Mutarotase in Higher Plants: Distribution and Properties

Abstract. *The enzyme mutarotase from mammalian tissues catalyzes interconversion of anomeric forms of glucose and structurally related sugars; it may be involved in transport of sugars. Isolation and species distribution of a similar enzyme in higher plants are described. The enzyme from green pepper* (Capsicum frutescens) *was purified 230-fold. It differs from the mammalian enzyme in both substrate specificity and lack of inhibition by 1-deoxyglucose and phloridzin.*

Keilin and Hartree demonstrated that preparations of glucose oxidase contain a second enzyme (which they named mutarotase) that accelerates the action of notatin by converting α-glucose to the beta form (*1*). Sugars related configurationally to D-glucose also were substrates. Comparable results were obtained by Levy and Cook (*2*), and Keston identified a similar enzyme in mammalian tissues (*3*). Because of the particularly high concentrations of this enzyme in kidney cortex, Keston proposed that it was involved in active transport of sugars.

We report the identification and species distribution of a similar enzyme in higher plants. The purified plant enzyme differs considerably from mammalian and bacterial enzymes both in substrate specificity and in behavior with certain inhibitors.

For measurement of enzyme activity, plant tissues were homogenized in three volumes of ethylenediaminetetraacetate (EDTA) buffer (0.005*M*, *p*H 7.4), with *p*H adjustment where necessary (*4*), and centrifuged for 20 minutes at 27,000*g*. Enzyme content of the supernatant was measured polarimetrically in an assay system containing freshly dissolved 0.3 percent α-D-glucose. Change in optical rotation with time was measured in the 1-ml cell of a Bendix automatic polarimeter (type 143A) coupled to a logarithmic pen recorder. Enzyme units were calculated from the increase in first-order mutarotational rate constants (*5*). A control containing boiled plant extract was run with each measurement to determine the possible influence of nonenzymatic components of extracts on the spontaneous mutarotation rate. Optical rotatory changes represented conversion of α- to β-glucose only, since assay by glucose oxidase showed that equivalent amounts of glucose were recovered from products of both spontaneous and enzyme-catalyzed mutarotation. Measurable quantities of the enzyme were found in 23 (*6*) of the 46 plant species surveyed (Table 1). Highest concentrations of enzyme, comparable to those in certain animal tissues (*7*, *8*), were found in green peppers (*Capsicum frutescens*), tulip bulbs (*Tulipa*), and sweet corn (*Zea mays*). The enzyme was most widely distributed in the families Solanaceae, Curcurbitaceae, and Liliaceae; it was absent, or below levels of detection, in all members of Rosaceae and Rutaceae that were tested. This absence of enzyme activity was not due to the presence of inhibitors, since activity of green-pepper enzyme was not decreased by addition of extracts of the Rutaceae.

Partial purification of enzyme from green peppers was carried out. Peppers (1 kg) were homogenized in 1 liter of EDTA buffer and centrifuged at 17,-

Table 1. Distribution of plant mutarotase. One unit of enzyme converts 1 μmole of α- to β-glucose per minute at 25°C. The enzyme was absent or less than 0.5 unit/g in members of the following families: Rutaceae (lemon, lime, orange, grapefruit); Umbellifereae (carrot, celery, parsley); Rosaceae (plum, pear, apple, strawberry); Ebenaceae (persimmon); Plantaginaceae (plantain); Convolvulaceae (sweet potato); Punicaceae (pomegranate); Vitaceae (grape); and Palmae (coconut).

Dicotyledons	Units per gram fresh weight	Monocotyledons	Units per gram fresh weight
Solanaceae		Liliaceae	
Bell (green) pepper	19.1	Asparagus	5.8
Red pepper	20.2	Hyacinth	11.3
Tomato	8.0	Tulip	23.9
Egg plant	3.5		
Potato (tuber)	<0.5		
Cucurbitaceae		Gramineae	
Cantaloupe	5.1	Corn	18.7
Cucumber	4.9		
Squash	6.2		
Compositae		Amaryllidaceae	
Lettuce	2.3	Green onion	3.4
Jerusalem artichoke	3.9	Snowdrop	3.8
Chrysanthemum	3.8	Narcissus	<0.5
Artichoke	<0.5		
Cruciferae		Iridaceae	
Broccoli	9.1	Iris	8.3
Cabbage	<0.5	Crocus	<0.5
Radish	<0.5		
Leguminoseae			
Common bean	10.4		
Lima bean	<0.5		
Garden pea	<0.5		
Lauraceae			
Avocado	12.0		

Table 2. Comparative specificities of inhibitors of plant and mammalian mutarotases. Inhibitor sugars were added in their mutarotational equilibrium form at the same concentration (0.3 percent) as the substrate. First-order rate constants were derived for mutarotation of α-D-glucose catalyzed by either purified green pepper or rat kidney mutarotase (*10*) in the presence and absence of the inhibitor. Percentage of inhibition was calculated from relative decreases in mutarotational rate constant; K_i, dissociation constant.

Green pepper mutarotase		Rat kidney mutarotase	
Inhibition (%)	K_i (m*M*)	Inhibition (%)	K_i (m*M*)
	D-*Glucose*		
	24*		30*
	L-*Arabinose*		
42	14	71	4.4
	α-Methyl-glucoside		
24	32	49	11.1
	D-*Galactose*		
12	78	55	8.8
	1-Deoxy-D-glucose		
0		30	23
	D-*Xylose*		
0		31	24
	Phloridzin (8×10^{-4} M)		
0		80	

*K_m.

000*g* for 15 minutes at 4°C. This crude extract had an enzyme activity of 7 units per milligram of protein. Supernatant was treated with ice-cold ammonium sulfate, and the bulk of the enzyme precipitated at concentrations between 38 and 60 percent saturation. After dialysis, redissolved protein was fractionated by column chromatography on hydroxylapatite (*9*). Enzyme was eluted as a single sharp peak by 0.2*M* phosphate buffer, *p*H 6.4. This protein concentrate represented 95 percent of the original enzyme activity (21,000 units) and had a specific activity of 1620 units per milligram of protein.

Substrate specificity was examined by measuring first-order rate constants for mutarotation of freshly dissolved crystalline sugars in the presence and the absence of enzyme; inhibitor specificity was determined by measuring inhibition of enzyme-catalyzed mutarotation of α-D-glucose (*10*). Michaelis constant (K_m) and dissociation constants (K_i) for the enzyme-inhibitor complexes were calculated by established procedures (*8, 10*).

Only D-glucose and D-galactose were substrates. Mutarotation of D-xylose and L-arabinose was not significantly accelerated. This is in marked contrast to the specificity of the mammalian enzyme (Fig. 1), for which the pentose sugars with configurations at carbon No. 2, related to glucose and galactose, are equally good substrates. Inhibitor specificity (Table 2) was different for the plant system; neither 1-deoxyglucose nor phloridzin inhibited the plant mutarotase.

A metabolic function for mammalian mutarotase has not been shown. It is unlikely that it operates merely in metabolic breakdown of glucose, since distribution of the enzyme in mammalian tissues shows no consistent parallelism with glycolytic capacity (*7*). Furthermore, activities of hexokinase, phosphohexose isomerase, and glucose-6-phosphate dehydrogenase do not appear to be significantly influenced by the anomeric configuration at carbon No. 1 (*11*), and glucose-6-phosphate itself is not a substrate for mutarotase. The high concentration in kidney cortex and in intestinal mucosa and liver (*7*), which release glucose to the bloodstream, indicates a role for the enzyme in membrane transfer of sugars. The role could be restoration of equilibrium after asymmetric transport of anomers of sugars, or direct participation in the transport process itself. That the enzyme may be part of a mammalian "permease" system for sugars is supported by observations that sugars which are efficient competitive inhibitors of enzyme action on glucose (*12*) also inhibit active transport of glucose by intestinal sacs incubated in vitro (*13*).

Plants have transport mechanisms for sugars (*14*) although their specificity has not been determined. Because of a possible relation of mutarotase to sugar transport, comparison of the range of specificity of the plant sugar-transport mechanism with that of plant mutarotase would be of interest.

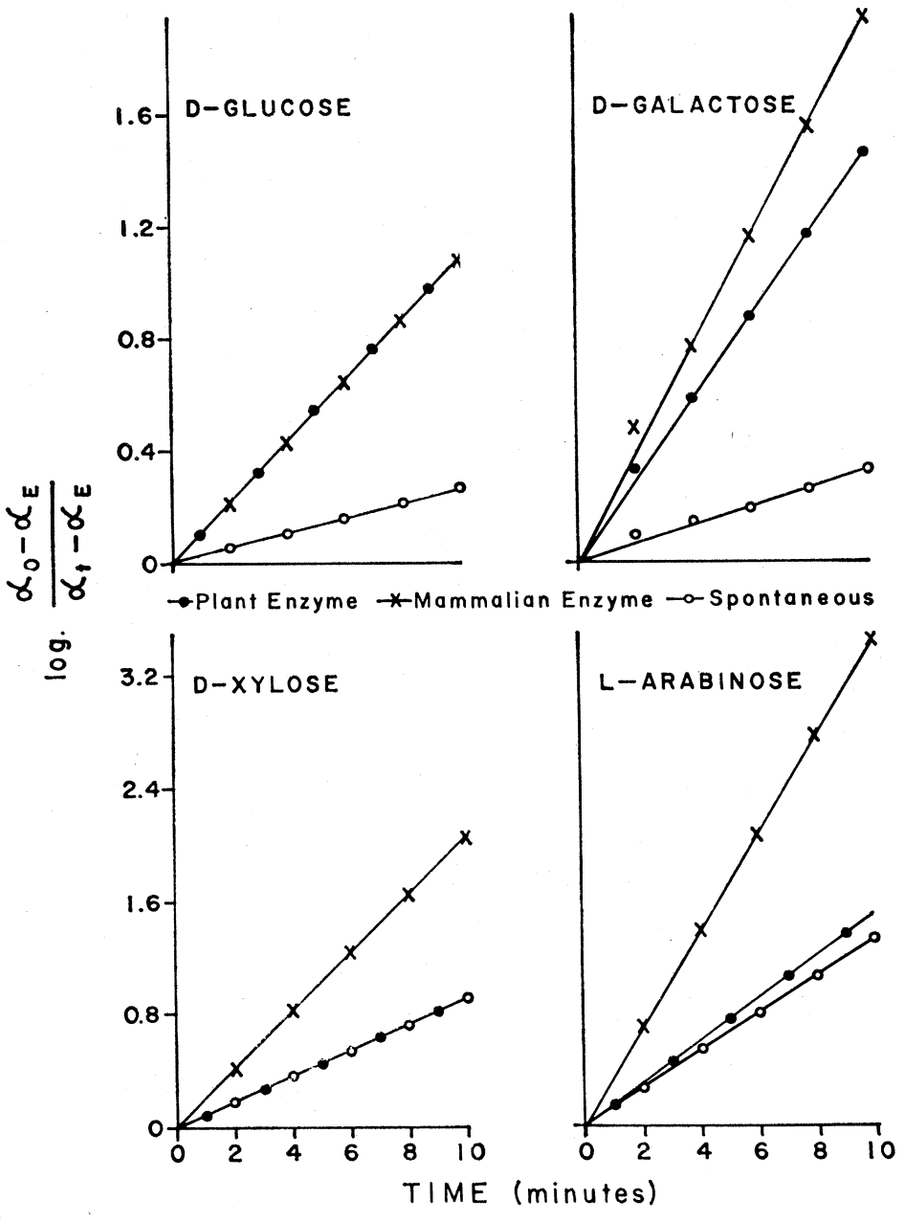

Fig. 1. Comparative specificities of substrates of plant and mammalian mutarotases. Mutarotation of 36 mg of freshly dissolved crystalline α-anomers of four sugars was measured in an assay system containing 40 units of either purified green pepper or rat kidney mutarotase in a total volume of 12 ml 0.005*M* EDTA buffer, *p*H 7.4, at 25°C. Optical rotation of the solutions was measured at intervals with the 1-ml cell of a Bendix photoelectric polarimeter. First-order rate curves were plotted as shown, where α_0, α_t, and α_E were the observed angular rotations at zero time, time t, and at equilibrium, respectively.

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D(—)-Lactate Dehydrogenase in Lower Fungi

Abstract. *A lactate dehydrogenase, specific for nicotinamide-adenine dinucleotide and D(−)-lactate, has been detected in extracts from two fungi of the order Leptomitales (Oomycetes). Several fungi of this order carry out a lactic acid fermentation under conditions of reduced oxygen tension.*

Although the fungi are generally an aerobic group of organisms, many of them grow readily under microaerobic or, as in the case of yeasts, anaerobic conditions. The energy required for anaerobic growth is obtained by fermentation of carbohydrates, with the production of lactic acid or ethyl alcohol. Although alcoholic fermentation has been studied extensively in yeasts, only a few investigators (*1*) have dealt with lactic fermentation in fungi. If the process of lactic acid formation is like that in animals, one would expect to find in fungi a lactate dehydrogenase whose coenzyme is nicotinamide-adenine dinucleotide (NAD) rather than a flavin (*2*).

Only about a dozen genera produce appreciable quantities of lactic acid during fermentation, but these are distributed randomly throughout the major groups of fungi. A NAD-dependent lactate dehydrogenase has been found in two of these genera, *Blastocladiella* (*3*) and *Rhizopus* (*1*).

In this report, we show that NAD-dependent lactate dehydrogenase (*4*), specific for the D(−) isomer of lactic acid, is present in high concentration in *Sapromyces elongatus* (strain 54-1) and *Mindeniella spinospora* (strain 57-14), two lactic acid–producing fungi belonging to the order Leptomitales (Oomycetes). Both were isolated in pure culture from decaying fruit by one of us (R.E.).

The *Sapromyces* mycelium was grown in a liquid medium consisting of peptone, yeast extract, and glucose (Cantino's PYG Broth, Difco); the organisms were harvested, washed on a Buchner funnel, and then stored in a freezer at −10°C. A cell-free extract was readily prepared by grinding 1 g (fresh weight) of mycelium in a glass homogenizer with 2 ml of cold 0.25*M* sucrose solution. The extract was clarified by centrifugation at 12,000*g* and then stored at −10°C. Samples of the extract were thawed, further diluted with 0.25*M* sucrose, and assayed spectrophotometrically for lactate dehydrogenase activity at 25°C. The reaction mixture contained sodium pyruvate ($3.3 \times 10^{-4}M$), the reduced form of nicotinamide-adenine dinucleotide (NADH) ($1.4 \times 10^{-4}M$), potassium phosphate buffer (0.1*M*, *p*H 7.5), and diluted extract in a final volume of 3.0 ml.

Oxidation of NADH, as measured by loss of absorbance at 340 mμ, proceeded linearly with respect to time and amount of extract added. No NADH was oxidized when pyruvate was omitted from the reaction mixture or when the extract had been boiled. The extract lost about one-half of its activity when incubated at 52°C for 20 minutes. The reduced form of nicotinamide-adenine dinucleotide phosphate (NADPH) was not a satisfactory replacement for NADH. Proteins in the extract were precipitated with cold 5-percent trichloroacetic acid, and their amount was estimated by the Lowry method (*5*) with bovine serum albumin as a standard. The specific activity of the lactate dehydrogenase present in the *Sapromyces* extract was 18 μmole of NADH oxidized per minute per milligram of protein. An extract of chicken breast muscle, prepared and assayed in the same way, had a specific lactate dehydrogenase activity of 10. The reaction rate was maximum at a pyruvate concentration of about $2 \times 10^{-3}M$; higher concentrations slightly inhibited the enzyme. The Michaelis constant (K_m), as determined from a Lineweaver-Burk plot, was $4 \times 10^{-4}M$.

The reverse reaction, namely the conversion of lactate to pyruvate, was studied at 25°C in a 3-ml reaction mixture containing lithium lactate (0.1*M*), NAD ($2 \times 10^{-4}M$), tris hydrochloride buffer (0.1*M*, *p*H 9.0), and extract. As shown in Fig. 1, NAD reduction was detected when the D(−) isomer of lithium lactate was used but not when L(+)-lactate was substituted for D-lactate. The K_m for D-lactate was found to be $5 \times 10^{-2}M$.

Samples of the *Sapromyces* extract were subjected to electrophoresis in starch gel at *p*H 7.0 (citrate-phosphate buffer) for 16 hours, with a voltage gradient of about 10 volt/cm, according to the procedure described by Fine and Costello (*6*). After electrophoresis, a slice of the gel was incubated for 30 minutes with a reaction mixture containing nitro-blue tetrazolium, phenazine methosulfate, NAD, lithium lactate, and tris buffer (*p*H 9.0) in order to find out where lactate dehydrogenase activity was located (*6*). A single, cathodic band of lactate dehydrogenase activity was revealed 2.3 cm from the application slit when D-lactate was present in the reaction mixture. No band appeared when another slice of the gel was incubated with a reaction mixture

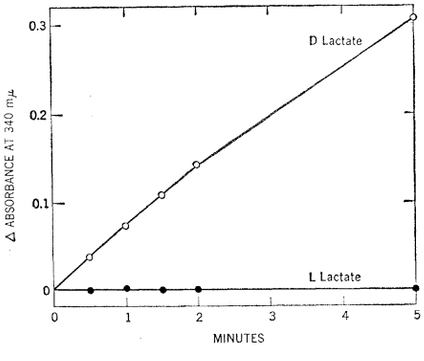

Fig. 1. *Sapromyces* lactate dehydrogenase assayed in the lactate to pyruvate direction. At 0 minutes, 0.02 ml of extract was added to the reaction mixture (see text for composition) which contained either lithium D(−)-lactate or lithium L(+)-lactate as substrate. Absorbance at 340 mμ was measured with a Zeiss spectrophotometer.