DIFFERENTIAL EFFECTS OF PURINE NUCLEOTIDES ON THE ACTIVITY OF TWO GLUTAMIC DEHYDROGENASES OF NEUROSPORA [1]

Chester S. Stachow and B. D. Sanwal
Department of Microbiology, University of Manitoba, Winnipeg, Canada.

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It has been demonstrated earlier that Neurospora possesses two glutamic dehydrogenases (Sanwal and Lata, 1961a), one specific for DPN and another for TPN. Such is also the case in some other organisms (Holzer, 1957; Kaplan, 1963). In most of the bacteria, higher plants, and animals, however, only one glutamic dehydrogenase is known to occur (Sanwal and Lata, 1964) which is specific for either DPN or TPN or, like the well known beef-liver glutamic dehydrogenase (Olson and Anfinsen, 1952), can utilize either of the pyridine nucleotide coenzymes as substrate. The important physiological question thus arises as to why Neurospora and yeast cells should possess two enzymes, where one enzyme alone would conceivably be sufficient to carry out the function normally assigned to glutamic dehydrogenase, viz., that of amination of α-ketoglutarate to glutamate. The equilibrium constants of both of the glutamic dehydrogenases of Neurospora (Sanwal and Lata, 1961a) favour the synthesis of glutamate in vitro.

It was suggested earlier (Sanwal and Lata, 1962a) that the reactions catalyzed by the glutamic dehydrogenases have two different physiological roles to fulfill in the cells, and each one of these functions is performed by a separate enzyme. The evidence indicated that the TPN-specific glutamic dehydrogenase primarily fulfilled a biosynthetic function, whereas the DPN-specific enzyme performed a catabolic role (Sanwal and Lata, 1961a; 1961b; 1962a). Following the report that purine nucleotides act as inhibitors

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or activators of the beef-liver glutamic dehydrogenase (Wolff, 1962; Frieden, 1963), we tested the effect of these compounds on the activity of the TPN and DPN-specific glutamic dehydrogenases of Neurospora. We describe below the interesting observation that only the DPN-specific enzyme is affected by various purine nucleotides in vitro, which leads to the surmise that a significant secondary role of the DPN-specific dehydrogenase may be to supply the precursor of glutamine which gives rise to purine nucleotides by a sequence of reactions described by Buchanan and Hartman (1959).

METHODS - The TPN-specific dehydrogenase was purified from strain STA 4 of Neurospora crass and assayed by methods described earlier (Sanwal and Lata, 1962c). For reaction velocity measurements at 340 mμ, a Gilford Model 2000 recording spectrophotometer was used. The DPN-specific enzyme was purified by a modification of our earlier procedure (Sanwal and Lata, 1961a) from glutamate induced cells of mutant strain am^3 which lacked the TPN-specific enzyme. The enzyme used here was approximately 330-fold purified compared with basal levels normally found in am^3 strain under uninduced conditions.

EXPERIMENTS AND DISCUSSION- In Fig.1 are presented the results of an experiment with TPN-specific dehydrogenase where TPNH was varied in the absence or the presence of 1.1×10^{-3}M GTP. It will be seen that TPNH shows substrate inhibition which is also the case with DPN-specific enzyme but GTP has no effect on the velocity of the TPN-specific dehydrogenase reaction. Similar results are obtained when ATP, ADP, AMP, CTP, CDP, UTP, and GDP (all tested at 10^{-3}M) are used in place of GTP. With the DPN-specific enzyme, however, GTP causes a marked inhibition of the reaction, when it is measured either in the oxidative deamination (Fig. 2) or reductive amination assay. The inhibition is competitive with the oxidized or reduced coenzyme and the K_i calculated according to Cleland (1963) is 6×10^{-5} M. ATP, ADP, CTP, CDP, UTP and UDP when tested separately at a

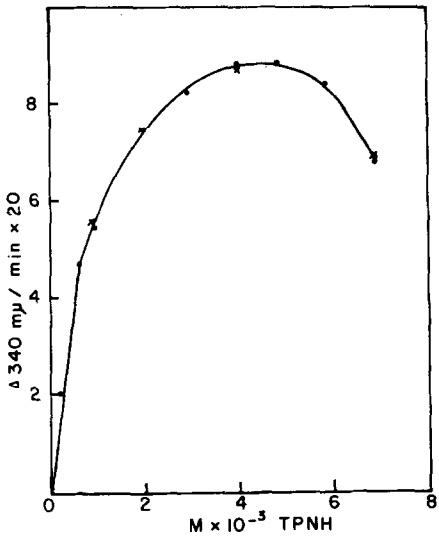

Fig. 1. TPNH inhibition of TPN-specific glutamic dehydrogenase catalyzed reductive amination of α-ketoglutarate. Assay mixture contained: 13 mM α-ketoglutarate; 80 mM $(NH_4)_2SO_4$; 0.02 M Tris-HCl buffer (pH 7.5) and 1.2 μg/ml enzyme. (●——●) minus GTP; (X——X) 1.1 x 10^{-3} M GTP.

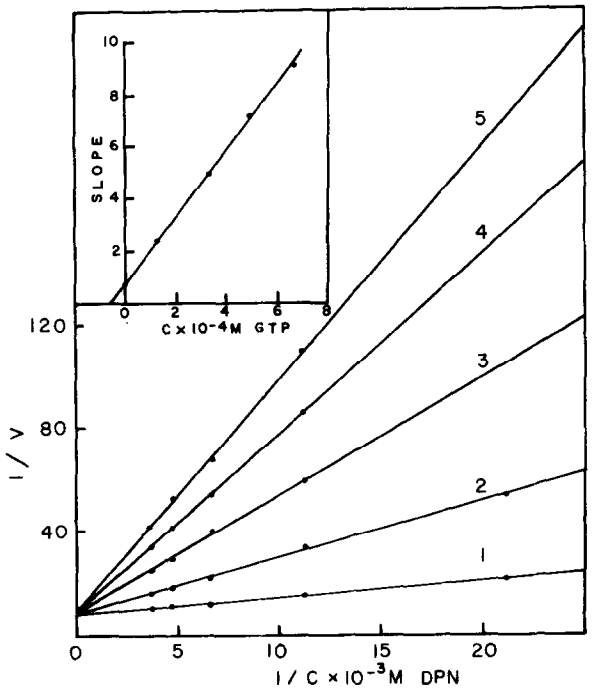

Fig. 2. Double reciprocal plots for DPN-specific glutamic dehydrogenase with DPN as varied substrate and GTP as inhibitor. L-Glutamic acid concentration, 25 mM. GTP concentrations: (1) none, (2) 0.13 mM (3) 0.33 mM (4) 0.49 mM (5) 0.66 mM. Enzyme concentration 1.2 μg/ml. The insert is a replot of slopes vs GTP concentration.

concentration of 10^{-3} M have no effect on enzyme activity, but GDP, GMP and IMP inhibit the enzyme competitively. The K_i values for GDP and GMP are nearly similar to GTP (2.5×10^{-5} M for GMP), but inhibition by IMP is considerably smaller ($K_i = 1.5 \times 10^{-4}$ M). Although ATP by itself does not alter enzyme activity, yet it must complex with the DPN-specific glutamic dehydrogenase because in its presence the inhibition caused by GTP is considerably alleviated (Fig. 3). Admittedly, however, the concentrations of ATP required to bring about this effect are considerably higher compared to GTP.

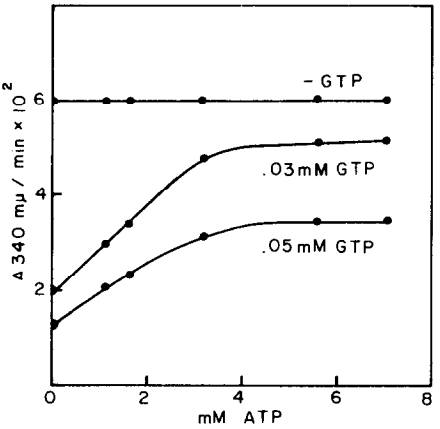

Fig. 3. Reversal of GTP inhibition of the DPN-specific glutamic dehydrogenase by ATP. Reaction mixture contained: 15 mM α -ketoglutarate; 40mM $(NH_4)_2SO_4$; 0.10 mM DPNH; 0.02 M Tris-HCl buffer (pH 8.5); 0.66 μg/ml enzyme, and concentrations of GTP and ATP as indicated.

The results obtained here are in many respects similar to beef-liver glutamic dehydrogenase. Frieden (1963) has reached the conclusion that the liver enzyme, in addition to an active coenzyme site, also has a highly specific purine nucleotide binding site, and the inhibition observed at high DPNH concentrations (Yielding et al, 1964) may be due to its binding at the purine nucleotide site. Also, some purine nucleotides (ADP, ATP) by binding at the latter site cause activation and others (GTP, ITP, etc.) cause inhibition. It is tempting to suggest that the DPN-specific glutamic dehydrogenase of Neurospora may also have two sites, one of which readily binds GTP and ATP and another of which is specific for the coenzyme,

but more sophisticated experiments would have to be performed to prove the conjecture.

More important than the binding sites on the enzyme surface, our investigations point to a possible metabolic control of the DPN-specific glutamic dehydrogenase activity by the purine nucleotide pools and perhaps also explain the necessity for the cell to produce two enzymes with seemingly identical functions. Since the purine nucleotide pools rather than individual nucleotides are specific in the inhibition of the enzyme, the most attractive possibility remains (Frieden, 1963) that the synthesis of purine nucleotides may be controlled by the availability of glutamine which in its turn may be generated largely by glutamate synthesized through the mediation of DPN-specific enzyme to the partial exclusion, perhaps, of the glutamate generated by the TPN-specific dehydrogenase. There is no direct experimental evidence yet to support the hypothesis. Such studies are in progress.

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