The author is a recipient of A.E.C. Grant AT(0.43)326-12. She thanks Dr. LEO VERNON for the spinach particle preparations, and Dr. BESSEL KOK for the frozen Scenedesmus chloroplasts; thanks go also to Varian Associates for use of an E-3 EPR spectrometer and to Spectraphysics for lending the laser (Fig. 1).

W. W. Hansen Laboratories of Physics, Stanford University, Stanford, Calif. (U.S.A.) ELLEN C. WEAVER*

1 B. KE, *Biochim. Biophys. Acta*, 88 (1964) 297.
2 H. BEINERT, B. KOK AND G. HOCH, *Biochem. Biophys. Res. Commun.*, 7 (1962) 209.
3 M. CALVIN AND G. M. ANDROES, *Science*, 138 (1962) 867.
4 L. P. VERNON, B. KE AND E. R. SHAW, *Biochemistry*, 6 (1967) 2210.
5 E. C. WEAVER, *Ann. Rev. Plant Physiol.*, (1968) 283.
6 H. BEINERT AND B. KOK, *Biochim. Biophys. Acta*, 88 (1964) 278.
7 D. I. ARNON, *Plant Physiol.*, 24 (1949) 1.
8 E. C. WEAVER AND N. I. BISHOP, *Science*, 140 (1963) 1095.

Received May 1st, 1968

* Present address: Exobiology Division, Ames Research Center, N.A.S.A., Moffett Field Calif. 94035, U.S.A.

BBA 43199

Absence of NADH:NAD$^+$ oxidoreductase activity in mitochondrial NAD(P) transhydrogenase

NAD(P) transhydrogenase (NADPH:NAD$^+$ oxidoreductase, EC 1.6.1.1, also known as TD-transhydrogenase) was first found in animal tissues by KAPLAN, COLOWICK AND NEUFELD[1]. Preparations of this enzyme always contain various amounts of NADH:NAD$^+$ oxidoreductase (also known as DD-transhydrogenase) activity[1,2].

It was assumed that a single lipoprotein was responsible for both activities[3]. However, the present communication shows that by treatment with a detergent, followed by gel filtration, fractions could be obtained containing the two activities separately.

Rat liver mitochondria (about 260 mg protein) prepared by WEINBACH's[4] method were treated with 7 ml of an ice-cold 0.016 M solution of Triton X-100 (0.05 M potassium phosphate, pH 7.4, 0.001 M EDTA) for 1 h, and centrifuged for 2 h at 38000 $\times$ *g*. In the clear supernatant both activities were assayed with oxidized 3-acetylpyridine–adenine dinucleotide (APAD$^+$) as hydrogen acceptor[5]. The specific activities were found to be 0.040 and 0.190 μmole/min per mg protein for the NADPH:APAD$^+$ and NADH:APAD$^+$ oxidoreductase reactions, respectively*. The supernatant was chromatographed on a Sephadex G-100 column, which was eluted

Abbreviations: APAD$^+$ and APADH, oxidized and reduced forms of 3-acetylpyridine–adenine dinucleotide.

* Mitochondria disintegrated in an analogous manner with 0.016 M digitonin[6] showed specific activities of 0.084 and 0.159, respectively.

with 0.05 M potassium phosphate (pH 7.0, 0.002 M EDTA). Fig. 1 shows the separation of the two activities. The recoveries were 107 % and 89 % for the NADPH: $APAD^+$ and NADH: $APAD^+$ oxidoreductase activities, respectively.

The fractions containing the separated enzymes were pooled and the dependence of the activities on pH and substrate concentration were measured in order to characterize both enzymes.

From a Lineweaver–Burk plot a K_m value for the reaction

$$NADPH + APAD^+ \rightleftharpoons NADP^+ + APADH \quad (1)$$

of $1.7 \cdot 10^{-4}$ M for NADPH was found in presence of a constant concentration of $2 \cdot 10^{-4}$ M $APAD^+$ under the conditions described in ref. 5. This value is of the same order as the various data found in the literature for NAD(P) transhydrogenases from different sources[5,7–9].

The K_m for the reaction

$$NADH + APAD^+ \rightleftharpoons NAD^+ + APADH \quad (2)$$

was found to be $1.4 \cdot 10^{-5}$ M for NADH ($APAD^+$ concentration, $2 \cdot 10^{-4}$ M). It agrees fairly well with the only value given in the literature[7], *viz.* for the leucocyte enzyme ($K_m = 5 \cdot 10^{-5}$ M).

The pH optima for Reactions 1 and 2 were determined in 1 M potassium phosphate buffers. For NADPH: $APAD^+$ oxidoreductase, maximal activity was found at pH 5.9–6.3, which is nearly identical with values for NADH: NAD^+ oxidoreductase found by other authors[1,7,10] in preparations from rat heart, beef heart and leucocytes. The pH range for maximum activity of the NADH: $APAD^+$ oxidoreductase is 5.3–5.8.

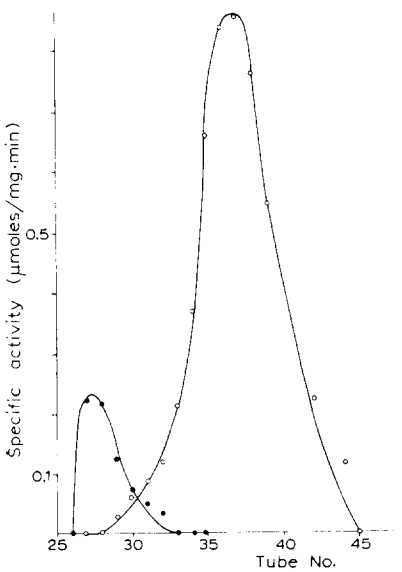

Fig. 1. Gel filtration of 5 ml mitochondrial extract (12.4 mg protein per ml) on a Sephadex G-100 column (57 cm × 3.5 cm, 0.9 ml eluate per min, 5-ml fractions). Assay of NADPH: $APAD^+$ and NADH: $APAD^+$ oxidoreductases according to STEIN, KAPLAN AND CIOTTI[5]. Specific activities are given in μmoles/mg protein·min. Protein was determined according to LOWRY *et al.*[6] in the eluate and by Kjeldahl's method in the other samples. ●—●, NADPH: $APAD^+$ oxidoreductase; ○—○, NADH: $APAD^+$ oxidoreductase.

SILBER, HUENNEKENS AND GABRIO[7] report a pH value of 5.8 for leucocytes, HUMPHREY[10] 6.3 for rat heart.

These results show that NAD(P) transhydrogenase is solubilized from liver mitochondria free of NADH:APAD$^+$ oxidoreductase activity by treatment with a nonionic detergent. Concerning the nature of the NADH:APAD$^+$ oxidoreductase activity which is eluted in the second peak, it seems reasonable to assume that it is due to lipoamide dehydrogenase (NADH:lipoamide oxidoreductase, EC 1.6.4.3)[11] which also catalyzes Reaction 2 by a mechanism proposed by MASSEY AND VEEGER[12]. Our findings (see also ref. 13) do not support the view of PESCH AND PETERSON[3] who suggested the existence of a single lipoprotein, which carries both activities but looses the NAD(P) transhydrogenase activity by change of protein conformation when lecithin is split off.

Institute of Medical Chemistry,
University of Vienna,
Vienna (Austria)

R. KRAMAR
M. MÜLLER
F. SALVENMOSER

1 N. O. KAPLAN, S. P. COLOWICK AND E. F. NEUFELD, *J. Biol. Chem.*, 205 (1953) 1.
2 B. KAUFMAN AND N. O. KAPLAN, *J. Biol. Chem.*, 236 (1961) 2133.
3 L. A. PESCH AND J. PETERSON, *Biochim. Biophys. Acta*, 96 (1965) 390.
4 E. C. WEINBACH, *Anal. Biochem.*, 2 (1961) 335.
5 A. M. STEIN, N. O. KAPLAN AND M. M. CIOTTI, *J. Biol. Chem.*, 234 (1959) 979.
6 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
7 R. SILBER, F. M. HUENNEKENS AND B. W. GABRIO, *J. Clin. Invest.*, 42 (1963) 1908.
8 Y. HORIE AND W. CHEFURKA, *Comp. Biochem. Physiol.*, 17 (1966) 1.
9 S. P. COLOWICK, N. O. KAPLAN, E. F. NEUFELD AND M. M. CIOTTI, *J. Biol. Chem.*, 195 (1952) 95.
10 G. F. HUMPHREY, *Biochem. J.*, 65 (1957) 546.
11 V. MASSEY, *Biochim. Biophys. Acta*, 37 (1960) 314.
12 V. MASSEY AND C. VEEGER, *Biochim. Biophys. Acta*, 48 (1961) 33.
13 T. KAWASAKI AND K. SATOH, *Federation Proc.*, 23 (1964) 432.

Received May 9th, 1968