific molecular variants of LPL further, the present study examined the comparative immunological reactivities of LPL prepared separately from cardiac muscle, lung tissue, adipose tissue, mammary gland and skeletal muscle of the rat, with a panel of polyclonal and monoclonal antibodies raised against either rat cardiac-muscle or bovine milk LPL. In addition to antibodies raised against the whole isolated molecule, a synthetic

peptide corresponding to the amino acid sequence of one of the heparin-binding regions in the LPL molecule (Kirchgessner et al., 1987; Cooper et al., 1989) was also used as an immunogen.

The similarity of reactivity between the anti-peptide antibody and the enzyme from the five sources, together with the consistent pattern of differences seen when the other polyclonal and monoclonal antibodies raised and screened against the whole molecule were studied, further substantiated the possible existence of tissue-specific molecular variants of LPL, as postulated previously (Soteriou and Cryer, 1993).

MATERIALS AND METHODS

Animals

Rats were of the MRC Hooded strain (body weights 200–300 g), and, once removed, tissues were subjected immediately to the procedures described below. Balb/c mice were used in the production of both the polyclonal and monoclonal antibody preparations.

Purification of LPL

LPL was isolated from the five rat tissue sources as described previously by Soteriou and Cryer (1993). Briefly, extracts of acetone/diethyl ether-dried tissue powders were subjected to two stages of affinity chromatography. The first stage involved heparin-Sepharose in 5 mM sodium barbital buffer, pH 7.5, containing 20% (v/v) glycerol, 0.1% (v/v) Triton X-100 and 1 mM phenylmethanesulphonyl fluoride, where LPL activity was eluted in the presence of 2.0 M NaCl. Fractions rich in LPL were applied to the second stage of affinity chromatography on

Abbreviation used: LPL, lipoprotein lipase.

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Concanavalin A–Sephadex as described in detail by Parkin et al. (1982).

LPL from bovine milk was purified by the method described by Olivecrona and Bengtsson (1984), which involved sequential affinity chromatography using heparin–Sephadex and then *N*-desulphated re-acetylated heparin–Sephadex. LPL was eluted by a gradient of NaCl concentration (1–2 M).

LPL activity was assessed by using a titration-based assay in which Intralipid (Vitrum, Stockholm, Sweden) was used as the substrate, as described by Riley and Robinson (1974) and later modified by Cryer and Jones (1978).

Production of antibodies to isolated LPL

Before its use as an immunogen, rat cardiac LPL was cross-linked into oligomers with glutaraldehyde by the method described by Reichlin (1970). For this, LPL was dissolved in 0.1 M potassium phosphate, pH 7.0, and glutaraldehyde (1.25 mM) added to give a final molar ratio of 11:1. A 5 μ l portion of 0.1 M aqueous solution of L-lysine hydrochloride was added, and the mixture was stirred for a further 1 h at room temperature. The material was then dialysed exhaustively against PBS (pH 7.4) at 4 °C.

The conjugated rat cardiac LPL and unconjugated bovine milk LPL immunogens (30 μ g) were each emulsified in Freund's complete adjuvant and injected subcutaneously into mice. The mice were given a further injection of immunogen (30 μ g) in incomplete Freund's adjuvant 28 days after the primary immunization and again after a further 21 days. Sera for screening were taken 7–10 days after this final booster injection.

Sera from all of the immunized mice were screened initially for the presence of anti-LPL antibodies by using an e.l.i.s.a. For this, isolated LPL in 0.1 M carbonate/bicarbonate buffer, pH 9.6, at a concentration of 2 μ g/ml was coated onto 96-well e.l.i.s.a. plates overnight at 4 °C. Previous studies (Vaughan-Thomas, 1988) have shown that such conditions produce maximal saturation binding of LPL to plates. After blocking the plates by incubation with 0.1% gelatin in PBS/Tween, the antibody-containing preparation was added to the coated wells. The binding of the primary antibodies was detected by using a horseradish peroxidase-linked second antibody (rabbit anti-mouse IgG antibody–horseradish peroxidase conjugate; Dako, Copenhagen, Denmark). Peroxidase activity was assessed at 492 nm with the chromogenic substrate *o*-phenylene diamine (40 μ g/ml).

The polyclonal antisera were also used to probe Western blots. For this, samples were separated on 8–20% gradient polyacrylamide gels (Siemankowski and Dreizen, 1978) in the buffer system described by Laemmli (1970). Transfer to membrane (Immobilon-P; Millipore) used a semi-dry Western blotting apparatus (Millipore) as described by Burnette (1981). Immobilized antigens were detected by using the putative antisera followed by a peroxidase/anti-peroxidase visualization system (Sigma, U.K.), with diaminobenzidine as enzyme substrate.

Production of monoclonal antibodies

Spleens from mice immunized with LPL were collected, 4 days after a final injection of immunogen, for fusion with a P3X63-Ag653 mouse myeloma cell line (Kearney et al., 1979). The cell fusion, hybrid selection and cloning were carried out by standard protocols identical with those described previously (see Liddell and Cryer, 1991). The hybridomas were screened for their

capacity to secrete anti-LPL antibodies, by using the e.l.i.s.a. system described above. Colonies of anti-LPL-secreting cells were cloned three times in order to ensure monoclonality.

Production of anti-synthetic-peptide antiserum

The sequence of the synthetic peptide equivalent to one of the heparin-binding regions of the LPL molecule was chosen from the published cDNA sequences for LPL, as summarized by Cooper et al. (1989). The technique of predicting surface accessibility (Thornton et al., 1986) was also used as the basis of antigenic-site prediction.

The synthesis of the peptide was kindly carried out by Professor B. M. Dunn (Department of Biochemistry and Molecular Biology, J. Hillis Medical Center, University of Florida, U.S.A.). The peptide was analysed by f.p.l.c. (Pharmacia) by reverse-phase chromatography on a Pep RPC column, and the amino acid composition of the eluted peptide was determined with a LKB 4150 amino acid analyser.

In order to raise antibodies against the synthetic peptide, the peptide was conjugated to either rabbit serum albumin with *N*-maleimidobenzoic acid *N*-hydrosuccinimide ester (Green et al., 1982; Yoshitake et al., 1982), or keyhole-limpet haemocyanin, with glutaraldehyde, before administration, or was administered in the unconjugated form.

Sera from immunized mice was tested for the presence of anti-peptide immunoreactivity by the standard e.l.i.s.a., except that, when unconjugated peptide was used as the immobilized antigen, 'Microelisa' plates M129B (Dynatech, Billingshurst, W. Sussex, U.K.) were employed.

The e.l.i.s.a. screening test was also used as the basis of a competition assay for determining whether the anti-peptide antibodies present in the sera were reactive specifically towards the heparin-binding region of the LPL. For this, LPL was bound to wells of e.l.i.s.a. plates as described above. After blockage of the sites that had not reacted, the bound LPL was incubated with low-molecular-mass heparin (4000–6000 Da; Sigma), up to 0.16 μ g per well, for 60 min at room temperature. Excess heparin was washed away, and the anti-LPL antibodies and the enzyme-linked second antibody were then added sequentially as described above, and the chromogenic substrate was used to monitor antibody binding.

RESULTS

Isolation of LPL

Rat tissue LPL preparations were isolated as described above and the preparations, each of which exhibited a single component on SDS/PAGE, of molecular mass 61 kDa, showed a range of specific activities, as described in detail in a previous study (Soteriou and Cryer, 1993). The specific activities of the replicated preparations from each tissue were as follows: cardiac muscle, 400 ± 30 units/mg (9); lung, 255 ± 7 units/mg (3); white adipose, 720 ± 23 units/mg (3); mammary gland, 450 ± 110 units/mg (3); skeletal muscle, 75 ± 1 units/mg (3) (means $\pm$ S.E.M.; *n* in parentheses). The preparations had pI values identical with those described previously (Soteriou and Cryer, 1993).

For the preparation of LPL from bovine milk, three separate isolations were carried out. The specific activities of the three preparations were 10795, 7737 and 8388 units/mg [mean 8973 ± 930 units/mg (S.E.M.)]. The mean purification factor was approx. 3500, and the single major band observed after SDS/PAGE displayed a molecular mass of 58.5 ± 2.1 kDa (S.E.M., *n* = 3).

Figure 1 The detection of anti-LPL antibodies in polyclonal sera, by an e.i.s.a.

Serum from mice immunized with bovine milk LPL (□), rat cardiac-muscle LPL oligomers (○) or rat cardiac-muscle LPL conjugated with keyhole-limpet haemocyanin (●) was tested for the presence of anti-LPL antibodies by use of an e.i.s.a. For the e.i.s.a., LPL was coated on to an e.i.s.a. plate at a concentration of 2 µg/ml in carbonate/bicarbonate coating buffer, pH 9.6, overnight at 4 °C. After blocking of residual binding sites, various dilutions of the serum to be tested were added. The bound antibodies were then detected with a horseradish peroxidase-conjugated anti-mouse IgG antibody. The A_{492} values shown are those from which the values for control wells incubated with non-immune serum have been subtracted.

Production of polyclonal antibodies to whole LPL

Antisera were raised successfully against LPL from both rat cardiac muscle and bovine milk. The antisera were tested by the e.i.s.a. screening test and found to exhibit positive reactivity at dilutions in excess of 2000- and 4000-fold respectively; see Figure 1. The bovine milk LPL was a somewhat stronger immunogen than the rat LPL oligomers in the mouse host, with both sera exhibiting clear dilution-related reactivity against immobilized rat cardiac muscle LPL. This was much less clearly the case when rat cardiac LPL conjugated to other protein carriers (e.g. keyhole-limpet haemocyanin) was used as immunogen, when insufficient immunological response was elicited.

In order to test the specificity of the antisera, immunoblots were carried out. Thus mouse anti-(rat cardiac LPL) and mouse anti-(bovine milk LPL) antisera were tested for their ability to detect LPL specifically in extracts of the five tissues listed above or in extracts of acetone/diethyl ether-dried powder of all five tissues. Firstly, Figure 2 shows a representative blot obtained when anti-(rat cardiac LPL) antiserum was used to probe Western blots derived from the electrophoretic separation of extracts of both fresh and acetone/diethyl ether-dried preparations of each tissue. The serum detected a single major molecular species in extracts of each of the tissue powders (Figure 2a). The mean molecular mass of this species was calculated, from three identical but independent immunoblots, to be 61 kDa (S.E.M. = 2.5 kDa) for cardiac muscle, 60 kDa (S.E.M. = 2 kDa) for adipose tissue, 62.5 kDa (S.E.M. = 4 kDa) for skeletal muscle, 60 kDa (S.E.M. = 2 kDa) for mammary tissue, and 60 kDa (S.E.M. = 2 kDa) for lung. Thus no significant difference between the molecular masses of the specifically detected component found with each of the tissues was revealed. The overall

Figure 2 Immunodetection of LPL in preparations of rat tissues by using anti-(rat cardiac LPL) antiserum

The Figure shows the outcome of immunoblotting experiments when anti-LPL serum was used to detect immunoreactive material from preparations of rat tissues Western blotted on to a transfer membrane. The membrane was probed with serum at a dilution of 1:250 in PBS/Tween. After washing, bound antibodies were detected by using a peroxidase/anti-peroxidase system and diaminobenzidine as enzyme substrate. (a) Pattern observed when extracts of acetone/diethyl ether-dried powders (10 µl of a 50 µg/10 µl homogenate) of the five rat tissues were used in the experiment. (b) Pattern observed when extracts of fresh rat tissues (10 µl of a 50 µg/10 µl homogenate) were used. In both cases, mammary tissue, adipose tissue, skeletal muscle, lung and cardiac muscle were applied to tracks 1–5 respectively.

mean molecular mass was 61 kDa, which was identical with that of the LPL isolated from each of the tissue preparations (see Soteriou and Cryer, 1993). In addition to the major species, a number of minor bands were also detected. When extracts of whole fresh tissues (Figure 2b) were examined, a very similar pattern of staining to that described above was observed. In three separate experiments, a major band revealed in this case had a mean molecular mass of 59.5 kDa ± 800 Da (S.E.M.) and a minor band of approx. 32–33 kDa.

When mouse anti-(bovine LPL) serum was used to probe blots of acetone/diethyl ether-dried powders of tissues, the pattern

Figure 3 Immunodetection of LPL in extracts of acetone/diethyl ether-dried powders by using anti-(bovine milk LPL) antiserum

Portions (10 μ l) of 50 μ g/10 μ l homogenates of acetone/diethyl ether-dried powders of the five rat tissues (lanes as in Figure 2) were Western-blotted and probed with mouse anti-(bovine milk LPL) antiserum. The protocol used was as described in the legend to Figure 2 and the Materials and methods section.

was as shown in Figure 3. In this case there were two major bands detected, with the mean molecular masses of these bands, determined in three separate experiments, being 60 kDa (S.E.M. = 2 kDa) and 51.5 kDa (S.E.M. = 650 Da) for each tissue. Again, certain minor bands with lower molecular masses were also detected.

Production of monoclonal antibodies to LPL

When rat cardiac tissue LPL was used as an immunogen, although hybridoma cells were produced, the success rate of the

production of lines secreting significant levels of anti-LPL antibodies was low.

By contrast, the production of hybridomas secreting anti-LPL antibodies from spleen cells taken from mice immunized with bovine milk LPL was considerably more successful. In the splenocyte/myeloma fusions that were carried out, at least 30 % of the resultant hybridoma populations were found to be positive with respect to the secretion of anti-LPL antibodies. Two of these populations were rendered monoclonal.

Comparison of the immunoreactivity of LPL from a range of rat tissues with anti-LPL antibodies

Mouse anti-(rat cardiac LPL) antiserum, mouse anti-(bovine milk LPL) antiserum and the two monoclonal antibodies were each compared for their reactivity with LPL isolated from the five rat tissue sources by using an e.l.i.s.a. system, in which LPL from the range of different tissue sources was coated on to plates and each was probed with the four different antibody preparations. The experiment was carried out on three separate occasions, each time revealing the same pattern of reactivities. Thus a hierarchy of immunoreactivity became apparent when the tissue-derived LPL preparations were compared. This hierarchy was identical for each of the antibody preparations. Table 1 shows the pattern of responses obtained in one of these experiments, together with the mean specific activities of the enzyme preparations used. The hierarchy of immunoreactivity was cardiac > lung > adipose > mammary > skeletal muscle as the comparative sources of LPL. There was no apparent relationship between the specific activity of any of the enzyme preparations and their respective immunoreactivity. The skeletal-muscle preparation was an exception, however, where both the enzyme specific activity and the level of immunoreactivity were the lowest of all the five tissues studied.

An identical hierarchy of immunoreactivity was revealed when enzyme preparations (15 μ g) were subjected to electrophoresis, immunoblotted and probed using the mouse anti-(rat cardiac LPL) antisera. The level of the immune response was quantified by scanning the developed blots with an LKB densitometer. A representative set of scans from one such experiment is shown in Figure 4. The major band [molecular mass 60 ± 1.5 kDa (S.E.M.)], derived from four independent experiments, each including LPL from all five tissue sources, had relative intensities of: cardiac, 1.0; lung, 0.76; adipose tissue, 0.46; mammary gland, 0.32; skeletal muscle, 0.22.

Table 1 Immunoreactivities of LPL isolated from various rat tissues as tested by e.l.i.s.a.

The results of a typical e.l.i.s.a. experiment are shown, in which sera obtained from mice immunized with rat cardiac LPL or bovine milk LPL and two anti-(bovine milk LPL) monoclonal antibodies were tested for reactivity toward immobilized LPL from rat cardiac muscle, lung tissue, adipose tissue, mammary gland and skeletal muscle. The protocol for the standard e.l.i.s.a. was as described in the text. Dilutions (in PBS/Tween) of the antibody preparations used were as follows: 1:500 for the anti-(rat cardiac LPL), 1:2000 for the anti-(bovine milk LPL) and 1:5000 for each of the monoclonals. The specific activities of the LPL preparation are also given. The Table represents one experiment in a replicated series of three such experiments.

Tissue source of LPL	Antiserum ...		A_{492}		Specific activity of antigen (unit/ μ g)
	Anti-(rat cardiac LPL)	Anti-(bovine milk LPL)	Anti-(bovine milk LPL) monoclonal 1	Anti-(bovine milk LPL) monoclonal 2	
Cardiac muscle	0.41	0.62	0.57	0.42	0.40
Lung	0.39	0.43	0.40	0.35	0.25
Adipose	0.33	0.39	0.38	0.29	0.72
Mammary	0.12	0.20	0.21	0.20	0.45
Skeletal muscle	0.1	0.11	0.12	0.11	0.07

Figure 4 Immunoreactivities of LPL isolated from various rat tissues as tested on Western blots

Samples of purified rat tissue LPL preparations (15 μ g) were subjected to electrophoresis and Western-blotted before being probed with mouse anti-(rat cardiac-muscle LPL) antiserum as described in the text. The developed blots were scanned with a laser densitometer, and the resulting scans showing the major immunoreactive species are shown in decreasing absorbance of the peaks. The scans corresponding to LPL purified from cardiac muscle, lung, adipose, mammary and skeletal-muscle tissue are shown from left to right, respectively. The absorbance is shown in arbitrary units, by using the same sensitivity and scale settings in each case.

Figure 5 Competition assay to investigate the binding of putative anti-(LPL-heparin-binding site) antibodies to immobilized LPL in the presence of low-molecular-mass heparin

The e.l.i.s.a. screening assay was modified for use as a competitive assay, in order to investigate the ability of low-molecular-mass heparin (0–0.16 μ g) to inhibit anti-peptide-antibody binding to LPL. Low-molecular-mass heparin was incubated with immobilized bovine milk LPL before addition of serum raised against the synthetic peptide. Other aspects of the e.l.i.s.a. were as described in the text.

Production and purification of synthetic peptide

F.p.l.c. analysis of the peptide (residues 143–155 inclusive: Ser-Arg-Thr-Asn-Thr-Lys-Val-Ser-Arg-Ile-Thr-Gly-Leu) revealed that the synthesized preparation was 93% pure, and therefore appropriate for the immunization of mice.

Production of antisera against the peptide corresponding to the heparin-binding site of LPL

Sera from three cohorts of mice immunized with unconjugated peptide, peptide conjugated to rat serum albumin or peptide conjugated to keyhole-limpet haemocyanin, were obtained and tested against cardiac LPL as described in the Materials and

Table 2 Examination of the immunoreactivity of anti-peptide antiserum with LPL purified from various rat tissues

Results are shown of an e.l.i.s.a. in which isolated LPL from five rat tissues (cardiac muscle, lung, adipose tissue, mammary gland and skeletal muscle) was coated on to an e.l.i.s.a. plate at 2 μ g/ml and probed with serum diluted 1:50 in PBS/Tween. The specific activities of the LPL preparation are also given as a guide.

	Tissue enzyme source				
	Cardiac muscle	Lung	Adipose	Mammary	Skeletal muscle
A_{492}	0.34	0.33	0.37	0.36	0.31
Specific activity (unit/ μ g)	0.38	0.24	0.70	0.44	0.07

methods section. Conjugation of the peptide before immunization was in neither case able to elicit a more intense response than did peptide administration alone (results not shown). Therefore the sera from mice immunized with unconjugated peptide were used subsequently.

Investigation of the reactivity of the anti-peptide antisera with LPL

The reactivity of the anti-peptide antiserum was then studied by using a competitive e.l.i.s.a. (see the Materials and methods section). By such an assay, it was found that, in the presence of low-molecular-mass heparin, the binding of the antisera (1:100 dilution in PBS/Tween) to bovine milk LPL was inhibited (Figure 5). The binding decreased by 20% when up to 0.04 μ g of heparin was added per well. However, further addition of heparin failed to produce any additional decrease in antibody binding.

Comparison of the immunoreactivity of the anti-peptide antiserum with LPL isolated from five rat tissue sources

The reactivity of each purified LPL, from the five rat tissues, with the anti-peptide antisera was compared. The resultant absorbancies (492 nm), together with the mean specific activities of the enzyme preparations, are shown in Table 2. By contrast with the data in Table 1, no significant differences between the reactivities of the LPL from each tissue was apparent when this anti-peptide antiserum was employed.

DISCUSSION

The use of antibodies as tools in the investigation of the properties of individual LPL preparations has been described previously (e.g. Vydelingum et al., 1986; Friedman et al., 1986). However, the present study compares the immunological characteristics of isolated LPL from five different tissue sources taken from a single species.

The molecular mass of the major species, identified on immunoblots by antibodies prepared against the whole LPL molecule, matched that of the isolated enzyme. This was in agreement with those studies reported by Vydelingum et al. (1986) and Friedman et al. (1986). In addition, and in relation to these previous studies, there was also agreement with the observation that a number of additional, lower-molecular-mass, bands were also revealed by the technique of Western blotting. It is most likely that the additional bands were representative of LPL degradation products (Socorro and Jackson, 1985) and/or incompletely processed forms of the enzyme (Amri et al., 1986).

Vydelingum et al. (1986) noted, however, that only one major species of the LPL revealed on immunoblots was glycosylated, and in the present case no such secondary bands were noted when enzyme isolated from each tissue was investigated.

There was similarity between the molecular mass of the major LPL species identified in each acetone/diethyl ether-dried powder extract or whole-tissue extract of the five rat tissues studied. This provided additional evidence that the molecular mass of LPL molecules derived from the different tissues from a single animal species did not vary significantly, as revealed in the previous study on the isolated enzyme preparations (Soteriou and Cryer, 1993).

The detection of two major species when the tissue powder extracts were probed with the anti-(bovine milk LPL) antiserum was distinct from the outcome seen when the anti-(cardiac LPL) antiserum was used. The molecular mass of the 52 kDa component, seen when anti-(bovine milk LPL) antiserum was used, corresponded to that immunodetected by other workers, such as Amri et al. (1986). These latter workers, for example, found that tunicamycin-treated Ob17 cells secreted an inactive LPL of this molecular mass, and it is possible that the species detected in the present study corresponds to this unglycosylated species. However, the reason why the band was detected to a greater degree with the anti-(bovine milk LPL) antiserum by comparison with the anti-(rat cardiac LPL) antiserum is not clear. Perhaps the species seen is more closely related to a prevalent species of inactive LPL found in milk, and therefore acted as a strong immunogen only when the LPL purified from milk was used.

When the immunoreactivities of the isolated enzyme preparations were compared by both the e.l.i.s.a. system and immunoblots, where the data were entirely consistent, a hierarchy of reactivity became apparent. This hierarchy was identical for all four of the antibody reagents that were prepared with whole LPL as immunogen, and was not obviously related to the specific activities of the enzyme preparations used in the assays. The fact that there was no obvious relationship between the specific activity and immunological reactivity of any given tissue enzyme preparation in the e.l.i.s.a. ruled out the possibility that the different immunological reactivities observed were due to variations in the amount of bound antigen, as did the supporting data derived from Western blots. Thus, although adipose-tissue LPL displayed the highest apparent specific activity, it did not display the highest level of immunoreactivity. The immunoreactivities of the LPL preparations derived from the different tissues may therefore be postulated to reflect real tissue-based molecular differences in the LPL molecules concerned. This would also be supported by our previous observation (Soteriou and Cryer, 1993) that the preparations retained the hierarchy of tissue-specific activities throughout the various stages of their purification to materials exhibiting single bands after PAGE.

The common hierarchy, irrespective of the antibody reagent, could lead to the speculation that the major part of the immune response elicited in the mice was due to the existence of only a few immunological determinants. That is, the monoclonal anti-(bovine milk LPL) antibodies were directed against the same major determinants on the LPL molecule as were the major antibodies species present in the polyclonal anti-(rat cardiac LPL) antiserum. However, it is unknown whether the two distinct clones produced different antibodies, since competitive binding studies were not carried out.

The data arising from the e.l.i.s.a. and from immunoblotting studies were identical, and this would suggest that the orientation, and hence availability of antigenic determinants, of LPL on both the e.l.i.s.a. plate surface and the blotting membrane were similar.

This result was unexpected, because Voyta et al. (1985) found that only two monoclonal antibodies, out of a total of ten produced by using bovine milk LPL and found to react with the enzyme in an e.l.i.s.a., reacted with enzyme that had been Western-blotted. It may be proposed that, in the study described by Voyta et al. (1985), the predominant antigenic determinants were masked by the immobilization process, but that this was not the case for the situation described in the present work.

The use of synthetic peptide immunogens as tools for the comparison of the immunoreactivity displayed by different tissue LPL preparations was a novel approach. With other antigens, Lerner (1982, 1984) found that anti-peptide antibodies, which reacted with the corresponding whole protein from which the peptide was derived, could be designated antibodies of predetermined specificity. That this general attribute was found to be the case in the specific case studied here, therefore, is not surprising. In addition, in this study, the anti-(heparin-binding-site peptide) antibodies could be demonstrated to be inhibited, to a degree, in their binding to LPL by low-molecular-mass heparin.

That the decrease in binding of anti-peptide antibody produced by low concentrations of heparin could not be made total by increasing the heparin concentration would suggest one of two possibilities: either the antibody had a greater affinity for the LPL than the heparin, or that, at high concentrations, heparin was binding to additional sites on the LPL molecule, other than the sequence corresponding to the sequence of the peptide. The observation that the binding of heparin to LPL could not be decreased below a threshold has been noted in other studies, for example Hata et al. (1993), where basic residues in segments of the primary sequence of human LPL were replaced with alanine. Thus Hata et al. (1993) found that five basic residues in two distinct segments were critical determinants for high-affinity heparin binding. Those residues were distinct from the peptide sequence of basic residues, postulated to be part of the heparin-binding site, and which was used as the immunogen in the present study. Thus it may be possible that, despite the low-molecular-mass heparin binding to the sequence of residues numbered 143–155, in the competitive e.l.i.s.a.-based assay, heparin could also bind to other regions on the LPL molecule (see also Davis et al., 1992). This additional binding, according to Hata et al. (1993), had at least an equal, if not greater, affinity for heparin and would explain how, when the concentration of heparin was increased from 0.04 to 0.16 μg per well, there was no further decrease in anti-peptide-antibody binding.

When the anti-peptide antibody was used in the e.l.i.s.a., to compare the immunological reactivities of a series of LPL preparations, there was no significant variation observed between the preparations of different tissue origins. This can be contrasted with the hierarchy seen when the antibodies raised against the whole molecule were used. Thus it appears that, with the tissue LPL species studied, the distinguishing antigenic determinants with which the monoclonal and anti-LPL antisera reacted were distinct from, and less highly conserved than, the postulated heparin-binding site (sequence and/or three-dimensional structure). This would be expected when considering the high degree of sequence conservation of the heparin-binding site of LPL from different species (Cooper et al., 1989) and, indeed different proteins (Persson et al., 1989).

In summary, this study has presented unique immunological evidence for the existence of molecular variations of LPL isolated from different tissues of a single species. These differences could arise from the same unknown molecular variations which gave rise to variations in the PI values discovered in our previous study (Soteriou and Cryer, 1993) or may arise from different distinguishing features. In either case, these differences are most

likely to be introduced post-transcriptionally. Other studies of interspecies immuno-cross-reactivities (I. M. George, A. Cryer and H. D. Griffin, unpublished work) indicate that immuno-detectable differences between LPL molecules of distinct species origin are not related to the glycosylation state of the molecule; however, this does not exclude this as a possible source of distinction when enzyme from different tissues of the same animal species are studied, as was the case here.

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