MITOCHONDRIAL ACTIVITY IN PEA COTYLEDONS DURING GERMINATION

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SUMMARY

Changes in activity of some mitochondrial enzymes from cotyledons of peas have been followed during germination. During the first day the activity of the succinate and malate oxidase system increased whereas succinate and malate dehydrogenase activity hardly changed. Oxidase and dehydrogenase activity both increased during the subsequent two days. The respiratory control of mitochondrial fractions from mature, air-dry cotyledons was very low but increased rapidly during the first day of germination. Mitochondrial activity decreased during the later phases of germination. It is suggested that the increase of the respiratory capacity of the mitochondria during the first day is effected by an increase of the capacity of the electron transfer chain.

1. INTRODUCTION

It is generally known that the respiration of developing seeds declines during their maturation phase to a hardly detectable level in air-dry, mature seeds and that it increases again during germination.

The mitochondrial electron transfer chain is in general closely involved in this respiration process. Previous experiments (KOLLÖFFEL 1970) showed in maturing pea cotyledons a strong decrease of the activity of the succinate and malate oxidase system and a relatively small decrease of succinate and malate dehydrogenase activity.

The object of the present study is to follow the further fate of these enzymes during subsequent germination of the seeds. It will be shown that the rate of increase of the oxidase activity was significantly greater than the rate of increase of the dehydrogenase activity.

2. MATERIALS AND METHODS

Germination conditions. Air-dry seeds of *Pisum sativum* L. cv. "Rondo" were soaked in tap water under aeration for 20–22 hrs. Next they were transferred to moist filter paper in large Petri dishes where they were allowed to germinate further for the appropriate time. The germination occurred in darkness at 23°. Only completely swollen seeds were used in the further experiments. Both seed coat and axis tissue were always carefully dissected from the cotyledons. The seeds used in the present experiments belonged to the same batches as those used in previous experiments (KOLLÖFFEL 1970).

Preparation of mitochondria. Preparation of the mitochondrial fractions was carried out as described previously (KOLLÖFFEL 1970), with only one change: the mitochondrial pellet which was obtained after recentrifuging an extract at

20,000 × g for 15 min was resuspended in a medium which, besides 0,2 M sucrose and 0.05 M KH_2PO_4 buffer (pH 7.2), also contained 2% (w/v) bovine serum albumin.

Enzyme assays. The oxidative and phosphorylative activity of the mitochondrial fractions were measured polarographically with succinate, malate and α-ketoglutarate as substrate in the presence of some cofactors. Malate dehydrogenase activity (EC 1.1.1.37) was estimated spectrophotometrically by measuring the reduction of oxaloacetate in the presence of NADH in a hypotonic medium. Succinate dehydrogenase activity (EC 1.3.99.1) was also estimated spectrophotometrically by measuring the reduction of 2,6-dichlorophenol-indophenol in the presence of phenazine methosulphate and succinate. The respiration rate of whole cotyledons was measured by the Warburg manometric technique. Details of these procedures are described elsewhere (KOLLÖFFEL 1970).

3. RESULTS

Air-dry cotyledons showed hardly any respiration. The respiration increased during germination and the course may be divided into several phases (*fig. 1*). A drop occurred after about 60 hrs.

The cotyledons of mature, air-dry pea seeds have a low water content (13%) which increased rapidly to about 49% of the fresh weight during the first 10 hrs of soaking and next slowly to about 57% during the subsequent 70 hrs. Thus, the rapid uptake of water during the first 10 hrs is accompanied by a marked rise in respiration rate. The radicle emerged about 20 hrs after the onset of soaking.

Reactivation of mitochondrial enzymes occurred *in vitro* by adding water to a powder of air-dry cotyledons. The powder was allowed to imbibe for 2 hrs at 0–5°. The mitochondrial fractions which were prepared from this showed a low O_2 consumption with succinate, malate and α-ketoglutarate (*table 1*). The O_2 uptake was slightly stimulated by ADP and decreased again when the added ADP had been converted to ATP. The respiratory control ratio (RCR), defined as the ratio between the ADP-stimulated or state 3 rate and the follow-

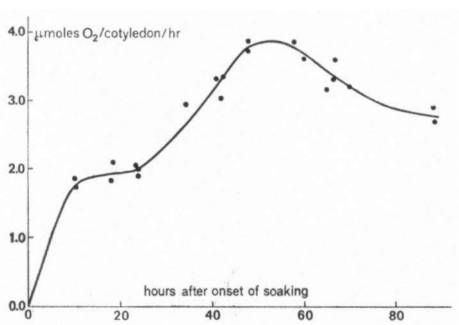

Fig. 1. Changes in respiration rate of pea cotyledons during germination.

Table 1. Comparison of O_2 uptake (state 3) and respiratory control ratios (RCR) of mitochondrial fractions from pulverized air-dry pea cotyledons and from cotyledons of peas soaked at 4° or 23°. O_2 uptake (Q_{O_2}) expressed as nmoles O_2/cotyledon/min.

treatment	substrate					
	succinate		malate		α-ketoglutarate	
	Q_{O_2}	RCR	Q_{O_2}	RCR	Q_{O_2}	RCR
air-dry	4	1.2	4	1.3	4	1.3
20 hr at 4°	6	1.2	6	1.4	5	1.3
20 hr at 23°	28	2.3	14	2.2	27	3.4

ing ADP-limited or state 4 rate, was low. Another addition of ADP was often without effect. The mitochondrial activity from cotyledons of seeds soaked for 20 hrs at 4° was also low (*table 1*). The *in vivo* respiration rate of these cotyledons, measured at 25°, was only about 1.1 μmoles/cotyledon/hr. Their water content was only somewhat lower (45% versus 52% at 20 hrs), but it is unlikely that this difference caused the relative low respiration rate *in vivo* and mitochondrial activity *in vitro*.

The O_2 consumption with succinate (*fig. 2*), malate and α-ketoglutarate (*table 2*) increased manifold during the first 65 hrs after the onset of soaking. Respiratory control and phosphorylation efficiency increased especially during the first 20 hrs. Highest values were obtained at about 40 hrs (*table 2*).

Fig. 2 further shows that succinate dehydrogenase activity scarcely changed during the first 20 hrs after the onset of soaking but doubled during the subsequent 40 hrs. Malate dehydrogenase activity increased somewhat less but further showed a similar developmental pattern.

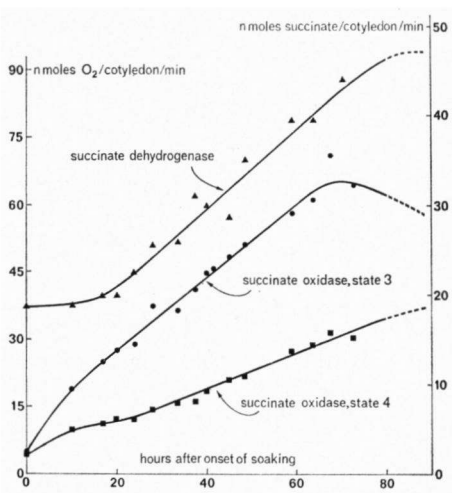

Fig. 2. Changes in the activity of the succinate oxidase system and succinate dehydrogenase activity of the mitochondrial fraction from pea cotyledons during germination.

Table 2. O_2 uptake (state 3), respiratory control (RCR), and phosphorylation efficiency (ADP/O) of mitochondrial fractions from pea cotyledons during germination. O_2 uptake (Q_{O_2}) expressed as nmoles O_2/cotyledon/min.

hours after onset of soaking	substrate					
	malate			α-ketoglutarate		
	Q_{O_2}	RCR	ADP/O	Q_{O_2}	RCR	ADP/O
0	4	1.3	—	4	1.3	—
18	12	2.1	1.2	25	3.3	1.8
40	35	3.2	1.6	36	3.4	1.9
65	42	2.1	1.2	42	2.3	1.5

The results obtained with mitochondrial fractions from cotyledons germinated for more than 90 hrs varied widely. None the less, they clearly indicated a decline of the state 3 O_2 consumption and a small increase of state 4 O_2 consumption. Consequently, the respiratory control decreased strongly and sometimes only uncoupled mitochondrial fractions were obtained. State 4 O_2 uptake decreased later also.

The decrease of the phosphorylation efficiency of pea cotyledons during germination was ascribed by ZEEVAART *et al.* (1968) to a mixture of long-chain fatty acids formed during homogenization of the tissue. The coupling could be restored largely by bovine serum albumin (BSA). In the present experiments, however, BSA had no effect on the oxidative and phosphorylative activity of mitochondria from cotyledons younger than 40 hrs, whereas the activity was enhanced more accordingly as the cotyledons were older. As the *in vivo* respiration (*fig. 1*) also decreased, it is likely that the decrease of oxidative and phosphorylative activity are manifestations of an *in vivo* desintegration of the mitochondria themselves. Comparable observations have been made with mitochondria from other seed reserve tissues (AKAZAWA & BEEVERS 1957b; YOUNG *et al.* 1960; HOWELL 1961; CHERRY 1963). Electron microscopic observations showed a desintegration of the mitochondrial ultrastructure in older peas (BAIN & MERCER 1966) and other seeds (CHERRY 1963; ÖPIK 1965).

4. DISCUSSION

Mitochondrial fractions from pulverized mature, air-dry cotyledons and those from cotyledons of seeds soaked for one day at 4° showed a very low respiratory control, a low activity of the succinate and malate oxidase system and a relatively high succinate and malate dehydrogenase activity. It is improbable that during the preparation of the extracts or during soaking a significant protein synthesis occurred, and consequently the observed activity reflects the potential activity of these mitochondrial enzymes in air-dry cotyledons. Obviously these enzymes are reactivated by hydration only during normal germination.

The biochemical integrity of the mitochondria as judged by their phosphorylation efficiency and respiratory control increased rapidly during the first 20 hrs. Concurrently, the activity of the succinate and malate oxidase system increased strongly whereas succinate and malate dehydrogenase activity increased only very little. Similar results were obtained by CHERRY (1963) and BREIDENBACH *et al.* (1967) in their experiments with germinating peanut seeds. The latter authors ascribed the low oxidase activity of their preparations to a low cytochrome content of the mitochondria.

The increase of the mitochondrial activity in germinating seeds has been attributed to two superimposed phenomena: 1. an increase in the respiratory capacity of the mitochondria themselves and 2. an increase in the number of mitochondrial. (AKAZAWA & BEEVERS 1957a, b; HOWELL 1961; CHERRY 1963; ALBERGONI *et al.* 1964; BREIDENBACH *et al.* 1966, 1967). Several electron microscopic observations showed an increase also in the mitochondrial ultrastructure during early germination (ÖPIK 1965; BAIN & MERCER 1966).

The present results are consistent with these findings but besides they strongly suggest that the increase in the respiratory capacity of the mitochondria themselves during early germination of the pea seeds must be attributed mainly to an increase of the biochemical integrity of the electron transfer chain. This conclusion is based on the finding that the oxidase activity and respiratory control strongly increased whereas the dehydrogenase activity increased only very little. It is not known whether the repair of the electron transfer chain is effected by a *de novo* synthesis of mitochondrial constituents or whether some kind of reactivation is involved. The increase of the mitochondrial activity in the first period of imbibition of castor bean seeds involves reactivation rather than *de novo* synthesis of enzymes (ALBERGONI *et al.* 1964; LADO 1966; see also MARRÈ 1967).

The present results are clearly related to those of previous experiments (KOLLÖFFEL 1970), which showed a strong decrease of the respiratory control and mitochondrial oxidase activity but only a slight decrease of the corresponding dehydrogenase activity in the cotyledons of maturing pea seeds. Thus, the activity of certain mitochondrial enzymes which was partly lost during maturation increased manifold during early germination.

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