FATTY ACID INHIBITION OF TRIACYLGLYCEROL LIPASE IN MITOCHONDRIAL FRACTIONS FROM BAKER'S YEAST

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1. Introduction

Membrane-associated enzymes present in mitochondrial fractions isolated from baker's yeast (*Saccharomyces cerevisiae*) are able to hydrolyze various emulsions of trioleoylglycerol at a high rate [1,2]. Since albumin is commonly introduced in the reaction mixture in studies dealing with enzymes acting on lipid substrates in order to increase the reaction rate [3] its effect on this particulate lipolytic activity has been investigated. A triacylglycerol emulsion with known particle diameter and free of excess emulsifying agent [2] has been used as substrate.

2. Materials and methods

2.1. *Preparation of mitochondria*

Commercial baker's yeast (*Saccharomyces cerevisiae*) was mechanically disrupted in a medium containing 0.25 M mannitol, 10 mM Tris–HCl, pH 7.5 and 0.1 mM EDTA using glass beads [4]. The mitochondria were isolated from the homogenates by differential centrifugation [1] in 0.25 M mannitol, 10 mM Tris–HCl, pH 7.5 and the final pellet of purified mitochondria was resuspended to a protein concentration of about 40 mg/ml and used on the day of preparation.

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2.2. *Preparation of gelatine stabilized trioleoylglycerol emulsions*

A 4% (w/v) gelatine in distilled water was brought to 50°C for 10 min and then allowed to cool to 25–35°C. To 3 ml 100 μl glyceryl tri([1-^{14}C]oleate), spec. act. 20 μCi/mmol was added and the mixture was sonicated at 25–35°C in a MSE 150 W ultrasonic disintegrator (3 × 45 s; power, low 1). The lipid droplets were isolated and sized by means of differential centrifugation [2]. Emulsions containing particles with an average diameter of about 0.5 μm were used.

2.3. *Lipase assay*

The amount of ^{14}C-labelled oleate released during incubation was determined by liquid scintillation counting after extraction of the incubation mixture with isopropyl alcohol/heptane/0.1 M H_2SO_4 (40 : 10 : 1, v/v/v) [5] and separation of the fatty acids according to [6]. Unlabelled free fatty acids were determined according to [7] after isolation by thin-layer chromatography (TLC). Palmitic acid was used as standard.

2.4. *Other methods*

The composition of the free fatty acid present in the mitochondrial preparations was determined by gas–liquid chromatography (GLC) on the methyl esters. Fatty acid free bovine serum albumin was produced by treatment of Cohn Fraction V albumin with charcoal [8]. Protein was determined by the method [9] with crystalline bovine serum albumin as the standard.

2.5. *Chemicals*

Glyceryl tri([1-^{14}C]oleate) was obtained from the Radiochemical Centre (Amersham) and unlabelled triolein from the Hormel Institute (Austin, MI). Crystalline and Cohn fraction V bovine serum albumin and gelatine was obtained from Sigma (St Louis). All other chemicals were of analytical grade.

3. Results and discussion

3.1. *Inhibition of the triacylglycerol lipase by free fatty acids*

The time course of the hydrolysis of gelatine stabilized trioleoylglycerol emulsions by the mitochondrial fractions, as measured by the rate of free fatty acid release, was followed in the presence of different amounts of defatted bovine serum albumin (fig.1). In the absence of albumin the fatty acid release rapidly decreased with time and only a small fraction of the added triglyceride was hydrolysed (the reaction was neither first nor second order). When albumin was added, the rate increased with the albumin concentration up to about 2 mg albumin/ml. At higher concentrations (up to 16 mg/ml) the period of constant initial velocity and the percentage of hydrolysis attained were approximately proportional to the albumin concentration.

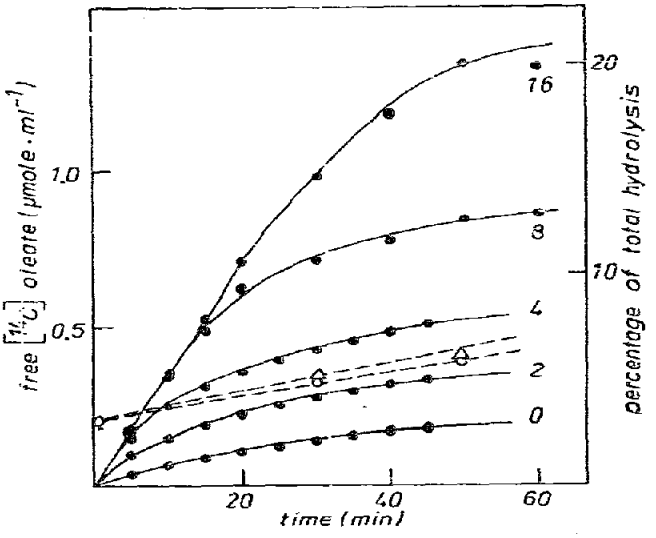

Fig.1. Time course of the hydrolysis of gelatine stabilized trioleoylglycerol emulsions by the mitochondrial fraction in the absence and in the presence of defatted bovine serum albumin (from 2.0–16 mg/ml). The reaction mixture contained 150 mM Tris–HCl, pH 7.5, 75 mM mannitol, 1 mM EDTA, 2.6 mg mitochondrial protein and 2.2 μmol trioleoylglycerol/ml. Temperature 30°C. Dotted lines: Free Fatty acids in the mitochondrial fraction in the absence (○) and in the presence of 4 mg albumin/ml (△).

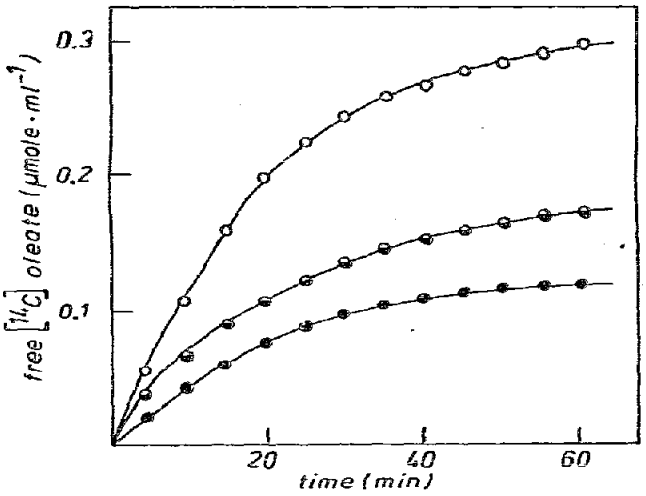

Fig.2. Time course of the hydrolysis in the presence of serum albumin (4 mg/ml) and 0 (○), 118 (⊖) and 235 (●) nmol oleate/ml added as the albumin complex. The reaction mixture contained 2.0 mg mitochondrial protein/ml and 2.05 μmol trioleoylglycerol/ml. Reaction conditions as in fig.1.

The activating effect of albumin decreased when albumin was replaced by albumin–oleate complexes (fig.2). This result clearly indicate that albumin acts as an acceptor for inhibitory free fatty acids [10].

A more precise analysis of the relation between albumin and free fatty acid effects was attempted by taking into consideration also the free fatty acid present in the mitochondrial fraction. The free fatty acid distributes itself (as long as equilibrium is maintained) between the albumin fatty acid binding sites, the aqueous phase, the triglyceride and the mitochondrial membranes according to affinity and volume of the appropriate phase. The amount of free fatty acid in the mitochondrial fractions immediately after preparation was 40–80 nmol/mg mitochondrial protein: palmitic (25%), palmitoleic (25%), stearic (10%) and oleic acid (30%). After 60 min

Table 1
Distribution of free fatty acids between the albumin fatty acid binding sites and the particles of the mitochondrial fraction

Albumin (mg × ml^{-1})	Free acid in 1 ml incubation mixture (nmol)	
	Supernatant	Pellet
0.0	10	188
2.0	125	55
4.0	138	40
8.0	143	10

The total amount of free fatty acid present in the supernatant and the pellet was measured after centrifugation at 50 000 × g for 30 min of a mitochondrial fraction incubated in a medium containing 150 mM Tris–HCl, pH 7.5, 75 mM mannitol, 1 mM EDTA and various amounts of defatted bovine serum albumin at 30°C for 5 min. Mitochondrial protein 4.6 mg/ml

incubation the total value was 70–150 nmol. These endogenous acids were shown to be readily removed from the particles and transferred to albumin (cf. table 1) and therefore competes with the labelled oleate released from the substrate for binding at the available fatty acid binding sites.

The distribution of free fatty acids as a function of incubation time and albumin concentration was therefore calculated on the following assumptions:

1 That labelled oleate as well as endogenous fatty acids were in rapid equilibrium with respect to binding to albumin and that all acids followed the binding isotherm of oleate [11].

2. A concentration-independent distribution of total free fatty acids between the lipid phase (trioleoylglycerol particles and membrane phospholipids) and the aqueous phase with an apparent overall distribution coefficient of 10^3 (the approx. value for palmitate and oleate in heptane–water systems at pH 7.45 [12]).

3. A linear increase in endogenous free fatty acids throughout the incubation period.

The equilibrium amount of unbound acids (total free fatty acids minus free acids bound to albumin)

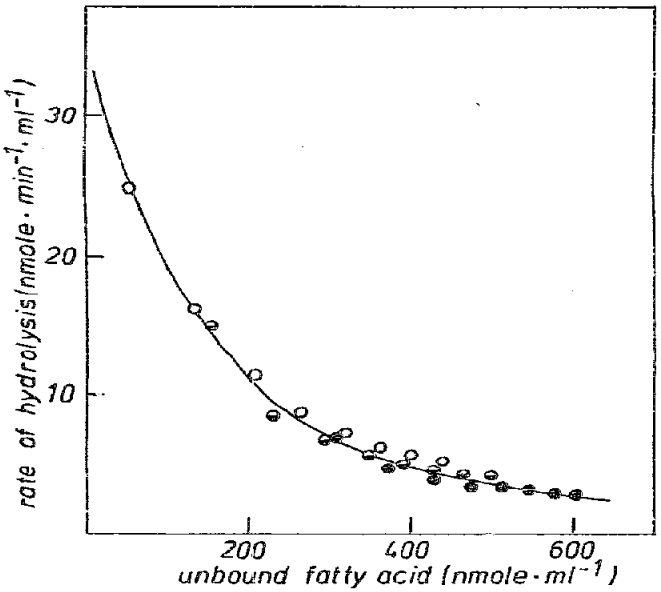

Fig.3. The correlation between the rate of hydrolysis and the calculated amount of unbound fatty acid (total free acid minus acids bound to albumin) in assay systems containing 0.0 (•), 2.0 (⊖) and 4.0 (○) mg albumin/ml. Data from fig.1.

was calculated with 5 min time intervals from the smoothed reaction curves in fig.1 with 0, 2 and 4 mg albumin/ml and correlated to the rates of labelled oleate release for each of these points. One single function, independent on the albumin concentration, was found to relate these two parameters within reasonable limits of uncertainty (fig.3). Therefore both the observed increase in initial rate and the apparent change in overall kinetics of the hydrolysis when albumin was added can be attributed to its ability to complex with inhibitory free fatty acids.

3.2. *Effect of Ca^{2+} and EDTA*

In the absence of albumin Ca^{2+} had an inhibitory effect and EDTA a stimulating effect on the hydrolysis of added trioleoylglycerol whereas these compounds had no effect in the presence of sufficient albumin to overcome inhibition by the fatty acids present (fig.4). It was found also (results not shown) that the observed release of endogenous fatty acids during incubation was enhanced by Ca^{2+} and depressed by EDTA. This indicates that the effects

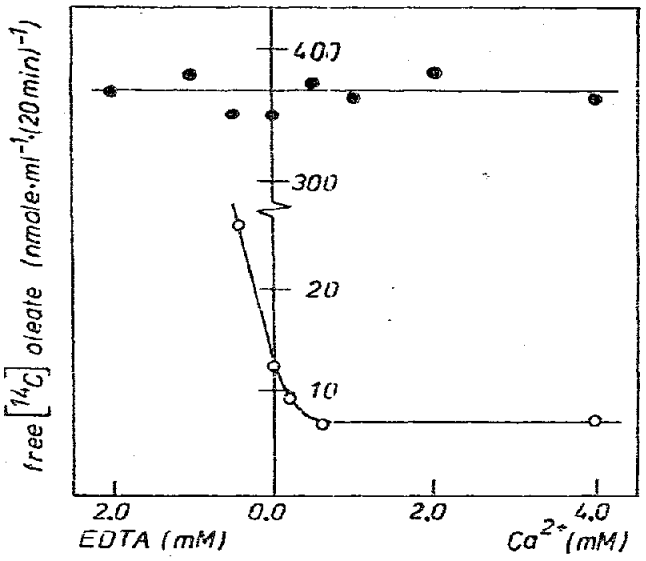

Fig.4. The effect of Ca^{2+} and EDTA on the hydrolysis of added trioleoylglycerol in the absence (o) and in the presence (•) of serum albumin (16 mg/ml). The reaction mixture contained 150 mM Tris–HCl, pH 7.5, 75 mM mannitol, 2.0 mg mitochondrial protein and 2.5 μmol trioleoylglycerol per ml. The reaction was carried out at 30°C for 20 min.

of Ca^{2+} and EDTA on the activity of the mitochondrial triacylglycerol lipase were indirect and mediated by the change in free fatty acids. These results also show that the membrane-bound triacylglycerol lipase differs from the soluble pancreatic lipase, which is also inhibited by fatty acids, but where Ca^{2+} reduces this inhibition by complex formation with the fatty acids [13].

3.3. *The mechanism of inhibition*

The course of the calculated relation between enzyme activity and inhibitor concentration (cf. fig.3) seems to indicate that the fatty acids act as a strong surface competitive inhibitor [14]. The presence of approx. 100 nmol unbound fatty acid/ml resulted in 50% inhibition of the triacylglycerol lipase. Quantitative electron microscopic examination of mitochondrial preparations and substrate emulsions indicated that if this amount of acid is distributed over both mitochondrial membranes and substrate particle interphase about 800 $Å^2$ is available for each molecule. Compared with the surface area of only 70 $Å^2$/ molecule for fatty acids in fatty acid micelles, these figures also argue against surface dilution as a cause of the inhibitory effect. An inhibition mechanism based on surface competition was on the other hand not indicated by replotting the values in fig.3 to give reciprocal rate versus concentration of unbound fatty acids. Such a plot showed a systematic deviation from a straight line and experiments at different substrate concentrations and substrate particle diameters are required for elucidation of the inhibitory mechanism.

The mechanisms controlling the interaction between intracellular particles and organelles are virtually unknown but the close association between lipid particles and mitochondria observed in heart muscle [15] and under special conditions in liver [15] and brown fat [16] indicate that such mechanisms might play a role in the regulation of fatty acid release from storage triglyceride. Further knowledge about the localization of the lipase in the mitochondrial fraction and its contribution to the hydrolysis of the triacylglycerol content of the lipid particles present in the yeast cell [17,18] is required before the significance of the present observations with respect to regulation of triacylglycerol utilization in the cell can be evaluated.

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