

Hepatic Arylsulfatases A and B Activities in Streptozocin-Induced Diabetic Rats

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• Abstract

Background—Reduction of sulfated glycosaminoglycans (GAG) in the liver and kidney of streptozocin-induced diabetic rats has been attributed to lowered synthesis and perhaps higher degradation of such compounds in these organs.

Objective—To measure hepatic lysosomal arylsulfatases A and B, (the enzymes responsible for the removal of sulfate groups from GAG), in starved and streptozocin-induced diabetic rats.

Methods—Diabetes was induced by streptozocin injection (40 mg per kg body weight) through the caudal vein in rats. After two weeks, the livers were removed and homogenized. Activities of arylsulfatases A and B were measured and compared with those of the liver homogenates from healthy and starved rats.

Results—The activity of liver arylsulfatase A in starved diabetic rats increased 2.15 fold as compared with normal starved animals, while that of fed diabetic rats was 3.16 fold higher than their respective control group. Increases of 1.70 and 1.94 fold in specific activities of arylsulfatase B was noticeable in the livers of diabetic animals under fed and starved conditions, respectively.

Conclusion—It appears that increased hydrolysis of sulfated GAG by liver lysosomal arylsulfatases A and B in streptozocin-induced diabetic rats may be among the contributing factors in the reduction of such sulfated compounds in this tissue.

Keywords • Hepatic arylsulfatases A and B • streptozocin-induced diabetes

Introduction

Increased degradation of heparan sulfate to large oligosaccharide fragments and the excretion of such fragments in the urine of patients with juvenile and adult onset diabetes has been described by Bonavita et al.¹ A significant decrease in the content of sulfated glycos-aminoglycans (GAG) was also demonstrated in the liver and kidney of streptozocin-induced diabetic rats.² Such animals demonstrated an increase in the activities of liver β -glucuronidase, β -N-acetyl-glucosaminidase together with increased excretion of uronic acid. In addition uptake of ³⁵S synthesis sulfate was diminished in the tissues of such animals. The data collectively suggested an altered and catabolism of GAG in streptozocin-induced diabetes.

Kjellen et al,³ demonstrated that heparan sulfate proteoglycans (HSPG) isolated from the livers of streptozocin-induced diabetic rats have a reduced net negative charge and contain a reduced number of sulfate groups. They suggested a decreased activity of glucosaminyl N-deacetylase, a regulatory enzyme for initial modification of the polymer prior to N- and O-sulfation, to be responsible for reduced HSPGs in the livers of diabetic rats. It was eventually shown by Unger et al.⁴ that N-deacetylase activity was actually 40% lower in diabetic hepatocytes as compared with control cells.

In this study experiments were conducted so as to demonstrate if the hepatic activities of two lysosomal sulfohydrolases i.e. arylsulfatases A and B, which are involved in the hydrolysis of sulfate

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esters and degradation of GAG, may also be affected by streptozocin-induced diabetes and starvation in rats.

Materials and Methods

Reagents

Nitrocatechol sulfate (dipotassium salt), sodium pyrophosphate and 4-nitrocatechol were obtained from Sigma Chemical Company (St. Louis, MO). Streptozocin was secured from the Upjohn Company (Kalamazoo, MI). Coomassie Brilliant Blue G-250 was purchased from Merck (Darmstadt, Germany). All other reagents were of analytical grade and obtained through other commercial sources.

Animal experiments

Four groups of adult male Sprague-Dawley derived rats (4-5 rats per group) with an average weight of 200 ± 2.0 g were used as experimental animals. The general conditions of animal quarters were as previously described.⁵ In all animal experiments, rats were housed individually and fed a rat chow diet (Pars Dam Co., Tehran, Iran) and water ad libitum. Two groups of rats were given a single injection of streptozocin (40 mg per kg of body weight) dissolved in 0.9% NaCl solution through the tail vein 2 weeks prior to the beginning of the experiments. The other two groups were not treated with streptozocin and were used as normal control groups.

Serum glucose levels of all groups were measured weekly by an enzymatic assay kit (Pars Azmoon Co., Tehran, Iran) upon collecting blood from the tails through heparinized microhematocrit tubes. After two weeks, animals with a serum glucose concentration of 16 mM or higher were considered as diabetic. One group of diabetic rats, together with a group of normal animals, were sacrificed at the end of the two weeks by decapitation and their livers were immediately removed, immersed in liquid nitrogen and kept at

-70° C prior to their use in enzymatic assays. They were designated as "diabetic fed" and "control fed" groups, respectively. Another group of diabetic animals and the normal controls were starved for 24 h prior to their sacrifice. The livers were removed and stored as above. They were designated as "diabetic starved" and "control starved", respectively.

Preparation of tissue homogenates

All operations were performed at 4° C unless stated otherwise. Liver samples (1g) were thawed and homogenized with 9 ml of a buffer containing Tris-HCl, 10 mM; sucrose, 0.25 mM; EDTA, 1mM, and dithiothreitol, 1mM; at pH 7.4. A glass-Teflon homogenizer with 20 up and down strokes at half-maximum speed was used for tissue homogenization. The homogenate was centrifuged at 34,800 g for 20 min and the supernatant was stored at -70° C. Prior to the measurement of arylsulfatases, the homogenate was thawed and diluted 10 fold with the above buffer mixture minus sucrose.

Assay of arylsulfatases A and B

Arylsulfatases A and B in the 34,800 g liver supernatants were assayed according to the procedure of Baum et al.⁶ with minor modifications. The modifications consisted of omitting the dialysis step and shortening the incubation times. In brief, the activity of arylsulfatase A was determined by adding 0.3 ml of 1: 10 diluted liver supernatant to 0.3 ml of reagent A (10 mM nitrocatechol sulfate, 0.5 mM sodium pyrophosphate, 1.7 M NaCl in 0.5 M sodium acetate buffer, pH 5.0) and incubated at 37° C for 30 min. The reaction was terminated with 0.3 ml of 1.0 N NaOH. The absorbance of liberated 4-

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nitrocatechol was measured using a Shimadzu spectrophotometer at 515 nm.

Arylsulfatase B activity was estimated by adding 0.3 ml aliquots of 1:10 diluted liver supernatants in each of 2 sets of assay tubes containing 0.3 ml of reagent B (50 mM nitrocatechol sulfate, 10 mM barium acetate in 0.5 M sodium acetate buffer, pH 6.0) incubated at 37° C. The reaction in one set was stopped after 10 min and that in the other set after 30 min by adding 0.3 ml of 1.0N NaOH. The absorbances of the liberated 4-nitrocatechol were measured as above. Correction for the residual activity of arylsulfatase A during arylsulfatase B measurements were done according to Baum et al.⁶ Control tubes in which the enzyme source was inactivated by addition of 1.0 N NaOH prior to incubation with reagents A or B was used to set the spectrophotometer at 0 absorbance.

Using various amounts of 4-nitrocatechol (0-300 nmol), a standard curve was established under the same condition as the experimental tubes. The amount of liberated nitrocatechol was calculated from the standard curve and the enzyme activities were expressed as nmol of nitrocatechol liberated per min per ml of the 34,000 g supernatant. Protein content was measured according to Bradford⁷ using bovine serum albumin as standard. Enzyme specific activities were expressed in terms of nmol nitrocatechol liberated per min per mg protein.

Student's t-test was used for the statistical analyses of the data.

Results

The effects of starvation and diabetes on the activities of hepatic arylsulfatases A and B are shown in [Table 1](#). As seen, both starvation and diabetes, increased liver arylsulfatases A and B specific activities. Streptozocin-induced diabetes produced a significant increase in arylsulfatase A activity of rats both in the fed state (3.16 fold, $p < 0.01$) and also in the starved state (2.15 fold, $p < 0.05$).

As shown in [Table 1](#), a 1.73 and a 1.94 fold increase in the specific activities of arylsulfatase B was noted compared with their fed and starved control animals, respectively.

Discussion

[Table 1](#) clearly demonstrates that both starvation and diabetes increase the specific activities of hepatic arylsulfatases A and B which are enzymes involved in the removal of sulfate groups from glycosaminoglycans (GAG). It was previously shown by Mohanam and Bose² that in addition to an increase in the activities of some liver enzymes involved in GAG catabolism such as β -glucuronidase and β -N-acetylglucosaminidase, a significant decrease in the uptake of 35-S sulfate was also noticeable in the liver and kidneys of diabetic rats, indicating an altered synthesis and catabolism of GAG to be the cause of significant decreases in the sulfated glycosaminoglycans in the liver and kidneys of streptozocin-induced diabetic rats. Our data ([Table 1](#)) also indicates that an increased degradation of sulfated GAG to be one of the possibilities for their reduction in the liver of diabetic rats in addition to their decreased synthesis suggested by others.^{3,4}

Pokrovsky et al.⁸ demonstrated a marked increase in the activities of arylsulfatases A and B in the liver homogenates of rats starved for 120-240 hours, while our data demonstrates that even a 24 h fast may result in approximately 3 fold increase in arylsulfatase A activity (1.31 vs. 3.85 nmol/min/mg protein, Table 1) and about 1.3 fold increase in arylsulfatase B activity (12.7 vs. 17.2 nmol/min/mg protein) of liver supernatants.

Activities of arylsulfatases during diabetes may be tissue dependent. Although Giacomelli et al.⁹ showed a decrease in the activities of arylsulfatases and other degrading enzymes of GAG catabolism in the myocardium of diabetic mice and indicated that such reduction in catabolic enzymes may be the cause of the accumulation of large residual bodies in the myocardium of such

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animals. Our data presents evidence for the increased activity of arylsulfatases in the liver as one of the contributing factors in the reduction of sulfated glycosamino-glycans in the livers of streptozocin induced diabetic rats.

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