SOLUBLE PYRIDINE NUCLEOTIDE DEHYDROGENASES OF BRAIN

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Aqueous extracts of brain homogenates contain enzymes which catalyze the oxidation of both DPNH and TPNH by a variety of electron acceptors including benzoquinones, naphthoquinones, thiazines, indophenols and ferricyanide (Englard and Strecker, 1956; Levine et al, 1960; Giuditta and Strecker, 1960). Two of these enzymes, which we have called brain diaphorases I and II, have been highly purified and some of their properties described (Giuditta and Strecker, 1961; Harper and Strecker, 1962). These two enzymes are unable to utilize as electron acceptors cytochrome b_5, cytochrome c, tetrazolium dyes, Janus Green B and lipoic acid, and are inhibited by very low concentrations of dicumarol.

A third pyridine nucleotide dehydrogenase present in aqueous extracts of brain homogenates has now been partially purified by the use of ammonium sulfate fractionation, cellulose ion-exchange adsorbents, and calcium phosphate gel. About half of this enzymatic activity was found in the soluble fraction after high speed centrifugation (105,000 x g. for 1 hour) of sucrose homo-

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genates of brain. The remainder of the activity was obtained in soluble form, as judged by non-sedimentation at 105,000 x g. for 1 hour, by washing the sedimented residue with sucrose, water or buffer solutions. This dehydrogenase catalyzed the oxidation of DPNH, but not TPNH, by electron acceptors including benzoquinones, thiazines, indophenols, ferricyanide and cytochrome b_5 (Table 1).

Table 1

DPNH-ferricyanide reductase activity with different acceptors

acceptor	final conc.	μmoles DPNH oxidized/ min/mg/protein
Potassium ferricyanide	$3x10^{-4}M$	.95
Methylene blue	$3x10^{-5}M$	.048
p-Benzoquinone	$9x10^{-5}M$	.048
2,5 Dimethyl-p-benzoquinone	$9x10^{-5}M$	.017
2,6 Dichlorophenolindophenol	$1x10^{-6}M$	.046
INT*	$1x10^{-3}M$	.0028
Cytochrome b_5	$2x10^{-6}M$	.078

*INT=2(p-iodophenyl)-3(p-nitrophenyl)-5 phenyl tetrazolium chloride.

Assay conditions: 150 μmoles of potassium phosphate pH=7.5, 1 μmole DPNH and .01 μmole dicumarol were present in a total volume of 3 ml. The reaction was initiated by the addition of a suitable aliquot of enzyme and the reaction rate determined from the change in absorbancy at 340 mμ for methylene blue, p-benzoquinone and 2,5 dimethyl-p-benzoquinone, at 420 mμ for ferricyanide, 600 mμ for 2,6 dichlorophenolindophenol and 500 mμ for INT.

Inactive as electron acceptors were naphthoquinones, tetrazoliums, cytochrome c (except in the presence of cytochrome b_5) Janus Green B and lipoic acid. Inactive as electron donors in addition to TPNH were hypoxanthine and acetaldehyde.

Since ferricyanide was the most active electron acceptor tested, we are provisionally referring to this enzyme as a DPNH-ferricyanide reductase. In contrast to the two brain diaphorases, the ferricyanide reductase was relatively insensitive to dicumarol and activity was measured in the presence of low concentrations of dicumarol to inhibit the diaphorases. Amytal and Antimycin A were also not inhibitory.

The intracellular distribution of this soluble DPNH-ferricyanide reductase is not yet known. All of the sub-cellular fractions obtained by differential centrifugation of brain homogenates catalyze the reduction of ferricyanide by DPNH, with greatest activity being present in the mitochondrial fraction. Most of this particulate-bound ferricyanide reducing activity remained intact after exhaustive treatment with aqueous solutions to remove all of the soluble activity. Total DPNH-cytochrome c reductase activity, both antimycin A sensitive and insensitive, and TPNH-cytochrome c reductase, were also relatively unaffected by the removal of the two soluble diaphorases and the soluble DPNH-ferricyanide reductase (Table II).

The residual particulate matter also catalyzed the reduction of tetrazolium dyes and Janus Green B by both DPNH and TPNH.

Treatment of the washed microsomal fraction with dilute sodium hydroxide or buffers of high pH brought into solution enzymatic activity which also catalyzed the reduction of ferricyanide and cytochrome b_5. The same enzyme could be extracted also from an acetone dried preparation of microsomes. This enzyme which appeared to be similar to the liver enzyme described

Table II

Cytochrome c reductase activity of washed homogenates of rat brain

	DPNH - cytochrome c reductase*	TPNH - cytochrome c reductase*
Initial homogenate	3.76	0.315
After H_2O extraction**	3.51	0.300
After extraction with phosphate buffer**	3.60	0.312

*μmoles of cytochrome c reduced per minute per g. wet wt. of tissue.

** The method of extraction has been previously described (Levine et al., 1960).

The incubation mixture contained 150 μmoles of potassium phosphate pH 7.5, 0.3 μmoles reduced pyridine nucleotide, 1 mg. of cytochrome c, and 1 μmole of KCN in a total volume of 3.0 ml. at 28-30°. Reaction was initiated by addition of a suitable aliquot of enzyme suspension and the rate determined from the change in absorbancy at 550 mμ.

by Strittmatter et al. (1956) and by Mahler et al. (1958) differed from the soluble DPNH-ferricyanide reductase in a number of physical characteristics. The soluble enzyme was not precipitated at hydrogen ion concentrations down to 1×10^{-5}M, and required 75% saturated ammonium sulfate for precipitation. It was adsorbed on DEAE-cellulose from aqueous solution and eluted by 0.05M potassium phosphate buffer, pH 6.5. The soluble enzyme was also adsorbed on calcium phosphate gel from aqueous solution and eluted by 0.01M potassium phosphate buffer pH 8.1. By contrast the microsomal enzyme was precipitated at pH5, and by 55% saturated ammonium sulfate, could not be recovered from DEAE-cellulose and was not adsorbed by calcium phosphate gel.

Treatment of the washed mitochondrial fraction with dilute sodium hydroxide or buffers of high pH, or with acetone resulted

in the solubilization of enzymatic activity which catalyzed the oxidation of DPNH by INT and Janus Green B. This enzyme is now being studied.

DISCUSSION

The soluble DPNH-ferricyanide reductase of brain differs from the two soluble diaphorases and from the DPNH dehydrogenase system in mitochondria in electron donor or electron acceptor specificity, and differs from the microsomal dehydrogenase in physical characteristics insofar as these have been examined.

Interest in electron transport from reduced pyridine nucleotides has been directed chiefly to the so called respiratory chain and to the DPNH-cytochrome b_5 reductase systems. These studies have usually been concerned with enzyme systems rather firmly bound to particulate matter which require digestion with hydrolytic enzymes or treatment with organic solvents to solubilize activity. More recently, the use of non-physiological acceptors has revealed DPNH and TPNH dehydrogenating enzymes of high activity in aqueous extracts of brain (Englard and Strecker, 1956; Levine et al., 1960; Giuditta and Strecker, 1960) and liver (Martius and Märki, 1957; Ernster and Navazio, 1958; Wosilait, 1960). The enzyme reported here also presumably belongs to the latter group, although discussion of the function of these dehydrogenases has been concerned almost exclusively with a possible interaction with the electron transport chain or with oxidative phosphorylation. There is as yet no conclusive evidence for such interaction. It is still entirely possible that these soluble enzymes function in some manner not yet considered.

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REFERENCES

Englard, S. and Strecker, H.J., Fed.Proc. 15, 248 (1956)
Ernster, L. and Navazio, F., Acta Chem.Scand. 12, 595 (1958)
Giuditta, A. and Strecker, H.J., Biochem.Biophys.Res.Commun., 2, 159 (1960)
Giuditta, A. and Strecker, H.J., Biochim.Biophys.Acta, 48, 10 (1961)
Harper, E. and Strecker, H.J., J.Neurochem. (in press)
Levine, W., Giuditta, A., Englard, S. and Strecker, H.J., J.Neurochem., 6, 28 (1960)
Mahler, H.R., Raw, I., Molinari, R. and Do Amaral, D.F., J.Biol.Chem., 233, 230 (1958)
Martius, C. and Märki, F., Biochem. Z., 329, 450 (1957)
Strittmatter, P. and Velick, S.F., J.Biol.Chem., 221, 277 (1956)
Wosilait, W.D., J.Biol.Chem., 235, 1196 (1960)