THE DISTRIBUTION OF POLYOL:NADP OXIDOREDUCTASE IN MAMMALIAN TISSUES

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SUMMARY

Polyol:NADP oxidoreductase and L-gulonate:NADP oxidoreductase in the soluble fraction of rabbit and rat tissues can be separated and identified by a vertical starch-gel electrophoretic technique. The validity of the method was established by comparing the electrophoretic mobilities of partially purified polyol:NADP oxidoreductases and L-gulonate:NADP oxidoreductases with the bands of enzyme activity detected in the soluble fraction of the tissues from which they were isolated. Polyol:NADP oxidoreductase was found to be widely distributed in the tissues of the rabbit and rat; this suggests that polyol synthesis from aldoses may occur in many mammalian tissues.

INTRODUCTION

Polyol:NADP oxidoreductase (E.C. 1.1.1.21) has been thought to have a restricted distribution in mammalian tissues. It was known to be present in seminal vesicles,[1] lens,[2,3] and placenta,[4] but was thought to be absent from other tissues. However, polyol:NADP oxidoreductases have recently been isolated from brain,[5] aorta,[6] sciatic nerve[7] and pancreas.[7] Interest in the distribution of this enzyme derives in part from evidence suggesting that increased reduction of glucose and galactose to their respective polyols is related to the development of pathological lesions in the lens and neural tissues in diabetes and galactosemia.[8,9,10,11] Cells containing polyol:NADP oxidoreductase (and in which the intracellular transport of an aldose substrate is not rate limiting) are thought to be at risk if the plasma level of the aldose is abnormally elevated.

The distribution of polyol:NADP oxidoreductase cannot be determined with certainty by demonstrating the NADPH dependent reduction of aldoses in the soluble fraction of tissues since L-gulonate:NADP oxidoreductase (E.C. 1.1.1.19) can contribute significantly to the observed activity. However, the in vivo

significance of polyol formation catalyzed by L-gulonate:NADP oxidoreductase is uncertain since the Km's for hexoses and pentoses of the enzymes isolated from brain and aorta are significantly greater than those of polyol:NADP oxidoreductase in the same tissues.[5,6] (Mano et. al[12] were unable to demonstrate the reduction of aldoses by L-gulonate:NADP oxidoreductase from rat liver, but assayed this activity only at low substrate concentrations.)

A vertical starch-gel electrophoretic technique permits the separation and identification of polyol:NADP oxidoreductase and L-gulonate:NADP oxidoreductase in the soluble fraction of rabbit and rat tissues; it has been employed to determine the distribution of these two enzymes.

METHODS

Adult, male, New Zealand rabbits and Wistar rats were decapitated; the tissues to be studied were homogenized in tris phosphate buffer (0.05 M), pH 7.3 containing 2-mercaptoethanol (0.5 mM) at 0°C. The ratio of buffer volume to tissue weight was: liver 2:1; kidney 5:1; lens 10:1, and for all other tissues 1:1. The homogenates were centrifuged at 100,000 x g for 30 minutes at 4°C. Aliquots of the supernatant (30 ul) were applied to a 15% starch-gel prepared with sodium phosphate buffer (33 mM), pH 6.3 containing 2-mercaptoethanol (0.5 mM). A specially constructed Buchler form with the gel length reduced to 22 cm was employed. The electrode buffer was sodium phosphate (0.13 M), pH 6.3 Vertical electrophoresis was carried out at 9 Volts/cm for four hours at 4°C. Enzyme activity was detected by incubating thin slices of the gel in carbonate-bicarbonate buffer (0.1 M), pH 9.6 containing L-gulonolactone (70 mM), NADP (2 mM), nitroblue tetrazolium (5 mM), and phenazine methosulfate (0.13 mM) at 37°C for 90 minutes in the dark. L-gulonolactone was chosen as substrate to permit the detection of L-gulonate:NADP oxidoreductase since Mano et. al[12] had reported that the enzyme isolated from rat liver would oxidize only L-hexonic acids and their lactones. Identical staining patterns were observed with L-gulonate prepared from the lactone obtained from Nutritional Biochemicals Corporation.

Polyol:NADP oxidoreductase was prepared from rabbit lens, sciatic nerve,

pancreas and aorta as previously described.[6,7] L-gulonate:NADP oxidoreductase was prepared from rabbit kidney and pancreas, and from rat kidney by the procedure reported for the rabbit aortic enzyme.[6] The Km L-gulonolactone of these enzymes were: rabbit kidney 30 mM; rabbit pancreas 2.0 mM; rabbit aorta 8.0 mM; and rat kidney 5.3 mM.

RESULTS

Two distinct, anodally migrating bands of NADP linked L-gulonolactone oxidizing activity were demonstrated in the starch-gel electrophoretograms of the soluble fraction of rabbit tissues. (Figure I) Neither band was observed in the absence of L-gulonolactone or L-gulonate. The slowly migrating band (Band I) has been identified as polyol:NADP oxidoreductase, and the more rapidly migrating anodal band (Band II) as L-gulonate:NADP oxidoreductase from the following observations.

Polyol:NADP oxidoreductase prepared from rabbit lens, sciatic nerve, and pancreas had the same electrophoretic mobility in the starch-gel system as Band I demonstrated in the soluble fraction of the same tissue. (Figure 1)

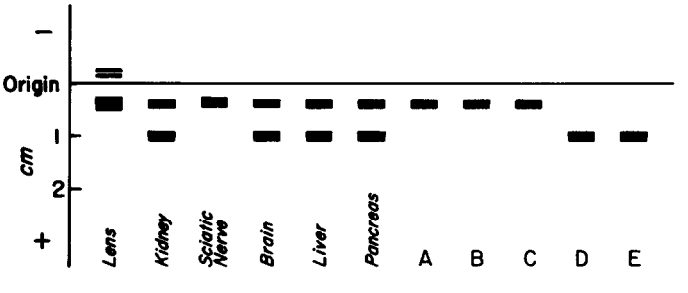

Figure 1: Diagram of vertical starch-gel electrophoretograms of the NADP linked L-gulonolactone oxidizing activity in the soluble fraction of rabbit tissues.

(A) Polyol:NADP oxidoreductase from rabbit lens.
(B) Polyol:NADP oxidoreductase from rabbit sciatic nerve.
(C) Polyol:NADP oxidoreductase from rabbit pancreas.
(D) L-gulonate:NADP oxidoreductase from rabbit kidney.
(E) L-gulonate:NADP oxidoreductase from rabbit pancreas.

The cathodally migrating activity observed in rabbit lens has not been characterized, it is not observed in the absence of substrate.

L-gulonate:NADP oxidoreductase prepared from rabbit pancreas and kidney had the same electrophoretic mobility as Band II demonstrated in the soluble fraction of rabbit pancreas and kidney. (Figure 1).

Polyol:NADP oxidoreductase and L-gulonate:NADP oxidoreductase can be separated by chromatography on DEAE-cellulose - one of the steps employed in the isolation of these enzymes.[5,6,7] When the 40 to 80% saturated ammonium sulfate fraction of rabbit sciatic nerve was chromatographed on DEAE-cellulose, only polyol:NADP oxidoreductase was recovered from the column. (Figure 2) As shown in Figure 1, only Band I was present in the starch-gel electrophoretogram of rabbit sciatic nerve.

There was also a good correlation between the identification of polyol NADP oxidoreductase and L-gulonate:NADP oxidoreductase in rabbit pancreas by the starch-gel method and by enzyme isolation. Both enzymes were recovered from the tissue by enzyme isolation (Figure 2), and both Band I and Band II

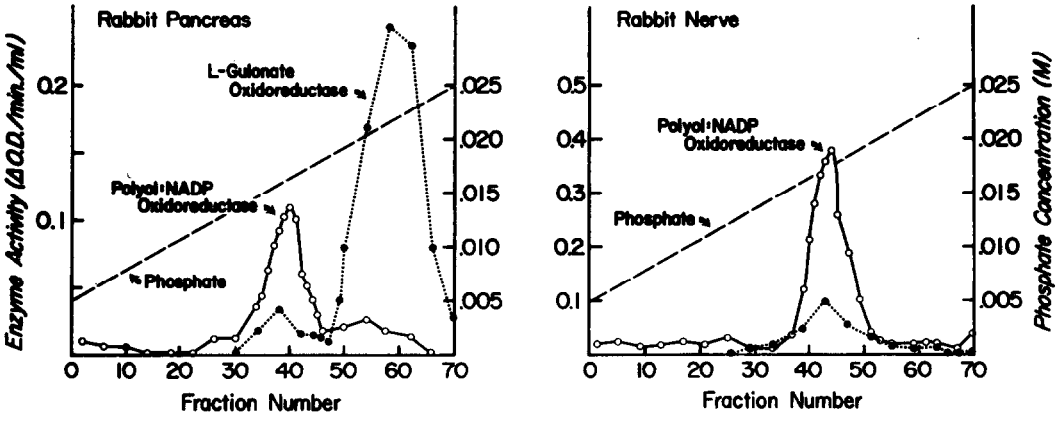

Figure 2: The desalted 40 to 80% saturated $(NH_4)_2$ SO_4 fractions (prepared as in reference 7) were pumped onto 2 x 25 cm DEAE-cellulose columns previously equilibrated with potassium phosphate buffer (5 mM) pH 6.8; the columns were washed with 200 ml of the same buffer, and developed with a linear gradient (700 ml) of the same buffer (5 mM to 25 mM). Ten ml fractions were collected. Enzyme activity was assayed spectrophotometrically at 340 mμ in 1.0 ml of phosphate buffer (67 mM) pH 6.2 containing NADPH (0.1 mM) and either: D-xylose (0.3 M) plus Li_2 SO_4 (0.4 M) indicated by open circles, or D-glucuronate (20 mM) indicated by closed circles. Assays were carried out at 30°C. (The polyol:NADP oxidoreductase peaks were subsequently chromatographed on Sephadex G-100).[7]

were present in the starch-gel electrophoretogram. (Figure 1)

Arsenis and Touster have recently reported that xylitol:NADP oxidoreductase (E.C. 1.1.1.10) is present in the cytosol as well as the mitochondria in guinea pig liver.[13] However, it is unlikely that this enzyme contributes to the activity of Band I or Band II, since xylitol:NADP oxidoreductase prepared from acetone powder of whole guinea pig liver[14] (which is largely cytoplasmic in origin)[13] was found to migrate cathodally in the starch-gel system.

Table I summarizes the distribution of these enzymes in rabbit tissues as determined by starch-gel electrophoresis and enzyme isolation.

Table II summarizes the distribution of polyol:NADP oxidoreductase and L-gulonate:NADP oxidoreductase in rat tissues as determined by vertical starch-gel electrophoresis. The electrophoretic mobilities of Band I and

Table I

Distribution of Polyol:NADP Oxidoreductase

and L-Gulonate:NADP Oxidoreductase in Rabbit Tissues

Tissue	Polyol:NADP Oxidoreductase	L-Gulonate:NADP Oxidoreductase
Lens	+ *	-
Kidney	+	+ *
Liver	+	+
Brain	+	+
Sciatic Nerve	+ *	-
Pancreas	+ *	+ *
Adrenal	+	+
Jejunum	+	+
Adipose Tissue	+	+
Diaphragm	+	+
Heart	+	+
Aorta	+' *	+' *

* The observation of a definite band of enzyme activity in the starch-gel electrophoretogram of a tissue is indicated by +; an asterisk (*) indicates that the enzyme has also been isolated from the tissue as indicated in the text and references 6 and 7. (+') Neither enzyme could be demonstrated in the unconcentrated soluble fraction of aortic homogenates, but polyol:NADP oxidoreductase and L-gulonate:NADP oxidoreductase isolated from rabbit aorta[6] had the appropriate electrophoretic mobilities.

Table II

Distribution of Polyol:NADP Oxidoreductase

and L-Gulonate:NADP Oxidoreductase in Rat Tissues

Tissue	Polyol:NADP Oxidoreductase	L-Gulonate:NADP Oxidoreductase
Lens	+	-
Kidney	+	+ *
Liver	+	+
Brain	+	+
Sciatic Nerve	+	-
Pancreas	+	+
Adrenal	+	+
Jejunum	+	+
Adipose Tissue	+	+
Diaphragm	+	+
Heart	+	+
Seminal Vesicles	+	-

See Table I for explanation of symbols.

Band II were the same in rat and rabbit tissues run in the same gels. Although extensive identification of the anodal bands observed in the starch-gel electrophoretograms of rat tissues has not been undertaken, rat kidney L-gulonate:NADP oxidoreductase was shown to have the same mobility as Band II in the soluble fraction of rat kidney.

It will be noted that polyol:NADP oxidoreductase was demonstrated in every tissue studied.

DISCUSSION

These studies suggest that polyol:NADP oxidoreductase is widely distributed in mammalian tissues and is not restricted to the few tissues in which it has previously been observed. The physiologic substrate or substrates for this enzyme in tissues other than the seminal vesicles (where it is glucose) is uncertain. However, the presence of significant concentrations of sorbitol in the lens,[8] brain,[10] nerve,[10,11] kidney,[15] and aorta[6] of normal animals suggests that glucose may be an important substrate for the enzyme in these tissues. Recent observations suggest that the sorbitol content of the aorta is subject

to hormonal regulation.[6] In addition, polyol:NADP oxidoreductases from rabbit lens, sciatic nerve, and pancreas have been found to have some of the properties of a regulatory enzyme.[7] It is therefore possible that polyol formation from aldoses, and in particular sorbitol formation from glucose, may be a normal metabolic activity in many mammalian tissues, and that this process may be subject to regulation by factors other than the concentration of substrate in the plasma.

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