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# The excellence of turnip mitochondrial fractions

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**Abstract**

Several mitochondrial fractions were screened for suitability in practical experiments designed for students, with the following issues in mind: avoiding the use of animals; minimal expenditure of labour and time; high enzyme activities; accessible instruments and low-cost materials. Turnips and potato tubers were identified as the best materials from which to extract purified mitochondria according to these criteria, with high respiratory activities and integrity maintained during five consecutive days. Mitochondrial respiration was assayed for succinate, exogenous NADH, malate/pyruvate and α-ketoglutarate oxidations with excellent results. At the fifth day, the respiratory control was still about 3, the integrity of the outer mitochondrial membrane maintained at values higher than 80%, and the enzymes retained more than 55% of the initial activities. 

## 1. Introduction

Most studies on mitochondrial bioenergetics are carried with the mitochondrial fraction of rat liver, owing to the large number of mitochondria and the simplicity of the preparation [1]. Less frequently, plant mitochondrial fractions have also been prepared to study mitochondrial bioenergetics [2]. Among the plant tissues used to extract mitochondria, potato tubers are often preferred owing to all the year round availability and low cost [3]. Other advantage over animal material is that potato mitochondria are much easier to isolate. Additionally, potato activities are better than those assayed with rat liver mitochondria, namely, one can obtain a more efficient oxidative phosphorylation and higher proton motive force. Furthermore, high activities are preserved over several days, as compared to animal preparations, specially if purification in Percoll gradient is carried out [3]. The advantages of potato tubers also apply to turnip roots. Laboratory practical work for undergraduate and graduate students on courses in Biochemistry, especially Bioenergetics, Metabolism and related areas, often require mitochondrial fractions that must be easy to prepare with suitable activities for performing class experiments with accessible techniques.

Among 30 different plant materials tested (results not shown), turnip mitochondrial fractions were selected, since the activities are preserved over five consecutive days. The activities of turnip preparation were very similar to those found in the potato tuber fraction, with turnip having some advantages relative to the isolation time and savings on reagents. This work is documented and illustrated with measurements of oxygen consumption upon oxidation of several suitable substrates, with calculation of respiratory indices to evaluate the bioenergetic competence of the isolated preparations. It is concluded that turnip preparations can be quite useful for experiments in laboratory classes for a full week, without the need to isolate fresh material for each day class.

## 2. Experimental

Turnip roots (*Brassica napus* L.) were peeled to expose clean tissue and kept in distilled water. All operations are performed at 0–4°C. The tissue was homogenized in a juicer (Moulinex) at 2 g fresh weigh per ml of homogenization medium containing 250 mM sucrose, 2 mM EDTA, 20 mM HEPES (pH 7.8) (or 40 mM HEPES for potato), 0.1% BSA and 4 mM cysteine (added just before homogenization) [3,4]. After filtration through 4–6 layers of cheesecloth, the homogenate was centrifuged at $3500 \times g$ for 15 min and the supernatant was centrifuged again at $10{,}000 \times g$ for 20 min. The final pellet was resuspended and applied to a 28% Percoll gradient (including 300 mM mannitol plus 10 mM HEPES, pH 7.2) and

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centrifuged for purification of the crude mitochondria [3]. The mitochondrial fraction was collected from the Percoll gradient with a Pasteur pipette and washed twice by centrifugation at 28,000 × g for 5 min in washing medium containing 250 mM sucrose, 0.1% BSA and 10 mM HEPES (pH 7.2). The pellet was gently resuspended in washing medium at a protein concentration of 20–30 mg/ml. In contrast to turnip, isolation from potato tubers requires elimination of large amounts of starch, partly sedimented by gravity during standing for 15 min, followed by a centrifugation at 400 × g for 5 min to eliminate a second fraction of starch. After this step, the process is as described for turnip. Protein was determined by the procedure of Bradford [5] calibrated with bovine serum albumin standards.

Oxygen consumption was monitored with a Clark oxygen electrode [6], at 25°C with stirring. The polarographic measurements were performed in 1.5 ml reaction medium containing 250 mM sucrose, 10 mM HEPES (pH 7.2), 20 mM KCl, 5 mM $K_2HPO_4$, 2 mM $MgCl_2$, 0.2 mM EDTA and 0.1% BSA. Uncoupled respiration was elicited by adding 75 ng/ml valinomycin. Respiration rates were calculated taking an oxygen concentration of 250 nmol $O_2$/ml in the experimental medium at 25°C. For good coupling of plant mitochondria, we always included 0.2 mM ATP in the assay medium. Mitochondrial protein was added at a concentration of 0.2–0.5 mg/ml, as indicated in the legends.

## 3. Results and discussion

The crude mitochondrial fraction isolated from root turnip was free from any visible contaminant to the naked eye (typical redish-brown of mitochondria), following purification on a Percoll gradient [3]. Washed crude mitochondria, stored at 0–4°C, exhibited good coupling for two consecutive days (results not shown). Purified mitochondria (PM), continuously stored at 0–4°C, also preserved very good coupling for at least five days.

Mitochondrial fractions in good coupling condition have a succinate-supported respiratory control (RC) of at least 2.5. Turnip and potato tuber purified mitochondria were assayed during a sequence of days of storage at 0–4°C. Fig. 1 shows that succinate-supported respiration maintains high activities and good energetic coupling state along the five days of assays. For succinate-dependent respiration, RC decays from higher values and, at the fifth day of assays, the RCs were still higher than 3 (Table 1). Very good energetic coupling for a period of at least 5 days was also observed, as illustrated in Table 1. Fig. 2 shows that these fractions exhibit excellent activities respiring succinate, exogenous NADH plus 0.5 mM $CaCl_2$ [7], malate/pyruvate and α-ketoglutarate plus the cofactors $NAD^+$ and thiamine pyrophosphate (TPP). Fig. 3 shows that addition of 0.1% BSA (Sigma, fraction V) to the reaction medium improves the coupling condition of mitochondria, binding fatty acids as products of the putative activity of lipases [8] and also by inhibiting the activity of the uncoupling protein [9].

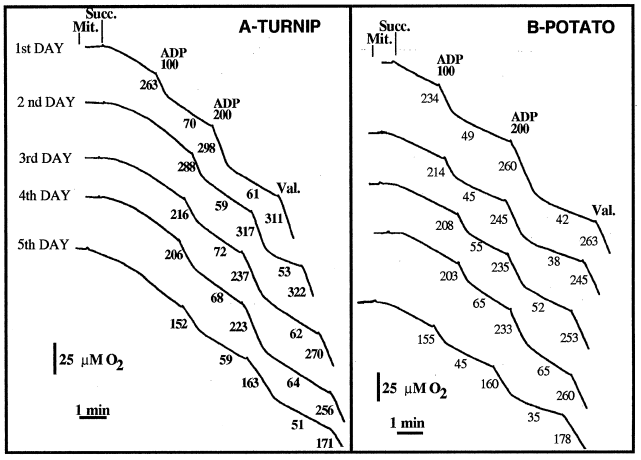


Fig. 1. Succinate-supported respiration of plant purified mitochondria over 5 consecutive days. Numbers represent activities expressed as nmol $O_2$. mg protein$^{-1}$. min$^{-1}$. Each day assays were repetitively performed, with additions as indicated for the first day assays. Mit., 0.2 mg/ml of purified mitochondria; Succ., 15 mM succinate; ADP 100, 100 μM ADP; ADP 200, 200 μM ADP; Val., 75 ng/ml valinomycin.

Table 1
Respiratory indices of the five days of assays with the same turnip and potato tuber mitochondrial fractions, corresponding to the data presented in Fig. 1

| Day of assay | Turnip | | Potato | |
|---|---|---|---|---|
| | RC | ADP/O | RC | ADP/O |
| First | 4.9 | 1.7 | 6.2 | 1.3 |
| Second | 6.0 | 1.6 | 6.5 | 1.8 |
| Third | 3.8 | 1.5 | 4.5 | 2.1 |
| Fourth | 3.5 | 1.5 | 3.6 | 2.1 |
| Fifth | 3.2 | 1.6 | 4.6 | 2.1 |

A reliable assay to check for the structural condition of purified mitochondria is the integrity of the outer membrane [3]. This is estimated from oxidation of cytochrome *c* (not permeable through the outer membrane), taking the oxygen consumption of the mitochondrial fraction in the presence of Triton X-100 (0.025–0.05%) as the 100% activity reference. Fig. 4 shows that mitochondrial outer membrane is preserved to an excellent degree. This was 97% after purification of turnip mitochondria, decaying to 88% at the fifth day. The maximal activity of turnip mitochondrial succinate-dependent respiration, obtained by uncoupling with valinomycin (75 ng/ml), was retained at the level of 55%, at the fifth day of assays.

Turnip roots are cheap, and their mitochondrial activities are higher and oxidative phosphorylation is more

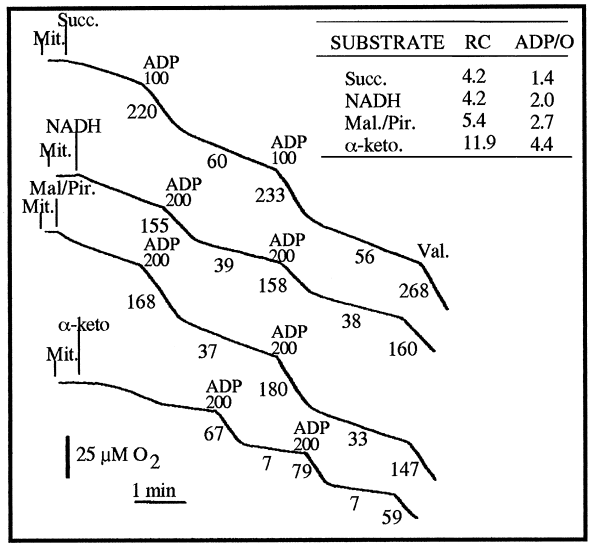


Fig. 2. Respiration of turnip mitochondria, with different substrates, during the day of mitochondrial isolation and purification (first day). Numbers represent activities expressed as nmol $O_2$. mg protein$^{-1}$. min$^{-1}$. Assays were performed in a similar way, with additions indicated at the top record. Mit., 0.2 mg/ml of purified mitochondria; Succ., 15 mM succinate; NADH, 1 mM NADH plus 0.5 mM $CaCl_2$; Mal/Pir., 30 mM malate + 6 mM pyruvate + 0.5 $NAD^+$ + 0.5 thiamine pyrophosphate; α-keto, α-ketoglutarate; ADP 100, 100 μM ADP; ADP 200, 200 μM ADP; Val., 75 ng/ml valinomycin.

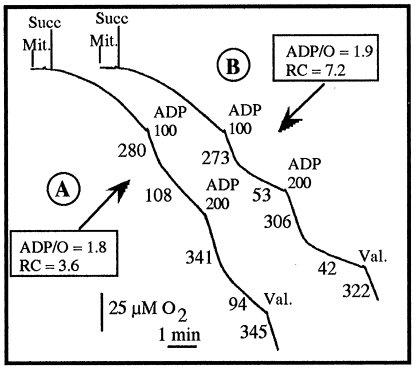


Fig. 3. Turnip mitochondrial coupling improvement by BSA. Assays were performed at the isolation day (first day), by using succinate (15 mM) as the respiratory substrate, in the absence (A) and in the presence (B) of 0.1% BSA in the reaction medium. Numbers represent activities expressed as nmol $O_2$. mg protein$^{-1}$ min$^{-1}$.

efficient, compared with laboratory animal mitochondria [1,2]. Furthermore, plant materials are easy to handle and their use avoids sacrificing animals. Recently harvested potatoes are perhaps the best material, but, in winter in Europe, only old tubers are available. However, turnips are available throughout the year. Furthermore, mitochondria isolation from turnip saves about 1 h of labour and requires less buffer, as the homogenization process results in less acidification and the negligible

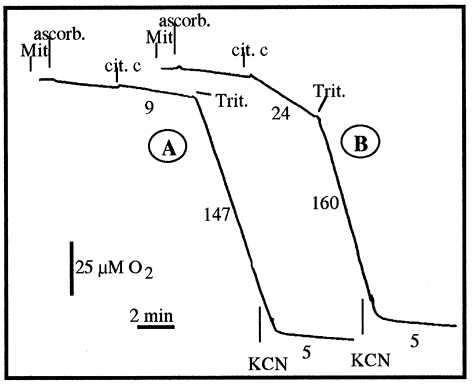


Fig. 4. Integrity of outer mitochondrial membrane ( = cytochrome *c* $O_2$ consumption of turnip mitochondria). Assays performed at the isolation day (A) and 5 days later (B). Numbers represent activities expressed as nmol $O_2$. mg protein$^{-1}$ min$^{-1}$. Assays were performed by adding 0.2 mg/ml protein (M.), 10 mM ascorbate (ascorb.), 30 μM cytochrome *c* (cit. c), 0.05% Triton X-100 (Trit.), and 0.5 mM KCN (KCN), as indicated on traces.

starch reserve facilitates laboratory operations during the isolation procedure. Thus, turnip root and potato tuber mitochondria are probably the best materials for use in class experimental work (and also for research, if the studies to be undertaken do not require the use of a particular species). This conclusion has been reached after the study of mitochondrial fractions isolated from about thirty different tissues or species, namely maize, beet root, oat, beans, wheat, cabbage, peas, broccoli, cauliflower, spinach, and others.

## Acknowledgements

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