ALLOSTERIC INHIBITION OF TPN-LINKED ISOCITRATE DEHYDROGENASE BY FOLATE

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During a survey of the effect of physiological compounds on a number of nicotinamide nucleotide dehydrogenases, we found that folic acid inhibits the reduction of TPN by porcine heart TPN-linked isocitrate dehydrogenase. Hitherto it has only been the DPN-linked isocitrate dehydrogenases from mammalian and other sources that exhibited control behavior when various substrates and effectors were added to them (Chen et al., 1964; Sanwal et al., 1963, 1964; Hathaway and Atkinson 1963). In view of the fact that folate does not resemble any of the substrates of isocitric dehydrogenase we decided to investigate the nature of this inhibition and have found that folate probably induces an allosteric transition in the enzyme.

TPN-linked isocitrate dehydrogenase from porcine heart was purchased from Sigma and purified further on C-50 (carboxymethyl sephadex). The enzyme was put on the column at 0.05 M potassium phosphate buffer pH 6.8. A gradient was then applied and the enzyme was eluted when the gradient reached 0.07-0.08 M. The final preparation could catalyze the reduction of 15-25 μmoles of TPN per mg protein at pH 7.5 (0.1 M tris-HCl buffer) at 340 mμ at 25°.

Enzyme activity was assayed in a Beckman spectrophotometer with a Gilford recorder attached. The slit width was opened 0.5 mm wider when the kinetics were done in the presence of folate. TPN, dl-isocitrate, DPNH, folic acid and tris buffer were obtained from Sigma. TPNH was purchased from Calbiochem. Folic acid was dissolved in water with the addition of NaOH to bring it to a pH of 7.5, the pH of the reaction mixture.

In Figure 1 we observe the effect of 0.9×10^{-4} M and 1.8×10^{-4} M folate on the reaction velocity at various isocitrate concentrations. In the presence of folate the normal hyperbolic curve is transformed into a sigmoid curve. Such a state of affairs has been interpreted by Monod et al. (1963, 1965) in terms of protein structure as follows: The protein must exist

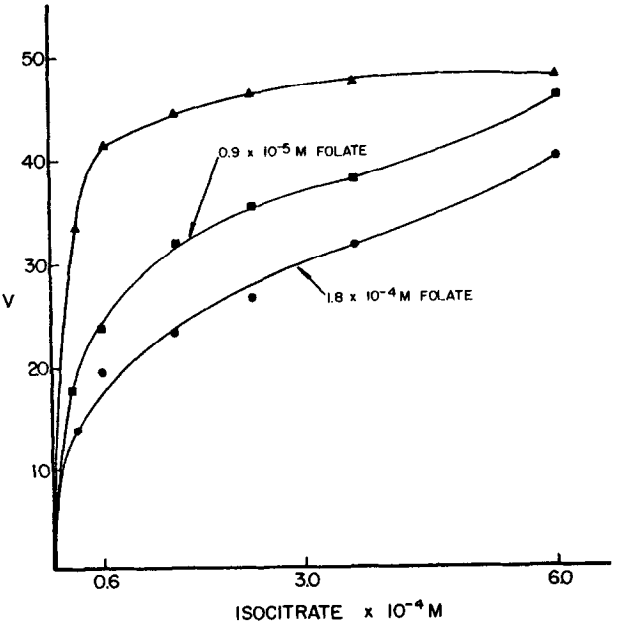

Figure 1. Reaction velocity at variable isocitrate concentration in the presence and in the absence of folate. TPN concentration 1.35×10^{-4}M; $MgCl_2$ concentration, 1.8×10^{-3}M. Reaction took place in 0.1 M tris-HCl buffer, pH 7.5 at 25°.

in two forms, one which contains all the subunits in one conformation and the other with the subunits in a different conformation. Atkinson et al. (1965), discussing DPN-linked isocitrate dehydrogenase and phosphofructokinase, have suggested that the binding of a ligand at one site can either increase or decrease the affinity of a ligand at a second site and that can affect the binding at still another site. Koshland et al. (1966) have mathematically treated several possible models of this type.

The nature of the folate inhibition, when measured at variable TPN concentrations, is shown in Figure 2. Double reciprocal plots indicate that the inhibition is neither competitive nor noncompetitive. A similar double reciprocal plot,

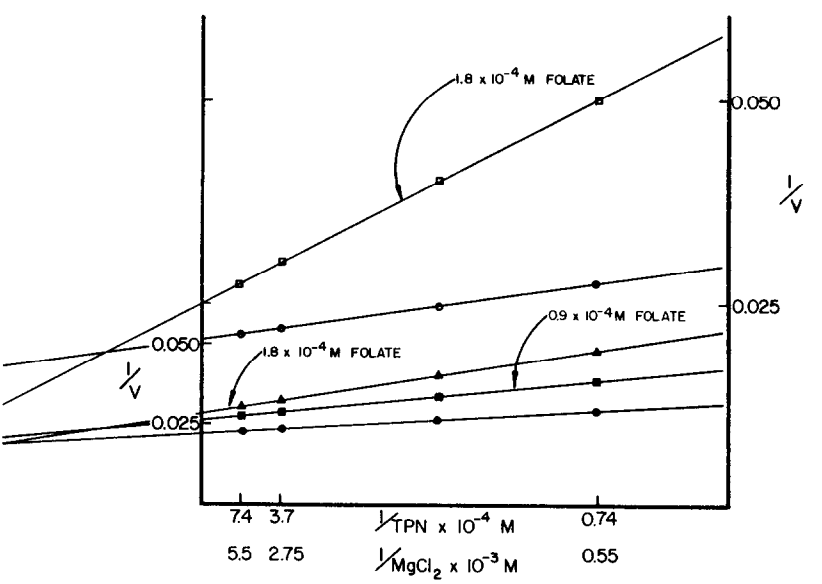

Figure 2. Double reciprocal plots at variable TPN and $MgCl_2$ in the presence and the absence of folate. Left ordinate and bottom three lines are varying TPN concentration in the presence and the absence of folate. dl-isocitrate concentration 6 x 10^{-4}M; $MgCl_2$ concentration, 1-8 x 10^{-3}M. Right ordinate and upper two lines are varying $MgCl_2$ concentrations in the presence and in the absence of folate. dl-isocitrate concentration 6 x 10^{-4}M; TPN concentration 1.35 x 10^{-4}M. Buffer and temperature same as previous figure.

but at variable magnesium chloride concentration, is shown in Figure 2 and indicates the same thing. These results tend to support an allosteric transition in the protein. The possibility that the inhibition by folate may be due to residual dihydrofolate reductase activity in the enzyme preparation (Futterman, 1957; Osborn and Huennekens, 1958), was excluded by performing the following experiment: 1×10^{-4} M DPNH or TPNH was added to 2×10^{-4} M folate in 0.1 M tris buffer, pH 7.5. The enzyme, at concentrations comparable to those used in the above experiments, was added to this mixture. No decrease in absorbancy at 340 mμ was observed for a ten minute period in both instances. Moreover, it does not seem likely from the method of preparation of both enzymes that there will be residual dihydrofolate reductase activity.

From the shape of the sigmoid curve in the presence of folate (Figure 1) and from the fact that folate does not competitively inhibit any of the substrates of isocitric dehydrogenase, we conclude that folate is probably inducing a conformational change in the protein (Monod et al., 1963, 1965; Koshland et al., 1966). Various DPN-linked isocitrate dehydrogenases have been shown to be regulated by substrates and effectors. Chen et al. (1964) have found that ADP activates DPN-linked isocitrate dehydrogenase from hog hearts and in the same fashion as its effect on glutamate dehydrogenase, ADP aggregates the enzyme. Sanwal et al. (1963, 1964) have found that the DPN-linked isocitrate dehydrogenase from Neurospora crassa gives parabolic kinetics when isocitrate is used as a substrate and this effect has been postulated as a mechanism for the regulation of the tricarboxylic acid cycle in those organisms. Lastly, Hathaway and Atkinson (1963) demonstrated the activation effect of AMP

on the DPN-linked yeast enzyme. No such effects were shown for the TPN-linked enzyme.

Porcine heart TPN-linked isocitrate dehydrogenase has a molecular weight of about 62,000 (Moyle and Dixon 1956; Seibert et al., 1957). To our knowledge nothing is known about its subunit structure. Consequently, we cannot (yet) propose cooperativity between the subunits as a basis for the action of folate. It is not necessary however, to resort to subunit interaction to explain the sigmoid curve in Figure 1. Sweeny and Fisher (1968) have shown that any enzyme model that conforms to a rate equation of $1/v = a + b[S]^x$ when x is equal to -2 or less will give a sigmoid curve. In a number of examples discussed by Fisher and Hoagland (1968) they have shown how an added inhibitor can modify the rate expression to give a rate that conforms to the equation given above. In those models a conformation change in the protein is implied without the necessity of involving subunits.

From a physiological point of view it is too early to tell what the significance of this effect may be. It is possible that it may be some link between one carbon atom and the tricarboxylic acid cycle. From the point of view of enzyme mechanism, it is not clear to us how folate exerts its action and while we can determine the effects of folate on the K_m's of various substrates from our double reciprocal plots the fact that the substrates of TPN-linked isocitrate dehydrogenases follow kinetics of random addition (Cleland, 1967) does not permit us to draw any conclusion regarding the influence of folate on the binding of the substrates. Had the enzyme reaction followed a compulsory order of addition of substrates, it might have been possible to obtain some information on the influence of folate on the binding constant of the first reacting substrate (Frieden, 1957).

Further studies on the effect of folate on the physical properties of the enzyme and the binding of its substrates are in progress.

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