THE ADENINE NUCLEOTIDE TRANSLOCATOR IN GENETICALLY AND PHYSIOLOGICALLY MODIFIED YEAST MITOCHONDRIA

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1. Introduction

Yeast cells offer a unique possibility to study function and biogenesis of components of the mitochondrial membrane in view of the fact that the yeast mitochondria are amenable to profound genetic or phenotypic modifications. This advantage has led to several studies on adenine nucleotide translocation in yeast mitochondria which, however, have brought some controversial results [1–6]. The present study was undertaken with the aim of helping to clarify these controversies. The study confirms and extends the previous data [1,2,7] showing that an ADP/ATP carrier is also present in mitochondria isolated from anaerobically grown yeast and from a cytoplasmic respiratory-deficient mutant. In addition, it provides further analysis of the genetically modified ADP/ATP carrier in yeast nuclear mutant [3].

2. Material and methods

Wild type yeast Saccharomyces cerevisiae DT XII, its cytoplasmic (ρ^-) respiration-deficient mutant DT XIIA and the diploid mutant strain $DH_1(op_1)$ were used in the study. The methods of aerobic and anaerobic cultivation and preparation of mitochondria were the same as described previously [2,3]. The measurements of translocation of binding ^{14}C-ADP and ^{35}S-carboxy-atractylate (CAT) to carrier specific sites were performed according to described procedures [8–10].

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** Abbreviations: ATR – atractylate, BKA – bongkrekate, CAT – carboxy-atractylate.

3. Results

The translocation of ADP and ATP in mitochondria isolated both from wild type yeast and from the cytoplasmatic respiratory deficient (ρ^-)-mutant was inhibited not only by atractylate (ATR) [1,2] but also by carboxy-atractylate (CAT) and bongkrekate (BKA). A concentration dependence of the inhibition by BKA and CAT of the exchange is shown in fig. 1 for the ρ^--mutant. Both inhibitors block the exchange up to 90%. Thus the exchange in ρ^--mutant mitochondria has similar sensitivity to the inhibitors as in the aerobically wild strain.

This is further supported by binding studies with ^{35}S-CAT shown in fig. 2a. A large portion of the CAT binds to the ρ^--mutant mitochondria with a high affinity ($K_d = 2.8 \cdot 10^{-8}$ M). Only a small portion of

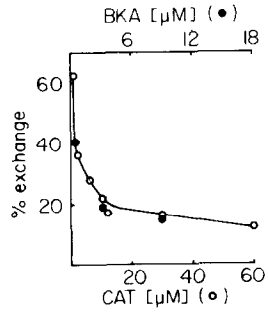

Fig. 1. Inhibition of adenine nucleotide translocation in ρ^--mitochondria. Mitochondria were prelabelled with ^{14}C-ADP for 60 min at 0° C in 0.6 M manitol and washed according to [8]. Back-exchange was started by addition of 200 μM ADP to the 0.5 ml incubation medium containing 0.45 M sorbitol, 1 mM EDTA, 10 mM MOPS, pH 6.4, 0.4 mg mitochondria protein and inhibitors in concentrations indicated on the abscissas. Incubation time 20 sec.

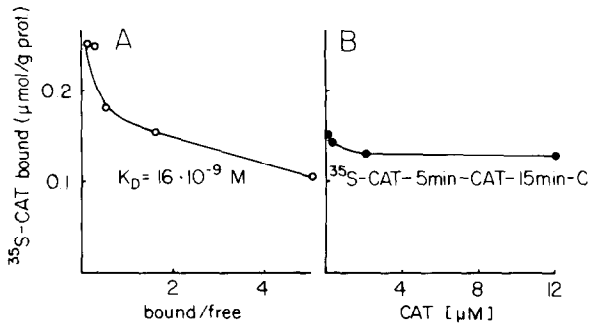

Fig. 2. Binding of ^{35}S-CAT to ρ^--mitochondria. (A) Scatchard plot of the binding of ^{35}S-CAT to ρ^--mitochondria. Mitochondria (0.8 mg) were incubated in 0.5 ml in the same incubation medium with increasing concentrations of ^{35}S-CAT at 20°C. After 5 min incubation the suspension was centrifuged and ^{35}S content was determined in both supernatant and sediments dissolved in 2% lubrol. (B) Conditions as in (A) but a fixed concentration of ^{35}S-CAT was used (0.4 μM) and after 5 min unlabelled CAT was added in concentration indicated on the abscissa.

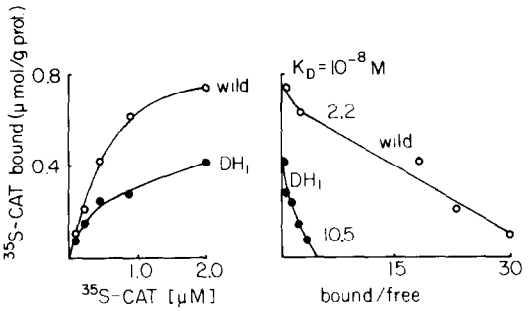

Fig. 4. Binding of ^{35}S-CAT to mitochondria isolated from the wild strain and from the nuclear DH_1 mutant. Conditions as in fig. 2a. Concentrations of mitochondria were 0.6 mg/0.5 ml for both the wild strain and for the mutant.

^{35}S-CAT, bound with low affinity, can be removed by unlabelled CAT (fig. 2b) demonstrating the very high affinity to the CAT binding. The affinity of the CAT binding to the ρ^--mutant mitochondria is about the same as the affinity of CAT to wild type mitochondria which is measured in fig. 4.

The competition between BKA and ^{35}S-CAT binding is illustrated in a comparison for wild type and ρ^--mitochondria in fig. 3. Similar as shown for animal mitochondria BKA is able to prevent the binding of ^{35}S-CAT only when added before CAT but not afterwards. This holds for both, wild- and ρ^--mitochondria. In general the inhibition of CAT binding by BKA is more complete than in the yeast mitochondria.

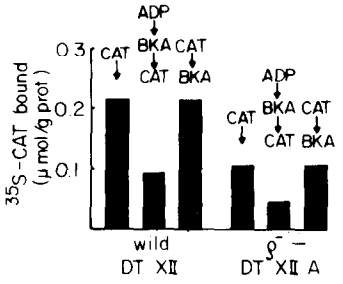

Fig. 3. The influence of bongkrekate on the binding of ^{35}S-CAT and the synergistic influence of ADP. Comparison of wild type and ρ^--mitochondria.

It was found previously that mitochondria from the nuclear mutant DH_1 (carrying the op_1 gene) possess a modified adenine nucleotide translocation system characterized by a low translocation rate and a decrease of the affinity of the carrier for substrates [3]. Therefore, it was of great interest to assess the affinity also of the CAT binding of these mitochondria. Experiment in fig. 4 shows the binding of CAT to the DH_1-mutant mitochondria as compared to the wild type. In the DH_1-mutant the number of binding sites is reduced as compared to the wild type mitochondria from about 0.8 to 0.4 ^{35}S-CAT μmoles/g protein and the affinity is decreased about 4-fold with a $K_d = 1 \cdot 10^{-7}$ M.

In this context the properties of the translocation system in mitochondria isolated from anaerobically grown wild type cells (promitochondria) were of interest. The mitochondria still have an active adenine nucleotide translocation system (data not shown) which, however, is reduced as compared to the aerobically grown cells: 0.68 μmol ADP/g protein/min as compared to 7 μmoles ADP/g protein/min. The ATR removable portion of ^{14}C-ADP binding to these mitochondria, however, is similar to that found previously for mitochondria from the aerobically grown cells (fig. 5). These mitochondria have an unusual pool of endogenous nucleotides which contains no ATP, 25% ADP and 75% AMP. The binding of CAT to the promitochondria is illustrated in fig. 6. There are low and high affinity binding sites with an approximate affinity

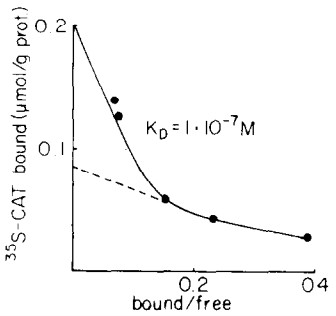

Fig. 5. Binding of ^{35}S-CAT to promitochondria. Concentration of promitochondria was 0.45 mg/0.5 ml.

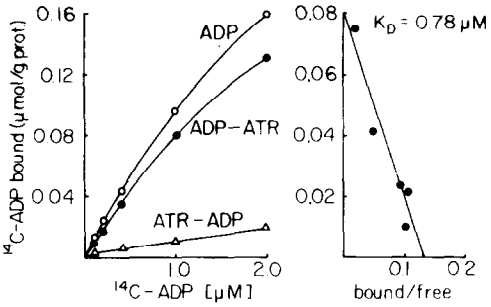

Fig. 6. Uptake of ^{14}C-ADP by promitochondria and binding of ADP to ATR-removable binding sites. Promitochondria (0.45 mg/0.5 ml) were incubated at 0°C for 2 min in a medium containing 0.45 M sorbitol, 1 mM EDTA, 10 mM MOPS, pH 6.4 and ^{14}C-ADP at concentrations indicated on the abscissa. ATR (100 μM) was either omitted from the medium (-○-○-) or added before (-△-△-) or 3 min after ^{14}C-ADP (-●-●-). ATR removable binding [9] was plotted against the individual bound/free ratios of ^{14}C-ADP. Endogenous content of adenine nucleotide in promitochondria was 3.75 nmoles AMP/mg protein, 1.0 nmoles ADP/mg protein and no ATP.

for the high affinity sites with $K_d = 1 \cdot 10^{-7}$ M, which corresponds to an about 5-fold decrease of the affinity as compared to aerobically grown mitochondria.

4. Discussion

The data on the ADP/ATP carrier system in the various type of yeast mitochondria are summarized in table 1, which permits a comparison with mitochondria from the aerobically grown wild type with those obtained from the cytoplasmatic ρ^--mutant, the promitochondria and the nuclear DH_1-mutant. For the mitochondria from the cytoplasmic respiratory deficient (ρ^-)-mutant the present results substantiate the earlier conclusions that the ADP/ATP carrier is preserved unmodified in these mitochondria although they lack protein synthesis [14,15]. Also the observation on the inhibitory effect of BKA on the multiplication of ρ^--yeast cells [13] is in line with this conclusion. These results are at variance with the report by Haslam et al. [5] who claim that the atractylate sensitive transloca-

Table 1
Some properties of adenine nucleotide translocation system in yeast mitochondria

Source of mitochondria	DT XII/ρ^+ aerobic	DT XIIA/ρ^- aerobic	DT XII/ρ^+ anaerobic	DH_1/ρ^+ aerobic
Number of CAT binding sites (μmoles/g protein)	0.7	0.18	0.10	0.4
Dissociation constant: K_d^* for CAT (10^{-8} M)	1.6	2.4	10	6.4
Translocation rate at 0° (v_T) (μmoles/g/min)	7	1.7	0.68	0.2
'Turnover' ratio (v_T/C_{CAT}) (min^{-1})	10	9.4	6.8	0.5

* mean value from several experiments

tion is absent in mitochondria of a cytoplasmic ρ^--mutant. Even though two different cytoplasmic ρ^--mutants were employed in the two studies, this would hardly explain the difference as mitochondrial protein synthesis is apparently absent in both mutants [14,15]. Insufficient kinetic resolution of the exchange in the experiments of Haslam et al. [5] might however explain the differences of the results.

The decrease in the number of binding sites in the ρ^--mitochondria as compared to the wild type may, however, be due to a significant change in the membranes of these mitochondria. It may also be explained, however, by lower purity of the mitochondria preparation from the respiratory deficient cells, which is difficult to assess since cytochrome aa_3 cannot be used as a common denominator because of its deficiency in the ρ^--mitochondria.

The case is similar in promitochondria. The number of binding sites for CAT is considerably diminished in these mitochondria as referred to protein content. Also the translocation rate is correspondingly decreased so that the turnover of the CAT binding sites is similar in the anaerobically as in the aerobically grown mitochondria. The unusual intramitochondrial adenine nucleotide pattern is understandable in view of the absence of the respiratory chain. The relatively high number of ATR removable ADP binding sites in yeast mitochondria may include some ATR induced leakage.

The results obtained with the ρ^--mutant mitochondria imply that the ADP/ATP carrier is nuclear coded. This is more directly demonstrated in the nuclear DH_1-mutant. Here the two characteristic parameters, the turnover of the ADP/ATP translocation rate and the affinity for CAT are strongly modified. A decrease in the affinity for ADP and ATP along with an unusual dependence of the translocation properties and its linkage to the energy state of the mitochondria was previously reported [3]. All these results seem to reflect a structural modification of the carrier as a result of the nuclear mutation. It seems unlikely that the carrier environment is responsible for these changes since neither phospholipid nor fatty acid composition were found to be different in the mutant as compared to the wild type mitochondria ([17], unpublished results).

In conclusion these results demonstrate that nuclear mutants may be of further great value in studying the ADP/ATP carrier. On the other hand, mitochondrial mutants are not to be expected to affect the ADP/ATP carrier directly [4,5]. Although no clear data are available from the literature, it might be possible that in cytoplasmatic mutants the carrier might be indirectly affected by changes of the lipid environment.

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