

OCCURRENCE OF A DPN-LINKED, N^5, N^{10} -METHYLENE TETRAHYDROFOLIC
DEHYDROGENASE IN EHRlich ASCITES TUMOR CELLS [‡]

K.G. Scrimgeour* and F.M. Huennekens

Department of Biochemistry, University of Washington
Seattle, Washington

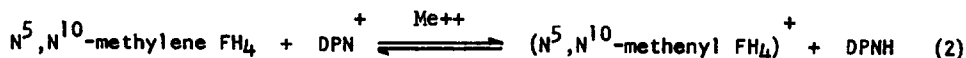
Received March 14, 1960

It has been demonstrated previously (Greenberg and Jaenicke, 1957; Hatefi, *et al.*, 1957; Osborn and Huennekens, 1957; Flaks, *et al.*, 1957; Whiteley, *in press*) that many tissues contain a TPN-linked, N^5, N^{10} -methylene tetrahydrofolic dehydrogenase (formerly called "hydroxymethyl tetrahydrofolic dehydrogenase") ^{‡‡}, which catalyzes the reaction^{**}:



In the metabolism of one-carbon units (Huennekens and Osborn, 1959), this enzyme is responsible for the interconversion of "active" C_1 units at the oxidation levels of formaldehyde and formate.

In Ehrlich ascites tumor cells (strain ELD, obtained from Dr. T.S. Hauschka, Roswell Park Memorial Institute) the above enzyme has been observed, and, in addition, it was found that crude extracts of these cells can carry out a DPN-linked, metal ion-dependent dehydrogenation of N^5, N^{10} -methylene FH_4 (equation (2))



[‡] Paper X in the series "Folic acid coenzymes and one-carbon units".

* Recipient of the Eli Lilly and Co. Predoctoral Award in the Department of Biochemistry, University of Washington, 1959-60.

^{‡‡} Since it has been shown recently that "active formaldehyde," the substrate in reaction (1), is actually N^5, N^{10} -methylene tetrahydrofolate (Blakley, 1958; Huennekens and Osborn, 1958), it would seem preferable to call this enzyme N^5, N^{10} -methylene tetrahydrofolic dehydrogenase.

^{**} The following abbreviations are used: DPN, DPNH, TPN, TPNH, oxidized and reduced di- and triphosphopyridine nucleotide; ATP, adenosine triphosphate; FH_4 , tetrahydrofolic acid; $f^5\text{-}^{10}\text{FH}_4$, N^5, N^{10} -methenyl FH_4 ; C_1 , one-carbon.

A comparison of these two activities is made in Table I. The substrate, N^5, N^{10} -methylene FH_4 , is generated in situ by the chemical interaction of $HCHO$ and FH_4 (Blakley, 1958; Huennekens and Osborn, 1958). Either reaction may be

Table I
Component Study for the TPN- and DPN-Linked
 N^5, N^{10} -Methylene FH_4 Dehydrogenases

| Additions | $f^{5-10}FH_4$ Formation |
|-----------------------------------|--------------------------|
| None | μmoles 0.0 |
| TPN | 0.101 |
| TPN + Mg^{++} | 0.095 |
| TPN (FH_4 omitted) | 0.0 |
| <hr/> | |
| DPN | 0.012 |
| DPN (Versene added) | 0.002 |
| DPN + ATP | 0.008 |
| DPN + Mg^{++} | 0.142 |
| DPN + Mg^{++} + ATP | 0.142 |
| DPN + Mg^{++} (FH_4 omitted) | 0.0 |

The complete system contained 10 μmoles of $HCHO$, 20 μmoles of 2-mercaptoethanol, 150 μmoles of potassium phosphate buffer, pH 7.5, 0.6 μmoles of DL- FH_4 , 0.03 ml. (1.0 mg.) of an ammonium sulfate fraction of ascites cell acetone powder extract, and water to make 3.0 ml. 0.6 μmoles of DPN or TPN, 10 μmoles of Mg^{++} , 1 μmole of ATP, and 1 μmole of Versene were added where indicated. The control vessel was identical except for the omission of $HCHO$. After 20 minutes incubation at 20° , the reactions were stopped by the addition of 0.4 ml. of 0.8 N HCl , and the amount of $f^{5-10}FH_4$ was determined from the optical density at 355 m μ . (Rabinowitz and Pricer, 1956).

followed in the forward direction by measuring the appearance of one of the products, $f^{5-10}FH_4$ or reduced pyridine nucleotide. It should be noted that the DPN enzyme, but not the TPN enzyme, requires Mg^{++} (or Mn^{++}) for full activity.

The DPN activity in the absence of added Mg^{++} is depressed markedly upon the addition of Versene.

That the DPN-linked activity is not due merely to the conversion of DPN to TPN via a metal ion and ATP-dependent kinase is shown by the following observations: (a) the DPN and TPN activities are additive; (b) added ATP is not required for the DPN system and there is no evidence that bound ATP is present in the partially purified preparation; (c) a well-dialyzed and aged preparation shows activity only with DPN; and (d) when DPN is used in reaction (2), DPNH is the product, as shown by its re-oxidation in the presence of acetaldehyde and crystalline alcohol dehydrogenase.

As required by the stoichiometry of equation (2), 0.11 μ moles of $f^{5-10}FH_4$ and 0.12 μ moles of DPNH were produced in a reaction carried out according to the

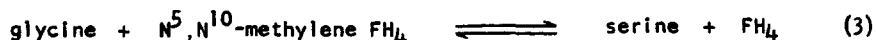
Table II

Reversal of the DPN-Linked N^5, N^{10} -methylene FH_4 Dehydrogenase

| Component Omitted | $f^{5-10}FH_4$ Disappearance |
|-------------------|------------------------------|
| | μ moles |
| None | 0.073 |
| DPNH | 0.006 |
| Mg^{++} | 0.019 |
| Glycine | 0.045 |

The assay system contained 0.28 μ moles of DL- $f^{5-10}FH_4$, 150 μ moles of potassium phosphate buffer, pH 6.5, 0.4 μ moles of DPNH, 50 μ moles of glycine, 30 μ moles of 2-mercaptoethanol, 20 μ moles of $MgCl_2$, 0.2 ml. of an aged acetone powder extract of ascites cells, and water to make to 3.0 ml. The control vessel was identical except for the omission of enzyme. After 30 minutes incubation at 20°, 0.3 ml. of 50% trichloroacetic acid was added and the residual $f^{5-10}FH_4$ estimated by the optical density at 355 $m\mu$.

protocol of Table I. The reversibility of the DPN-linked system is shown in Table II. DPNH and $f^{5-10}FH_4$ were used as substrates, and glycine was added in order to facilitate reversal of the reaction by trapping N^5, N^{10} -methylene FH_4 via the endogenous enzyme, serine hydroxymethylase (equation (3)):



The K_m values for the reactants in equation (2) are: DPN ($1.2 \times 10^{-4}M$); DL-FH₄ ($4.2 \times 10^{-5}M$); and Mg^{++} ($3 \times 10^{-3}M$). The K_m value for TPN in reaction (1) is $4.0 \times 10^{-5}M$.

The DPN enzyme has been partially purified from phosphate buffer extracts of acetone-dried ascites tumor cells by removal of nucleic acids with protamine, followed by precipitation of the enzyme with saturated ammonium sulfate, pH 8, (0-48 per cent fraction), and with solid ammonium sulfate (0-35 per cent fraction). In contrast to the TPN enzyme, the DPN enzyme cannot be detected in normal mouse liver, in the liver of mice bearing the ascites tumor, or in chicken, rabbit, cod, salmon and human liver.

This investigation was aided by grants from the American Cancer Society, (P-203), the U.S. Public Health Service, (CY-3310), and the Life Insurance Medical Research Fund.

References

- Blakley, R.L., Nature, **182**, 1719 (1958).
- Flaks, J.G., Warren, L., and Buchanan, J.M., J. Biol. Chem., **228**, 215 (1957).
- Greenberg, G.R., and Jaenicke, L., in Ciba Foundation Symposium on Chemistry and Biology of Purines, G.E.W. Wolstenholme and C.M. O'Connor, eds., Little, Brown and Co., Boston, 1957, pp. 203-232.
- Hatefi, Y., Osborn, M.J., Kay, L.D., and Huennekens, F.M., J. Biol. Chem. **227**, 637 (1957).
- Huennekens, F.M., and Osborn, M.J., 3rd International Congress of Biochemistry, Vienna, September 1958, Abstracts, p. 54; see also Osborn, M.J., Talbert, P.T., and Huennekens, F.M., J. Am. Chem. Soc. in press.
- Huennekens, F.M., and Osborn, M.J. Advances in Enzymology, **21**, 369 (1959).
- Osborn, M.J., and Huennekens, F.M., Biochim. Biophys. Acta, **26**, 646 (1957).
- Rabinowitz, J.C., and Pricer, W.E., Jr., J. Am. Chem. Soc. **78**, 5702 (1956).
- Whiteley, H.R., Comp. Biochem. Physiol., in press.