BIOCHEMISTRY

Rapid Isolation of Active Mitochondria from Plant Tissue

MOST of the methods which have been described for the isolation of mitochondria from a variety of tissues[1-3] are essentially very similar. The cells are gently disrupted in a suitable medium, the homogenate is then filtered through a coarse filter and the supernatant obtained is fractionated into nuclear, mitochondrial, microsomal(+) soluble components by differential centrifugation. The mitochondrial fraction, which is obtained by centrifugation at 5,000–15,000*g* for 15–30 min, is then washed and resedimented. One of the drawbacks of this conventional technique is that it is both time and labour consuming, making it impractical to isolate mitochondria from several different sources (for example, tissue receiving different experimental treatments) on the same day, or to compare the effects of different buffers, in the isolation media, on the subsequent activity of the mitochondria.

The results presented here were obtained from a series of experiments which were carried out to investigate whether it might be possible, by changing the method of filtration and centrifugation, substantially to reduce the time necessary to isolate biochemically active mitochondria from Jerusalem artichoke tubers. In all the experiments which I have described, the metabolic activity of the mitochondria was assayed at 25° C in 2·0 ml. of a medium consisting of 0·25 molar sucrose, 0·05 molar *tris* (*p*H 7·2), 0·05 molar potassium hydrogen phosphate, 0·01 molar malate, 0·1 per cent bovine serum albumin and 2 mg of mitochondrial protein (measured using biuret reagent) using a vibrating platinum electrode polarograph (G.M.E. 'Oxygraph'). The ADP : O ratio and the value for the respiratory control were measured by adding aliquots of 5 μl. of a solution containing 0·5 μmoles of ADP to the reaction medium.

Experiments were then carried out with the aim of significantly accelerating the isolation procedure. A method of isolating mitochondria similar to that described by Wedding and Black[4] was adopted as a reliable basis for obtaining active mitochondria. This basic method of isolation takes 70 min to carry out. The results are shown in Table 1, column *A*. All the solutions and apparatus used in the isolation procedures were maintained at 1°–4° C. Using a stainless steel vegetable grater (holes 2 mm in diameter), 60 g of peeled Jerusalem artichoke tubers was grated in 50 ml. of a medium consisting of 0·5 molar sucrose and 0·05 molar *tris* (*p*H 7·8). The homogenate was then stirred and filtered through four layers of muslin and the resulting solution was centrifuged at 3,000*g* for 10 min to remove cell debris and nuclei. The supernatant was then further centrifuged at 15,000*g* for 25 min and the pellet which formed was resuspended in 25 ml. of 0·4 molar sucrose and 0·05 molar *tris* (*p*H 7·2) using a loose fitting Teflon homogenizer. The mitochondria were then sedimented at 15,000*g* for 25 min, and finally resuspended in 1·0 ml. of 0·4 molar sucrose and 0·05 molar *tris* (*p*H 7·2) and their respiratory activity was measured. Columns *B*, *C* and *D* in Table 1 show the results which were obtained when the basic method of isolation was modified in various ways. In treatment *B* the tissue was homogenized as already described and the homogenate was then filtered through two layers of nylon fabric (mesh 50 strands/cm) (ref. 5). No precipitate was found after centrifugation at 3,000*g* for 10 min, so this step was omitted. Mitochondria were removed from the supernatant after they had been centrifuged at 15,000*g* for 15 min. The pellet was resuspended in 25 ml. of the washing medium and recentrifuged at 15,000*g* for a further 15 min. The mitochondria were then suspended in 1·0 ml. of medium and their respiratory activity was measured. Treatment *C* was similar to *B* except that the supernatant and the washed mitochondria were centrifuged at 40,000*g* for 5 min. In treatment *D*, the homogenate was filtered through the nylon fabric and the supernatant was centrifuged at 40,000*g* for 1·5 min. The mitochondrial pellet was not washed, but was suspended in 1·0 ml. of 0·4 molar sucrose and 0·05 molar *tris* (*p*H 7·2) and assayed for respiratory activity.

Table 1. RESPIRATORY ACTIVITY OF MITOCHONDRIA ISOLATED BY MEANS OF DIFFERENT PROCEDURES

	Isolation procedures			
	A	*B*	*C*	*D*
Time elapsed during the isolation procedure	70 min	37 min	15 min	7·5 min
Total protein in the mitochondrial fraction	46 mg	32 mg	35 mg	31 mg
Rate of oxygen uptake "state 3" (μmoles oxygen/mg protein/min)	0·026	0·043	0·044	0·043
Rate of oxygen uptake "state 4" (μmoles oxygen/mg protein/min)	0·009	0·011	0·009	0·008
Respiratory control value	2·8	3·9	4·9	5·3
ADP : O ratio	2·9	2·9	2·8	2·9

Isolation treatments were: *A*, filtration through muslin and sedimentation between 3,000–15,000*g* for 25 min, followed by washing and resedimentation at 15,000*g* for 25 min; *B*, filtration through nylon fabric and sedimentation between 0–15,000*g* for 15 min, followed by washing and resedimentation at 15,000*g* for 15 min; *C*, the procedure was the same as for *B* except that sedimentation in both cases was carried out at 40,000*g* for 5 min; *D*, filtration through nylon fabric and sedimentation at 40,000*g* for 1·5 min only.

The new method reduced the time taken for isolation of active mitochondria from 70 min to just over 7 min. This was achieved by more efficient filtration, by increasing the speed of centrifugation and by omitting the final washing procedure. The values for the respiratory control increased from 2·8 to 5·3 as the time taken to isolate the mitochondria was decreased. The increase in respiratory control was chiefly the result of a significant increase in the rate of oxygen uptake during "state 3" respiration, although a slight decrease was apparent in the rate of oxygen uptake during "state 4" respiration. The ADP : O ratio remained constant in all the preparations. The increase in the rate of oxygen uptake per unit of protein in the mitochondrial fraction could be the result of rapid removal of the mitochondria from the supernatant which may contain endogenous inhibitors like quinones and fatty acids. A further advantage of this rapid isolation technique is that when it is used in conjunction with a polarographic method of assay, it is possible for mitochondria to be isolated and assayed for rate of oxygen uptake, ADP : O ratio and respiratory control can be obtained in less than 10 min, thus enabling many different samples to be compared in one day.

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Determination of Free Radicals in Gamma Irradiated Proteins

THE long-lived free radicals produced by gamma radiolysis of dry proteins at room temperature have been previously studied by electron spin resonance[1] (ESR). The carbon radicals which can be observed by ESR have been interpreted[2] as being predominantly located on glycine. Recently, the use of tritiated hydrogen sulphide as interceptor of free radicals has been proposed[3] as a new experimental technique for determining the distribution of free radicals located on carbon atoms. The method involves exposing the gamma irradiated lyophilized proteins to tritiated hydrogen sulphide in order to form carbon–tritium bonds in the protein. Tritium distribution among