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Levels of Glucose Dehydrogenases with Different Substrate Specificities in Rat and Beef Livers

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Summary

The relative substrate specificities of glucose dehydrogenases (E.C. 1.1.1.47) from beef liver and rat liver are very different. The beef enzyme oxidizes glucose more rapidly than either glucose-6-phosphate or galactose-6-phosphate. On the other hand, the dehydrogenase from rat liver prefers the hexose phosphates to glucose.

A procedure for estimating the level of glucose dehydrogenase in rat and beef liver is described. The glucose-6-phosphate dehydrogenase activity attributed to glucose dehydrogenases is estimated to be about one-fifth and one-third that of cytoplasmic glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49) in female and male rat liver respectively.

A fluorometric adaptation of the less sensitive spectrophotometric assay for glucose dehydrogenase is described.

Introduction

Nearly fifty years ago Harrison (1) described an enzyme from beef liver, glucose dehydrogenase, which catalyzes the reversible oxidation of glucose and xylose by NAD. This enzyme is widely distributed in mammalian tissues (2,3) and is found in an inactive form in the microsomal fraciton from which it is released in an active form by treatments which disrupt microsomes (1-7). Several years ago Beutler and Morrision (6) found the enzyme to oxidize also some hexose-6-phosphates including glucose-6-phosphate in the presence of NADP or NAD. Glucose dehydrogenase however differs from glucose-6-phosphate dehydrogenase (E.C. 1.1.1.1.49) in its subcellular distribution (7), substrate specificity (3,6), electrophoretic mobility (3,8), immunochemical properties (9), and mode of inheri-

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tance (10-12). Its metabolic role is obscure though its kinetic properties would suggest that it is likely functioning as a hexose-6-phosphate dehydrogenase (6).

While studying the intracellular location of the enzyme, we noted that the relative substrate specificities of the enzyme in detergent extracts prepared from beef and rat liver microsomes are strikingly different. The data here indicate that a single enzyme in each extract is responsible for these enzymatic activities and that although the levels of glucose dehydrogenase in the two livers differ greatly, hexose-6-phosphate activities are about the same in both species.

Methods and Materials

The dehydrogenases were assayed fluorometrically in a Farrand fluorometer by following the emission of light at 460 nm. Light was adsorbed at 340 nm. The system for glucose dehydrogenase contained in a vol of 1.5 mL: 100 umoles Tris pH 8.0; 0.15 μmoles NAD; 800 μmoles glucose; 0.4 μmole EDTA; 0.4 mg NaN_3. The EDTA and NaN_3, which had no effect on the activity of the dehydrogenase, were added only because they effectively prevented growth of microorganisms in the glucose solution used. The systems for glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and galactose-6-phosphate dehydrogenase contained in 1.5 mL: 100 umoles glycine, pH 10.0; 0.4 umoles NADP; 4 μmoles hexose-phosphate or hexanoic-phosphate. Under these conditions, the rates of formation of reduced pyridine nucleotide formation were directly proportional to time and enzyme concentration after corrections were made for reduced pyridine nucleotide formation independent of added substrate. Corrections for phosphogluconate dehydrogenase activity were made essentially by the method of Glock and McLean (13) when glucose-6-phosphate dehydrogenase was measured. The fluorometric assay was about 8 times more sensitive than the corresponding optical assays (5,6).

The microsomes and soluble supernatant fractions were obtained from 20% homogenates of fresh rat liver or frozen beef liver prepared according to a slight modification (14) of the method of deDuve et al (15). The microsomal fraction was washed twice in 0.25 M sucrose. The washed microsomes were suspended in water (1.0 mL water was used for the microsomes from 25 g liver) by homogenization. The microsomes were extracted for enzymes by shaking 0.72 mL microsomal suspension with 0.3 mL 0.7 M Tris-glycine, pH 7.5, 0.9 mL 5% ICI Americas G-7181 and 1.08 mL water for 30 min in an ice bath. The clear supernatant fraction obtained after centrifugation of the detergent mixture for 30 min at 105,000 x g was either used directly or dialyzed vs. 0.1 M NaCl, 0.01 M Tris, pH 8.0 for two days in the cold. There is no loss of glucose dehydrogenase activity during dialysis.

The levels of glucose dehydrogenase in liver homogenates were determined as follows. Various amounts of 20% homogenates of fresh rat liver prepared in a Waring blender in 0.25 M sucrose were shaken for 30 min in an ice bath with different concentrations of ICI Americas G-7181 in a total vol of 1.01 mL. The supernatant fractions obtained after centrifugation of the treated homogenates for 30 min at 105,000 g were dialyzed vs. 0.1 M NaCl, 0.01 M Tris, pH 8.0 for 7 days in the cold. The precipitate which formed during dialysis was removed by centrifugation. Glucose dehydrogenase in beef and rat liver extracts was found to be completely stable to another dialysis for 7 days. Dialysis of the extracts was necessary to remove endogenous substrates which interfered with

TABLE I

Relative Substrate Specificities of Extracts of Beef Liver and Rat Liver Microsomes

	Glucose Dehydrogenase	Glucose-6-phosphate Dehydrogenase	Galactose-6-phosphate Dehydrogenase
Beef	100	21	18
Rat	100	208	150

The conditions are described in the text. The units in the table are relative rates.

the assay for glucose dehydrogenase. The values which are presented are uncorrected for possible small changes in volume during dialysis.

Glucose-6-phosphate, 6-phosphogluconate, galactose-6-phosphate, NAD,NADP, phenazine methosulfate, NADH and nitroblue tetrazolium were obtained from Sigma Chemical Co. The nonionic detergent G-7181, a polyoxyethylene (8) dodecyl alcohol, was the gift of Dr. G.J. Stockburger, ICI Americas, Inc. The concentrations of stock solutions of glucose-6-phosphate and galactose-6-phosphate were measured optically at 340 nm by using NADP and various preparations of glucose dehydrogenase.

Separations by isoelectric focusing were carried out essentially as described (14) except that no detergent was used. The separation was carried out at 400 V for 5 hrs. Gel electrophoresis was carried out essentially as described (14). No detergent was used and the gels were run at 250 V for 1.5 hrs.

The dehydrogenases were detected in gels by staining with a tetrazolium dye. The solutions used contained (per 10 mL): for glucose dehydrogenase: 3 mg nitroblue tetrazolium, 0.3 mg phenazine methosulfate, 6 mg NAD, 180 mg glucose, 2.5 mmoles Tris pH 7.3; for glucose-6-phosphate dehydrogenase: 3 mg nitroblue tetrazolium, 0.3 mg phenazine methosulfate, 8 umoles NADP, 40 umoles glucose-6-phosphate, 2.5 mmoles Tris pH 7.3. The solution used for galactose-6-phosphate dehydrogenase was identical to that used for glucose-6-phosphate dehydrogenase except that 40 umoles galactose-6-phosphate replaced glucose-6-phosphate. The gels were stained for 2 hrs at room temperature in the dark. The stained gels were washed in water and stored in 1 M acetic acid. In order to account for occasional staining of gels independent of carbohydrate substrate, it was necessary to compare the staining patterns obtained with the complete staining mixture with those obtained using a staining mixture without carbohydrate substrate.

Results and Discussion

The data in Table I indicate the relative substrate specificities of detergent extracts of washed beef liver and rat liver microsomes. Under the conditions used, rat liver microsomes have more glucose-6-phosphate dehydrogenase activity than galactose-6-phosphate dehydrogenase activity. Both hexose-6-phosphate activities are considerably greater than that of glucose dehydrogenase. These results are in close agreement with those of Beutler and Morrison (6) who found much less glucose dehydrogenase than hexose-6-phosphate dehydrogenase in mouse liver microsomal extracts. Rat liver microsomes have some preference for glucose-6-phosphate

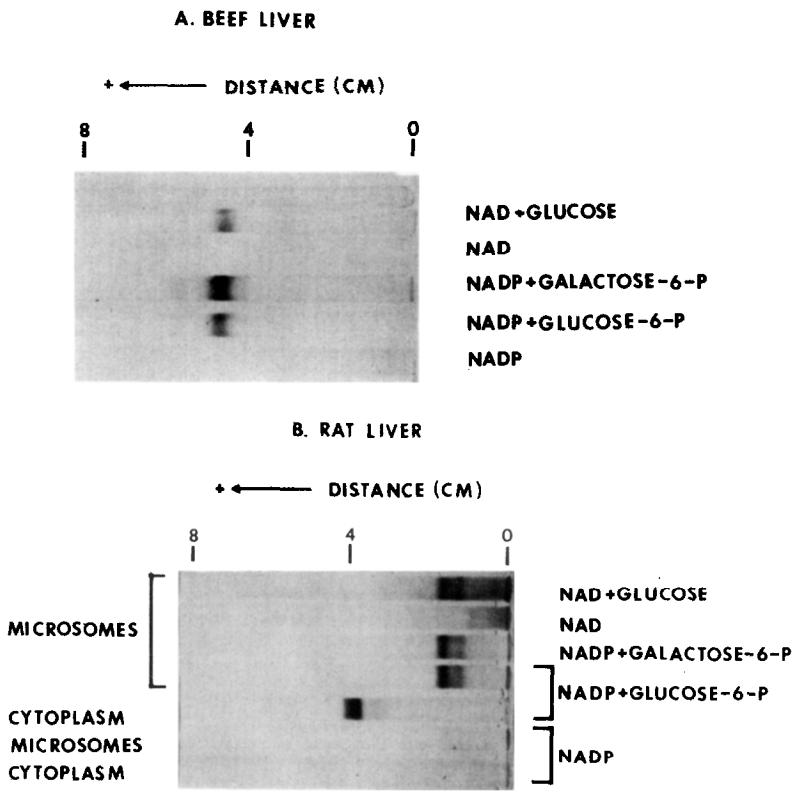

Fig. 1. Electrophoretic separation of various dehydrogenases in dialyzed supernatant fractions and microsomal extracts from beef and rat liver. The conditions are described in the text. Beef liver extracts were used in Fig. 1A and rat liver extracts in Fig. 1B. The fractions which were added and staining mixtures used are as indicated.

over galactose-6-phosphate while mouse liver microsomes show the reverse preference. However, the enzyme in beef liver microsomes has a different substrate specificity. The glucose dehydrogenase activity is much stronger than either hexose phosphate dehydrogenase in beef liver microsomal extracts. This result was unexpected since Beutler and Morrison (6) reported that the relative substrate specificities of the beef enzyme remained constant over a 1500-fold purification of the enzyme without indicating any difference in substrate specificities between the murine and bovine enzymes. Thus, the beef liver enzyme has a different substrate specificity than the enzymes from mouse or rat liver if these activities can be attributed to a single enzyme in each extract.

The data in Fig. 1 show that electrophoresis in gels of extracts of washed

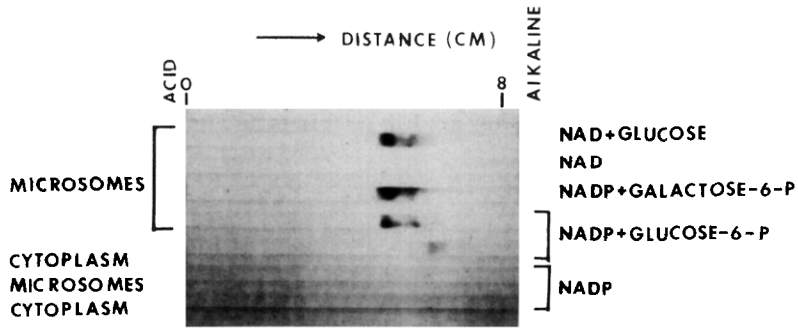

Fig. 2. Isoelectric focusing of beef liver microsomal extract. The conditions are described in the text.

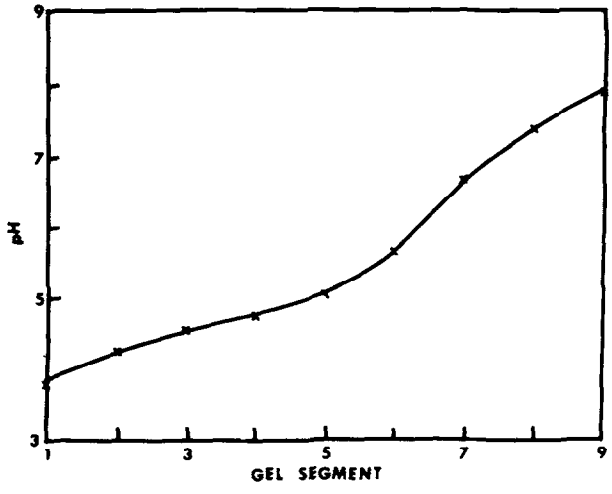

Fig. 3. pH gradient developed in gels.

microsomes from both rat and beef liver failed to separate these three enzymatic activities. These results confirm those of Beutler and Morrison (6) who were unable to separate these activities from mouse liver extracts by starch gel electrophoresis. The data in Fig. 2 show that isoelectric focusing in gels also does not separate these three activities from beef liver microsomes. Thus, a single enzyme in each extract is responsible for all three enzymatic activities. The pH gradient formed in these gels is in Fig. 3.

Although glucose dehydrogenase has been detected in many animal tissues (23), no quantitative data of the levels of the enzyme were given. The levels in micro-

somes from various tissues determined by Mandula et al (2) are low because extraction of the enzyme by their method using cholate yields only about 60% as much enzymes as extraction by Triton X-100 or ICI Americas G-7181. Two problems in determining the levels of this enzyme are the choice of substrates to be used in the enzymatic assay and the requirement to release quantitatively the active enzyme from its inactive, particulate form. The assay of choice for this purpose appears to be glucose dehydrogenase, since no other enzyme(s) in dialyzed extracts of animal tissues catalyzes this reaction. The more sensitive fluorometric reaction was used to detect this reaction which is rather weak in rat liver extracts.

The determination of the levels of glucose dehydrogenase in tissues depends upon the complete release of active enzyme from its latent or inactive form. Although there is no completely satisfactory way in which this can be done, the following approach seemed likely to provide a reasonably valid estimate of the levels in liver of this soluble enzyme. Preliminary experiments showed that extraction of microsomes by Triton X-100 released more enzyme than vigorous sonication, freezing and thawing or treatment by hypotonic solution. Triton X-100 extracted more enzyme than cholate, deoxycholate, or laurylsarcosinate. However, ICI Americas G-7181 was at least as effective as Triton X-100 in extracting glucose dehydrogenase. The ability of this detergent to extract active enzyme showed no significant dependence on pH or ionic strength. It was then found that a relatively low level of this most effective detergent extracted from different concentrations of homogenates amounts of glucose dehydrogenase which were directly proportional to the concentration of homogenate extracted. In order to be more certain that all the enzyme was extracted by this relatively low level of detergent, higher concentrations of detergent were shown not to extract additional dehydrogenase. Applications of this method to rat and beef liver are described in Table II. Since the detergent treatment of the microsomes also releases additional substrates reducing NAD, it was convenient to dialyze the extracted enzyme.

The levels of glucose-6-phosphate and galactose-6-phosphate dehydrogenase activities due to glucose dehydrogenase can be calculated from the level of

TABLE II

Glucose Dehydrogenase Released by Various Concentrations of ICI Americas G-7181 from Different Levels of Rat and Beef Liver Homogenates

	Rat Liver			Beef Liver		
ICI Americas G-7181 Added	10	20	30	10	20	30
Homogenate Extracted (ml)						
0.33	8	9	6	65	50	65
0.66	20	18	19	115	110	115
0.99	30		23	135	145	150

The conditions are described in the text. The values in the Table are those of glucose dehydrogenase (units of fluorescence change per 5 min).

glucose dehydrogenase and the ratios of these three enzymatic activities determined under conditions where a single enzyme was responsible for all the activities (Table I). These data are presented in Table III. A comparison between the glucose-6-phosphate dehydrogenase activity due to glucose dehydrogenase and the cytoplasmic dehydrogenase (E.C. 1.1.1.49) is uncertain because Glock and McLean (13) used a different procedure in measuring the level of the later enzyme. When differences in assay procedures are neglected, it is estimated that the glucose-6-phosphate dehydrogenase activity attributed to glucose dehydrogenase is about one-third and one-fifth that of cytoplasmic glucose-6-phosphate dehydrogenase in male and female rat livers respectively. This estimate assumes that the levels of glucose dehydrogenase are the same in male and female rats. Since glucose dehydrogen-

TABLE III

Levels of Various Dehydrogenase Activities Attributed to Glucose Dehydrogenase in Beef and Rat Livers

	Glucose Dehydrogenase	Glucose-6-phosphate Dehydrogenase	Galactose-6-phosphate Dehydrogenase
Beef	720	166	130
Rat	30.2	63	45

The units above are nmoles/min/g liver.

ase is inherited autosomally (12), a sex difference in the levels of this enzyme is unlikely. Glucose-6-phosphate dehydrogenase is sex-linked (10,11).

The metabolic function of glucose dehydrogenase remains unsolved. Beutler and Morrison (6) suggest that the enzyme is a hexose-phosphate dehydrogenase rather than a glucose dehydrogenase because the apparent km's for the hexose phosphates are much lower than that for glucose. The results presented here showing approximately the same leveels for hexose phosphate dehydrogenase rather than glucose dehydrogenase in beef and rat livers support this suggestion.

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