Lipoprotein lipase: size of the functional unit determined by radiation inactivation

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Abstract Radiation inactivation was used to determine the functional molecular weight of lipoprotein lipase (LPL) in rat heart and adipose tissues. This technique reveals the size of the smallest unit required to carry out the enzyme function. Supernatant fractions of the tissue homogenates were exposed to high energy electrons at −135°C. LPL activity showed a simple exponential decay in all samples tested. Because changes in nutritional state shift the distribution of LPL between the capillary endothelial and parenchymal cells within heart and adipose tissues, fasted and refed rats were used for the radiation studies. The functional molecular weight was calculated to be 127,000 ± 15,000 (mean ± SD) daltons for heart and adipose. Thus, the smallest unit required for enzyme function was the same in both of these tissues and did not vary with nutritional state. The data suggest that, compared with LPL monomer sizes reported in the range 55,000 to 72,000, this active unit constitutes a dimer.—**Garfinkel, A. S., E. S. Kempner, O. Ben-Zeev, J. Nikazy, S. J. James, and M. C. Schotz.** Lipoprotein lipase: size of the functional unit determined by radiation inactivation. *J. Lipid Res.* 1983. **24:** 775–780.

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An obligatory step in the transport of triglyceride fatty acids from circulating chylomicrons and VLDL into tissues is the hydrolysis of the triglyceride core of these lipoprotein particles by the enzyme, lipoprotein lipase (LPL) (1). It is well documented that LPL is located both at capillary endothelial surfaces and in parenchymal cells of heart and adipose tissues. Presumably, it is the fraction of LPL at the luminal surface of the capillary that functions in vivo in triglyceride hydrolysis. The intracellular LPL is considered to be the precursor of the endothelial enzyme (2). However, the precise relationship between the LPL fractions at the separate tissue sites is not clear. A difference in molecular size, possibly reflecting different subunit composition, has been proposed (3). Subunit molecular weights for LPL determined under denaturing conditions have been reported generally in the range of 55,000–72,000 (4–10). In contrast, the native enzyme apparently has a molecular weight of the order of 100,000–120,000 (4, 11–15) and may be a dimer in solution (15).

Among the methods available for determining the molecular size of enzymes, the technique of radiation inactivation (16) provides a special advantage in that the enzyme does not need to be purified. With this technique, loss of biological activity with increasing radiation exposure is measured, and the size of the functional unit, the "functional molecular weight", rather than a structural object is measured.

We have employed target analysis of radiation inactivation to determine the functional molecular weight of LPL in rat heart and adipose tissues. The data reveal only one functional molecular weight, 127,000 daltons, for LPL in these tissues. Thus, this is the smallest structure required to carry out the hydrolytic function of the enzyme. Based on monomeric molecular weights in the 55,000–72,000 range, this active unit would constitute a dimer.

MATERIALS AND METHODS

Enzyme preparation

Tissues were obtained from male Sprague-Dawley rats weighing 160–250 g. The animals were either fasted overnight or fasted overnight and refed for 18 hr with rat chow plus 20% glucose in the drinking water. After decapitation of the rats, the epididymal adipose tissue and hearts were excised and homogenized at a ratio of 100 mg or 50 mg/ml buffer, respectively. The buffer used was 5 mM barbital, pH 7.2, containing 20% glycerol. Homogenates pooled from four rats were centrifuged at 25,000 *g* for 1 hr at 4°C. The supernatant

Abbreviations: LPL, lipoprotein lipase; SDS, sodium dodecylsulfate.

fraction of the heart preparations and the infranate of the adipose tissue preparations were used as enzyme sources.

Glucose-6-phosphate dehydrogenase ("Zwischenferment" from Baker's yeast, Type VII, Sigma Chemical Co., St. Louis, MO) was added to the supernatant fractions (5 U/ml) and 0.5-ml aliquots were dispensed into 2.0-ml glass ampoules, frozen immediately in dry ice, sealed, and shipped in dry ice to Bethesda, MD.

Radiation inactivation

The frozen samples were irradiated at the Armed Forces Radiobiology Research Institute (Bethesda, MD) employing a linear accelerator generating electrons with an energy of 13 MeV at a dose rate of 30 Mrads/hr. The logarithm of the fraction of LPL activity remaining after increasing radiation doses was a linear function of the radiation dose (in rads), and the slope of this function, K, was determined by linear regression analysis constrained to pass through 1.0 at zero dose. The molecular weight of the enzyme unit was calculated from the relation (17) molecular weight = $6.4 \times 10^{11}\ S_t K$, where S_t is a temperature factor. The temperature was maintained during irradiation at $-135 \pm 2°C$ with a stream of cold nitrogen gas, and at this temperature S_t has been experimentally determined to have the value of 2.8 (18). Alternatively, the temperature factor for a given irradiation was determined from radiation inactivation of several internal standard proteins.

Enzyme assay

LPL was assayed (19) using as substrate serum-activated trioleoylglycerol containing a trace of tri [9,10(n)-^{3}H]oleoylglycerol (111.1 Ci/mmol, New England Nuclear, Boston, MA). One mU of LPL activity represents the release of 1 nmol free fatty acid per min. Glucose-6-phosphate dehydrogenase was assayed as described by Olive and Levy (20). Protein was determined by the method of Bradford (21).

RESULTS

Adipose tissue

LPL activity of the tissue homogenates employed was stable to freezing and storage at temperatures ranging from −40° to −135°C. When frozen supernatant fractions of epididymal adipose homogenates from glucose-refed rats were exposed to up to 60 Mrads from a linear electron accelerator, adipose LPL activity showed a simple exponential decay with radiation dose (**Fig. 1**). The adipose LPL functional molecular weight was calculated to be 126,000 ± 24,000 (mean ± SD). The single slope described by the data points indicates that most of the enzyme activity resides in a single specie. The experiments shown in Fig. 1 reveal that less than 5% of the initial activity could have been due to functional units of other sizes (16).

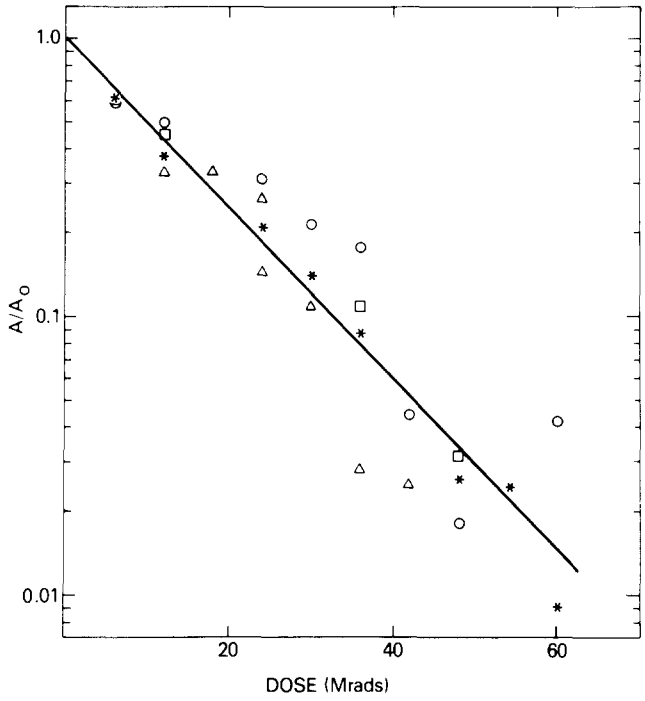

Fig. 1. Inactivation of lipoprotein lipase activity from adipose tissue of fed rats by high energy electron irradiation. Frozen samples of the supernatant fraction of epididymal adipose tissue homogenates were irradiated at −135°C. The fitted line is from constrained least squares analysis. Symbols (*, □, △, ○) represent four separate experiments.

Heart tissue

On exposure of LPL extracts from hearts of fasted rats to ionizing radiation (**Fig. 2**), a simple exponential decay with radiation dose was again observed. The calculated size of the heart LPL specie, 129,000 ± 8,000 daltons (mean ± SD), did not differ significantly from that of adipose tissue (**Table 1**).

Fed vs. fasted

Since LPL activity in heart and adipose tissues varies in an opposite manner in different nutritional states, the LPL functional molecular weight was also determined in hearts of fed rats and in adipose tissue of fasted animals. In each case a simple exponential decay of enzyme activity was observed with increasing radiation dose. In neither tissue did the result differ from that obtained with the opposite nutritional state (Table 1). Thus, the size of the active LPL unit apparently does not vary with the nutritional condition of the animal, and the size is also the same in both adipose and heart tissues.

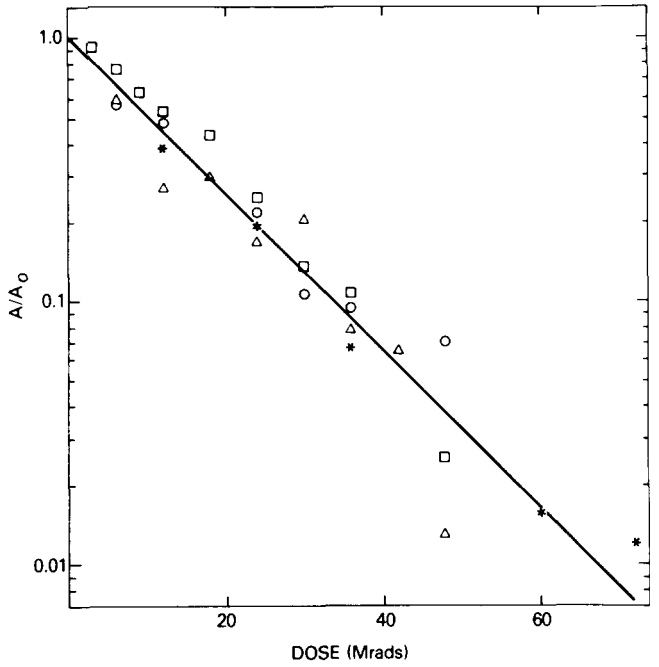

Fig. 2. Inactivation of lipoprotein lipase activity from heart tissue of fasted rats by high energy electron irradiation. Frozen samples of the supernatant fraction of heart tissue homogenates were irradiated at −135°C. The fitted line is from constrained least squares analysis. Symbols (*, □, ○, △) represent four separate experiments.

It should be noted that the similar functional molecular weights (Table 1) in both fed and fasted adipose tissue and heart were obtained from tissues in which the LPL specific activity (mU/mg protein) differed by 3- to 5-fold (**Table 2**).

Table 1 summarizes the entire series of experiments. The collective data show the functional molecular weight of LPL in heart and adipose tissues to have a single value of the order of 127,000 ± 15,000 daltons (mean ± SD).

The functional molecular weight obtained for the internal standard, glucose-6-phosphate dehydrogenase, was 114,000 ± 13,000 daltons (mean ± SD) (**Fig. 3**). The molecular weight of this enzyme apoprotein obtained from sedimentation equilibrium studies in the presence of guanidinium chloride is reported to be 102,000 (22).

TABLE 1. Functional molecular weight of LPL

Tissue	Molecular Weight	
	Fed	Fasted
	daltons	
Heart	134,000 ± 22,000 (2)[a]	129,000 ± 8,000 (3)
Adipose	126,000 ± 24,000 (4)	123,000 ± 16,000 (3)

[a] Values shown are means ± SD for the number of experiments in parentheses.

TABLE 2. LPL activity in tissues of fed and fasted rats

Tissue	Exp	LPL Activity	
		Fed	Fasted
		mU/mg protein	
Heart	1	1.6 ± 0.3[a]	5.4 ± 0.8
	2	1.7 ± 0.2	6.5 ± 1.1
Adipose	1	26.3 ± 3.7	9.1 ± 0.5
	2	36.7 ± 8.1	9.3 ± 0.7

[a] Values shown are means ± SD for four animals per group.

DISCUSSION

The technique of radiation target theory analysis (23), which we have employed to estimate the LPL functional molecular weight, shows the size of the active enzyme unit to be 127,000 ± 15,000 daltons. This technique has the major advantage that enzyme purification is not required. Thus, any artifacts introduced by various purification methods are eliminated. Further, the radiation sensitivity of enzymes is the same in vivo as in cell extracts or purified preparations, thus permitting examination of membrane-bound systems (24).

Our data are substantial evidence that active LPL in both rat heart and adipose tissue is a single unit of approximately 127,000 daltons. A diversity of values has

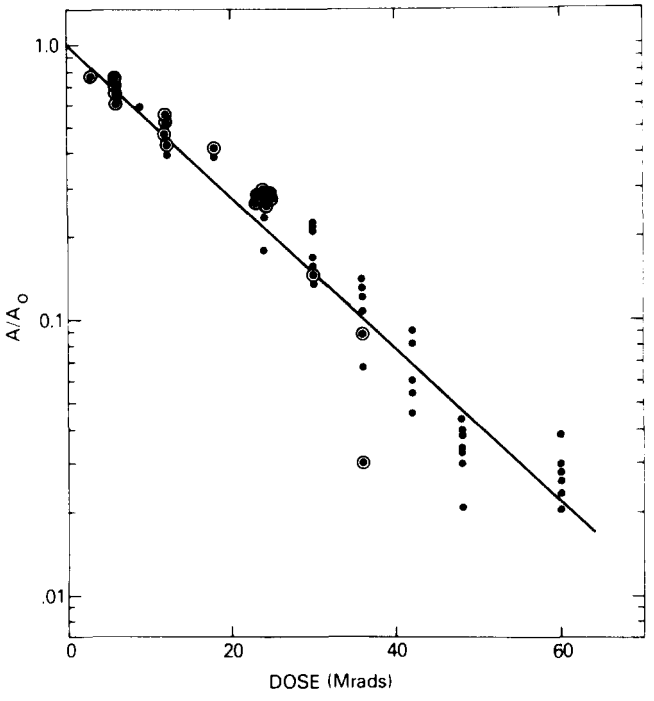

Fig. 3. Yeast glucose-6-phosphate dehydrogenase activity after increasing doses of radiation. This enzyme, added to supernates of rat tissue homogenates as an internal standard, was irradiated in the frozen samples at −135°C. The fitted line is from constrained least squares analysis. Data from fourteen separate experiments.

been reported for the molecular weight of LPL, perhaps resulting from the fact that the purification methods are dissimilar in some aspects. Also, the possibility of actual species and tissue differences cannot be excluded, since there is incomplete agreement in reported amino acid composition of the LPL enzymes that have been analyzed (1).

The reported values for the molecular weight of LPL fall into three general categories, one category in the range 55,000–73,000 (4–10). A second category of two considerably lower values, 34,000 (25) and 37,500 (4), has been reported. With two possible exceptions (4, 8), these values were obtained under denaturing conditions. Under such conditions where no enzyme activity is measurable, identification of the fraction(s) representing LPL protein frequently becomes uncertain (7). Compared with that range of molecular weights obtained for LPL under denaturing conditions, a third category of values, 100,000–150,000 (4, 11–15), approximately twice the first set, has been found for the "native enzyme". Our determination of 127,000 for the functional molecular weight clearly coincides with the last group and in relationship to the 55,000–73,000 category would constitute a dimer.

In a study in which enzyme activity could be measured, Bensadoun et al. (8) found the monomer molecular weight of pig adipose LPL to be 62,000 by gel filtration in a buffer containing 1 M NaCl. The authors point out that 1 M NaCl may dissociate the enzyme and with appropriate handling reassociation is not prevented. In another study, Fielding, Shore, and Fielding (4) reported an LPL monomeric molecular weight of 37,000, determined by several procedures including ultracentrifugation and sedimentation equilibrium centrifugation. Eighty percent of this enzyme activity was lost during preparation. In addition to the 37,000 monomer "not excluding the existence of an even smaller molecular weight of 17,000", a dimer was found of 72,000 and an oligomer of 145,000. The 145,000 molecular weight was obtained for lipase "not pretreated with detergent".

In further studies of the native enzyme, Iverius and Ostlund-Lindqvist (15), employing sedimentation and diffusion coefficients, determined native bovine milk LPL to be a dimer of molecular weight 98,000. This value was confirmed by Bengtsson and Olivecrona (12); in their study milk LPL was eluted from Sephadex G-150 columns in a position corresponding to that of a globular protein of molecular weight 100,000–110,000. This is in contrast to the finding of Bensadoun et al. (8) that pig adipose LPL treated in a similar manner eluted at 60,000. However, Bengtsson and Olivecrona (12) point out that, since LPL is a glycoprotein and binds detergents, the elution position in 1.5 M NaCl and 5 mM deoxycholate does not accurately estimate the molecular weight. In addition, these workers suggest that smaller LPL units may represent enzyme proteolytically cleaved in vivo or during preparation (cf. Ref. 4).

Utilizing sucrose gradient centrifugation and a crude LPL extract from acetone powder of rat adipose tissue, Ashby, Tolson, and Robinson (14) recovered the enzyme activity quantitatively in a single peak. Comparison with the sedimentation rates of marker proteins indicated a molecular weight for LPL in the region of 120,000. Similar results were obtained from adipose tissue of either fed or fasted rats. Therefore, these workers suggested that rat adipose preparations contained only one LPL specie similar in size to the form b (11) that we described previously.

The rationale for examining tissues from both fed and fasted animals is based on the generally accepted view that in heart tissue of carbohydrate-fed rats LPL activity is almost entirely in the parenchymal cells (2), whereas in the fasted animal a large proportion of the enzyme is bound to the luminal surface of the capillary endothelium (26). In adipose tissue the enzyme location is reversed under these nutritional conditions; that is, in fasted rats the adipocytes contain most of the LPL activity, while a large proportion of the enzyme is outside of the adipocytes in the fed state (3). If two active LPL units of differing molecular weights (e.g., an "active" monomer and an "active" dimer) were present in the tissues, they would be most likely to be detected in the hearts of fasted rats and in adipose tissue of fed rats. The inactivation of a mixed population of active monomers and dimers can be predicted (16). Comparison with the data shown in Figs. 1 and 2 indicates that any putative active monomers cannot have contributed more than 5% to the original activity. The present results suggest that in both heart and adipose tissues a single active LPL unit, a dimer, is present. If a monomer exists in these tissues, it may either be inactive or constitute a small percent of the active enzyme protein. From the radiation data presented here we can find no evidence for a functionally active monomer in any tissue under any nutritional state.

It is generally agreed that LPL is a glycoprotein. From studies of the glycoprotein, invertase, it was reported (27) that covalently-bound oligosaccharides were not detected by target analysis, a result supported by radiation studies of several other glycoproteins.[1] Thus the target size reported here should be interpreted as

[1] Kempner, E. S. Unpublished observations.

only the protein fraction of the LPL enzyme, and comparisons should be made with the conventional molecular weight determinations of carbohydrate-free preparations.

The observed target size can depend on the physical state of the sample during irradiation (28, 29) as well as on the conditions during assay (23). In all these cases, the target sizes determined under various conditions were different multiples of some basic monomeric structure. Therefore, it should be emphasized that, as in all other scientific enquiries, the observed measurements are valid for the stated experimental conditions. The interpretation of these target sizes for LPL is consistent with a dimer of the monomer band seen in SDS polyacrylamide gel electrophoresis (4–10). Under our experimental conditions, the expression of LPL hydrolytic activity requires the presence of two intact monomer polypeptides.

Our data provoke the interesting speculation that inhibition or inactivation of LPL activity by 1 M NaCl, classically a prime criterion for identifying LPL, may reflect dissociation of the active dimer into inactive monomers, the reaction being more or less reversible depending on the experimental conditions selected. The effects of NaCl on LPL have been studied extensively (30–33). There is fairly general agreement that NaCl affects the interaction between the enzyme and its apoprotein cofactor, although the mechanism of this interaction is not at all clear. Hypothetically, an easily dissociated dimer suggests a possible regulatory mechanism for the enzyme. We expect to test this hypothesis by seeking an inactive monomeric LPL precursor by means of radiolabeling and radioimmunoassay.

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