ALDOSUGAR DEHYDROGENASES FROM *NEUROSPORA CRASSA*

Partial purification and characterization of D-arabinose:NAD dehydrogenase

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Received 4 December 1972

1. Introduction

The pyridine nucleotide-linked oxidation of free sugars to their corresponding lactones has been described in several organisms [1–14]. The enzymes acting on free aldopentoses seem to catalyze the first reaction of a pathway leading to intermediates of the citric acid cycle [3] but the possibility of their involvement in biosynthetic pathways should not be lightly dismissed. Despite their general occurrence [15] only the arabinose dehydrogenases from *Pseudomonas* [3, 5–7] and a related enzyme from mammalian liver [11–13] have been studied with some detail. We wish now to report the presence in *Neurospora crassa* of several sugar dehydrogenases, and the partial purification and properties of a D-arabinose:NAD dehydrogenase.

2. Methods

Wild type strain 74 A of *Neurospora crassa* was grown in Vogel's medium [16] containing 2% sucrose as carbon source. The flasks were shaken on an Eberbach rotatory shaker at about 140 rpm at 25°. Approx. 8 g (wet weight) of mycelia were suspended in ice-chilled 0.05 M Tris-HCl pH 7.2 buffer and grinded in an ice chilled mortar. The suspension was centrifuged at 4° at 4,000 rpm for 20 min in a Sorvall RC 2 centrifuge. The supernatant liquid was further centrifuged at 40,000 rpm during 60 min in a Hitachi ultracentrifuge. The sediment was discarded.

Dehydrogenase activity was assayed through the reduction of NAD or NADP at 340 nm in the presence of sugar. The reaction mixtures contained 10 mM KCl, 100 mM Tris HCl buffer (pH 7.5), 1.6 mM EDTA, 0.5 mM NAD^+ or $NADP^+$, 100 mM sugar and enzyme in a final volume of 0.5 ml. A similar system without sugar was used as a blank. A Gilford spectrophotometer thermostatted at 30° provided with a recording attachment was used. One unit of activity is the amount of enzyme catalyzing the reduction of 1 μmole of $NAD(P)^+$ per min at 30°.

Polyacrylamide gel electrophoresis was performed at about 10° exactly as described by Davis [17] at 4 mA per column. Histochemical localization of enzyme activities were done by staining the gels per 10 min in the medium described above plus 0.04 mg/ml phenazine methosulfate and 0.4 mg/ml nitroblue tetrazolium.

Partial purification of D-arabinose dehydrogenase was accomplished by DEAE-cellulose (Whatman DE-52) chromatography in a 1 × 16 cm glass column. A linear gradient (240 ml) from 0 to 0.5 M KCl was applied to the column and 2-ml fractions were collected in a Gilson microfractionator. Protein was estimated by measuring the absorbance of the effluent at 280 nm.

3. Results

Direct measurement of dehydrogenase activity in high-speed supernatant liquids revealed routinely very

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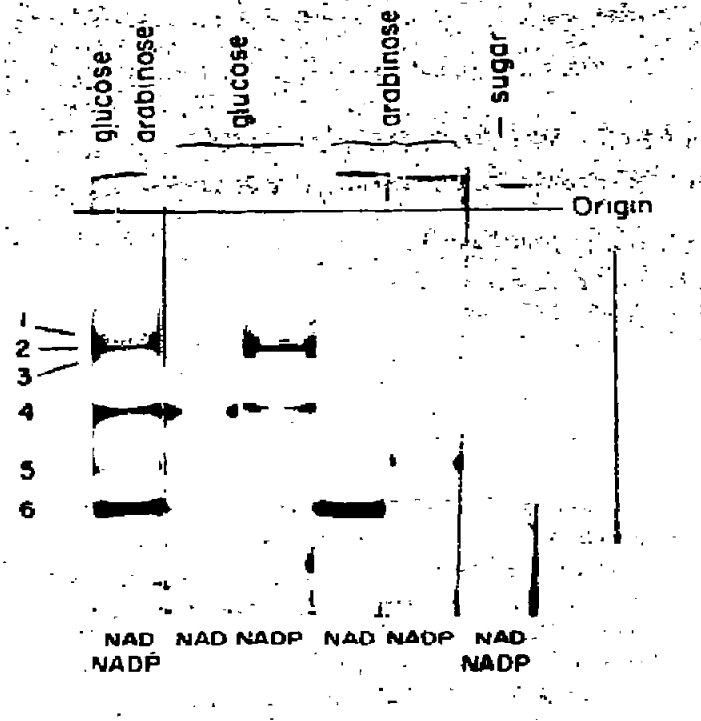

Fig. 1. Polyacrylamide gel electrophoresis of a high speed supernatant liquid from *N. crassa*. 100 µl of extract were charged in each column. After electrophoresis the tubes were immersed for 10 min in the assay medium containing mixtures of sugars and pyridine nucleotides as indicated, and then fixed in 7% acetic acid.

weak reduction of pyridine nucleotides with high blank values. Acrylamide gel electrophoresis however, revealed at least 6 distinct bands of enzyme activity (fig. 1). Bands 1 to 3 were visible when the medium contained D-glucose and $NADP^+$. The pair D-xylose–$NADP^+$ was also active although at a much lower rate. Band 4 appeared with glucose as hydrogen donor with either NAD^+ or $NADP^+$ as acceptor. Band 5 was observed with the pair D-arabinose–$NADP^+$ only when high amounts of extract were electrophoresed. Band 6 was always present as a rapidly and heavily stained band when D-arabinose (or L-fucose) and NAD^+ were present in the reaction mixture.

Isolation and partial purification of the enzyme corresponding to band 6 was accomplished by DEAE-cellulose column chromatography (fig. 2). A thin, symmetrical peak eluted from the column at about 0.13 M KCl. Attempts to concentrate the enzyme activity by ammonium sulfate precipitation or freeze-drying resulted in the almost complete loss of the activity. Thus, without further purification the fraction were used for enzyme characterization.

Table 1

Substrate specificity of D-arabinose dehydrogenase from *Neurospora crassa*.

Sugar	Relative activity*
D-Arabinose	1.00
L-Fucose	0.44
D-Ribose	0.11
L-Arabinose	0.06
D-Glucose	0.05
D-Xylose	0.02
2-Deoxy-D-ribose	0
2-Deoxy-D-glucose	0
D-Fucose	0
L-Rhamnose	0
L-Xylose	0
6-Phosphogluconate	0

Concentration of substrates, 100 mM; NAD^+ concentration, 0.5 mM.

* D-arabinose = 1.00.

Substrate specificity studies are shown in table 1 using NAD^+ as hydrogen acceptor inasmuch as $NADP^+$ was completely inactive. D-Arabinose is by far the best substrate, although L-fucose and D-ribose were also attacked but to a much lesser extent.

The effect of D-arabinose concentration on the velocity of NAD^+ reduction and viceversa is shown in fig. 3. Classical Michaelis–Menten kinetics were obtained. The calculated K_m values were 6.7×10^{-4} M for D-arabinose and 2.96×10^{-4} M for NAD^+. We have not performed detailed analysis of the reaction products but since the addition of hydroxylamine to the reaction mixture at pH 6.5 resulted in hydroxamate production we presume that an arabonolactone is the immediate product of the oxidation reaction.

4. Discussion

This report adds several enzymes to the growing list of dehydrogenases that catalyze oxidation–reduction reactions of free sugars. The enzymes from *Neurospora* are present in very small amounts. For instance, hexo-

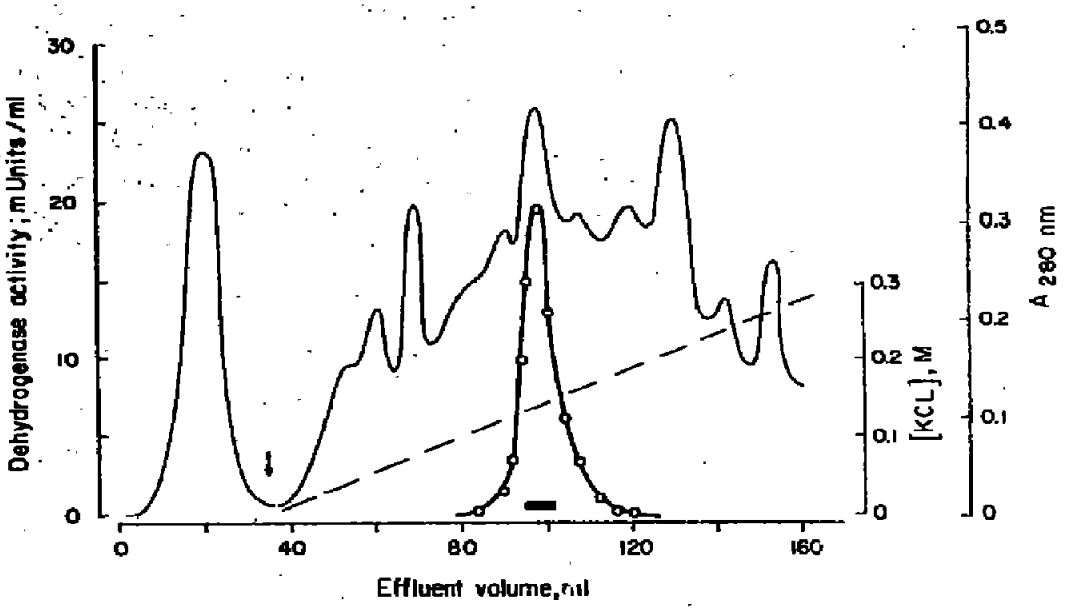

Fig. 2. DEAE-cellulose column chromatography of a high speed supernatant fluid from *N. crassa*. Every other tube was assayed for D-arabinose dehydrogenase activity (o—o—o) and absorbance at 280 nm (———). The arrow indicates the beginning of the KCl gradient (– – –). The heavy horizontal line under the peak of enzyme activity indicates the fractions pooled for subsequent studies.

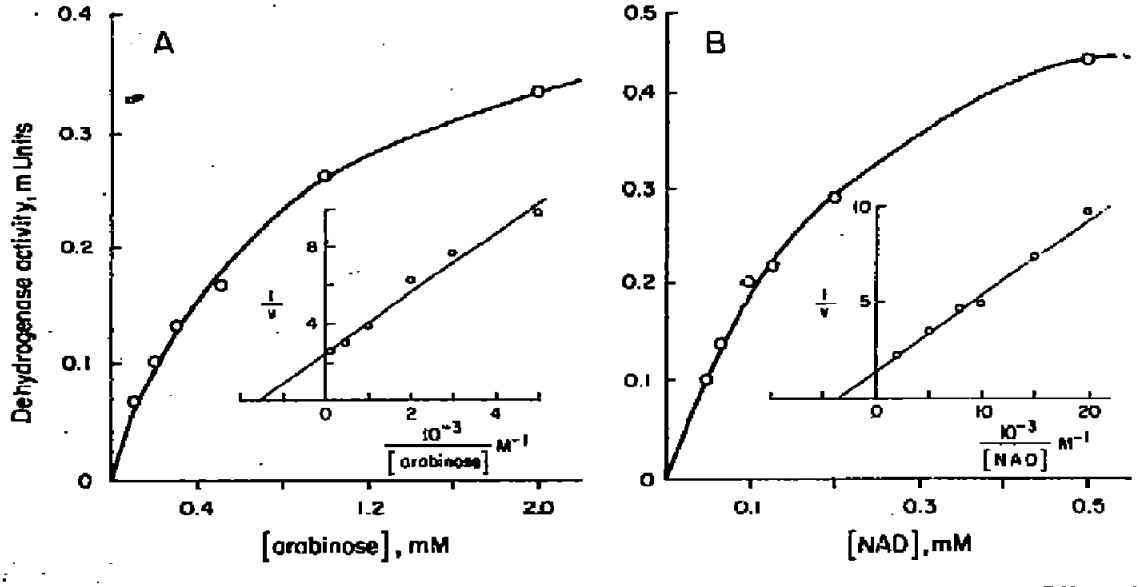

Fig. 3. The effect of substrate and acceptor concentrations on the velocity of D-arabinose dehydrogenase. A) Effect of D-arabinose concentration at a fixed level (0.5 mM) of NAD^+. B) Effect of NAD^+ concentration at a fixed level (100 mM) of D-arabinose. Insets are double reciprocal plots.

kinase and glucose 6-phosphate dehydrogenases are at least one thousand times more active than D-arabinose dehydrogenase. Thus, the pathways in which these dehydrogenases are involved must be of minor quantitative importance to the cell, at least from the point of view of energy production. However, the possibility of their being involved in biosynthetic pathways has not been ruled out (see for instance [18]).

Our studies with D-arabinose dehydrogenase from *N. crassa* reveal striking differences with other D-arabinose dehydrogenases so far reported. For instance, the enzyme from *Pseudomonas* [6] is able to utilize both NAD^+ and $NADP^+$. Also, the *Neurospora* dehydrogenase is different from the pig enzyme whose preferred substrate is L-fucose [12]. Finally, it is also different from the D-arabinose dehydrogenase from rat liver which is active only with $NADP^+$ as hydrogen acceptor [11, 15].

Preliminary experiments on colonial mutants of *Neurospora* have shown interesting quantitative differences in the amount of arabinose dehydrogenase and will be soon reported.

Acknowledgements

This work was supported by grants from the Comisión de Energía Nuclear, Comisión Investigación Científica, Facultad de Medicina, y Comisión Central de Investigación Científica, Universidad de Chile. We thank Miss Jasna Radojković and Miss María Riquelme for their help in the early phases of this study.

References

[1] D.C. Harrison, Biochem. J. 25 (1931) 1016.
[2] N.G. Brink, Acta Chem. Scand. 7 (1953) 1081.
[3] R. Weimberg and M. Doudoroff, J. Biol. Chem. 217 (1955) 607.
[4] R.P. Metzger, S.S. Wilcox and A.N. Wick, J. Biol. Chem. 239 (1964) 1769.
[5] A.L. Cline and A.S.L. Hu, J. Biol. Chem. 240 (1965) 4488.
[6] A.L. Cline and A.S.L. Hu, J. Biol. Chem. 240 (1965) 4493.
[7] A.L. Cline and A.S.L. Hu, J. Biol. Chem. 240 (1965) 4498.
[8] P. Cuatrecasas and S. Segal, J. Biol. Chem. 241 (1966) 5904.
[9] P. Cuatrecasas and S. Segal, J. Biol. Chem. 241 (1966) 5910.
[10] E. Beutler and M. Morrison, J. Biol. Chem. 242 (1967) 5239.
[11] R.P. Metzger and A.N. Wick, Biochem. Biophys. Res. Commun. 26 (1967) 742.
[12] H. Schachter, J. Sarney, E.J. McGuire and S. Roseman, J. Biol. Chem. 244 (1969) 4785.
[13] F.W. Mobley, R.P. Metzger and A.N. Wick, Arch. Biochem. Biophys. 139 (1970) 83.
[14] T. Ureta and J. Radojković, FEBS Letters 9 (1970) 57.
[15] T. Ureta, J. Radojković and M. Riquelme, in preparation.
[16] H.J. Vogel, Microbial Genetics Bull. 13 (1956) 42.
[17] B.J. Davis, Ann. N.Y. Acad. Sci. 121 (1964) 404.
[18] R.L. Katzman, E. Lisowska and R.W. Jeanloz, Biochem. J. 119 (1970) 17.