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SHORT COMMUNICATION

A NITRATE REDUCTASE FROM APPLE ROOTS

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Abstract—Inhibitors of nitrate reductase in extracts of fine roots of apple were removed by dialysis against phosphate buffer or by passing the extracts through Sephadex G-25. Reduced benzyl viologen, generated from reduced nicotinamide adenine dinucleotide by an enzyme from *Azotobacter*, was used as the hydrogen donor for nitrate reductase.

INTRODUCTION

NITRATE reductase mediates the first step reduction of nitrate to nitrite in bacteria, fungi and green plants.[1–5] This enzyme, a flavoprotein containing molybdenum, utilizes a variety of hydrogen donors. It was first extracted from *Neurospora crassa* and subsequently from a variety of plants.

Nitrate reductase has not hitherto been detected in extracts of fruit tree tissues. We now report that cell-free preparations of the roots of apples contain a potent inhibitor of the enzyme which can be removed either by a prolonged dialysis or by passage through Sephadex G-25. In this way an active enzyme can be prepared from this source.

RESULTS

The undialysed brown-coloured extracts from the roots were devoid of nitrate reductase activity. The addition of small amounts of this material to a purified nitrate reductase from *Micrococcus denitrificans* produced an inhibitory effect. When the root extracts were dialysed for 42 hr, or passed through a Sephadex G-25 column, the inhibitor(s) were removed and nitrate reductase was detected as shown in the table.

NITRATE REDUCTASE ACTIVITY OF EXTRACTS OF FINE ROOTS OF APPLE

	Extracts dialysed (hr) against 0·067 M phosphate buffer				
	0	24	42	64	84
Nitrate reductase activity mμmoles NO_2^- formed per mg protein (see text)	0	0	71	71	58

Enzyme assay as in text.

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Thus about 71 mμmoles nitrite per mg protein was produced in the nitrate reductase assay after either dialysis or Sephadex column treatment. The molecular weight of the inhibitor was under 5000 since it was adsorbed on to the Sephadex G-25 and the enzyme activity was detected in the effluent fractions. No inhibitor of nitrate reductase however was found in extracts of wheat leaves extracted by the method described here.

EXPERIMENTAL

Samples of thin roots of Jonathan trees on Malling M-104 rootstock grown in water cultures containing nitrate were thoroughly washed in ice-cold distilled water, dried on filter paper and stored at −17° for 24 hr. The material, cut with surgical scissors, was macerated in three times its weight of cold 0·067 M phosphate buffer (pH 7·5) containing 0·01 M L-cysteine for 1·5 min at top speed in a Bühler blendor (Bühler, Tübingen). The brown-coloured homogenate was squeezed through muslin and the resulting extract put into a Visking tubing and dialysed in the cold by stirring against 0·067 M phosphate buffer (pH 7·5). Both muslin and tubing were soaked in 0·067 M phosphate buffer (pH 7·5) containing 0·001 M L-cysteine hydrochloride before use. After dialysis or Sephadex treatment, the enzyme activity was assayed under anaerobic conditions in Thunberg tubes at 30°. The reaction mixture in a final volume of 2 ml was as follows: *tube:* 1 ml 0·05 M phosphate buffer (pH 7·5), 0·1 ml 0·01 M KNO_3, 0·1 ml 0·02 M $NADH_2$ and 0·1 ml 0·01 M benzyl viologen. *sidearm:* 0·4 ml root extract (3 mg protein/ml), 0·1 ml *Azotobacter* enzyme (2 mg protein/ml) and 0·2 ml 0·05 M phosphate buffer (pH 7·5). The azotobacter enzyme was used to generate reduced benzyl viologen from reduced nicotinamide adenine dinucleotide as described by Naik and Nicholas.[6] The reduced dinucleotide alone does not serve as a reductant for the nitrate reductase enzyme. After incubating for 15 min the reaction was stopped by adding zinc acetate and ethanol[7] and then centrifuging for 10 min at 5000 *g* nitrite was determined in an aliquot of the supernatant solution using the sulphanilamide method.[3]

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