Effects of glycerol on human adipose tissue triglyceride lipase activity

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Summary Glycerol fully protects the human adipose tissue triglyceride lipase against the denaturing effects of high and low temperatures. Under such protection, storage of crude preparations at −10°C or incubation at 50°C resulted in a 1.5–3-fold increase of the measured lipase activity. This increase was shown to be related to enzyme newly released from tissular microparticles present in the samples. Advantage may be taken of these observations to improve greatly the conditions of extraction and storage of this lipase activity.

Supplementary key words heat denaturation · cold denaturation · saccharose · propanediol · extraction · storage

The triacylglycerol acylhydrolase activity present in human adipose tissue is often referred to as "hormone-sensitive" lipase (1, 2). In vitro determination of its level of activity as well as attempts to carry out its purification have been especially hindered by the very low levels of enzyme extractable from human fat and by the high susceptibility of this lipase to numerous physical and chemical agents. We show in this paper that glycerol, rather specifically among other polyols, has a protective effect on the activity of this lipolytic system when it is exposed to high and low temperatures.

Materials. Glycerol tri[9,10-^{3}H]oleate (0.5 Ci/mmole) (Radiochemical Centre, Amersham) and unlabeled triolein (Sigma Chemical Co.) were purified first by

chromatography on Florisil (3). The formation of methyl and/or ethyl oleate by a Florisil-catalyzed transesterification reaction was avoided by using freshly distilled solvents and reducing the duration of the elution steps to less than 4 hr (4). The triglycerides eluted from Florisil were then subjected to thin-layer chromatography on silica gel using a system previously published (5). The final radiochemical purity was virtually 100% when the labeled triolein was rechromatographed on thin-layer plates in the same system as that used for purification. Bovine serum albumin, cyclic 3′,5′-AMP, and ATP were obtained from Sigma Chemical Co.

Methods. Specimens of subcutaneous adipose tissue were obtained from human subjects undergoing surgery. The samples were immediately rinsed in 0.05 M sodium phosphate buffer (pH 7.4) and homogenized in the same medium at 4°C in a glass–Teflon tissue grinder (4 ml/g of tissue). The homogenates were centrifuged at 4°C either at 12,000 *g* for 30 min or at 100,000 *g* for 1 hr. The solid fat cakes were carefully removed from the tops of the homogenates after centrifugation, and the infranatant fluid was used as source of enzyme. The protein concentrations were determined by the method of Lowry et al. (6); crystalline bovine serum albumin served as standard. Appropriate concentrations of glycerol were added to the reference samples when glycerol-containing extracts were measured. All concentrations of solvents are expressed as final concentrations.

Emulsification of the substrate [^{3}H]triolein (0.5 mM) was achieved by sonication in 0.05% bovine serum albumin in a Branson Sonifier (microtip, about 10 W/ml) for 30 sec at 4°C. The assays were performed at 37°C in a final volume of 10 ml containing the substrate, ATP (10^{-4} M), cyclic 3′,5′-AMP (2×10^{-6} M), $MgCl_2$ (3.6×10^{-3} M), sodium phosphate buffer (10^{-2} M), pH 7.0, and the enzyme (usually 2 ml of extract). Under these experimental conditions, the enzyme shows a maximum activity at pH 7.2 ± 0.2. Sodium taurocholate was not included in the assay system because it was found to be either without effect or inhibitory, depending on the concentration used. Assays were performed with constant agitation of the incubation mixture. 1-ml aliquots of the medium were removed 0, 5, and 10 min after the addition of enzyme. The content of [^{3}H]oleic acid was determined after isolation of the acids by using a liquid–liquid partition system previously described (7, 8). Traces of [^{14}C]-oleic acid were added to each assay system and served to evaluate the recovery of [^{3}H]oleic acid from each aliquot (60–90%). The technique for counting ^{3}H and ^{14}C has been previously described (4). 1 unit of activity corresponds to the release of 1 μmole of acid/min.

Under the assay conditions, enzymatic kinetics are of zero order for at least 20 min within the range of activity used (0.2–0.5 mU) and give a linear dose-response curve, whether or not glycerol is present in the assay mixture. Boiling the extracts for 2 min completely abolished the measured activity. The use of a neutral pH and the absence of cofactor lipoprotein in the incubation mixture reduce to a minimum the contribution of lipoprotein lipase (if any) to the lipolytic process.

TABLE 1. Influence of various thermal treatments on human adipose tissue lipase activity in presence or absence of polyols

Addition to Extracts	Lipase Activity			
	Control 4°C	Cold Treatment −10 ± 2°C	Heat Treatment 50°C	Heat Treatment 55°C
		%		
None	100	0–10	20	0
Glycerol				
5%	100	150	190	
25%	100	200	250	52
50%	100	225	320	
Propanediol, 25%	100	46	0	
Saccharose (w/v), 25%	100	50	110	0

The extracts (12000 *g* infranatant fractions) were prepared at 4°C in 0.05 M sodium phosphate (pH 7.4). Lipase activity (mU/ml of extract) refers to the initial reaction rates determined graphically by plotting the amounts of [^{3}H]oleic acid released 0, 5, and 10 min after the addition of enzyme. The active preparations contained 0.3–1.2 mU of lipase/mg of protein, depending on the samples of adipose tissue used. The activity did not vary when assayed shortly after the addition of glycerol, propanediol, or saccharose at the indicated concentrations to the extracts kept at 4°C. For each extract this value was taken as control value (100%). The cold treatment was carried out by placing the samples at −10 ± 2°C for 24 hr and then in an ice bath prior to assay. The observed changes of enzymatic activity could not be related to the physical state (frozen or not) of the samples. The heat treatment was carried out by incubating the extracts at 50 or 55°C for 90 min. The percentages shown derive from values obtained in individual experiments. Each value was determined by duplicate assays, which were reproducible within 5% of [^{3}H]oleic acid released. Two to four experiments of each type were performed, showing variations of the degree of increase of the lipase activity ranging from 30 to 50%.

Results and Discussion. The lipolytic activities of the different extracts prepared at 4°C were measured in all cases before thermal treatment, and the levels of activity were taken as control values (100%).

It can be seen in Table 1 that, in the absence of a polyol, storage at −10°C resulted in a complete or almost complete loss of the lipase activity contained in a 12,000 *g* infranatant fraction. In contrast, the activity was markedly enhanced when the same treatment was applied to extracts that had been supplemented with different concentrations of glycerol. The increase of activity was roughly proportional to the concentration of added glycerol. Propanediol and saccharose offered some protection against the cold inactivation. Somewhat different results were obtained when a 100,000 *g* infranatant fluid was subjected to the same treatment. In the absence of added glycerol, the lipase activity again underwent irreversible denaturation. This loss could be entirely prevented by inclusion of 25% glycerol (final concentration) in the extract, but no increase of activity was observed.

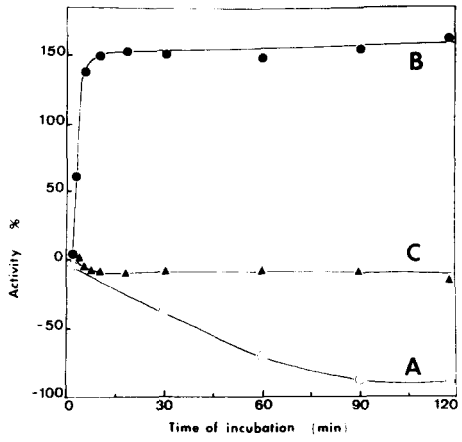

Fig. 1. Variation of the measured lipase activity of human adipose tissue extracts incubated at 50°C in the presence or absence of 25% glycerol. Aliquots were pipetted from the extracts at the indicated times and immediately assayed at 37°C. Activities (mU/ml of extract) are expressed as percentages of control values measured in the extracts kept at 4°C, before incubation. Curve *A*, 12,000 *g* infranatant fraction without glycerol; curve *B*, 12,000 *g* infranatant fraction with 25% glycerol; curve *C*, 100,000 *g* infranatant fraction with 25% glycerol. Values are those derived from individual experiments as indicated in the legend to Table 1.

As shown in the table, a 12,000 *g* infranatant fraction prepared in 0.05 M sodium phosphate buffer (pH 7.4) lost about 80% of its lipase activity by incubation at 50°C for 1 hr. The same heat treatment applied to extracts that contained glycerol initially not only preserved the enzyme but markedly raised the levels of the measured lipase activity. Other polyols were not equally effective. Saccharose protected the activity against the inactivation at 50°C, whereas propanediol seemed to increase the susceptibility of the enzyme to heat. Glycerol-free extracts incubated at 55°C showed a complete inactivation, and a much lesser degree of protection was obtained by glycerol.

At 4°C, a glycerol-free extract still contained 80–100% of its initial lipase activity after 8 days. In 25% glycerol, a slight increase of activity (10–20%) was occasionally observed.

Fig. 1 shows the effects of incubation at 50°C on the lipase activity as a function of the duration of incubation and the nature of the enzymatic preparation. Without glycerol, the enzymatic activity contained in a 12,000 *g* infranatant fraction was almost completely lost after 1 hr (curve *A*). After 1 hr of incubation, the addition of 25% glycerol to this extract did not restore the original activity. In the presence of glycerol, the effect of incubation at 50°C depended on whether the enzymatic preparation was a 12,000 *g* (curve *B*) or a 100,000 *g* (curve *C*) infranatant fraction. Within 5 min, the former showed a sharp increase of the measured lipase activity. In contrast, glycerol protected the latter from inactivation, but no enhancement of activity was observed.

The effect of glycerol as a potential lipase activator in dry foods has been studied by Guardia and Haas (9), who used hog pancreatic lipase as a model. Temperatures and glycerol concentrations were varied in the assay system, so that it is impossible to know from their experiments whether the observed changes of activity were due to effects bearing on the chemical process of catalysis or on the levels of enzyme in the lipolytic medium. Our data show that, in the case of the human triglyceride lipase, glycerol does not interfere in the hydrolytic process catalyzed by the enzyme at 37°C but is associated with apparent changes of activity in the extracts.

First, glycerol protects the enzyme against cold and heat denaturation by a mechanism unknown at this time. Very recently, Bradbury and Jakoby (10) presented indirect evidence that a glycerol-induced conformational change was responsible, at least in part, for the stability at 2°C or upon freezing and thawing of yeast aldehyde dehydrogenase.

Second, when crude extracts were used as a source of lipase, heating at 50°C or freezing and thawing in the presence of glycerol always produced a substantial augmentation of activity. Since such changes were not observed in particle-free preparations, they could be ascribed to enzyme newly released from tissue microparticles present in the crude extracts under the physico-chemical conditions of the experiment.

The remarkable property of glycerol to stabilize human adipose tissue lipase is of practical importance. Glycerol permits the prolonged storage of active preparations in the cold. One may also take advantage of a well-controlled heat and/or cold treatment to markedly improve the yield of the enzyme from tissue samples.

Finally, the demonstration of the heterogeneous condition of triglyceride lipase in aqueous extracts of adipose tissue points to the uncertainty of the in vitro determinations of the lipolytic activity of such crude preparations.

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