SHORT COMMUNICATIONS

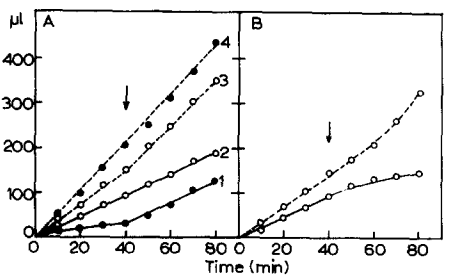
BBA 43262

Oxidative phosphorylation in yeast

VII. Inhibition of oxidative phosphorylation and of respiratory enzyme synthesis by oligomycin in intact cells

Oligomycin has been an important tool in studies of oxidative phosphorylation in subcellular systems[1-3]. Additional information can be gained by studying the action of oligomycin at the level of the intact cell. Such a study is presented in this communication, complementing data from another laboratory[4].

Fig. 1 shows that respiration of aerobically grown yeast can be inhibited up to 67 % by oligomycin, the inhibition being relieved by uncoupling agents. The extent of the respiratory inhibition by oligomycin has varied considerably (in the range of



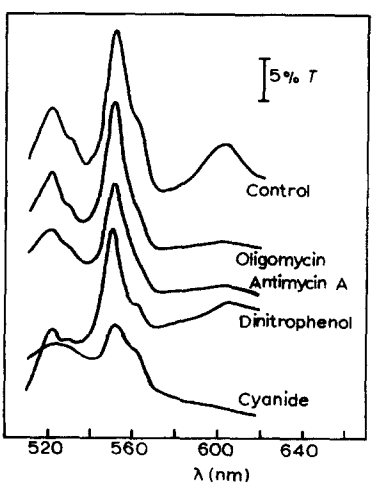


Fig. 1. Effect of oligomycin on respiration of aerobically grown yeast. *S. cerevisiae* DT XII was cultured aerobically at 30° in a semi-synthetic medium[5] with 0.25 % glucose for 24 h. Respiration was measured manometrically at 30°. Main compartments of Warburg vessels contained in 2.5 ml: 80 mM citrate phosphate buffer (pH 4.3), 50 mM glucose and 0.8 mg yeast (dry weight). Inhibitors were used at the following final concentrations: oligomycin, 40 µg/ml (+ 1 % methanol); 2,4-dinitrophenol, 50 µM. Full lines represent O_2 consumption, dotted lines CO_2 production. A. 2 and 3, control without oligomycin; 1 and 4, oligomycin present in the main compartment. At arrow, 2,4-dinitrophenol was added from the side arm. B. At arrow, oligomycin was added from the side arm.

Fig. 2. Difference spectra of yeast grown in the presence of inhibitors. The cells grown as described in Table I were washed and suspended in an equal volume of water. Spectra were recorded in the SF 10 spectrophotometer; suspension in the reference cuvette was oxidized with 60 mM H_2O_2 and in the sample cuvette reduced either with endogenous substrates (in control and in oligomycin-cultured cells) or with 10 mM DL-lactate + solid dithionite (in other cases).

30–67 %) depending on the growth phase from which the cells were harvested and on the yeast strains used. Aerobic fermentation of glucose was increased by oligomycin to the anaerobic level, indicating that the Pasteur effect had been abolished by oligomycin. Preincubation of cells with oligomycin was necessary to obtain a full effect, showing that the permeation of oligomycin into the yeast cells (or possibly into yeast mitochondria, as shown in ref. 5) is a relatively slow process.

TABLE I

RESPIRATION OF CELLS GROWN IN THE PRESENCE OF INHIBITORS

S. cerevisiae DT XII was cultured aerobically at 30° for 30 h in a semi-synthetic medium[6] with 0.5 % glucose either in the absence of inhibitor (control) or in the presence of inhibitors in the following concentrations: oligomycin, 10 μg/ml; antimycin A, 0.5 μg/ml; 2,4-dinitrophenol, 0.1 mM; KCN, 1–0.12 mM (concentrations at the beginning and end of cultivation, respectively). Respiration was measured polarographically in a buffer containing 50 mM potassium glutarate, 10 mM potassium phosphate and 100 mM KCl (pH 4.3) in the presence of 20 mM glucose.

Inhibitor	*Respiration* ($\mu l\ O_2 \cdot h^{-1} \cdot mg^{-1}$ *dry wt.*)	
	Without CCCP	*In the presence of 10 μM CCCP*
None (control)	92	159
Oligomycin	6	12
Antimycin A	1	2
2,4-Dinitrophenol	9	14
KCN	2	3

Abbreviation: CCCP, carbonylcyanide *m*-chlorophenylhydrazone.

Aerobic growth of *Saccharomyces cerevisiae* DT XII in liquid complex medium with 2 % lactate as carbon source was arrested by oligomycin in concentrations higher than 1 μg/ml. The yield of cells growing aerobically in the complex medium with 2 % glucose was also considerably lower in the presence of oligomycin, being only slightly higher than under anaerobic conditions (see also ref. 6). When aerobically grown cells were plated on solid complex medium with 2 % glucose and 10 μg/ml oligomycin, only small colonies were formed which, unlike large colonies formed on control oligomycin-free medium, did not stain with triphenyltetrazolium chloride. This was suggestive that the cells might have been converted into respiration-deficient mutants[7] in the presence of oligomycin; however, no mutant formation by oligomycin over the spontaneous rate (less than 1 %) could be detected by appropriate tests[8]. This was in variance with a previous report[4] indicating induction of respiratory mutants by oligomycin in another strain.

Another explanation for the lack of stainability with triphenyltetrazolium chloride would be the phenotypic suppression of the formation of the respiratory system. This has been in fact proved, in accord with a previous report[4]. The question arises as to whether such a suppression is specific for oligomycin or is exerted by other mitochondrial inhibitors. As shown in Table I, the respiration of cells was considerably suppressed when they were grown aerobically on glucose in the presence of the oxidative phosphorylation inhibitor oligomycin, the uncoupler 2,4-dinitrophenol, or the respiratory inhibitors cyanide and antimycin A. As shown in Fig. 2, the cells grown in the presence of the inhibitors contained almost the same or a slightly reduced

amount of cytochrome *c* (except for cells grown in the presence of cyanide), less cytochrome *b* and almost no cytochrome *a*. The extent of the diminution of the respiratory pigments parallels the sensitivity of the pigment formation toward catabolic repression[10]. The inhibition of cytochrome *a* formation in cells grown in the presence of antimycin A has already been observed by YČAS[9]. The fraction of respiratory-deficient mutants was not increased over the spontaneous rate by any of the inhibitors.

It should be recalled that, although suppressing the formation of the complete respiratory system when present during aerobic growth, neither cyanide[11], antimycin A (ref. 9) or oligomycin[12] was found to prevent the induced synthesis of respiratory enzymes when anaerobically grown yeast was incubated in air under resting conditions. Uncoupling agents inhibited the respiratory adaptation only at higher concentrations[12].

The results lead to the following conclusions: (1) Oligomycin does function as an inhibitor of oxidative phosphorylation in appropriate yeast strains and under appropriate conditions. (2) The "tightness" of coupling between respiration and ATP synthesis[13] may vary in intact yeast cells but may be as high as 67 %. (3) The abolishment of the Pasteur effect by oligomycin is in line with the role of mitochondrial phosphorylation in the control of glycolysis[14]. (4) Compounds which prevent ATP formation in mitochondria, either by inhibiting the respiration or by uncoupling or inhibiting oxidative phosphorylation, suppress the formation of the complete respiratory system, cytochrome *a* synthesis being affected the most. This effect is most likely due to the release of the Pasteur effect by the compounds and the subsequent increase of catabolic repression. The observation that the inhibitors do not considerably inhibit respiratory adaptation in yeast is in line with a finding (K. KOLLÁR AND L. KOVÁČ, unpublished) that the catabolic repression of the respiratory enzyme synthesis is considerably stronger in growing yeast than in anaerobically grown cells undergoing respiratory adaptation under resting conditions. (5) No induction of respiratory-deficient mutants in the strain studied with the inhibitors used was observed. Since other investigators did find induction of respiratory mutants by oligomycin[4] and by 2,4-dinitrophenol (N. YANAGISHIMA, unpublished, quoted in ref. 8), described an increased formation of the respiratory-deficient mutants in some anaerobically grown strains[15] but not in others[16], and isolated a mutant which gives rise to 100 % respiratory-deficient mutants under conditions of catabolic repression[17], it is possible that there is a relation between the extent of catabolic repression of respiratory enzyme synthesis to which individual yeast strains can be submitted and the ease with which they form respiratory-deficient mutants (see also refs. 18, 19).

Department of Biochemistry, L. KOVÁČ
Komensky University, E. HRUŠOVSKÁ
Bratislava (Czechoslovakia) P. ŠMIGÁŇ

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The Ca^{2+}-dependent contraction and relaxation of actomyosin fibers

Since the discovery of native tropomyosin[1] or complex of tropomyosin and troponin[2,3], this protein system has been regarded as necessary for sensitizing actomyosin to Ca^{2+} (*cf.* ref. 4). Thus far, the Ca^{2+} sensitivity of actomyosin has been tested by the inhibition of superprecipitation or the inhibition of the Mg^{2+}-activated ATPase activity[2,5,6]. A partial reversibility of the contracted actomyosin suspension by the removal of Ca^{2+} has recently been reported[7]. However, a direct test of contraction or relaxation of actomyosin fibers controlled by Ca^{2+} has not yet been performed. The present study is concerned with the Ca^{2+}-dependent contraction and relaxation of actomyosin fibers and the requirement of the troponin–tropomyosin complex for the Ca^{2+} sensitivity.

Fibers were made from surface-spread actomyosin layers on a trough solution, containing 20 mM KCl, 1 mM $MgCl_2$ and 25 mM Tris–HCl buffer (pH 7.0) as described by HAYASHI[8], except that a loop of fiber was made[9]. Tension development was measured by a sensitive quartz lever as described previously[10].

Fibers made from natural actomyosin (myosin B) of rabbit skeletal muscle contracted maximally within one minute after addition of 5 mM ATP in a medium containing 0.08 M KCl, 8 mM $MgCl_2$, and 0.02 M Tris–histidine buffer (pH 7.0) (Fig. 1). When 3 mM (ethylene glycol bis-(β-amino ether)-*N,N'*-tetraacetic acid (EGTA)) was added to the contracted fiber to remove free Ca^{2+}, an immediate decrease in tension occurred. The extent of the drop in tension or the relaxability differed from preparation to preparation, ranging from 15 to 100%; the average of 12 runs was 50%. A typical result is seen in Fig. 1. On the other hand, when myosin B was washed 5 times by repeated suspension and sedimentation in a low ionic strength solution containing 1 mM $NaHCO_3$ and 2 mM Tris buffer (pH 7.6) to remove native tropomyosin[5], this washed myosin B became completely insensitive to Ca^{2+}; namely, it

Abbreviation: EGTA, ethylene glycol bis-(β-amino ether)-*N,N'*-tetraacetic acid.