GMP-stimulation of the cyanide-insensitive mitochondrial respiration in heat-shocked conidia of *Neurospora crassa*[1]

M. Michéa-Hamzehpour[2] and G. Turian[3]

Laboratoire de Microbiologie générale, Université de Genève, 3, Place de l'Université, CH-1211 Genève 4 (Switzerland), 5 August 1986

Summary. In mitochondria of heat-shocked conidia of *Neurospora* exogenous NADH and succinate were oxidized mainly via the alternative, hydroxamate-sensitive pathway (70%) and only 30% via the cytochromic, cyanide-sensitive pathway which was predominant in untreated conidia; the alternative oxidase pathway was markedly stimulated by guanosine 5'-monophosphate (GMP).

Key words. Neurospora crassa; conidia; heat-shock; mitochondria; cyanide-insensitive respiration; guanosine 5'-monophosphate (GMP).

Cyanide-insensitive respiration is preeminent in heat-shocked conidia of wild type *Neurospora*[4,5]. Such conidia developed a highly hydroxamate-sensitive respiration with profound alteration of their mitochondrial structure between 8 and 15 h of treatment at 46°C. The alternative pathway has been shown to be stimulated by nucleotides, especially by guanosine-5'-monophosphate or GMP[6,7]. It was therefore of interest to check this effect on our heat-shocked mitochondrial system.

Materials and methods. The wild type strain Lindegren 853 A of *Neurospora crassa* was obtained from the Fungal Genetic Stock Center (FGSC, Humboldt State University Foundation, Arcata, CA, USA).

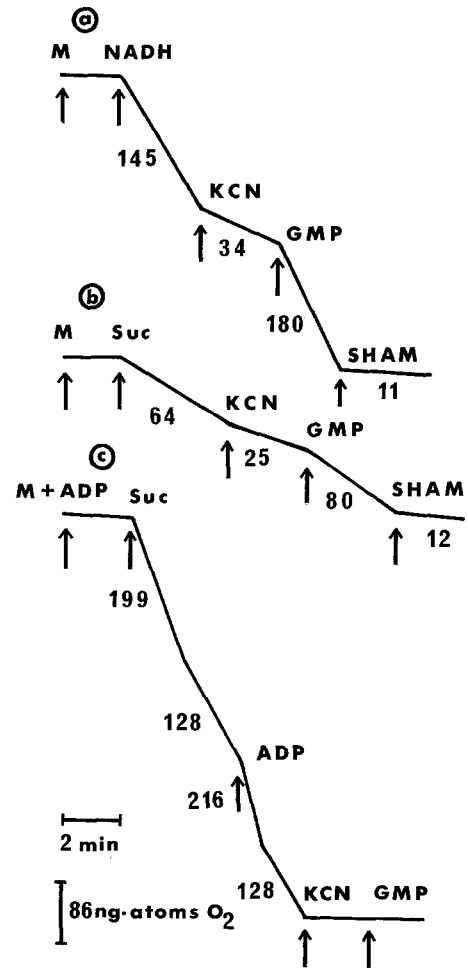
Large batches of wild type conidia were produced on solid nitrate minimal medium[8]. Fernbach flasks (1.8 l) containing 250 ml of medium were inoculated with 2.5×10^8 conidia and kept in the dark at 25 °C during two days. Humidified air was then introduced continuously into the flasks maintained during three days under fluorescent light. Conidia were inoculated at a final concentration of 5×10^6/ml in liquid Vogel's medium[9] containing 2% sucrose and incubated in a reciprocal shaker at 25 °C or at 46°C for different periods, as described previously[4].

Heat-shocked wild type conidia (10 h at 46°C: swollen without outgrown germ tubes) were harvested at room temperature by rapid filtration on a Millipore filter (mesh 1.2 µm). Untreated wild type conidia were harvested from Fernbach flasks with distilled water, filtered through four layers of cheesecloth and centrifuged at 2500 × g for 10 min. The conidial pellets were further washed with distilled water and reconcentrated by low speed centrifugation as before.

The washed conidia were then disrupted as described by Ortega Perez et al.[10] Briefly, this method consists of breaking the conidia with glass beads (0.4–0.5 mm diam.) on a modified Vortex mixer at maximal speed. The extraction buffer contained 0.44 M sucrose, 25 mM Tris-acetate, 0.7 mM EGTA and 0.5% bovine serum albumin (BSA), pH 7.2. The time of disruption for heat-shocked conidia was increased from 90 to 120 s. The centrifugation steps after disruption were as described previously[10]. The final mitochondrial pellet was suspended in the respiratory medium to a final concentration of about 20 mg of protein/ml. All operations were performed at 4°C. Mitochondrial protein was measured by the Coomassie Blue G250 method[11] using BSA as standard. O_2 consumption was measured polarographically with a Clark-type oxygen electrode (Gilson Oxygraph model K-10) in a stirred 1.7 ml reaction volume at 25°C. For each assay, 0.4–0.8 mg of mitochondrial protein was used. The respiratory medium contained 0.44 M sucrose, 25 mM Tris-acetate, 1 mM EDTA, 5 mM $MgSO_4$, 10 mM KH_2PO_4 and 2% BSA, pH 7.2.

Results and discussion. In mitochondria isolated from untreated *Neurospora* conidia, the NADH and succinate-oxidase activities were usually stable for periods up to 5-6 h. However, with the usual concentration of BSA in the extraction buffer (0.3%), the oxidative activities of mitochondria isolated from heat-shocked conidia decreased rapidly during the first hour of preparation. By increasing the concentration of BSA to 0.5%, the stability of the respiration rates was extended to a period of 4 h.

All mitochondria showed a high rate of oxygen consumption when NADH was used as substrate. The rates of oxygen uptake with NADH and succinate were lower in mitochondria of heat-shocked conidia compared to those observed in mitochondria of untreated ones. A 48 and 35% decrease in NADH and succinate oxidase activities, respectively, was observed when compared with those obtained from mitochondria of untreated conidia. When mitochondria were incubated with GMP, the rate of oxygen uptake with both substrates was markedly increased (up to 100%).



Effect of GMP on the alternative oxidase mediated *a* NADH and *b* succinate oxidase activities of mitochondrial (M) preparations from conidia of *Neurospora* heat-shocked for 10 h at 46°C. *c* Effect of GMP on succinate oxidase (Suc) activity of mitochondria isolated from untreated conidia of *Neurospora*. Oxidase activities measured polarographically at 25 °C in a reaction chamber, vol. 1.7 ml. Final concentrations: mitochondrial protein 0.4–0.8 mg; NADH 2.5 mM; succinate 10 mM; ADP 60 µM for succinate oxidation; KCN and GMP 1 mM; SHAM 2 mM. Numbers adjacent to traces are respiration rates in ng-atoms of O_2/min/mg of protein.

Stimulation of the alternative oxidase activity by GMP was found to be highest when the cytochromic pathway was inhibited by 1 mM KCN (fig.). When NADH was used, the stimulation was about 500% (fig., a) while the stimulatory effect of GMP on the succinate oxidase activity was only about 300% (fig., b). Both nucleotide activations were sharply inhibited by 2 mM SHAM or salicyl hydroxamic acid, a specific inhibitor of the mitochondrial cyanide-insensitive oxidase[12]. GMP was totally without effect on the oxygen consumption of mitochondria isolated from untreated conidia both in the presence of succinate (fig., c) or NADH as respiratory substrates. AMP, ADP, ATP and GTP were also tested and the highest stimulation (about 100%) was obtained with AMP.

The total activity of the alternative oxidase in mitochondria of heat-shocked conidia was assayed in the presence of 1 mM KCN and 1 mM GMP. Maximum inhibition of the alternative oxidase activity was in the range of 60–70% when 2 mM SHAM was used. KCN and SHAM together inhibited 90% of the oxidase activities. The residual respiratory activity is of unknown origin.

To germinate, the heat-shocked conidia have to be shifted-down to 25°C, at which temperature they efficiently recover an efficient ATP-producing cyanide-sensitive pathway[5].

1 We are grateful to Mrs A. Cattaneo for her skillful technical assistance.

2 Present address: Département de Microbiologie, CMU, 9 ave. Champel, CH-1211 Geneva, Switzerland.
3 To whom reprint requests should be addressed.

4 Michéa-Hamzehpour, M., Grange, F., Ton That, T.C., and Turian, G., Archs. Microbiol. *125* (1980) 53.
5 Michéa-Hamzehpour, M., and Turian, G., Experientia *40* (1984) 1441.
6 Vanderleyden, J., Peeters, C., Verachtert, H., and Bertrand, H., Biochem. J. *188* (1980) 141.
7 Vanderleyden, J., Kurth, J., and Verachtert, H., Biochem. J. *182* (1979) 437.
8 Davis, R.H., and De Serre, F.J., Meth. Enzymol. *27* (1970) 79.
9 Vogel, H.J., Am. Nat. *98* (1964) 435.
10 Ortega Perez, R., Michéa-Hamzehpour, M., and Turian, G., J. gen. Microbiol. *127* (1981) 309.
11 Spector, T., Analyt. Biochem. *86* (1978) 142.
12 Schonbaum, G.R., Bonner, W.D., Storey, B.T., and Bahr, J.T., Pl. Physiol. *47* (1971) 124.

0014-4754/87/040439-02$1.50 + 0.20/0

Developmental forms of human skeletal muscle AMP-deaminase

K. Kaletha, J. Spychala and G. Nowak

Department of Biochemistry, Medical School, ul. Debinki 1, PL-80-211 Gdansk (Poland), 22 July 1986

Summary. Chromatography on phosphocellulose revealed the existence of two well-separable forms of skeletal muscle AMP-deaminase in the tissue extracts of 11- and 16-week-old human fetuses. One of these forms elutes from the column at the same salt concentration as the muscle isozyme found in the skeletal muscle extract from adult man, and seems to have similar kinetic properties. The second form, which was found only in vestigial amounts in adult human tissue extract, represents different kinetic properties and seems to be a form characteristic for the fetal period of ontogenesis.
Key words. AMP-deaminase.

AMP-deaminase (E.C. 3.5.4.6; AMP-aminohydrolase) catalyzes irreversible hydrolytic deamination of 5'-adenosine monophosphate. The enzyme is widespread in animal tissues, although a considerably higher concentration is found in skeletal muscle than in other tissues, including heart and smooth muscle[3]. The enzyme of skeletal muscle is closely associated with myosin[4], participates in the purine nucleotide cycle[5], and catalyzes the reaction which is the major source of ammonia in the working muscle[6]. The total amount of AMP-deaminase in skeletal muscle varies during the course of development[7,8] and the changes in immunological, chromatographic and kinetic properties of the enzyme observed during this process indicate a developmental shift in the isozymic pattern[9–11].

Recently Fishbein et al.[12], using a new histochemical method, reported that the skeletal muscle of some patients is practically devoid of AMP-deaminase activity. The enzyme deficiency has been found in about 1% of muscle biopsies, with approximately equal frequency in males and females. All negative histochemical stains showed less than 5% of normal specific activity of the enzyme[13]. Sabina et al.[14] have found recently, that AMP-deaminase deficiency reduces the entry of adenine nucleotides into the purine nucleotide cycle during exercise, resulting in disruption of this cycle, and leading to muscular dysfunction. The deficiency appears to occur in two forms[15].

Table 1. AMP-deaminase activity in human fetal and adult human skeletal muscle extracts

Source of the enzyme	Activity (units*/per mg of protein)
11-week-old fetus (5)	0.03 ± 0.01
16-week-old fetus (1)	0.06
Adult man (1)	0.16

* One unit of the enzyme activity was defined as the amount of enzyme which catalyzes the formation of 1 μmole of ammonia per minute at 1 mM substrate concentration. Data are means from the number of determinations given in parentheses.

The primary form seems to be inherited as a complete gene block in an autosomal-recessive pattern. This form of deficiency is usually not symptomatic until middle or adult age, when muscle cramping and exercise intolerance develop. The patients have normal levels of AMP-deaminase in other tissues. Since the gene defect is not rare, in some cases it is associated with other neuromuscular disease.

The secondary, carrier form is quite common (10% in the muscle biopsy specimen population) and in this form of deficiency, the residual AMP-deaminase level is considerably higher, although clearly still deficient. Other muscle enzymes are also depleted, although not as severely.

Even in the primary form of AMP-deaminase deficiency one could find a trace of residual activity in the muscle tissue. In Fishbein's opinion such activity may represent a fetal muscle isozyme, or a blood-cell isozyme contaminating the muscle biopsy.

In this paper data are presented which indicate a developmental shift in the isozymic pattern of AMP-deaminase in human skeletal muscle.

Materials and methods. Material: Samples of human fetal leg muscle at the ages of 11 and 16 weeks of gestation were obtained from the Obstetric and Gynaecological Department of the Academic Medical School in Gdansk. Samples of 11-week-old hu-