DAMPED OSCILLATORY CONTROL OF MITOCHONDRIAL RESPIRATION AND VOLUME

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Damped oscillations of respiration and volume have been found under conditions of energized ion transport by EDTA-treated mitochondria (Azzi and Azzone, 1966). The large oscillatory volume changes which we have observed appear to reflect the increased permeability of the mitochondrial membrane but do not require the presence of antibiotics such as valinomycin (Pressman, 1965), gramicidin (Chappell and Crofts, 1965), or nonactin homologs (Graven, Lardy, and Rutter, 1966), reagents which increase the permeability of mitochondria to monovalent cations. Hence the present results may provide a simpler system for the study of the damped oscillatory control of mitochondrial processes under the conditions of energized ion transport.

Energy Sources for Mitochondrial Swelling -- The Steady State

Swelling supported by electron and energy transfer systems in rat liver mitochondria under steady state conditions is illustrated in Figure 1. Rat

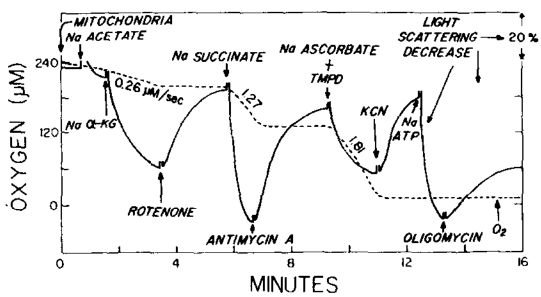

FIGURE 1.

Energy dependence of mitochondrial swelling. Rat liver mitochondria were prepared in 0.33 M sucrose 1 mM Tris-EDTA, pH 7.5 by usual procedures. The basic reaction mixture contained sucrose (100 mM), Tris-HCl (10 mM, pH 7.5), Tris-EDTA (0.3 mM) and rat liver mitochondria (2.2 mg protein in a 3 ml volume). Other additions as indicated are: acetate (50 mM), α-ketoglutarate (10 mM), succinate (5mM), ascorbate (5 mM) and ATP (4 mM) all as sodium salts; KCN (1 mM), rotenone (0.1 μg/mg protein), antimycin A (0.1 μg/mg protein), and oligomycin (0.15 μg/mg protein). Swelling was measured as decrease of 90° light scattering at 540 mμ; the initial scattering level was taken as 100 % and swelling was related to diminution of scattering from the initial level. A downward deflection of the solid trace corresponds to a decrease of light scattering.

liver mitochondria are suspended in a Tris-sucrose-EDTA medium. The addition of sodium acetate to the mitochondrial suspension provides the anion necessary for the swelling process. Hence, addition of α-ketoglutarate, as substrate, induces a large swelling which reaches a steady state in several minutes. Treatment of aerobic oxidation of α-ketoglutarate by rotenone inhibits respiration, and causes reversal of swelling. However, swelling can be reinitiated by succinate which bypasses the rotenone inhibited site as shown by the stimulation of electron transfer and induction of swelling. Succinate induced electron transfer if inhibited by antimycin results in another reversal of swelling. If electron transfer over the rotenone and antimycin inhibited sites is now bypassed by ascorbate-TMPD, swelling is once again induced as electron flow is re-established. Inhibition of electron flow through the terminal region by cyanide again inhibits electron transfer and mitochondrial swelling is again reversed. Under these circumstances electron flow is no longer possible because rotenone, antimycin, and cyanide are present. However, ATP can be employed to supply energy for the reinitiation of swelling. Oligomycin interferes with the effectiveness of ATP, once again resulting in reversal of swelling. This single experiment shows that electron transfer through all three sites of the respiratory chain associated with phosphorylation are capable of supporting swelling, and that in the absence of electron flow ATP is also an effective energy source for this purpose. Swelling brought about by the various conditions shown in this experiment results in steady state changes of mitochondrial volume, i.e., after the swelling state is achieved, volume will remain unchanged as long as the aerobic steady state persists. This is to be contrasted with the experiments shown below where a simple change in the pH in the suspending medium results in the loss of this steady state characteristic.

Energy Sources for Mitochondrial Swelling -- The Oscillatory State

Inhibition of the steady state and the induction of oscillatory changes in mitochondrial respiration and volume is illustrated in Figure 2 for rat liver mitochondria suspended in Tris-sucrose-EDTA medium at pH 8.2. The experiment was begun by suspending mitochondria in the presence of substrate, sodium succinate and rotenone. In this condition mitochondria experience a slow rate of electron transfer in the aerobic steady state. On the addition of a permeant anion, sodium acetate, respiration is increased more than two fold and a rapid and extensive swelling is initiated. After about forty seconds, the mitochondria undergo a reversal of the swelling process, and a series of damped oscillations in volume ensues over the next several minutes. These are reflected by oscillations in respiratory rate. The oscillations terminate and swelling is reversed when the mitochondria become anaerobic.

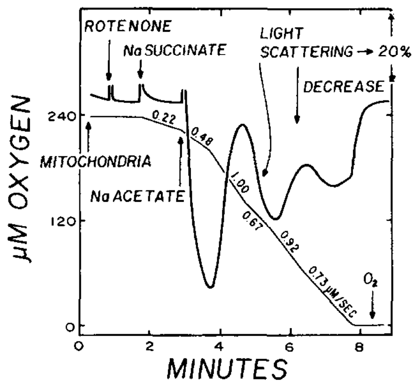

FIGURE 2.

Oscillation of mitochondrial volume and respiration. Basic conditions as in Figure 1, except pH 8.2 and rat liver mitochondria (7.7 mg protein in a 7 ml volume). Other additions as indicated are Na succinate (3.2 mM), Na acetate (43 mM) and rotenone (0.1 μg/mg protein). Light scattering change was measured at 575 mμ and the conventions are the same as in Figure 1.

Several other points of interest in this experiment are: a) the respiratory rate undergoes an abrupt two fold acceleration when the mitochondria begin the contracting phase, b) the changes in volume and respiration both show damped oscillatory characteristics, c) the average period of an oscillation is dependent upon temperature of the medium and is about 100 seconds at 25°C.

The anion and cation specificity for oscillation in electron transport and swelling is shown in Table I. The comparison shows that lithium and sodium salts of various organic acid anions, and phosphate, are more effective in inducing oscillatory responses than are the corresponding potassium or ammonium salts. The respiratory and volume changes follow a similar pattern with all effective substances. In general those substances such as sodium acetate or sodium phosphate which result in the most rapid initial swelling change are those which are capable of inducing a train of oscillations.

Some other features surrounding the occurrence of damped oscillations of mitochondrial volume which have been observed are as follows: 1) under appropriate conditions oscillation of the respiratory rate is accompanied by oscillation in the oxidation-reduction state of the respiratory carriers as judged by pyridine nucleotide fluorescence studies, 2) the oscillations can be energized by ATP under conditions where electron transport is inhibited, 3) after a series of damped oscillations has faded, the response can be reinitiated by restoring the energy supply. When energy is being supplied by

TABLE I

RELATIVE EFFECTIVENESS OF IONS IN INDUCING OSCILLATIONS OF VOLUME AND RESPIRATION IN MITOCHONDRIA

Basic conditions as in Figure 1, except pH 7.8. Other additions: succinate (3 mM), rotenone (0.1 μg/mg protein) and as indicated 50 mM each of formate, acetate, propionate or butyrate and 20 mM each of phosphate or arsenate. Volume changes were measured as 90° light scattering changes (at 540 mμ) as described in legend to Figure 1. Phases of oscillation are indicated by I → V with data taken at the peak amplitudes for swelling cycles; respiration was computed from the corresponding swelling or shrinking portion of the cycle as shown in Figure 2.

Conditions	Swelling-Shrinkage Cycle: Per cent Scattering Level					Respiration Cycle: μM O_2/sec				
	I	II	III	IV	V	I	II	III	IV	V
Li acetate	68	51	62	58	61	0.32	0.50	0.35	0.40	0.36
Li propionate	69	49	63	57	61	0.44	0.66	0.48	0.58	0.49
Na formate	40	18	21	--	--	0.24	0.36	0.23	--	--
Na acetate	64	38	53	49	--	0.49	0.91	0.68	0.79	--
Na propionate	67	40	55	50	52	0.46	0.72	0.51	--	--
Na butyrate	66	34	54	49	52	0.48	0.60	0.42	--	--
Na phosphate	48	17	39	31	37	0.44	1.12	0.78	0.98	0.70
Na arsenate	43	37	39	38	--	0.38	0.66	--	--	--
K acetate	52	--	--	--	--	0.31	--	--	--	--
K propionate	51	46	--	--	--	0.32	--	--	--	--
K phosphate	30	24	--	--	--	0.35	--	--	--	--
NH_4 acetate	16	--	--	--	--	0.32	--	--	--	--
Methylamine acetate	19	--	--	--	--	0.32	--	--	--	--
Cyclohexylamine acetate	17	--	--	--	--	0.31	--	--	--	--
Tris acetate	27	--	--	--	--	0.34	--	--	--	--

substrate, reinitiation involves the transition from the anaerobic to aerobic state by the rapid admission of oxygen to the reaction system. When the energy source for the oscillation is ATP, a second addition of ATP reinstitutes the oscillatory response.

pH Dependence and Role of EDTA

A question which arises is why an increase in the pH of the suspending medium from 7.5 to 8.2 should give rise to oscillatory conditions. Detailed studies on pH dependence of the occurrence of oscillations indicate inhibition below pH 7.4 - 7.5 and a full response at pH 8.2. At pH 7.8 the amplitude and train of oscillations are about two-thirds of the maximal response. It occurred to us that the increasing effectiveness with increase in pH might be related to the enhanced chelation of divalent cations by EDTA at high pH. Azzi and Azzone (1966) have suggested that the mitochondrial membrane is more permeable to monovalent cations in the presence of EDTA. Azzi, Rossi, and Azzone (1966) have reported that Mg^{++} inhibits the permeability of rat liver mitochondria to univalent cations while Mg^{++} chelating agents such as EDTA and citrate increase permeability. These investigators have suggested that the permeability of mitochondria may be physiologically controlled by membrane bound magnesium. In support of this suggestion we have found that a) omission of EDTA from the reaction medium permits steady state rather than oscillatory state responses, b) mitochondria capable of manifesting damped oscillations of volume and respiration in the presence of EDTA lose this characteristic if Mg^{++} is added to the system in a concentration which exceeds its binding by the chelating agent, and c) EDTA and magnesium are competitive with regard to the occurrence of mitochondrial oscillations.

The observation of oscillatory changes in mitochondrial volume observed in the presence of certain antibiotics (Pressman, 1965), (Graven, Lardy, and Rutter, 1966) show a marked resemblance to the characteristics of the oscillatory phenomenoma reported here. Observations of the oscillations in the activity of the mitochondrial respiratory chain accompanying oscillations of volume, and the inhibition of this process by Mg^{++} indicate a close association between the process of respiratory control and ion permeability in determining steady state or oscillatory state characteristics of mitochondrial energy linked processes.

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