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SHORT REPORTS

SUBSTRATE SPECIFICITY OF PEROXISOMAL ACYL-CoA OXIDASE

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Key Word Index—*Spinacia oleracea*; Chenopodiaceae; *Vigna radiata*; Fabaceae; acyl-CoA oxidase; kinetic constants; plant peroxisomes.

Abstract—Using peroxisomes as an enzyme source, we have determined the K_m and V_{max} values of two plant peroxisomal acyl-CoA oxidases for various acyl-CoAs. Plant peroxisomal acyl-CoA oxidases seem to be active on long and short acyl-chain CoAs.

It has recently been shown that the enzymes of the β-oxidation pathway are also associated with plant peroxisomes which do not belong to the glyoxysomal type of these organelles [1–4]. The initial enzyme of this peroxisomal β-oxidation pathway is an acyl-CoA oxidase which transfers electrons directly to O_2, producing H_2O_2 [3]. Neither glyoxysomal nor plant peroxisomal acyl-CoA oxidases have been characterized. This report describes the substrate specificity of two plant acyl-CoA oxidases using peroxisomes as an enzyme source.

The isolation and purification of peroxisomes from spinach (*Spinacia oleracea* L. cv Früremona) leaves and mung bean (*Vigna radiata* L.) hypocotyls using sucrose density gradient centrifugation has been described [1, 3]. Acyl-CoA oxidase activity was assayed following H_2O_2 formation as in ref. [3] except that acyl-CoAs of different chain lengths were used as substrates. Acyl-CoAs were purchased from Sigma (Munich, F.R.G.). Acyl-CoA oxidases were active with saturated acyl-CoAs (50 μM) having carbon chain lengths of 4–18. The oxidases utilized lauroyl-CoA and myristoyl-CoA most effectively. With butyryl-CoA or octanoyl-CoA as substrate, the enzymes showed at least half the activity obtained with palmitoyl-CoA, the usual substrate of acyl-CoA oxidase assays. The activity of the enzymes with oleoyl-CoA or linoleoyl-CoA was comparable to or even higher than that obtained with stearoyl-CoA. The apparent K_m values and the V_{max} values, estimated by Lineweaver–Burk plots, of the acyl-CoA oxidases studied for various acyl-CoAs are shown in Table 1.

Table 1. Substrate specificity of acyl-CoA oxidase of peroxisomes from spinach leaves and mung bean hypocotyls

	Enzyme source			
	Spinach leaf peroxisomes		Mung bean hypocotyl peroxisomes	
Acyl-CoA substrates	K_m (μM)	V^*_{max}	K_m (μM)	V^*_{max}
Butyryl-CoA	32	0.43	55	2.67
Octanoyl-CoA	26	0.44	23	0.78
Lauroyl-CoA	12	1.20	13	1.73
Myristoyl-CoA	11	1.27	20	2.02
Palmitoyl-CoA	23	0.89	22	1.19
Stearoyl-CoA	23	0.95		
Linoleoyl-CoA	19	0.89	20	1.37

* V_{max} is expressed in nkat/mg organelle protein.

The activity of the plant peroxisomal acyl-CoA oxidases towards short acyl-chain CoAs distinguishes them from the mammalian peroxisomal acyl-CoA oxidase. This enzyme shows very low activity with short acyl-chain CoAs in comparison with its activity on long acyl-chain CoAs [5–7]. In addition, the K_m value of rat liver acyl-CoA oxidase for octanoyl-CoA is *ca* five times higher than that for palmitoyl-CoA (12 μM) [8] while the K_m values of the plant peroxisomal acyl-CoA oxidases are roughly the same for both these substrates (Table 1). Mammalian systems probably handle long and short acyl-chain CoAs by dual compartmentation of the β-oxidation sequence [6, 9, 10]. Long acyl-chain CoAs are oxidized in peroxisomes and mitochondria while short acyl-chain CoAs are oxidized in mitochondria only. Acyl-CoA dehydrogenase, which catalyses the first oxidative reaction of the mitochondrial β-oxidation sequence, has not yet been demonstrated for plant mitochondria and the existence of a β-oxidation system in plant mitochondria has yet to be proven [2, 3]. Nevertheless, based on the data presented in this report, plant peroxisomes appear to be capable of oxidizing long and short acyl-chain CoAs.

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SPECIFIC DETECTION OF α-AMYLASE ACTIVITY IN CRUDE PLANT EXTRACTS AFTER ISOELECTRIC FOCUSING

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Key Word Index—Procion Red MX 2B amylopectin; α-amylase; β-amylase; isoelectric focusing.

Abstract—A new method which utilizes Procion Red MX 2B amylopectin for the detection of α-amylase in crude plant extracts is described. The substrate is specific only against α-amylase hydrolysis and β-amylase does not attack it. Paper containing Procion Red MX 2B amylopectin applied to gels after isoelectric focusing reveals α-amylase isoenzymes as white bands. When this technique is used, heat-inactivation of β-amylase is not required.

INTRODUCTION

Amylases are involved in the hydrolysis of starch during the germination of seeds. Two distinct types of amylases are present in cereal grains: α-amylase, synthesized *de novo* during the germination process; and β-amylase, present in the starchy endosperm in bound and free forms [1]. Isoenzymes of both α- and β-amylases have been identified and described in the literature [2–5].

It is difficult to assay α-amylase activity in crude plant extracts because of the presence of the β-enzyme; however, it is possible to inactivate malt β-amylase selectively by heating under suitable conditions [6] or alternatively to utilize chromogenic substrates specific for α-amylase [7, 8]. While chromogenic substrates can be utilized for enzymatic determinations of activity, their application to isoelectric focusing is limited because of their low sensitivity when they are included in agar gels and in the film used as an overlay [9].

The incubation of focused gels in starch solutions and staining with iodine represents a specific method for α-amylase detection, only if β-amylase is inactivated by heating of enzyme samples [10]. In this way, one or more unstable α-amylase isoenzymes extracted from some cereal seeds are lost [11, 12]. In addition, such heat treatment cannot be applied to α-amylase determination in other plant tissues (e.g. soybean and alfalfa tissues) in which α-amylase activity is labile [8].

In this paper we describe a method for specific detection of α-amylase isoenzymes after isoelectric focusing without previous heat-inactivation of β-amylase. The method utilizes a soluble chromogenic and very sensitive substrate, Procion Red MX 2B amylopectin, previously used in clinical [13] and forensic science [9].

RESULTS AND DISCUSSION

Sax *et al.* [13] demonstrated that Procion Red MX 2B amylopectin is a soluble and very sensitive substrate for human salivary amylase, but they did not consider its specificity against β-amylase hydrolysis because animals have no β-amylase. We tested the substrate with commercial β-amylase using from 1 to 3000 U: no coloured soluble sugars were released from the red-dyed amylopectin. On the contrary, the substrate is specific and very sensitive (< 0.5 U) against α-amylase hydrolysis. This property of the synthetic substrate can be easily utilized for α-amylase assay in the presence of β-amylase. In particular, the solubility of Procion Red MX 2B amylopectin allows paper sheets to be made on which the substrate can be precipitated. The pink paper can then be