MODULATION OF MAMMALIAN POLYOL:NADP OXIDOREDUCTASE ACTIVITY BY ADP AND ATP

Rex S. Clements, Jr. and Albert I. Winegrad
George S. Cox Institute
Department of Medicine
University of Pennsylvania
Philadelphia, Pennsylvania 19104

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SUMMARY

The xylose-reducing activity of polyol:NADP oxidoreductases isolated and partially purified from rabbit lens, sciatic nerve, and pancreas were found to be stimulated by ATP and to be inhibited by ADP. With the lens enzyme these effects of ATP and ADP appear to be associated with changes in Vmax without significant changes in the Km's for xylose or NADPH.

INTRODUCTION

Polyol formation in mammalian tissues containing polyol:NADP oxidoreductase (E.C.1.1.1.21) has been thought to be regulated primarily by the intracellular concentration of aldose substrate.[1,2,3] We have recently observed that the addition of dibutyryl-3'5'-cyclic AMP, epinephrine and isoproterenol *in vitro* results in a rapid increase in the sorbitol content of the rabbit aorta.[4] Intracellular transport is not rate limiting for glucose utilization in the aorta, and glucose-6-phosphatase activity is not present.[5] The demonstration of acute hormonal effects on aortic sorbitol content therefore raises the possibility that the formation and/or metabolism of polyols in mammalian tissues may be subject to types of regulation not previously suspected. This led us to examine the properties of polyol:NADP oxidoreductase isolated and partially purified from rabbit lens, sciatic nerve and pancreas. The results suggest that the activity of this enzyme *in vivo* may be subject to regulation by the intracellular concentration of ADP, ATP and other nucleotides.

MATERIALS AND METHODS

Polyol:NADP oxidoreductase was isolated and partially purified from rabbit lens, sciatic nerve, and pancreas. Two hundred grams of frozen tissue (Pel-Freez, Rogers, Arkansas) was homogenized in 5 x volume of potassium phosphate buffer (5 mM) pH 6.8 containing 2-mercaptoethanol (1 mM) in a Virtis homogenizer at 4°C. Trasylol® (100,000 u.) a protease inhibitor, was added to the buffer in which the pancreas was homogenized. Following centrifugation at 20,000 x g for 30 minutes, the supernatant fraction was treated with 2 grams of aged calcium phosphate gel per gram of protein, and the gel removed by centrifugation. The supernatant fluid was subjected to ammonium sulfate fractionation with solid ammonium sulfate. The protein which precipitated in the 40 to 80% saturated fraction was dissolved in a small volume of potassium phosphate buffer (5 mM) pH 6.8, desalted by passage through a 2.5 x 40 cm column of Sephadex G-50, and pumped onto a 2.5 x 25 cm column of DEAE cellulose previously equilibrated with the same phosphate buffer. The column was washed with 200 ml of potassium phosphate buffer (5 mM) pH 6.8 and developed with 700 ml of a linear gradient of the same buffer (5 to 25 mM). This method of elution was chosen to avoid the inhibition of polyol:NADP oxidoreductase caused by exposure to chloride ions. Polyol:NADP oxidoreductase was eluted as a single peak between 14 and 18 mM phosphate. L-gulonate:NADP oxidoreductase (E.C.1.1.1.19) is present in rabbit aorta and pancreas and was eluted from the DEAE cellulose column as a single peak between 20 and 24 mM phosphate. The pooled fractions of polyol:NADP oxidoreductase were concentrated by pressure dialysis and were applied to a 2.5 x 80 cm column of Sephadex G-100 equilibrated with phosphate buffer (5 mM) pH 6.8. The column was developed with the same buffer at 20 ml per hour. Polyol:NADP oxidoreductase activity was associated with a single peak of protein from the Sephadex G-100 column. However, the partially purified enzymes from lens and pancreas gave 4 bands of protein on disc electrophoresis.[6] On starch-gel electrophoresis the lens, sciatic nerve, and pancreatic polyol:NADP oxidoreductases

gave a single band of enzyme activity, and had mobilities which could be clearly distinguished from that of L-gulonate:NADP oxidoreductase.

Polyol:NADP oxidoreductase activity was assayed spectrophotometrically at 340 mu in 1.0 ml of potassium phosphate buffer (67 mM) pH 6.2 containing D-xylose (300 mM) and NADPH (0.1 mM) at 30°C. The specific activities (international units per mg) of the partially purified preparations of polyol:NADP oxidoreductase were: lens 0.11, sciatic nerve 0.36, and pancreas 0.05.

RESULTS AND DISCUSSION

Polyol:NADP oxidoreductases from rabbit lens, sciatic nerve, and pancreas were found to have the broad substrate specificity for aldoses previously observed with polyol:NADP oxidoreductases isolated from sheep seminal vesicles,[7] bovine lens,[8] bovine brain,[9] and *Candida utilis*.[10] The Km's for D-xylose, D-glucose, D-glucuronate, D-glucuronolactone, and NADPH of the rabbit polyol:NADP oxidoreductases are listed in Table 1.

Table 1

Substrate	Lens	Sciatic Nerve	Pancreas
D-xylose	25 mM	26 mM	40 mM
D-glucose	190 mM	90 mM	120 mM
D-glucuronate	36 mM	9 mM	30 mM
D-glucuronolactone	7 mM	3 mM	7 mM
NADPH	0.04 mM	0.03 mM	0.01 mM

Table 1. Assays performed in 1.0 ml of phosphate buffer (67 mM) pH 6.2 with 0.1 mM NADPH at 30°C. Km NADPH was determined under similar conditions with 0.3M D-xylose as substrate.

These rabbit enzymes are similar to the enzymes from sheep seminal vesicles[7] and calf lens[8] in that they are inhibited by NaCl (0.1 M) and stimulated by Li_2SO_4 (0.4 M) when assayed with D-xylose (0.3 M) as substrate under the standard conditions. The rates of reaction with NADH (0.1 mM) relative to that with NADPH (0.1 mM) for the lens, nerve and pancreatic enzymes were 0%, 9% and 10%, respectively.

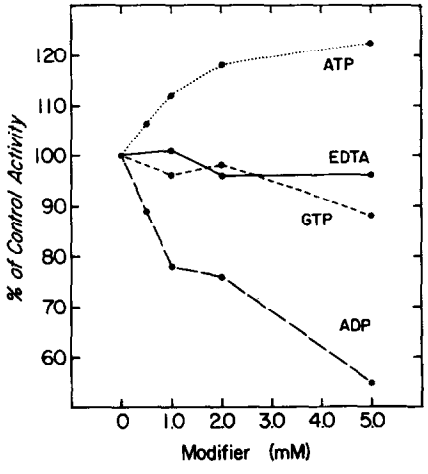

Figure 1 The rate of NADPH oxidation by polyol:NADP oxidoreductase from rabbit lens was determined in 1.0 ml of phosphate buffer (0.067M) pH 6.2 containing xylose (0.3M) and NADPH (0.1 mM) at 30°C, with the additions indicated.

As shown in Fig. 1, ATP (0.5 to 5.0 mM) increased the rate of D-xylose reduction catalyzed by rabbit lens polyol:NADP oxidoreductases. Disodium EDTA (1.0 to 5.0 mM) had no significant effect on the activity of this enzyme. However, ADP (0.5 to 5.0 mM) caused progressive inhibition of the D-xylose-reducing activity. GTP (5 mM) produced slight inhibition, while 5'AMP (0.05 to 1.0 mM) and 3'5'-cyclic AMP (0.001 to 0.02 mM) had no significant effect on the rate of D-xylose reduction by this enzyme.

As shown in Fig. 2, ADP (5 mM) had no effect on the Km's for D-xylose or NADPH of rabbit lens polyol:NADP oxidoreductase. Similarly, ATP (2 mM) did not affect the Km's for these substrates. The effects of ATP and ADP on the

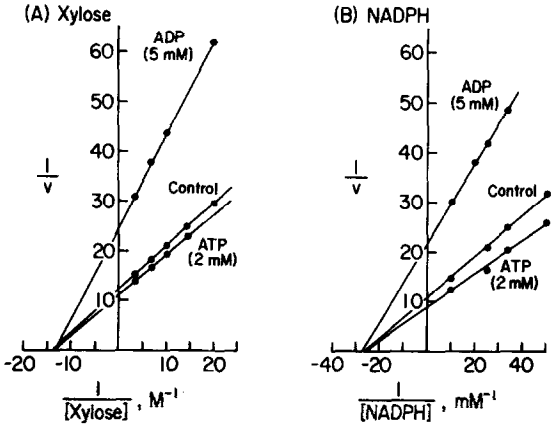

Figure 2 The Km xylose and Km NADPH of rabbit lens polyol:NADP oxidoreductase were determined under conditions noted in Methods.

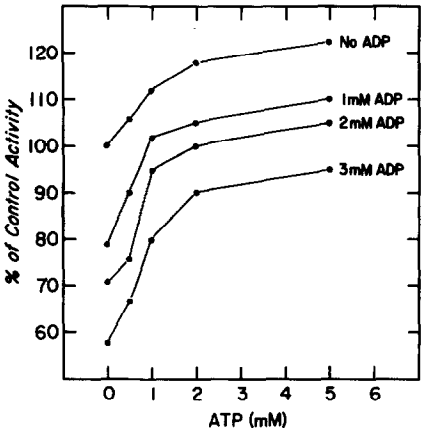

Figure 3 The rate of NADPH oxidation by rabbit lens polyol:NADP oxidoreductase was determined in 1.0 ml phosphate buffer (0.067M) pH 6.2 containing xylose (0.3M) and NADPH (0.1 mM) at 30°C with the ADP and ATP concentrations indicated.

rate of D-xylose reduction by this enzyme appear to be primarily upon the Vmax.

The effects of increasing ATP concentration on the rate of D-xylose reduction by rabbit lens polyol:NADP oxidoreductase in the presence of constant concentrations of ADP are shown in Fig. 3. ATP consistently increased the D-xylose-reducing activity in the presence of inhibitory concentrations of ADP, and the magnitude of the effect produced by low concentrations of ATP (1.0 to 2.0 mM) was increased in the presence of ADP.

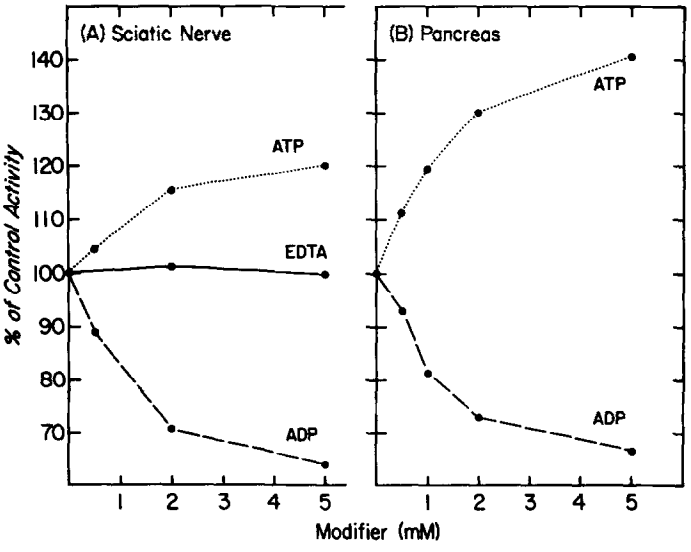

Figure 4 The rate of NADPH oxidation by polyol:NADP oxidoreductase from rabbit sciatic nerve and pancreas was determined in 1.0 ml phosphate buffer (0.067M) pH 6.2 containing xylose (0.3M) and NADPH (0.1 mM) at 30°C with the additions indicated.

Modulation of polyol:NADP oxidoreductase activity by ATP and ADP appears to be characteristic of this enzyme in rabbit tissues. As shown in Fig. 4, the activities of partially purified polyol:NADP oxidoreductases from rabbit sciatic nerve, and pancreas were also stimulated by ATP and inhibited by ADP.

Although there is no consensus concerning the physiologic substrate for polyol:NADP oxidoreductase in other tissues, this enzyme catalyzes the essentially irreversible reduction of glucose to sorbitol in seminal vesicles.[7] The observation that the activity of polyol:NADP oxidoreductase from lens, sciatic nerve and pancreas can be modulated by ADP and ATP therefore raises the possibility that it may function as a regulatory enzyme.

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