Fatty acids induced uncoupling of *Saccharomyces cerevisiae* mitochondria requires an intact ADP/ATP carrier

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Abstract Fatty acids stimulate the oxidation rate of mitochondria isolated from the wild-type *Saccharomyces cerevisiae*, but do not affect significantly the respiration of mitochondria isolated from mutants, in which the ADP/ATP carrier (AAC) was either modified (R96H) or deleted (Δ*aac2*). Similarly as in mammalian mitochondria, the transmembrane electrical potential difference (Δψ) in the wild-type yeast mitochondria was dissipated by low concentrations of free fatty acids, and this was partially inhibited by bongkrecate. In contrast to the wild-type mitochondria, the addition of increasing concentrations of fatty acids to the *op1* (R96H) mutant mitochondria abolished only a small portion of Δψ, as compared to the change induced by classical uncouplers. The different effects of fatty acids on both, the respiration and the Δψ of mitochondria isolated from the wild-type and the *aac* mutants, respectively, demonstrates that the intact AAC is essential for the fatty acids induced H^+ permeability of mitochondrial membrane.

Key words: Yeast mitochondria; Fatty acids; ADP/ATP carrier; *S. cerevisiae*

1. Introduction

Two organelles, mitochondria and peroxisomes, participate in the β-oxidation of free fatty acids in eucaryotic cells. In mammals, the oxidation takes place predominantly in mitochondria where either large amount of ATP, or heat is produced. It is believed that the thermoregulatory mechanism in the later case includes a physiological uncoupling of mitochondria by fatty acids [1,2]. The uncoupling is necessary to ensure a rapid re-oxidation of reducing equivalents that are produced during the mitochondrial β-oxidation. Fatty acids themselves, exhibited relatively weak protonophoric activity in artificial phospholipid membranes [3] and therefore membrane proteins have been implicated in the fatty acids-induced uncoupling of mitochondria [4]. In experiments with isolated mitochondria [5,6] it was shown that inhibitors and substrates of ADP/ATP carrier (AAC) suppressed to some extent the fatty acids effect on the respiratory rate and on the mitochondrial transmembrane potential (Δψ). In addition, it was recently shown that the fatty acids-induced H^+ transport can be demonstrated in the proteoliposomes containing AAC and cytochrome *c* oxidase from bovine heart mitochondria [7].

In yeast, degradation of fatty acids takes place exclusively in peroxisomes [8], and it is not expected that yeast possess similar thermoregulatory mechanisms as mammals. Yeast mitochondria participate at the metabolism of the end products of peroxisomal oxidation [9], but it is not clear, whether the fatty acids could influence mitochondrial metabolism in a similar way as they do in mammalian mitochondria.

In the present work we demonstrate, that the free fatty acids-mediated H^+ transport is not restricted to the organelles of thermoregulation possessing organisms. The different effects of free fatty acids on the membrane potential of wild-type and *aac* mutant mitochondria, in a direct way demonstrate that intact ADP/ATP carrier is required for fatty acids-mediated H^+ transport. The results of the present study offer a new approach in the study of the interactions between fatty acids and AAC exploring the potential of yeast molecular genetics.

2. Materials and methods

2.1. Strains, media and isolation of yeast mitochondria

The following *S. cerevisiae* strains were used: W303-1B (*MATα*, *ade2*, *leu2*, *his3*, *trp1*, *ura3*, *can1*) provided by B.L. Trumpower (Dartmouth Medical School, Hanover, NH); the *AAC2* disrupted strain JLY-73 (*MATα*, *ade2*, *his3*, *trp1 ura3leu2*, *can1*, *aac2::HIS3*) provided by M. Douglas (University of North Carolina), the triple *aac* mutant JL-1-3 (*MATα*, *ade2*, *trp1*, *leu2*, *ura3*, *his3*, *can1*, *aac1::LEU2*, *aac2::HIS3*, *aac3::URA3*) prepared in this laboratory [10], and the *op1* (*pet9*) mutant in which a R96H substitution is present in the product of *AAC2* gene was described before [11]. Yeast cells were grown on standard YP medium (1% yeast extract, 2% Bacto-Peptone, Difco) supplemented with either 0.25% or 0.5% glucose for the wild-type and the mutants, respectively. The cells were harvested, converted to protoplasts by enzymatic digestion with zymolyase and further processed for isolation of mitochondria as described earlier [12]. After the final centrifugation mitochondria were resuspended in a small volume of 0.6 M mannitol, 2 mM EDTA, pH 7.5.

2.2. Mitochondrial Δψ assay and oxygen consumption

The mitochondrial transmembrane potential (Δψ) was estimated as absorbance red shift of safranine T [13] by dual wavelength spectrophotometry at 533 nm minus 510 nm using a Hitachi Perkin-Elmer 356 spectrophotometer. Mitochondria (0.4 mg protein/ml) were resuspended in the medium containing 0.65 M manitol, 5 mM phosphate, 20 mM Tris-maleat pH 6.8, 1 mM safranin T and energized by addition of 5 mM citrate. The concentrations of the uncouplers, bongkrecate and respiratory substrates are given under the figures.

Oxygen consumption was measured at 30°C in a 1 ml thermostatically controlled chamber equipped with a Clark electrode in the medium described above supplemented with 2 mM EDTA and 1 mM $MgCl_2$.

3. Results and discussion

The respiration of wild-type yeast mitochondria oxidizing citrate is completely uncoupled by addition of 50 μM oleate (Fig. 1a) to the incubation medium. Palmitate had the same uncoupling effect (data not shown). On the contrary, the same or higher concentrations of fatty acids did not significantly

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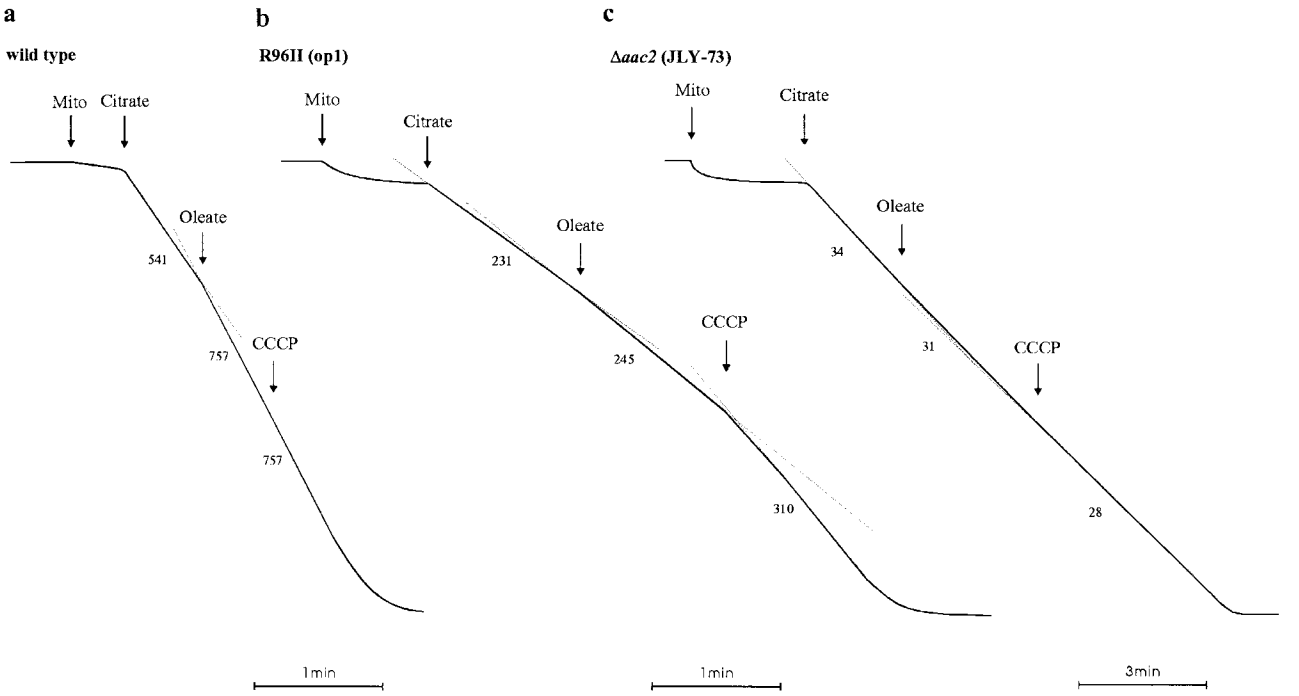

Fig. 1. Effect of oleate on the respiration of mitochondria with either functional or defective ADP/ATP transport. Mitochondria isolated from the wild-type (0.4 mg), the *op1*(R96H) mutant (0.4 mg), and the Δ*aac2* deleted strain (1.5 mg) were suspended in buffer described in the Methods (see Section 2) in an 1 ml chamber containing oxygen electrode. Where indicated, 10 mM citrate, 50 μM oleate and 10 μM CCCP were added. The numbers under the traces are respiratory rates in nmol (O) min^{-1} mg^{-1} protein.

influenced the respiratory rate of mitochondria isolated from the *op1* (R96H) mutant (Fig. 1b). The *op1* mutation is in the *AAC2* gene encoding the major mitochondrial ADP/ATP carrier and the mutant mitochondria exhibited a very low rate of ADP/ATP exchange across the mitochondrial membrane [11,12] as compared to the wild-type. Fig. 1 further shows that the respiration of mitochondria from another yeast mutant, JLY-73 (Δ*aac2*), which did not contain AAC in the mitochondrial membrane due to deletion of *AAC2* gene, is not significantly influenced neither by oleate, nor by CCCP (Fig. 1c).

The different effect of free fatty acids on the respiratory rates of mitochondria with either a functional, or an impaired ADP/ATP exchange was further investigated by following the mitochondrial Δψ using safranin T as a probe [13]. As shown in Fig. 2a, the increasing concentrations of oleate induced progressive collapses of Δψ in the wild-type mitochondria, and the effect of 50 μM oleate on the Δψ resembles the action

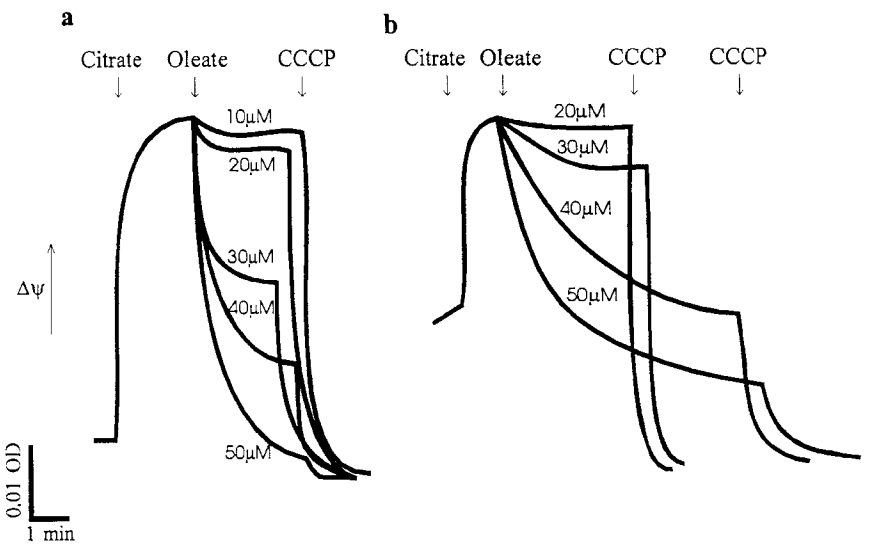

Fig. 2. Effect of oleate and bongkrecate on the Δψ in the wild-type mitochondria. The Δψ was estimated as described in the Methods (see Section 2). Mitochondria were energized by addition of 5 mM citrate and where indicated uncoupled by 10 μM CCCP. In (b) the mitochondria were pretreated with 10 μM bongkrecate.

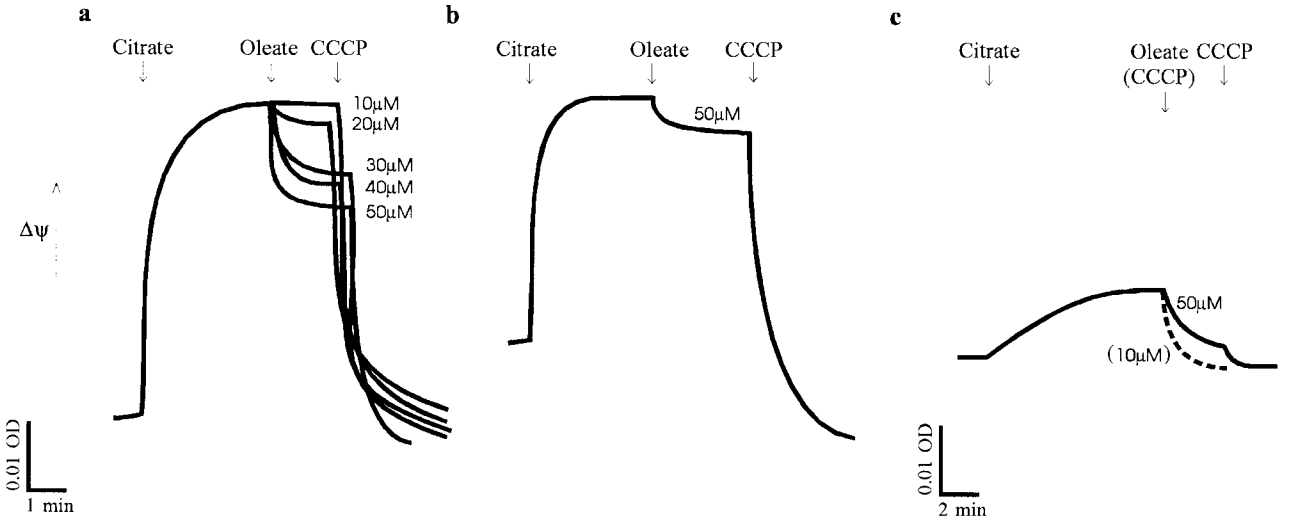

Fig. 3. Effect of oleate on the Δψ in mitochondria from the *op1* (R96H) (a, b) and the Δ*aac2* deletion mutant (c). In (b) the mitochondria from the *op1*(R96H) mutant were pretreated with 10 μM bongkrecate.

of standard uncoupler CCCP. Again, palmitate had the same effect on the Δψ as oleate (data not shown).

The pretreatment of wild-type mitochondria with bongkrecate, a specific inhibitor of ADP/ATP translocation, partially reduced the rate of fatty acids-induced depolarization (Fig. 2b). Similar effect of ADP/ATP carrier inhibitors on the fatty acids induced uncoupling of mammalian [5,6] and plant [14] mitochondria has been interpreted to indicate that the carrier is involved in the fatty acids mediated H^+ permeability of inner mitochondrial membrane. The sensitivity of fatty acids-induced uncoupling to the different ADP/ATP transport inhibitors markedly varies with the nature of the inhibitor and with the source of mitochondria used [5,6,14,15]. In addition, it might also reflect an indirect action of these compounds on Δψ. It was, therefore, of interest to see if the mutations impairing the function of the carrier would also affect the fatty acids-induced H^+ permeability of mitochondrial membrane. Fig. 3 shows the effect of oleate on Δψ in mitochondria isolated from both, the *op1*(R96H) and Δ*aac2* deletion mutants. It should be noted that *op1*(R96H) mutation strongly diminished the rate of adenine nucleotide translocation across the membrane and, to a less extent, reduced the affinity of the carrier to specific ligands [12,16,17]. As shown at Fig. 3, the effect of fatty acids on the Δψ of mutant mitochondria is quantitatively different. A relatively small portion of Δψ formed during the substrate oxidation in *op1* (R96H) mutant mitochondria was dissipated by addition of free fatty acids. Neither higher concentrations of oleate and palmitate, nor other respiratory substrates used (data not shown), added significantly to the extent of depolarization observed with 50 μM oleate (Fig. 3a). The saturation obtained with the increasing oleate concentrations in both, the mutant and wild-type mitochondria (Fig. 2a) suggests clearly that a protein component is involved in the effect of fatty acids, and most probably, it is the AAC. This is further strengthened by the pretreatment of the *op1* mutant mitochondria with bongkrecate, which inhibits further the uncoupling effect of oleate (Fig. 3b). This is in agreement with the increased sensitivity of ADP/ATP exchange in the *op1* mutant mitochondria to bongkrecate observed earlier [12].

As a control, we have also used mitochondria isolated from the *AAC2* disrupted strain, JLY-73, as well as from the triple deletion mutant, JL-1-3, in which all three genes, *AAC1*, *AAC2*, and *AAC3*, encoding the ADP/ATP carrier were deleted [10]. Both types of mutant mitochondria responded exactly in the same way to the all additions of substrates, fatty acids and uncouplers (Fig. 3c). This demonstrates that the products of the other two isogenes, *AAC1* and *AAC3*, do not participate at the uncoupling, as they do not participate at the mitochondrial ADP/ATP transport [10]. Mitochondria from both deleted strains have reduced contents of respiratory enzymes and exhibited low rates of respiration [17,18] (see also Fig. 1). Accordingly, both the rate and the extent of polarization of mitochondrial membrane are very low (Fig. 3c). Addition of oleate also produced a very slow response, different from that observed with both, the wild-type and *op1*(R96H) mutant mitochondria (Figs. 2a and 3a), and from that induced by CCCP. This AAC-independent fatty acids-induced uncoupling could be mediated by other anion carriers as it has been recently suggested [19,20].

Taken together the above results demonstrate the ability of free fatty acids to induce changes in the Δψ of yeast mitochondria in a similar manner as they do in the organelles of termoregulation possessing organisms. Using *S. cerevisiae* mutants with either defective or deleted AAC, we have shown that the fatty acids-induced proton permeability of inner mitochondrial membrane requires specific interactions between the fatty acid and AAC. We presume that these interactions could be further investigated by the means of molecular genetics and mutagenesis techniques, exploring the tools and the approaches described in this study.

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