RESOLUTION OF LACTIC DEHYDROGENASE IN BEEF CORNEA EPITHELIUM ON DIETHYLAMINOETHYL CELLULOSE*

Bernard Wortman and Aleene Schneider

Department of Ophthalmology, Washington University Medical School
St. Louis, Missouri

Received January 25, 1960

Kinoshita (1957) demonstrated that the addition of pyruvate to beef cornea epithelial cells stimulated the production of $C^{14}O_2$ from glucose-1-C^{14}, and further observed that LDH** in cornea epithelial homogenates may function with TPNH. This implies that TPNH linked reduction of pyruvate to lactate may be coupled with the TPN-dependent hexosemonophosphate oxidative pathway. Therefore, LDH may enhance this pathway by serving as an efficient system for the oxidation of TPNH. In this communication DEAE cellulose anion-exchange chromatography will be used to demonstrate two forms of LDH in beef cornea epithelial extract, and also that both forms contain DPNH- and TPNH-linked activities.

EXPERIMENTAL

Beef cornea epithelial extract - Epithelial cells were scraped from beef cornea, homogenized in 4.5 volumes (w/v) of 0.1 M Tris-HCl, pH 7.4

*This study was supported in part by a grant (B-1911) from the National Institute of Neurological Diseases and Blindness, United States Public Health Service.

**Abbreviations used are: LDH, lactic dehydrogenase; DEAE, diethylaminoethyl; Tris, tris(hydroxymethyl) aminomethane; and DPN, DPNH, TPN, TPNH respectively for oxidized and reduced forms of di- and tri- phosphopyridine nucleotides.

and centrifuged for 1 hour at 34, 800 g. The supernatant fluid was dialyzed against 0.005 M Tris-HCl, pH 7.4 and centrifuged for 1 hour at 34, 800 g. The extract was assayed for protein concentration (Lowry et al., 1951) and used for chromatography.

Column chromatography - Anion-exchanger columns were prepared in a manner similar to that previously reported (Peterson and Sober, 1956, and Moore and Wortman, 1959). DEAE cellulose (Distillation Products Industries, Rochester, New York) was found to have an absorption capacity of 1.0 mEq/mg after it was washed with 1 N NaOH, water, and dried in ethanol and in ether. Columns (1 x 20 cm) were packed with a 2% suspension of DEAE cellulose (approximately 2 gm) under pressure (10 psi) and equilibrated with 0.005 M Tris-HCl, pH 7.4. Five ml of beef cornea epithelial extract were applied to the column and eluted with a linear gradient to 0.1 M NaCl in 0.05 M Tris-HCl, pH 7.4.

Enzyme assay - Aliquots from collected column fractions were diluted 1:100 with 0.1 M Tris-HCl, pH 7.4 and assayed for DPNH-linked LDH activity as follows. Two μl aliquots were incubated for 15 minutes at 38° with 26.8 μmoles of Tris-HCl, pH 7.4, 0.28 μmoles of Na pyruvate, 0.02 μmoles of DPNH (Sigma Chemical Co., St. Louis, Mo.) and 0.14 μgm of bovine plasma albumin in a total volume of 275 μl. TPNH-linked LDH activity was measured in undiluted column fractions which were found to contain DPNH-linked LDH activity as follows. Fourteen μl aliquots were incubated 15 minutes at 38° with 24.3 μmoles of Tris-HCl, pH 7.4, 0.23 μmoles of Na pyruvate, 0.02 μmoles of TPNH (Sigma Chemical Co., St. Louis, Mo.), and 0.13 μgm of bovine plasma albumin in a total volume of 289 μl. The amount of nucleotide oxidized was calculated on the basis of a_M=6270 at 340 mμ.

Unless otherwise stated, all operations were conducted in the cold.

RESULTS

Two forms of DPNH-linked LDH activity were found in beef cornea epithelial extract. The first was weakly bound to DEAE cellulose and eluted from the column before application of the NaCl gradient (fractions 5 and 6, Figure 1); the second was eluted with 0.02 M NaCl (fraction 48, Figure 1).

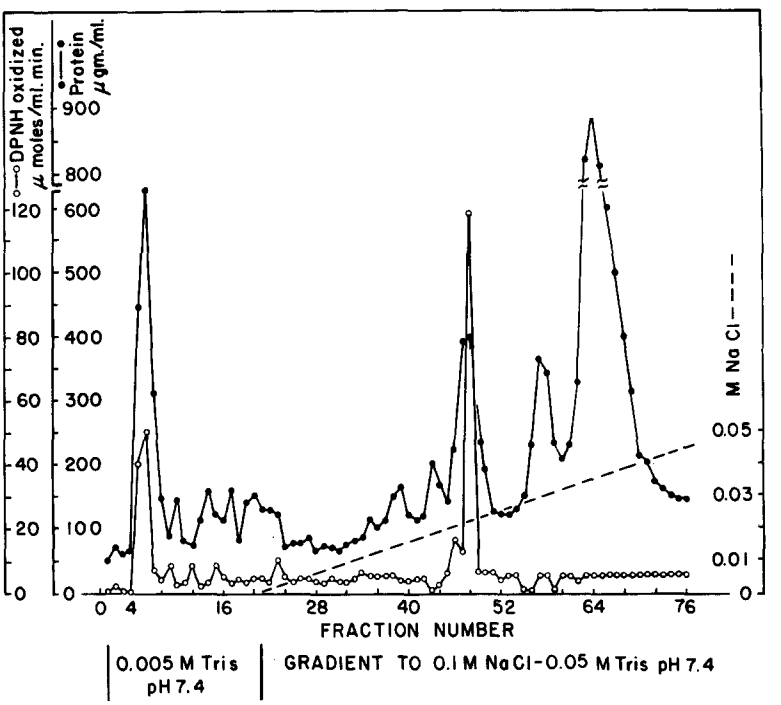

Figure 1. Chromatography of beef cornea epithelial extract on DEAE cellulose (2 gm) anion-exchanger column (1 x 20 cm). Five ml of prepared epithelial extract (15.3 mg protein/ml) were applied to the column and eluted with a linear NaCl gradient and fractions (3.3 ml) collected.

Column fractions 5, 6 and 48 were found to contain TPNH-linked LDH activity. TPNH was oxidized at a lower rate than was DPNH, (Table I). The ratio of DPNH to TPNH oxidation by LDH was 2196, 850,

888 and ca. 181,000 in starting extract, and in column fractions 5, 6 and 48, respectively.

TABLE I

Lactic Dehydrogenase In Beef Cornea Epithelial Extract After Resolution on DEAE Cellulose Anion-Exchanger

Preparation	DPNH	TPNH	$\frac{DPNH}{TPNH}$ ratio
	(mμmoles/min/mg protein) [a]		
Starting extract	6.1×10^3	2.78	2196
Fraction 5	91.8×10^3	108.0	850
6	81.0×10^3	91.2	888
48	299.0×10^3	1.65 [b]	ca 180,000

[a] Calculated from nucleotide oxidized in 15 min at 38°.
[b] Too low to be measured accurately.

The protein pattern shown in Figure 1 represents approximately 65% of the starting extract protein applied to the column. The recovery of DPNH- and TPNH-linked LDH activity was approximately 110% and 159%, respectively.

LDH activity was unstable after chromatography, and by 24 hours in the cold, activity was reduced in fractions 5 and 6 and lost in 48.

DISCUSSION

These data confirm the previous observation that TPNH-linked LDH activity is present in beef corneal epithelium. Furthermore, both DPNH- and TPNH-linked LDH activities were found in the same column fractions. The high ratio of TPNH- to DPNH-linked LDH activities previously reported was not found under the experimental conditions of this study and are more in accord with that reported for liver (Navazio et al., 1957). These

data do not necessarily reduce the value of the hypothesis that TPNH-linked LDH may be coupled with the hexosemonophosphate oxidative shunt in corneal epithelium in much the same manner as has been proposed for liver (Navazio et al., 1957).

SUMMARY

Multiple forms of lactic dehydrogenase (LDH) in beef cornea epithelial extract were studied by use of diethylaminoethyl (DEAE) cellulose anion-exchange chromatography. After passage through DEAE cellulose anion-exchange column, two forms of diphosphopyridine nucleotide (DPNH) linked LDH were detected. Both forms used triphosphopyridine nucleotide for the reduction of pyruvate to lactate but at a much lower rate than DPNH.

ADDENDUM

Since completion of this work another laboratory has demonstrated multiple forms of LDH in beef cornea epithelium and retina by different techniques (Futterman and Kinoshita, 1959).

REFERENCES

Futterman, S. and Kinoshita, J., (1959). J. Biol. Chem., 234, 3174

Kinoshita, J., (1957). J. Biol. Chem., 228, 247

Lowry, O. H., Rosebrough, N. J., Farr, A. I. and Randall, R. J., (1951). J. Biol. Chem., 193, 265

Moore, B. W. and Wortman, B., (1959). Biochim. Biophys. Acta, 34, 260

Navazio, F., Ernster, B. B. and Ernster, L., (1957). Biochim. Biophys. Acta, 26, 41ĉ

Peterson, E. A. and Sober, H. A., (1956). J. Am. Chem. Soc., 78, 751