Adenine Phosphoribosyltransferase Activity in Mitochondria of *Catharanthus roseus* Cells

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Subcellular localization of adenine phosphoribosyltransferase was examined in *Catharanthus roseus* cells. At least 13% of the activity was observed in the particulate fraction with the rest observed in the cytosol. Both differential and Percoll density gradient centrifugation indicated that particulate adenine phosphoribosyltransferase was predominantly located in the mitochondria.

Introduction

Adenine phorphoribosyltransferase (APRTase, EC 2.4.2.7) is one of the purine salvage enzymes and the importance of its role in nucleotide synthesis during cell growth [1 – 3] and during seed germination [4, 5] has been previously postulated. Intracellular location of APRTase has not yet been reported apart from the fact that membrane-localized APRTase was detected in *Escherichia coli* [6].

In this study, we determined the subcellular localization of APRTase in *Catharanthus roseus* cells and obtained results to show that APRTase activity was recovered in both the cytosolic and mitochondrial fractions. This is the first report of APRTase being located in mitochondria.

Materials and Methods

Five-day-old cultures of *Catharanthus roseus* cells were used as the materials for this work. The culture conditions were as described in a previous paper [7].

The cells (6 g fresh weight) were homogenized for 10 sec in a glass homogenizer with 50 mM imidazole buffer (pH 7.8) containing 0.25 M sucrose, 0.1% 2-mercaptoethanol and 2 mM EDTA. After an initial centrifugation at $170 \times g$ for 5 min to remove cell debris, particulate fractions were separated by differential centrifugation as described previously [8].

In the case of Percoll density centrifugation, the crude mitochondrial fraction (600 – 12000 g particulate fraction) was suspended and washed in 25 mM HEPES buffer (pH 7.6) containing 0.25 M sucrose, 5 mM $MgCl_2$, 5 mM KCl and 1 mM dithiothreitol. After centrifuging, the resulting pellet was

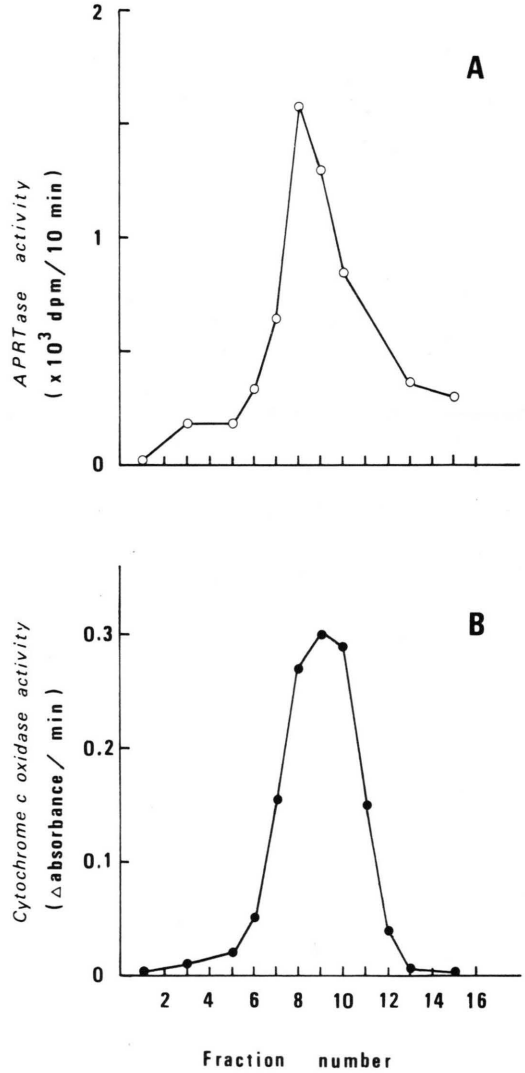

Fig. 1. The distribution of adenine phosphoribosyltransferase (APRTase) (A) and cytochrome c oxidase (B) in Percoll density gradients of the crude mitochondrial preparation. Activities of APRTase and cytochrome c oxidase are expressed as AMP produced (dpm) per 10 min and cytochrome oxidized (Δ absorbance at 550 nm) per min, respectively.

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Table I. Subcellular distribution of *Catharanthus roseus* adenine phosphoribosyltransferase. Enzyme activity is expressed as nmol of AMP formed per 1 h.

Fraction	Total protein	Total activity		Specific activity
	[mg]	[nmol/h]	[% of total]	[nmol/h/mg protein]
$600-12000 \times g$	1.54	124.3	(13.3)	80.7
$12000-100000 \times g$	1.36	12.9	(1.4)	9.5
$100000 \times g$ supernatant	17.07	799.1	(85.3)	46.8

resuspended in 1 ml of the same medium and then layered onto a discontinuous gradient consisting of four steps of 5, 28, 45 and 60%, v/v Percoll (10 ml each) containing 25 mM HEPES buffer (pH 7.6), 0.25 M sucrose, 5 mM $MgSO_4$, 5 mM KCl and 1 mM dithiothreitol. The gradients were centrifuged using an angle rotor at $18000 \times g$ for 30 min. After separation, 2.5 ml fractions were collected and sonicated with an ultrasonic disintegrator as shown in a previous paper [8].

Activity of APRTase was determined radiochemically according to our previous report [3] and cytochrome c oxidase activity was assayed essentially as described by Sottocasa *et al.* [9].

Results

Table I shows that about 85% of APRTase activity was recovered in the cytosolic fraction and about 15% of the enzyme was located in the particulate fraction. Since specific activity of APRTase in the $12000 \times g$ precipitate fraction was higher than that in the cytosolic fraction, the enzyme activity detected in the particulate fraction seems to be not attributable to contamination by the cytosolic enzyme. This was further confirmed by the Percoll density centrifugation experiments (Fig. 1). APRTase activity appeared in a single peak coinciding with the activity of cytochrome c oxidase, a marker enzyme of mitochondria.

The results obtained here suggest that APRTase is localized in both the cytosol and mitochondria of *Catharanthus roseus* cells.

Duscussion

A number of enzymes which participate in purine metabolism have been reported, but only a few investigations of the subcellular distribution of these enzymes have been carried out, especially in higher plants [10]. Cellular localization of enzymes of purine salvage in mitochondria has not been reported in any organisms.

This work demonstrated the presence of APRTase in *Catharanthus* mitochondria. Since a large amount of ATP is produced by oxidative phosphorylation within the mitochondria, the demand for the total adenine nucleotides should be large. The localization of APRTase in the mitochondria indicates that there is operation of adenine salvage which may satisfy the demand for rapid biosynthesis of adenine nucleotides in this organelle.

Our previous work revealed that approximately 30% of 5-phosphoribosyl-1-pyrophosphate synthetase was located in the mitochondrial fraction of *Catharanthus* cells [8]. Therefore, 5-phosphoribosyl-1-pyrophosphate, a substrate of APRTase, may also be generated within the mitochondria.

It may also presumed that adenine salvage in mitochondria can serve for nucleic acid synthesis in the organelle.

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