CHROMATOGRAPHIC AND ELECTROPHORETIC EVIDENCE FOR SEVERAL SUGAR DEHYDROGENASES IN MAMMALIAN LIVER

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1. Introduction

Some enzymes catalyzing the pyridine nucleotide-linked oxidation of free monosaccharides have been described in the mammalian liver. These include the microsomal glucose dehydrogenase first described by Harrison [1] later purified and characterized by several investigators [2–5], and the soluble galactose dehydrogenase reported by Cuatrecasas and Segal [6, 7]. In bacteria, several dehydrogenases acting on free pentoses have been described, for instance L-arabinose dehydrogenase [8], D-arabinose dehydrogenase [9–11], and an aldose dehydrogenase with broad specificity [9–11]. To our knowledge none of these enzymes have been described so far in the mammalian liver, with the possible exception of L-fucose dehydrogenase from pork liver, acting also on D-arabinose [12].

This communication presents evidence for the presence in pig liver of several enzymes catalyzing the general reaction

$$\text{Aldopentose} + \text{NAD(P)} \rightarrow \text{aldopentonic acid} + \text{NAD(P)H}_2$$

with varying aldose and pyridine nucleotide specificities. At least five enzymes have been detected by chromatography and polyacrylamide electrophoresis differing in several ways from the previously described mammalian and bacterial sugar dehydrogenases.

2. Methods

Freshly excised pig liver was obtained in a local slaughtherhouse. All operations were performed at 0–4° unless otherwise stated. The liver was homogenized in a Waring blendor with one volume of a 10 mM tris-HCl-1 mM EDTA, pH 7.0 buffer. The homogenate was centrifuged at 30,000 rpm for 60 min in a Spinco preparative ultracentrifuge. The supernatant fluid was treated batchwise with CM-Sephadex equilibrated in the same homogenizing buffer. The non-absorbed fraction was recovered by filtration through Whatman # 1 filter paper. This fraction (S_{cm}) was chromatographed through a DEAE-cellulose column equilibrated with homogenizing buffer. After washing, the column was resolved with a linear gradient to 0.2 M KCl in the same buffer.

Dehydrogenase activity was assayed by measuring spectrophotometrically the reduction of NAD or NADP at 340 nm in the presence of the suitable sugar. A Gilford spectrophotometer provided with a thermospacer to operate at 30° was used. The final composition of the assay medium was: 10 mM KCl, 85 mM tris-HCl buffer (pH 7.5), 12.6 mM $MgCl_2$, 1.6 mM EDTA, 0.5 mM NAD or NADP, 200 mM sugar substrate, and 0.05 to 0.2 ml enzyme fraction in 1 ml cuvettes. The same system without sugar was used as blank. One unit is the amount of enzyme that catalyzes the reduction of 1 nmole of NAD(P) per min at 30°.

Polyacrylamine gel electrophoresis was performed at about 10° using the system described by Davis [13]. Running time was about 60 min at 4 mA per column. Dehydrogenase activity was revealed by staining the gels in a medium similar to that described above except for the inclusion of 0.04 mg/ml phenazine methosulfate and 0.4 mg/ml nitro blue tetrazolium.

Control tubes without sugar were always included to distinguish nonenzymatic bands.

3. Results and discussion

Measurement of sugar dehydrogenase activity in crude pig liver extracts showed a small but definite activity especially when D-arabinose and NAD were used as substrates. D-Xylose and D-ribose in the presence of NADP were also active. Gel electrophoresis of crude extracts revealed several weak bands of enzyme activity not suitable for photography.

Column chromatography of the S_{cm} fraction revealed several peaks with dehydrogenase activity (fig. 1). The peak tubes were combined and concentrated by freeze drying. After dyalisis against distilled water the pooled fractions were subjected to electrophoresis and enzyme activity staining.

The breakthrough peak A contained a dehydrogenase acting on D-xylose and to a lesser extent on D-ribose, using only NADP as hydrogen acceptor. No bands were observed when sugar was omitted from the staining mixture (fig. 2A). D-Glucose, D-mannose, D-fructose, 2-deoxy-D-ribose, D- or L-fucose and D-lyxose were not able to reduce NADP or NAD. This enzyme has been purified to a homogeneous state and its properties will be reported elsewhere [14].

Peak B was resolved by electrophoresis in two bands (fig. 2B). Band II stained only with NADP as hydrogen acceptor, and the preferred sugars were D-xylose, D-ribose and D-arabinose. D-Glucose and L-arabinose were active to a limited extent. This enzyme has also been purified [15].

Band III was active either with NAD or NADP. Glucose was the best substrate with both nucleotides, but D-xylose was also attacked. D-Arabinose and D-ribose were not substrates, neither with NAD nor NADP (fig. 2B).

Electrophoresis of fraction C revealed a dehydrogenase (Band IV) acting on D-arabinose, L-fucose and weakly on D-ribose (fig. 2C). Other sugars were not substrates. The enzyme uses only NAD as hydrogen acceptor, and it may well be the same enzyme described by Schachter et al. [12] as L-fucose dehydrogenase.

Peak D was resolved by electrophoresis in two very close bands with identical substrate specificity to

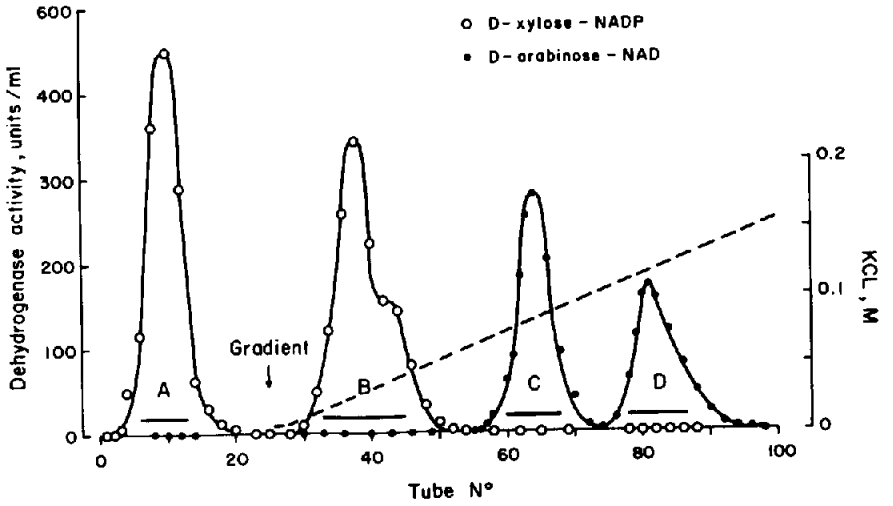

Fig. 1. DEAE-Cellulose column chromatography on the S_{cm} fraction. 10 ml of the S_{cm} fraction corresponding to 4 g of liver were used. Column size was 21 X 1.2 cm. 3.5 ml fractions were collected. A linear gradient (200 ml in each flask) to 0.2 M KCl (- - - - -) in 10 mM tris-HCl-1 mM EDTA, pH 7.0 started at tube 25. Enzyme activity was measured with several combinations of sugar and pyridine nucleotide but only the combination with the highest activities are shown. Tubes with activity were pooled as shown by the horizontal lines under each peak.

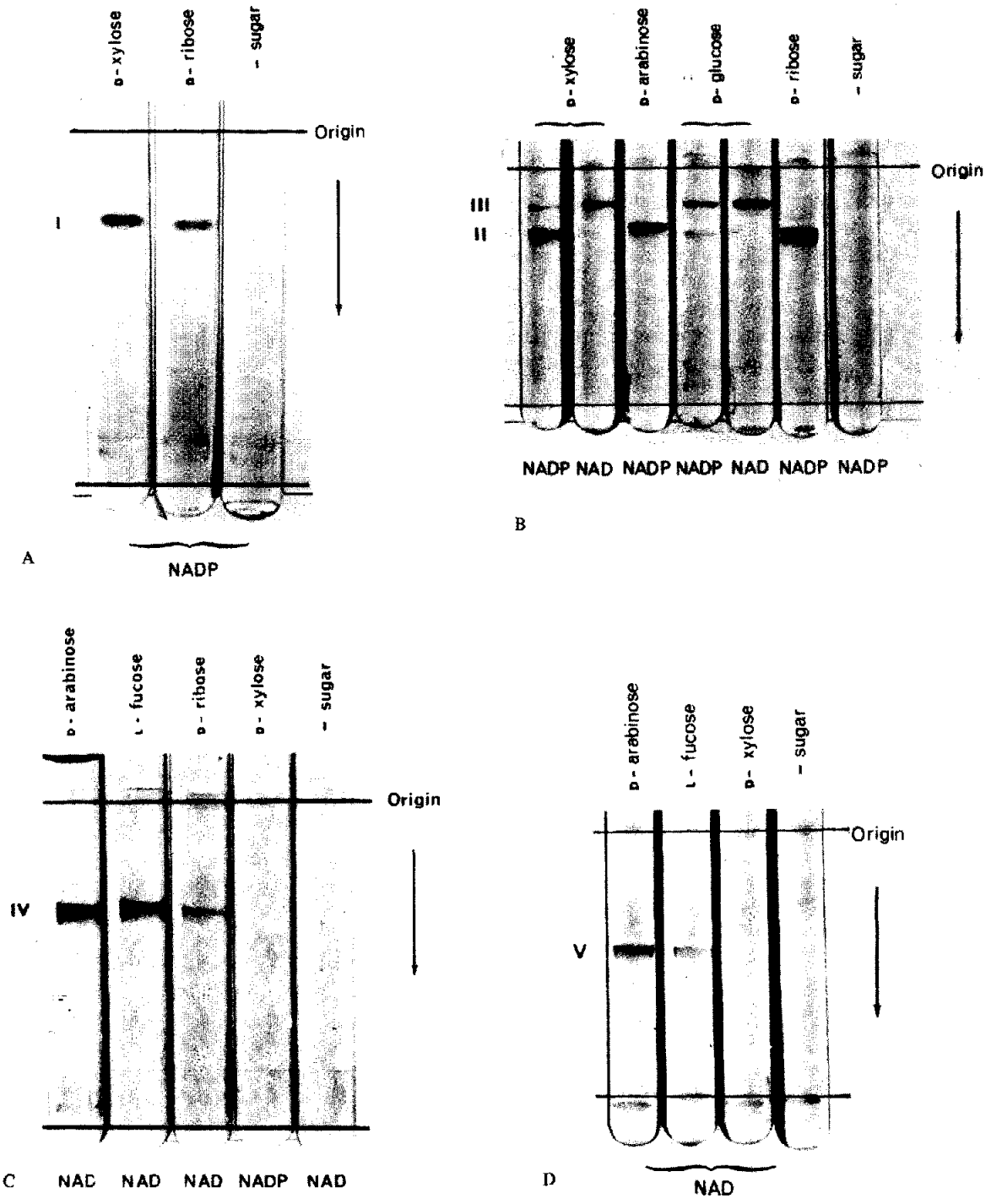

Fig. 2. Acrylamide gel electrophoresis of fractions A–D. Procedure was as described in Methods. Origin is indicated by the upper horizontal line; the direction was toward the anode. The lower horizontal line marks the distance traversed by the dye marker. The gels were stained for activity during 10 min at room temperature in darkness.

Band IV (fig. 2D). Photography does not show the separation between both bands as clearly as inspection of the gels. We have not yet further characterized these enzymes.

The general properties of the six enzymes are very different from those of glucose dehydrogenase [1–5] and galactose dehydrogenase [6, 7]. Alcohol was not a substrate for any dehydrogenase, thus precluding its identity as alcohol dehydrogenase as suggested in the case of galactose dehydrogenase [16]. None of the enzymes were active against 6-phospho or 5-phosphosugars. When hydroxylamine was added to a reaction mixture similar to that described for the spectrophotometric assay, an hydroxamic acid derivative was detected suggesting an aldopentonolactone as the immediate product of the reaction.

It is surprising that these enzymes have not been described earlier. Perhaps this is due to the fact that they are not common to all mammals. We have found only very small, barely detectable amounts of dehydrogenase I in guinea pig, rat and hamster liver. On the other hand, the Chilean rodent *Octodon degus* (Rodentia, Octodontoidea) showed appreciable amounts of the pentose dehydrogenases.

The presence of so many sugar dehydrogenases makes one wonder about their physiological role in the catabolism of free pentoses. A working hypothesis is that some mammals use oxidative reactions on free pentoses bypassing the glycolytic and Warburg-Dickens-Lipmann pathways to feed the tricarboxylic acid cycle, via α-ketoglutarate, as is the case in some bacteria [8]. We are at present working on this problem.

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