PARTIAL PURIFICATION OF RAT LIVER D-ARABINOSE DEHYDROGENASE

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Received February 17, 1967

Breusch (1943) reported 24 years ago that cat liver homogenates had the capacity to oxidize D-arabinose in the presence of methylene blue. Wainio (1947) using lamb liver preparations later confirmed that the mammalian liver was capable of D-arabinose oxidation. Since that time, there have been no reports of attempts to characterize the enzyme responsible for this activity. In this paper, we report the first partial purification of the enzyme D-arabinose dehydrogenase from a non-bacterial source. We have purified the enzyme from rat liver 46 fold over that activity of the crude homogenate. D-arabinose dehydrogenase activity is localized in the rat liver soluble fraction. The enzymatic activity is $NADP^+$ specific. The pH of maximal activity is 9.4 and the K_m for D-arabinose is 0.053 M. D-lyxose also served as a substrate, yielding about one-tenth of the activity of D-arabinose. The product appears to be D-arabonolactone.

METHODS

Chemicals -- $NADP^+$ was purchased from Boehringer Mannheim Corp., NAD^+ from the Sigma Chemical Company. Solutions of these coenzymes were adjusted to pH 7.2 before use. D-arabonolactone

was obtained from K and K Laboratories, Inc., and was twice recrystallized from acetone before use.

Assay Procedure -- The assay employed in these studies consisted of following the formation of NADPH using a Beckman DB spectrophotometer set at 340 mμ, coupled with a Sargent model SRL recorder. Two cuvettes containing a total volume of 1 ml were employed. Each cuvette contained 0.05 M buffer of the appropriate pH, either 0.45 mM $NADP^+$ or 0.89 mM NAD^+, and the enzyme solution to be tested. The reaction was begun by the addition of a substrate solution to one of the cuvettes. All assays were conducted at 25°C. Protein concentration was estimated using its absorbancy at 280 and 260 mμ, as described by Layne (1957).

Purification Procedure -- Sprague-Dawley rats were decapitated, their livers removed and immediately homogenized in 5 parts of 0.15 M KCl, using a Potter-Elvehjem apparatus. The homogenate was centrifuged at 25,000 x g for 2 hrs. The resulting supernatant contained all of the D-arabinose dehydrogenase activity.

Ammonium sulfate was added to the soluble fraction to 55% saturation; the solution was then adjusted to pH 8.0 and stirred 20 min. After centrifugation for 20 min. at 13,000 x g, the precipitate was discarded and the supernatant adjusted to 75% saturation with ammonium sulfate, as above. The precipitate, which contained most of the activity, was dissolved in 0.03 M pH 7.0 Tris-acetate buffer.

The enzyme preparation was applied to a 6.4 x 91 cm Sephadex G-25 column and eluted with 0.05 M Tris-HCl buffer, pH 8.4. The protein eluant was applied to a 2 x 41 cm DEAE column equilibrated with 0.03 M Tris-acetate buffer at pH 8.0. Protein was eluted

from the column by application of a KCl gradient resulting from allowing 500 ml of 0.5 M KCl in 0.03 M Tris-acetate buffer to siphon slowly into 500 ml of the same buffer with constant stirring. The resultant elution pattern is shown in Figure 1. The next step must be carried out as soon as possible because the enzyme is unstable at this point.

The fractions showing D-arabinose dehydrogenase activity were combined and fractionated with ammonium sulfate as above, except that the solutions were not adjusted to pH 8.0. Most of

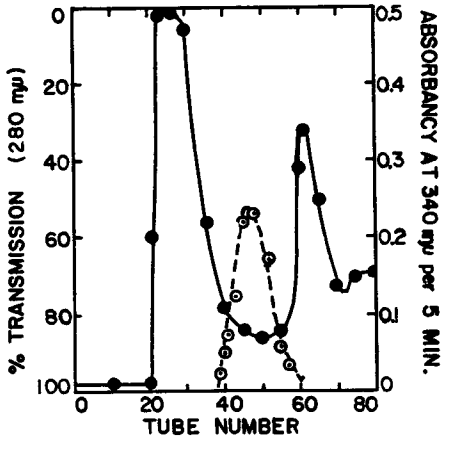

Figure 1: Elution pattern of D-arabinose dehydrogenase from a DEAE column. The solid line represents the % transmission of the eluted protein. The broken line shows the D-arabinose dehydrogenase activity expressed in terms of the absorbancy increase at 340 mμ per 5 min. using pH 8.4 Tris buffer.

the activity was found in the precipitate formed between 90-100% saturation. The precipitate was dissolved in buffer as above.

All of the above operations were performed between 0-4°. The procedure is summarized in Table I.

TABLE I

PURIFICATION OF D-ARABINOSE DEHYDROGENASE

Procedure	Volume (ml)	Activity (units/ml)	Protein (mg/ml)	Specific Activity (units/mg)	Purification
Crude supernatant	55	0.24	22	0.011	--
Ammonium sulfate	10	0.46	32	0.014	1.28
Sephadex G-25	14.5	0.45	26	0.017	1.57
DEAE column	75.5	0.043	0.54	0.080	7.3
Ammonium sulfate	2	0.79	1.56	0.51	46

The units employed are μmoles NADPH formed/min.

RESULTS

The purified D-arabinose dehydrogenase was $NADP^+$ specific. Of the sugars tested, D-lyxose was the only other substrate for the enzyme besides D-arabinose, being oxidized at about 10% of the rate of the latter. D-xylose, D-ribose, L-arabinose, D-galactose, D-glucose, and 6-deoxy-D-glucose were not substrates for this enzyme.

The graph of D-arabinose dehydrogenase activity versus pH shows a sharp peak of maximal activity at pH 9.4, as shown in Figure 2. At this pH, the K_m was 0.053 M for D-arabinose and 0.061 mM for $NADP^+$. At pH 8.4, the D-arabinose K_m was 0.027 M.

Paper chromatography of the reaction mixture yielded a compound which migrated similarly to arabonolactone in either ethyl acetate:formic acid:water (3:1:1) or n-butanol:acetic acid:water (4:2:1). Detection of the compounds was accomplished with either aniline-oxalate reagent for reducing sugars or methanolic hydroxylamine and $FeCl_3$ for lactones (Hais and Macek, 1963).

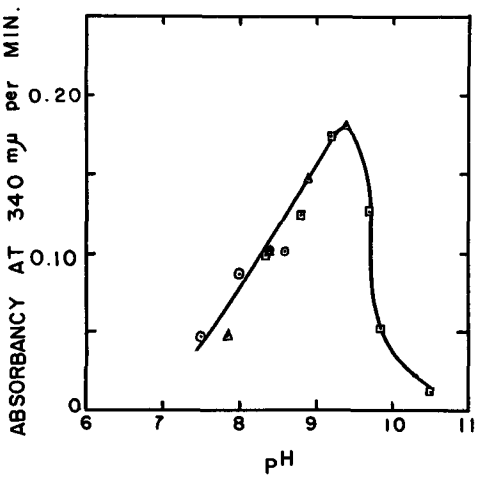

Figure 2: Variation in D-arabinose dehydrogenase activity with pH. Enzyme purification 46 fold was assayed as described using either Tris-HCl (⊙), glycylglycine (△), or glycine (⊡) buffer of the appropriate pH.

DISCUSSION

D-arabinose dehydrogenase has been isolated previously from Pseudomonas saccharophilia by Palleroni and Doudorff (1956, 1957). To our knowledge, this paper reports the first partial purification of the enzyme from a mammalian system. The bacterial enzyme is NAD^+ specific, whereas the rat liver enzyme requires $NADP^+$ for activity. This $NADP^+$ specificity, as well as differences in the pH of maximal activity, also distinguishes D-arabinose dehydrogenase activity from the D-arabinose oxidation reported by Cautrecasas and Segal (1966a) by their preparation of partially purified D-galactose dehydrogenase and NAD^+. In addition, there are differences between the two enzymes in their behavior on ion exchange columns, D-arabinose dehydrogenase being retained by DEAE while galactose dehydrogenase is retained by CMC (1966b).

It is clear that there are two enzymes in mammalian liver which are capable of catalyzing the oxidation of D-arabinose.

Acknowledgment -- This work was partially supported by Grant AM-5685 from the United States Public Health Service.

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