GUANOSINE-5'- TRIPHOSPHATE AS THE ALLOSTERIC EFFECTOR OF FRUCTOSE 1,6- DIPHOSPHATASE IN RHODOPSEUDOMONAS PALUSTRIS

C. S. Stachow and C. F. Springgate

Department of Biology, Boston College.
Chestnut Hill, Massachusetts, 02167.

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SUMMARY: The inhibition of 300-fold purified fructose 1,6-diphosphatase from Rhodopseudomonas palustris by guanosine-5'-triphosphate is described. Kinetic data illustrates that GTP inhibition is specific, allosteric in nature and non-competitive. Guanosine-5'-triphosphate was not utilized in the enzymatic reaction. Evidence presented suggests that GTP levels play an important role in regulation of the photo-synthetic reductive carbon cycle in Rhodopseudomonas.

Adenosine-5'-monophosphate (AMP) has been demonstrated to be an allosteric inhibitor (Taketa and Pogell, 1963, 1965; Underwood and Newsholme, 1965) of fructose 1,6-diphosphatase from a wide variety of organisms. However, Mukkada and Bell (1969) have reported a fructose diphosphatase (FDPase) of Acinetobacter which is inhibited by ATP and citrate but AMP-insensitive. Studies on the photosynthetic carbon reduction cycle (Pedersen et. al., 1966; Bassham et. al., 1968, 1969) have provided evidence that the fructose diphosphatase reaction plays a key role in regulating biosynthesis and the light-dark transition. In this communication the allosteric inhibition of fructose 1,6-diphosphatase by GTP is reported and partially characterized in the facultative photoheterotroph Rsp. palustris.

MATERIALS AND METHODS:

Approximately 300-fold purified FDPase was obtained from Rsp. palustris, grown photoheterotrophically in synthetic medium (Cohen-Bazire et. al., 1957). Glutamate, malate, and acetate were used as carbon sources. Cells were disrupted in 0.1 M Tris-HCL buffer, pH 7.4, containing 1.0 mM dithiotheitol (DTT) by means of a French pressure cell operated at 10,000 psi. FDPase was purified by $MnCl_2$, ammonium sulfate and streptomycin sulfate precipitation, alumina C-gamma adsorption-elution and DEAE-cellulose column chromatography. Details of the purification procedure will be published elsewhere. The resulting enzyme preparation was stable for two weeks when stored at -15° and was free of the following contaminating enzymes: NADPH oxidase, acid and alkaline phosphatase, phosphofructokinase and aldolase. Fructose 1,6-diphosphatase activity was determined spectrophotometrically by following the rate of NADP reduction at 340 mμ in the presence of excess coupling enzymes. The assay mixture contained: 1.0 mM FDP, 1.0 mM NADP, 1.0 mM $MnCl_2$, 10 μg phosphoglucoisomerase, 2 μg glucose 6-phosphate dehydrogenase and 100 mM Tris-HCL buffer (pH 8.0) containing 1.0 mM DTT in a final volume of 1.0 ml. All reagents were analytical grade and purchased from Sigma Chemical Co. Uniformly labelled ^{14}C-GTP was purchased from New England Nuclear Corp.

RESULTS AND DISCUSSION:

Inhibition of fructose 1,6-diphosphatase activity (figure 1.) was found to be specific for guanosine-5'-triphosphate as the following compounds tested at a concen-

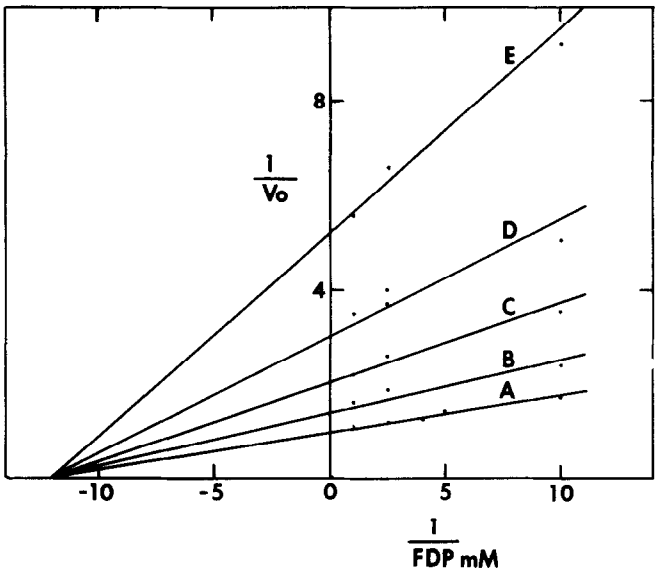

Figure 1. Double reciprocal plots of velocity versus FDP concentration at several GTP concentrations (fixed variable). Curve (A) minus GTP; (B) 1.0 mM GTP; (C) 1.25 mM GTP; (D) 1.5 mM GTP and (E) 2.0 mM GTP.

tration of 3.0 mM had no effect on FDPase activity: ATP, ADP, AMP, cyclic 3',5'-AMP, CTP, CDP, CMP, UTP, UDP, ITP, IDP, IMP, GDP, GMP, cyclic 3',5'-GMP, reduced and oxidized NAD and NADP, citrate, acetate and pyridoxal phosphate. Levels of GTP used in our studies had no effect on the coupling enzyme system.

Reciprocal plots of velocity versus fructose 1,6-diphosphate concentration (figure 1.) at several concentrations of GTP (fixed variable) resulted in a non-competitive inhibition pattern. From this data it can be seen that GTP does not influence the Km of FDP which was calculated to be 8.3×10^{-5} M. These results are similar to the AMP inhibition of mammalian fructose 1,6-diphosphatase (Taketa and Pogell, 1965) but differ from the competitive inhibition reported for the plant enzyme (Scala, 1969). GTP inhibition could be

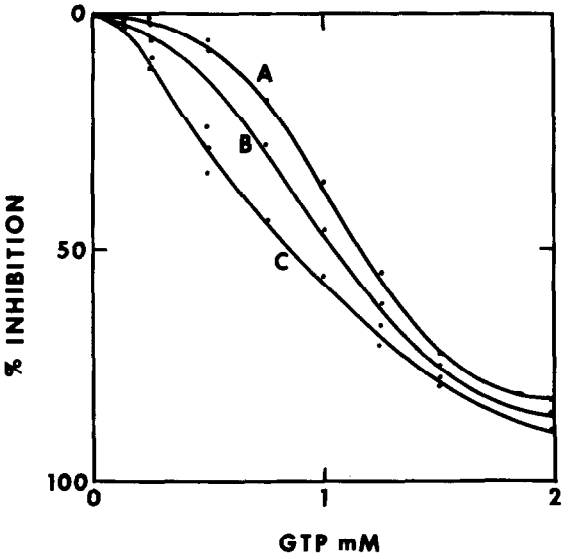

Figure 2. Effect of guanosine -5'-triphosphate on fructose 1,6-diphosphatase activity. Percent inhibition of FDPase activity is plotted against varying GTP concentrations at several fixed variable concentrations of FDP. Curve (A) 1.0 mM FDP; (B) 0.4 mM FDP; (C) 0.1 mM FDP.

completely reversed by dilution but not by substrate concentrations as high as 20 x Km.

Plots of percent inhibition versus GTP concentration (figure 2.) yield sigmoid curves which demonstrate the inhibition does not follow simple mass action theory. The concentration of GTP which resulted in 50% inhibition of enzymatic activity at varying substrate levels ranged from 7.4×10^{-4} M at 0.1 mM FDP to 12×10^{-4} M at 1.0 mM FDP or approximately ten times Km.

A series of straight lines was obtained when log V_o-v/v was plotted against log GTP concentration (figure 3.). The Hill coefficient, n was calculated to be 2.9 at 25° indicating more than one GTP binding site per enzyme molecule. The determination of the precise number of GTP sites must await further binding studies.

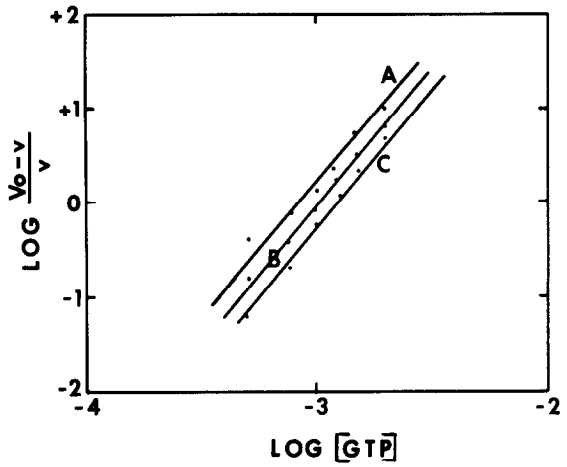

Figure 3. Hill plots of GTP inhibition at various FDP concentrations. Plotted as log V_o-v/v versus log (GTP) where V_o represents velocity in the absence of GTP and v represents velocity in the presence of GTP. Curve (A) 0.10 mM FDP; (B) 0.40 mM FDP; (C) 1.0 mM FDP.

To determine whether GTP was utilized in the reaction, ^{14}C labelled GTP (5.0 mM containing 3 x 10^6 DPM) was included in the standard reaction mixture and incubated for 15 min. Subsequent identification and percent recovery of guanosine-5'-triphosphate by paper chromatography (System IV, Pabst Laboratory Circular OR-10) and scintillation counting demonstrated that the inhibitor, GTP was not hydrolyzed during the reaction.

The regulatory nature of fructose 1,6-diphosphatase, which is reflected in the sigmoid inhibition data shown in figure 2, suggests that the levels of GTP in the cells play an important role in controlling the flow of carbon in the photosynthetic reductive carbon cycle of Rhodopseudomonas palustris. Studies to further characterize this control mechanism are now in progress at this laboratory.

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