Glutathione-Insulin Transhydrogenase of Human Kidneys

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SUMMARY

Data are presented which show that an enzyme having properties very similar to those of glutathione-insulin transhydrogenase of liver occurs in human kidneys. The enzyme has been purified partially and some of its properties are described. DIABETES *18*:176-78, March, 1969.

It is generally believed that, after liver, the kidneys may be a major site for the metabolism of insulin. This viewpoint is supported by observations of physiological and clinical situations as well as by metabolic studies. Rubenstein and Spitz[1] have reviewed the literature on this subject recently and demonstrated a physiological significance of the renal destruction of insulin.

One of the factors responsible for the degradation of insulin in liver[2,3] and pancreas[4] has been shown to be the action of an enzyme, glutathione-insulin transhydrogenase. This enzyme inactivates insulin by cleaving the hormone at the disulfide bonds. However, in the case of kidneys, the nature of the insulin-degrading activity has not been known. The present study was undertaken to determine whether an enzyme similar to the glutathione-insulin transhydrogenase of liver is present in human kidneys.

MATERIALS AND METHODS

The procedure for the purification of the enzyme was that of Tomizawa and Halsey.[2] Kidneys were obtained from a normal twenty-three-year-old female who had died instantly of a severe head injury; purification of GSH-insulin transhydrogenase from the liver obtained from the same person has previously been reported.[3] Autopsy was performed eighteen hours after death and processing of the kidneys for the preparation of acetone-dried powder was begun within two hours after autopsy.

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The acetone powder was stored at —15°. Initial assays for comparison with liver were carried out after one month and purification was undertaken after three years. Enzyme activity was followed by the use of an I-125-insulin assay.[4] A unit of enzyme was defined as the amount of enzyme that solubilized in trichloroacetic acid 1 per cent of the total radioactivity.[4]

RESULTS AND DISCUSSION

Enzyme purification

Twenty-six grams of acetone powder were subjected to fractionation; a summary of the results is presented in table 1. A 386-fold purification of the activity in the extract of the acetone powder was achieved. Distribution of protein and of enzyme activity after the starch-block electrophoretic separation of the enzyme, the last step in the purification scheme, is shown in figure 1. Even though a 386-fold purification of the activity was achieved, the enzyme is still contaminated with other proteins. The enzymes obtained from human liver, beef liver and beef pancreas were only 115, 234 and 71-fold pure, respectively, and yet, in each case, it was apparently uncontaminated with other proteins. The fact that the human kidneys enzyme can be purified by the same procedure previously used for the isolation of GSH-insulin transhydrogenase from human liver,[3] beef liver[2] and beef pancreas[4] indicates that the four enzymes may have essentially the same physical properties.

The active protein in fraction 61-63 (figure 1) was eluted from the starch block and used for further studies.

Requirement for simple sulfhydryl compound. The enzyme has absolute requirement for a simple sulfhydryl compound such as GSH; when GSH was omitted from the incubation mixture, no degradation of insulin took place (table 2). In separate experiments, it was established that GSH could be substituted by 2-mercaptoethanol and 2-mercaptoethylamine. A similar requirement for a simple sulfhydryl compound has been

TABLE 1

Purification and recovery of enzyme

Preparation	Total weight of protein (mg.)	Total activity (units)	Specific activity (units/mg.)	Degree of purification	Recovery (per cent)
Dialyzed extract*	5,784	9,430	1.6	1	100
XE-64 eluate	234	3,430	14.7	9	36
Saturation, 0.60-0.85, with $(NH_4)_2SO_4$	58	4,710	81.6	50	50
Ethanol, 0.12-0.18	4	806	201.5	123	8.5
Starch block peak	0.41	259	632.8	386	2.8

*Acetone powder of human kidney, 26 gm.

shown for GSH-insulin transhydrogenase isolated from three different sources.[2-4]

Presence of sulfhydryl group on the enzyme for activity

The procedure used was the same as previously described.[5] When the enzyme was treated with iodoacetate prior to incubation with GSH and substrate, there was some loss (25 per cent) in activity (table 3). When preincubated with GSH and then treated with iodoacetate, there was complete loss of activity. These data indicate that there is present a "buried" sulfhydryl residue(s) on the enzyme, which after reaction with GSH, participates in the catalytic reaction. A similar effect of iodoacetate was observed with the beef pancreatic enzyme[5] except for a slight difference: In the case of pancreatic enzyme, treatment with iodoacetate prior to incubation with GSH resulted in no loss in enzyme activity, whereas in the present study there was a loss of 25 per cent activity. This difference might occur because the kidney enzyme was not as pure as the pancreatic enzyme, and another enzymatic activity might have been present.

These data demonstrate the occurrence in human kidneys of an enzyme which is very similar to GSH-insulin transhydrogenase, previously shown to be present in human liver,[3] beef liver,[2] and beef pancreas.[4]

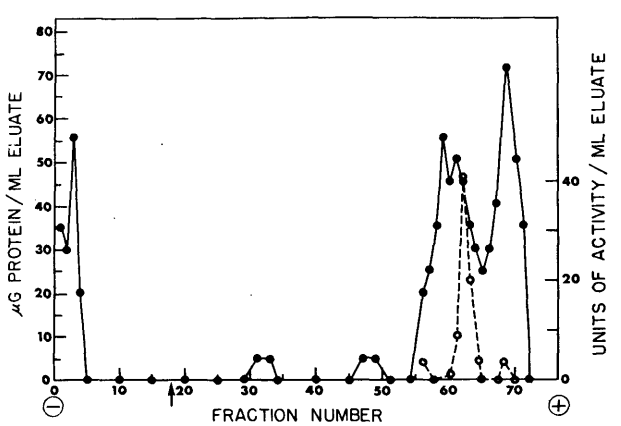

FIG. 1. Starch-block electrophoretic purification of the enzyme from ethanol-fractionated kidneys protein. The arrow indicates the point of application of sample. ●———●, protein; O- - - - O, enzyme activity.

TABLE 2

Requirement for a sulfhydryl compound

(The enzyme activity was determined with radioactive assay.[4] GSH was replaced with the compound shown; the final concentration of each compound was 1 mM. Five μg. of enzyme was used per incubation. All experiments were carried out in duplicate.)

Sulfhydryl compound	Relative enzyme activity
None	0
GSH	100
Mercaptoethanol	96
Mercaptoethylamine	89

TABLE 3

Effect of iodoacetate on the activity of the enzyme

(All incubations were carried out at 37°. In Experiment I, the enzyme (5 μg.) was incubated alone, and in Experiment 2 in 1 mM. GSH in a total volume of 0.5 ml. After five minutes, 0.1 ml. of buffer or 0.1 ml. of iodoacetate (10 mM.), was added and the incubation was continued for another five minute period. The reagents were removed by dialysis against the buffer for seventy-two hours in the cold room. During the dialysis the buffer was changed every eight to ten hours. The buffer used for all the experiments was 0.1 M potassium phosphate (pH 7.5) containing 5 mM EDTA. The resulting solutions were assayed for enzymatic activity by the radioactive assay.[4] All experiments were carried out in duplicate.)

Experiment No.	Enzyme treatment	Relative enzymatic activity
1.	A. Buffer	100
	B. Iodoacetate	75
2.	A. GSH + buffer	95
	B. GSH + iodoacetate	0

Comparison of insulin degrading activity of human kidneys and liver.

Since the liver and kidneys were obtained from the same human subject, it was of interest to compare total insulin-degrading activity of the two organs. Dialyzed extracts were prepared from the acetone powders and their insulin-degrading activity was determined using the same preparation of I-125-insulin. Kidneys were found to possess only one tenth of the insulin-degrading activity per unit protein compared with liver.

ACKNOWLEDGMENT

This work was supported by a Research Grant A-3854 from the National Institute for Arthritis and Metabolic Disease, U.S. Public Health Service.

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