

Regulation of glycerol 3-phosphate oxidation in mitochondria by changes in membrane microviscosity

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The inhibition of glycerol 3-phosphate oxidation by oleic acid correlates with changes in membrane microviscosity monitored by the steady-state fluorescence anisotropy of DPH. The dynamic measurements indicate that the changes of both the limiting anisotropy and rotational relaxation time occur in a concentration range where the enzyme activity is strongly inhibited.

(Brown fat) Mitochondria Glycerol 3-phosphate oxidation Oleic acid Limiting anisotropy
Rotational relaxation time

1. INTRODUCTION

In brown adipose tissue cells the glycerol 3-phosphate shuttle plays an important role in the regulation of cell metabolism [1]. Its function may be regulated by free fatty acids acting on mitochondrial glycerol 3-phosphate oxidation [2]. Their effect was explained by inhibition of reducing equivalent transfer between glycerol 3-phosphate dehydrogenase and coenzyme Q [3] but the molecular mechanism of fatty acid action is unknown. The crucial problem that has to be elucidated is whether free fatty acids act directly on the enzyme protein molecule or whether their effect is due to modification of the state of the lipid bilayer and only consequently the enzyme function.

To obtain more information on the molecular mechanism of fatty acid action we measured simultaneously the inhibitory effect of oleic acid on glycerol 3-phosphate oxidation in brown adipose tissue mitochondria and the effect of fatty acids on the state of the lipid bilayer determined from steady-state anisotropy measurements of the hydrophobic fluorescence probe 1,6-diphenyl-1,3,5-hexatriene (DPH). Because the value of

steady-state anisotropy included contributions of both the structural properties of the lipid bilayer (expressed as limiting anisotropy r_∞) and its dynamic state (expressed as the rotational relaxation time τ_c) we measured both these parameters.

Here, we describe a decrease of both the limiting anisotropy and the rotational relaxation time induced by oleic acid.

2. MATERIALS AND METHODS

Mitochondria were isolated from brown adipose tissue of cold-adapted hamsters [4]. The rate of glycerol 3-phosphate oxidation was measured with a Clark oxygen electrode in a medium containing 0.25 M sucrose, 10 mM Tris-HCl, 1 mM EDTA and 2 mM CaCl_2 , pH 7.4 [3], and expressed as $\text{ng atom O} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$. Mitochondrial proteins were determined by the method of Lowry et al. [5].

The fluorescent probe DPH was dissolved in acetone and mitochondria were labelled according to Shinitzki and Barenholz [6]. The excitation and emission wavelengths were 360 and 450 nm, respectively. The fluorescence lifetime was measured by the phase-modulation method [7] on

an SLM 4 800 S subnanosecond spectrofluorometer. Operating in the T-format this spectrofluorometer was used for measurements of both the differential tangent and the steady-state anisotropy.

Weber's theory of hindered rotation [8] was used, yielding the limiting anisotropy r_∞ and the rotational relaxation time τ_c . Eqn 1 applies for these quantities:

$$r = \frac{r_0 - r_\infty}{1 + \frac{\tau}{\tau_c}} + r_\infty \quad (1)$$

where r is steady-state anisotropy and r_0 the fluorescence anisotropy observed in the absence of depolarizing rotations (r_0 was taken as 0.39). The apparent microviscosity η_a value can be obtained from eqn 2:

$$\eta_a = \tau_c C_{(r)} T \quad (2)$$

where T is the absolute temperature and $C_{(r)}$ is a complex structural parameter of the probe. All measurements were performed at 22°C.

Glycerol 3-phosphate was obtained from Genzyme (England) and other chemicals from Sigma (USA).

3. RESULTS

In agreement with our previous observation [3] fig.1A demonstrates that sodium oleate has only a weak inhibitory effect on glycerol 3-phosphate oxidation up to 100 nmol oleate/mg mitochondrial protein. At higher oleate concentrations a much stronger inhibition was observed.

Under the same experimental conditions we measured the steady-state anisotropy of DPH and found lower values as the concentration of sodium oleate increased. The decrease of the anisotropy fitted well with the inhibition of enzyme activity (fig.1B).

The question remained as to whether the changes of steady-state anisotropy were due to changes of the structural or dynamic properties of the lipid bilayer. To obtain more information we measured the limiting anisotropy (r_∞) and the rotational relaxation time (τ_c) which were used for characterizing the structural and dynamic parameters of the lipid bilayer.

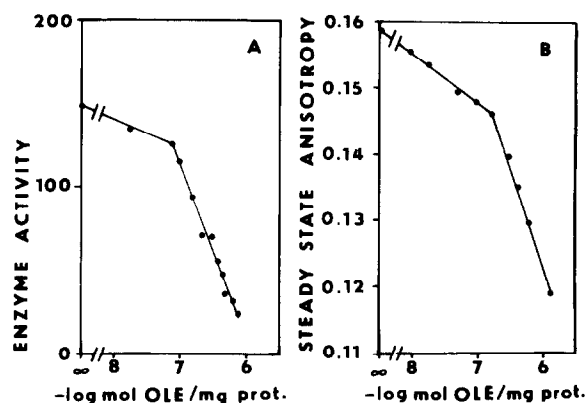


Fig.1. The effect of sodium oleate on glycerol 3-phosphate oxidation (A) and on the steady-state anisotropy of DPH (B) measured according to Shinitzki and Barenholz [6] in brown adipose tissue mitochondria. Enzyme activity was expressed as ngatom oxygen $\cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$. Anisotropy values are expressed in arbitrary units.

As shown in fig.2 at low oleate concentrations (below 100 nmol/mg protein) only the limiting anisotropy (r_∞) is slightly modified whereas the rotational relaxation time (τ_c) remained unchanged. At higher oleate concentrations where a stronger inhibition of enzyme activity occurred

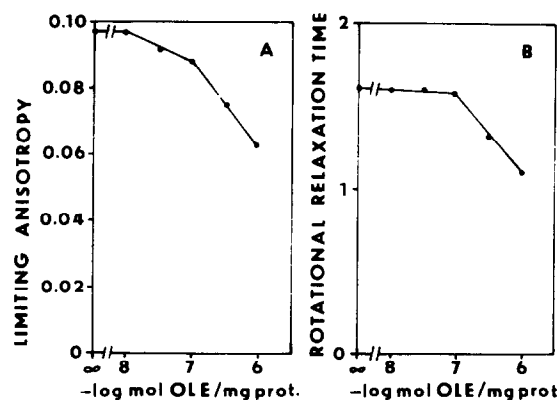


Fig.2. The effect of sodium oleate on limiting anisotropy (A) and rotational relaxation time (B) obtained by phase fluorometric measurements according to Lakowicz et al. [11]. The limiting anisotropy values are expressed in arbitrary units and values of the rotational relaxation time in ns.

both the limiting anisotropy and the rotational relaxation time values decreased.

4. DISCUSSION

The hydrophobic lipid bilayer plays an important role in maintaining the structural organization of protein molecules incorporated into the membrane lipid phase. Moreover, the protein molecules in biological membranes are in a dynamic state. The whole protein molecule or at least its parts move periodically over a wide time scale [9]. The state of the lipid membrane phase can thus play a role in regulating the functional activity of various membrane-bound enzymes [10], and substances that can modify microviscosity of the lipid membrane phase may through this mechanism regulate various metabolic processes.

Free fatty acids can be considered not only as substrates for oxidation but also as one of the regulatory factors. The present data clearly show that the molecular mechanism of their inhibitory effect on the transfer of reducing equivalents from glycerol-3-phosphate dehydrogenase to ubiquinone can be easily explained by modification of the membrane lipid phase, characterized by steady-state fluorescence anisotropy data. This inhibitory effect is completely reversible which indicates that properties of the lipid phase return to the original state when bound free fatty acids are removed by β -oxidation or fatty-acid-binding proteins.

However, steady-state anisotropy is a function of both the limiting anisotropy and the rotational relaxation time. Changes in these parameters permit alternative possibilities: The inhibitory effect of free fatty acids is due to changes of both parameters (r_∞ , τ_c) or due to one of them being accompanied by the other. The better correlation between changes of enzyme activity (fig.1A) and limiting anisotropy (fig.2A) indicates that the structural parameter is more important.

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