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Kinetic studies of purified malate dehydrogenase in liver of streptozotocin-diabetic rats and the effect of leaf extract of *Aegle marmelose* (L.) Correa ex Roxb.

P V Seema, B Sudha, Pius S Padayatti, Asha Abraham, K G Raghu & C S Paulose*
Molecular Neurobiology and Cell Biology Unit,
Department of Biotechnology,
Cochin University of Science and Technology,
Cochin 682 022, India

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The functional basis of diabetes-mellitus to a certain extent, can be elucidated by studying diabetes-induced changes in metabolic enzymes. Malate dehydrogenase (MDH), is an enzyme directly involved in glucose metabolism. The kinetic parameters of MDH and its purified cytosolic isozyme, S-MDH, have been studied in the liver of streptozotocin-diabetic rats; also the potential of the leaf extract of *A. marmelose* as an anti-diabetic agent was investigated. The K_m of the liver enzyme increased significantly, in both crude and purified preparations in the diabetic state when compared to the respective controls. Insulin as well as leaf-extract treatment of the diabetic rats brought about a reversal of K_m values to near normal. V_{max} of purified S-MDH was significantly higher in the diabetic state when compared to the control. Insulin and leaf extract treatment did not reverse this change. Since MDH is an important enzyme in glucose metabolism, the variation in its quantitative and qualitative nature may contribute to the pathological status of diabetes. The fact that leaf extract of *A. marmelose* was found to be as effective as insulin in restoration of blood glucose and body weight to normal levels, the use of *A. marmelose* as potential hypoglycemic agent is suggested.

Diabetes mellitus is a metabolic disorder associated with altered glucose metabolism as well as with the changes in protein and lipid metabolism[1]. Malate dehydrogenase (MDH) (EC 1.1.37) is an important enzyme in glucose metabolism. The cytosolic isozyme of MDH, S-MDH plays an important role in gluconeogenesis. In diabetes, gluconeogenesis is accelerated. Experimentally induced diabetes has been shown to cause changes in metabolic enzymes in various tissues[2-4]. Enzymes of glucose metabolism have also been studied in diabetic subjects[5].

Indigenous remedies have been used long before the use of insulin for treatment of diabetes-mellitus. Plant extracts have been used by various investigators as hypoglycemic agents[6,7]. *Aegle marmelose* (L.) Correa ex Roxb. has been reported to have antidiabetic effect in alloxan-diabetic rats[8].

This study attempts to examine the alterations in the activity of crude MDH and purified S-MDH in the liver of rats during streptozotocin-induced diabetes-mellitus. Also the effect of the leaf extract of *A. marmelose* on streptozotocin-diabetic rats and its effect of MDH activity in the diabetic state have been studied.

Age-matched, adult male Sprague Dawley rats (~200 g body wt) were used in experiments. The rats were divided randomly into two groups. One group received single intrafemoral vein injection of streptozotocin (STZ) (45 mg/kg body weight), dissolved in a citrate buffered vehicle (*p*H 4.5)[9]. A second group injected with vehicle alone served as control. First group of diabetic rats was given daily insulin (Lente) injection, intraperitoneally at 24 hr intervals according to a reported protocol[10]. On the first day 1 unit/kg body weight was given. The dose was increased and up to 6 units was given when required to maintain glucose levels to near normal levels. The last injection was given 24 hr before sacrificing the diabetic rats. Another group of diabetic rats were given the leaf extract of *A. marmelose* orally. Fresh leaves of *A. marmelose* were dried in shade and powdered. Leaf powder (10 g) was mixed with 100 ml of distilled water and stirred for 2 hr. It was kept overnight at 4°C and the supernatant was collected. This was used as the crude leaf extract and it was given orally to the second group of diabetic rats in the dosage of 1 g/kg body weight[8] at 24 hr intervals. The blood glucose was estimated using glucose oxidase enzyme kits (MERCK). The animals were given food and water *ad libitum*. Blood glucose levels and body weights of these animals were noted regularly on every 3rd day. After a period of 2 weeks, animals were sacrificed and the liver tissue was collected and stored at −70°C till analysis.

Malate dehydrogenase was assayed according to Mehler *et al.*[11] in crude and purified S-MDH and the kinetic parameters were determined. MDH was purified from rat liver based on the method of of Straub[12]. Acetone powder extraction and ammonium sulphate fractionation (50-65%) yielded the isozyme mixture. S-MDH was isolated by DEAE ion-exchange chromatography[13]. This was used for kinetic study. Puri-

*Correspondent author

rophoresis on a 12.5% resolving gel. The enzyme was visualised on the gel by enzyme-specific staining[14].

The data were analysed by one-way analysis of variance (ANOVA) and least significant difference (lsd)[15].

General characteristics of the four experimental groups are given in Table 1. Streptozotocin administered rats showed significant elevation of blood glucose and loss of body weight. Administration of insulin as well as leaf extract reversed the blood glucose to normal levels. The body weight also showed recovery in both the treated groups.

In STZ-induced diabetes mellitus, the values of K_m of oxaloacetate of total MDH (crude preparation) in the liver increased significantly (+43%) when compared with controls (Table 2). It is reversed by insulin and leaf extract treatment. K_m (oxaloacetate) of purified S-MDH showed significant increase (+33%) over control and this increase was reversed by insulin and leaf extract treatment (Table 3). V_{max} of purified S-MDH showed significant increase in diabetic state (+181%) when compared to control (Table 3). Insulin and leaf extract treatment did not reverse the increased V_{max} values of purified liver S-MDH (Table 3). The V_{max} of crude preparations of total MDH did not show any significant change (Table 2).

The significant elevation of blood glucose level and decrease in body weight in streptozotocin-induced diabetic rats may be due to altered carbohydrate metabolism and oxidation of lipids respectively. This is brought about by the selective destruction of islet β cells by the toxin[1].

A. marmelose leaf extract was comparable to insulin in reversing the body weight and blood glucose levels to normal levels. The leaf extract treated animals appeared healthier and were less prone to the frequent hypoglycemic condition observed in their insulin-treated counterparts.

The experimental diabetes altered the activity of MDH in liver. An increase in K_m of oxaloacetate of crude preparation of MDH and of purified S-MDH is observed. This signifies a decreased affinity of the enzyme for oxaloacetate which may be due to the conformational change of the enzyme MDH[16]. S-MDH is important for gluconeogenesis in the cytoplasm where it converts malate to oxaloacetate which is then converted to phosphoenolpyruvate. Decreased affinity of S-MDH to oxaloacetate implies an enhanced rate of conversion of malate to oxaloacetate, a substrate for gluconeogenesis. This enables increased gluconeogenesis, a conspicuous event in diabetes. Increase in V_{max} is observed in purified fraction of S-MDH. Thus the changes in K_m and V_{max} can favour by insulin or leaf extract administration. This shows absence of proper carbohydrate metabolism.

Normal and diabetic malic enzymes have been reported to be biochemically identical. The K_m and V_{max} values of diabetic and normal MDH for NADP were different[17]. Our results show the changed K_m and V_{max} values of MDH for oxaloacetate. The activity of soluble and particulate fractions of MDH has been reported to increase in liver of diabetic rats and insulin treatment did not restore the enzyme to control levels in the liver[18]. The increased V_{max} of purified S-MDH in liver in our experiments was not reversed by insulin or leaf extract administratin. This shows that this change is independent of the changes brought about by the absence of insulin or may be regulated by some other factor(s). The decrease in the affinity of the enzyme for oxaloacetate is reversible by insulin and leaf extract treatment. The decrease in K_m thus

Table 1—General characteristics of experimental rats
[Values are mean ± SD of 6-7 separate estimations]

Experimental group	Body weight (g)	Blood glucose (mg/dl)
Control	183.0±9.0	111.7±14.0
Diabetic	172.0±6.0*	532±28.0**
Diabetic with insulin treatment	190.0±12.0	151.8±36.0
Diabetic with leaf extract treatment	185.0±21.0	111.7±26.0

*P<0.05 compared to initial weight; and ** <0.05 with respect to control.

Table 2—Kinetic parameters of crude liver malate dehydrogenase
[Values are mean ± SE of 4-6 separate determinations]

Animal status	V_{max} (Units/min/mg protein)	K_m (m*M*)
Control	618.75±6.22	1.90±0.19
Diabetic	637.13±14.9	2.71±0.08*
Diabetic & insulin treated	622.75±17.39	1.97±0.08
Diabetic and leaf extract treated	583.7±18.64	2.19±0.17

*P<0.05 compared with control.

Table 3—Kinetic parameters of purified S-MDH in rat liver

Animal status	V_{max} (×10³) (Units/min/mg protein)	K_m (m*M*)
Control	215	2.19
Diabetic	605*	3.04**
Diabetic & insulin treated	572*	2.26
Diabetic and leaf extract treated	600*	2.07

*P<0.05 compared with control.
**P<0.05 compared with other groups.

may be an "accommodation" by the enzyme in the absence of proper carbohydrate metabolism.

Insulin and other hypoglycemic agents such as leaf extract of *A. marmelose* reverse the diabetic state by maintaining the glucose level, but the metabolic function is not totally reversed by treatment as seen by the change prevailing in MDH activity.

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