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THE ISOLATION OF MITOCHONDRIA FROM POTATO-TUBER TISSUE USING SODIUM METABISULPHITE FOR PREVENTING DAMAGE BY PHENOLIC COMPOUNDS DURING EXTRACTION

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Abstract—Products of the oxidation of endogenous phenolics formed during extraction uncouple mitochondria isolated from potato-tuber (RCR = 1, P/O = 0 for all substrates tested). Potato mitochondria prepared in media containing low concentrations of metabisulphite are as active as those prepared by Verleur using cysteine. Since metabisulphite acts only by inhibiting phenoloxidases, then the method described should apply to the extraction of mitochondria from all tissues free from endogenous tannins, for the method is independent of concentration of endogenous phenolics and activity of phenoloxidase in the tissue.

INTRODUCTION

QUINONES formed from endogenous phenolics† through action of phenoloxidases, and the tannins† formed by condensation of these quinones, damage mitochondria during extraction by combining with them to form inactive or uncoupled complexes.[2-4] Polyvinylpryrrolidone (PVP) forms complexes with tannins and quinones[5] and has been used for protecting mitochondria during extraction.[3,4,6-9] However, the results of Hulme *et al.*[10] show that mitochondria, prepared from apple fruit with PVP, are poorly coupled and exhibit poor respiratory control compared with the best preparations of plant mitochondria.[11-14] Furthermore, PVP does not protect mitochondria extracted from potato-tuber tissue.[4] Evidence that PVP does not quantitatively bind the high concentrations of chlorogenic acid and other phenolics occurring in plant tissues has been reviewed by Anderson.[15]

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† A phenolic is defined (for the purpose of this paper) as a compound which is a substrate for any of the phenoloxidases. A tannin is defined by Swain[1] as a compound of MW 500–3000 with 1–2 phenolic hydroxy groups per 100 MW.

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[4] A. C. HULME and J. D. JONES, in *Enzyme Chemistry of Phenolic Compounds* (edited by J. B. PRIDHAM), p. 97, Pergamon Press, Oxford (1963).
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[11] J. D. VERLEUR, *Plant Physiol.* **40**, 1003 (1965).
[12] J. T. WISKICH and W. D. BONNER, *Plant Physiol.* **38**, 594 (1963).
[13] J. T. WISKICH, R. E. YOUNG and J. B. BIALE, *Plant Physiol.* **39**, 312 (1964).
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[15] J. W. ANDERSON, *Phytochem.* in press.

In tissue containing phenolics, but not tannins, thiols protect mitochondria either by condensing with quinones or by reducing them again to phenolics, and Verleur[11] has prepared coupled mitochondria from potato-tuber tissue by including cysteine in the extracting medium. However, Verleur points out that when the concentration of phenolics in the tissue is high, equally high concentrations of cysteine must be used. Cysteine is oxidized rapidly by plant mitochondria[16] and to avoid possible high background oxidation in his experiments, Verleur[11] restricted the tissue he used to xylem parenchyma in which the concentration of chlorogenic acid is lower than in the cortex.

Anderson and Rowan[17] have shown that stronger, non-thiol reducing agents such as metabisulphite, act by inhibiting phenoloxidases completely and are more effective than thioglycollate in protecting peptidase extracted from leaves of tobacco. In this paper we show that metabisulphite also protects mitochondria extracted from potato-tuber tissue.

RESULTS

Characteristics of the Mitochondria Prepared from Potato-Tuber Tissue

The two criteria used in judging integrity of the mitochondria were the values of the P/O ratio (the ratio of orthophosphate atoms incorporated into ATP to atoms of oxygen consumed) and the respiratory control ratio (RCR).[18] A typical trace (Fig. 1) shows oxidation of

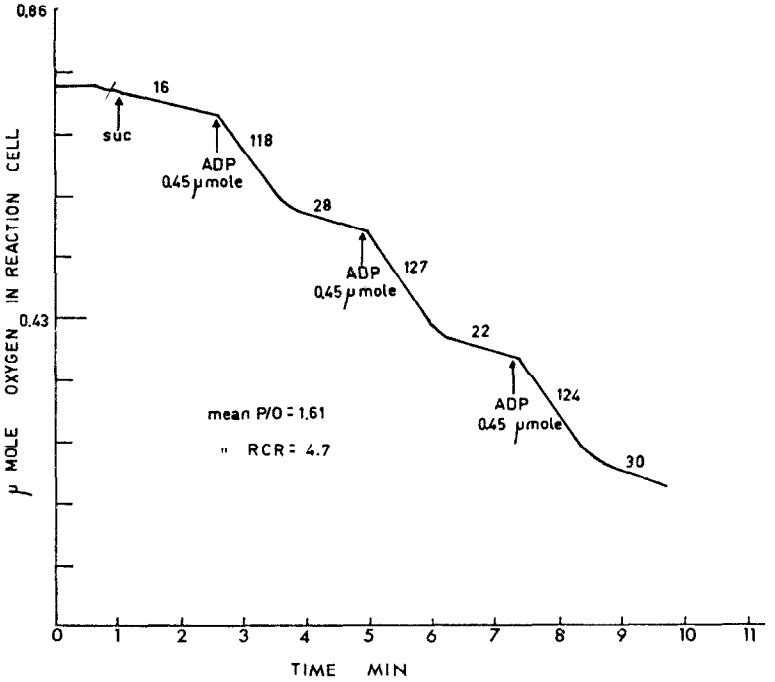

FIG. 1. A TRACE FROM THE OXYGEN ELECTRODE SHOWING UPTAKE OF OXYGEN OF POTATO MITOCHONDRIA OXIDIZING SUCCINATE (10·8 mM).

Mitochondria were prepared by the standard method described in the Experimental section (equivalent to treatment 4 in Table 1).

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[18] B. CHANCE and G. R. WILLIAMS, *Advan. Enzymol.* **17**, 65 (1956).

succinate (10·8 mM); the rate of uptake of oxygen increased immediately after adding ADP (State 3) and decreased rapidly to State 4 after all ADP was esterified to ATP. The P/O ratio was close to 2, the theoretical maximum, and the RCR (about 4) was high for plant mitochondria.[11,14] Using citrate (10·8 mM) as substrate, the rate of oxygen uptake in State 3 was lower than with succinate, but the average values of the RCR and P/O ratio were 3·30 (range 2·2–4·3) and 2·44 (range 1·9–3·0) respectively. The range of results in seventeen determinations with succinate as substrate are shown under treatment 4, Table 1.

TABLE 1. THE EFFECT OF CONCENTRATION OF SODIUM METABISULPHITE IN MEDIA 1 AND 2 ON RCR AND P/O RATIO OF POTATO-TUBER MITOCHONDRIA OXIDIZING SUCCINATE (10·8 mM)

Treatment:	1	2	3	4	5	6
	Concn of sodium metabisulphite (mM)					
Medium 1	0	1·0	2·0	2·0	3·0	4·0
Medium 2	0	0·5	0·5	1·0	0·5	1·0
RCR (mean)	1	1	1	4·44	2·38	3·74
(range)				2·8–5·7	2·2–2·6	3·4–4·3
P:O (mean)	0	0	0	1·66	1·44	1·31
(range)				1·4–2·2	1·4–1·5	1·2–1·4

The Effect of Concentration of Sodium Metabisulphite in Media 1 *and* 2

Table 1 shows the effect of increasing the concentration of metabisulphite in media 1 and 2 on the RCR and the P/O ratio. Increasing the concentration of metabisulphite in either medium above that in treatment 3 produced coupled mitochondria.

DISCUSSION

Since the level of metabisulphite in medium 2 is critical (cf. treatment 3 and 4, Table 1) we concluded that in treatment 4 (but not in treatment 3) sufficient metabisulphite is carried forward from medium 1 to medium 2 to give a concentration of metabisulphite inhibiting phenoloxidases. Since medium 3 contained no metabisulphite we conclude that either the enzyme was inactive by this stage, or that the concentration of substrate was too low for phenoloxidase to form sufficient oxidation products to inhibit the mitochondria. Since treatment 4 gave higher RCR and P/O ratios, this was adopted as the standard method for extracting mitochondria.

The minimum concentration of metabisulphite preventing formation of phenoloxidase products during extraction of enzymes is independent of the concentration of phenolics and activity of phenoloxidase in the tissue since this compound acts only by inhibiting phenoloxidase and not by combining with quinones.[17] Therefore, the method described here (treatment 4) for extracting undamaged mitochondria from potato-tuber tissue should apply without change to any plant tissue containing phenolics (but not tannins).

EXPERIMENTAL

Potato tubers (*Solanum tuberosum* L., cultivar Sequoia) were bought from retailers and stored in darkness at room temperature.

Na_2H citrate, D(+)-mannitol, $Na_2S_2O_5$ and EDTA (Laboratory Reagent Grade) were supplied by British Drug Houses Ltd., or Hopkins and Williams Ltd. Other chemicals were of Analytical Reagent grade. Bovine

serum albumin (Cohn fraction No. V) was supplied by the Commonwealth Serum Laboratories, Parkville, Victoria, Australia. ADP was supplied as the sodium salt by Sigma Chemical Co., St. Louis, Missouri, U.S.A.

The concentration of oxygen in suspensions of mitochondria was measured in a "Perspex" reaction chamber (3·6 ml) maintained at 25° with a water jacket, using a Clark oxygen electrode (Yellow Springs Instrument Co., Yellow Springs, Ohio, U.S.A.), a circuit producing an appropriate polarizing voltage (0·8 V) and a linear recorder (Model EUW 20-A, Heath Co., Benton Harbour, Michigan, U.S.A.). The recorder was adjusted so that the full scale was equivalent to the concentration of oxygen in solution at 25°. The contents of the reaction chamber were mixed with a magnetic stirrer. Additions to the reaction chamber were made with "Agla" syringes.

Extraction of Mitochondria

Chilled potatoes were peeled thickly and 100 g of tissue passed through a juice extractor (Braun AG, Frankfurt, Main, W. Germany)[19] into 300 ml of medium 1. Unless stated otherwise, this contained mannitol (0·35 M), sucrose (0·35 M), phosphate buffer (pH 6·5, 10 mM), 0·1% (w/v) bovine serum albumin, EDTA (1·0 mM) and $Na_2S_2O_5$ (2·0 mM). The extract was pressed through cheese-cloth and cell debris removed by centrifugation (600 *g* for 10 min). The fraction containing mitochondria was recovered by centrifugation at 8000 *g* for 10 min, and resuspended in 70 ml of medium 2; unless stated otherwise, this was similar to medium 1, except that EDTA was omitted and the concentration of metabisulphite was reduced to 1 mM. The mitochondrial fraction was recovered again by centrifugation (8000 *g* for 10 min) and resuspended in 70 ml of medium 3, similar to medium 2, but without metabisulphite. The mitochondrial fraction was recovered again by centrifugation as before and resuspended by hand with a Potter–Elvejhem glass homogenizer in 6 ml of medium 3. Usually 0·6 ml of this suspension was added to the reaction chamber containing medium 4 (similar to medium 3 but with 0·5 mM EDTA added). All operations were carried out in a cold room at 1°.

[19] D. L. MILLARD, J. T. WISKICH and R. N. ROBERTSON, *Plant Physiol.* **40**, 1129 (1965).