THE ROLE OF TRANSHYDROGENASE IN THE ENERGY-LINKED REDUCTION OF TPN‡

Fritz Hommes* and Ronald W. Estabrook**
Department of Biophysics and Physical Biochemistry
Johnson Foundation, University of Pennsylvania
Philadelphia 4, Pennsylvania

Received February 25, 1963

The energy-linked reduction of the endogenous TPN of mitochondria was first described by Klingenberg and Schollmeyer (1962) and by Estabrook et al. (1961, 1963). Recently Danielson and Ernster (1963) have extended these studies by demonstrating the ATP requirement for the reduction of exogenous TPN by DPNH employing submitochondrial particles. These transhydrogenase type reactions of mitochondria therefore appear to be related intimately to the associated enzymes of oxidative phosphorylation and therefore are analogous to the energy driven succinate-linked reduction of pyridine nucleotide (Chance and Hollunger, 1961; Löw et al., 1961). The results of Danielson and Ernster (1963) in which exogenous pyridine nucleotides are employed now permits a definitive study into the relationship of the enzymes involved in the energy-linked reduction of TPN with the classical transhydrogenase described by Kaplan and co-workers (1953).

The studies of Ball and Cooper (1957) as well as those of Stein et al. (1959) have established the inhibition of transhydrogenase by thyroxine and triodothyronine (TIT). Indeed, these compounds are the only known specific inhibitors for the transhydrogenase reaction. Chance et al. (1963) have recently shown a similar inhibition by thyroxine

---

‡ Supported by U.S. Public Health Service Grant RG 9956.
* Fulbright Scholar, 1961-1963.
** This work was carried out during the tenure of a USPHS Research Career Development Award (GM-K3-4111).

for the succinate-linked reduction of the endogenous pyridine nucleotides of mitochondria. Therefore it was of interest to determine whether the comparable reaction for the energy-linked reduction of TPN was inhibited in a similar manner. In addition the temperature coefficient for the energy-linked reactions shows a remarkably high activation energy comparable to that observed by Kaplan (1963) with the isolated transhydrogenase. These properties support the hypothesis of a role for an asymmetrical as well as a symmetrical transhydrogenase reaction operative with an activated pyridine nucleotide, presumably associated with the enzyme system of oxidative phosphorylation. Other studies on the inhibitory influence of magnesium ion upon the pattern of the energy-linked reduction of TPN introduce further insight into the relationship of this reaction to the enzymes of oxidative phosphorylation.

Methods. Submitochondrial particles were prepared by sonic treatment of frozen beef heart mitochondria as described previously (Hommes, 1962) following the procedure described initially by Linnane and Zeigler (1958). The extent of pyridine nucleotide reduction was monitored fluorometrically using a constant temperature reaction chamber in an Eppendorf fluorometer adapted for continuous recording (Estabrook and Maitra, 1962). Pyridine nucleotides and alcohol dehydrogenase were purchased from Boehringer and Sohne. Triodothyonine was a gift from Smith, Kline and French Company, Philadelphia, Pennsylvania.

Results. When experiments of the type described by Danielson and Ernster (1963) were carried out in the presence of varying concentrations of triodothyronine (TIT), the rate of TPN reduction decreased with increasing concentrations of TIT. This is illustrated in Fig. 1 where the fluorometric tracings of pyridine nucleotide reduction were obtained using submitochondrial particles derived from heart muscle together with ethanol and alcohol dehydrogenase for the reduction of a catalytic concentration of DPN. In agreement with the results of Danielson and Ernster (1963), TPN is not reduced under these conditions until ATP

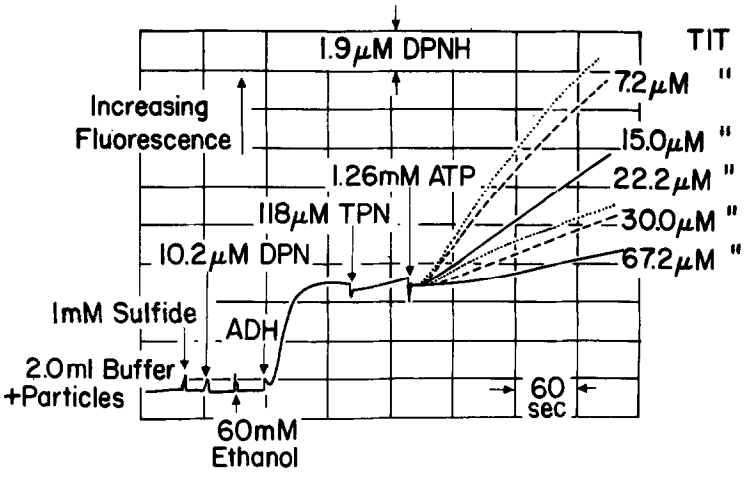

Fig. 1. Kinetics of the energy-linked TPN reduction. Submitochondrial particles derived from beef heart were suspended in buffer containing 80 mM KCl, 10 mM TRA, 10 mM $MgCl_2$, pH 7.5, to a final protein concentration of 1 mg per ml. Sulfide was used as a terminal inhibitor. Other additions to the reaction system are indicated in the figure.

is added to the system. The extent of inhibition is plotted as a function of TIT concentration in Fig. 2. In addition, results obtained on the TIT inhibition of the ATP requiring reaction for the succinate-linked reduction of exogenous DPN, employing the same submitochondrial particles, are also included in Fig. 2. The extent of inhibition of pyridine nucleotide reduction obtained in these studies corresponds nearly exactly with the extent of inhibition observed by Ball and Cooper (1957) as well as Stein et al. (1959) on the transhydrogenase mediated reaction of TPNH oxidation by DPN.

The studies by Chance et al. (1963) indicate the antogonistic role of magnesium in determining thyroxine effects on reductive reactions. Since the above studies (Fig. 1) were carried out in the presence of 10 mM $MgCl_2$, it was necessary to determine whether a comparable effect of magnesium ion could be observed here. When experiments of the type illustrated in Fig. 1 were carried out in the absence of magnesium it was observed that ATP was not required to initiate the reduction of TPN. The subsequent addition of magnesium, however, did inhibit TPN reduction and this inhibition was readily reversed by ATP but not versene. The

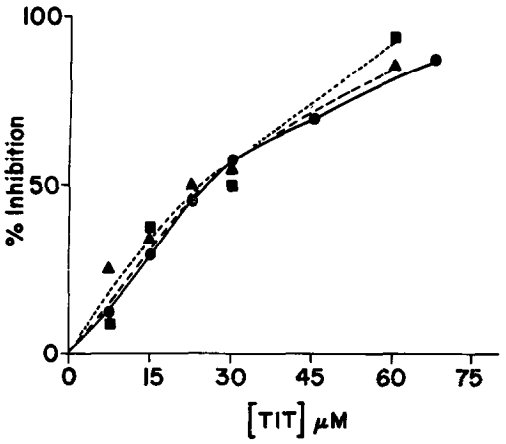

Fig. 2. Inhibition of the energy-linked TPN and DPN reduction as a function of triodothyronine (TIT) concentration. ●——●: energy-linked TPN reduction in the presence of 10 mM $MgCl_2$ and 1.25 mM ATP. Experimental conditions as in Fig. 1. ▲——▲: energy-linked TPN reduction in the absence of magnesium ions and ATP. Experimental conditions as in Fig. 1. ■ —— ■: succinate-linked DPN reduction. To the particles suspended in buffer (10 mM $MgCl_2$) was added 1 mM sulfide, 5 mM succinate, TIT, 67 μM DPN and 0.5 mM ATP to start the reaction.

---

influence of TIT on the rate of TPN reduction in the absence of magnesium is included in Fig. 2. In contrast to the study (Chance et al., 1963) on the succinate-linked reduction of endogenous pyridine nucleotides, no significant difference in the inhibitory effect of TIT was obtained in the presence or absence of magnesium in the present studies. Furthermore, a ten-fold increase in TIT concentration was necessary to give 50 % inhibition in submitochondrial particles compared with intact mitochondria. The ability to obtain TPN reduction in the absence of ATP is presumable a consequence of the incomplete inhibition of DPNH oxidation by the concentration of sulfide employed. The resultant slow rate of respiration permits the generation of an intermediate of oxidative phosphorylation which replaces ATP in the reaction (cf. Danielson and Ernster, 1963). The addition of magnesium inhibits the reaction presumably by stabilizing a high energy intermediate. The addition of ATP restores a sufficiently high steady state concentration of intermediate to facilitate reduction of TPN.

As indicated above, in addition to its sensitivity to thyroxine and triodothyronine, the transhydrogenase of Kaplan is characterized by a high temperature coefficient (Avi-dor et al., 1962; and Kaplan, 1963). The influence of temperature on the rate of TPN reduction from DPNH as activated by ATP was measured at varying temperatures as was the comparable reaction for the ATP dependent succinate-linked reduction of DPN. As shown in Fig. 3, the temperature dependency for the reaction is pronounced with a calculated Ahrennius activation energy of greater than 20 kilocalories. This temperature coefficient applies to both energy-linked reactions.

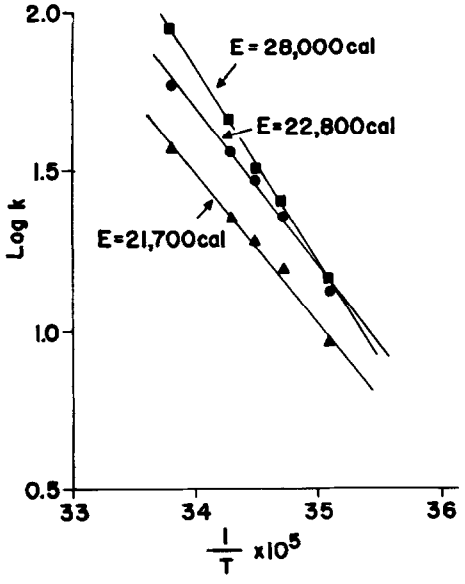

Fig. 3. Determination of the Arrhenius activation energy (E) of the energy-linked TPN reduction in the presence of magnesium and ATP (●——●), in the absence of magnesium and ATP (▲——▲) and of the succinate-linked DPN reduction (■ —— ■). Experimental conditions as in Fig. 2.

Summary. Danielson and Ernster (1963) have concluded from their studies that the energy-linked reduction of TPN observed with submitochondrial particles "is different from the transhydrogenase reaction between TPNH and DPN" described originally by Kaplan et al. (1953, 1959).

In contrast to this conclusion the present studies point to the similarity of the transhydrogenase of Kaplan to the transhydrogenase reaction mediating the energy-linked reduction of TPN. This is supported by the similarity in sensitivity to triodothyronine as well as the unusually high temperature coefficient for both types of reactions. Similar properties for the energy-linked succinate reduction of DPN suggests the activation of DPN or DPNH which can then participate in a symmetrical or asymmetrical transhydrogenase reaction. This interpretation also affords an explanation for the second order kinetics with respect to DPN observed by Hommes (unpublished results) for the succinate-linked reduction of DPN.

REFERENCES

Avi-Dor, Y., Olson, J. M., Doherty, M. D., and Kaplan, N. O. (1962). J. Biol. Chem., 237, 2377.

Chance, B. and Hollunger, G. (1961). J. Biol. Chem., 236, 1534.

Chance, B. and Hollunger, G. (1963). J. Biol. Chem., 238, 445.

Ball, E. G., and Cooper, O. (1959). Proc. Natl. Acad. Sci. U.S., 43, 357.

Danielson, L. and Ernster, L. (1963). Biochem. Biophys. Res. Comm. 10, 91.

Estabrook, R. W., Fugmann, U., and Chance, E. M. (1961). Proceedings of the Vth International Congress of Biochemistry, Pergamon Press, 464.

Estabrook, R. W. and Maitra, P. K. (1962). Anal. Biochem., 3, 369.

Estabrook, R. W. and Nissley, S. P. (1963). in "Die funktionelle und morphologische organisation der telle," Symp. Ges. deut. Naturforsch. u. Arizte, Rottach-Egern, Springer Verlag, Heidelberg, in press.

Hommes, F. A. (1962). Biochem. Biophys. Res. Comm., 8, 248.

Kaplan, N. O., Colowick, S. P., and Neufeld, E. F. (1953). J. Biol. Chem., 205, 1.

Kaplan, N. O. (1963), personal communication.

Klingenberg, M. and Schollmeyer, P. (1961). Proceedings of the Vth International Congress of Biochemistry, Pergamon Press, 5, 46.

Linnane, A. W. and Zeigler, D. M. (1958). Biochim. Biophys. Acta, 23, 630.

Löw, H., Kruger, H., and Zeigler, D. M. (1961). Biochem. Biophys. Res. Comm., 5, 231.

Stein, A. M., Kaplan, N. O., and Ciotti, M. M. (1959). J. Biol. Chem., 234, 973.