Glucose Dehydrogenase Activity of Yeast Glucose 6-Phosphate Dehydrogenase. Inhibition by Adenosine 5′-Triphosphate and Other Nucleoside 5′-Triphosphates and Diphosphates*

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ABSTRACT: The inhibitory effects of a variety of nucleoside 5′-triphosphates, -diphosphates, -monophosphates, and related compounds on glucose dehydrogenase and glucose 6-phosphate dehydrogenase activities of highly purified (Boehringer) yeast D-glucose 6-phosphate:nicotinamide–adenine dinucleotide phosphate oxidoreductase (EC 1.1.1.49) preparations have been investigated. The results of studies of inhibition of both activities considered with respect to the various substrates followed classical kinetic patterns. Inhibitions were noncompetitive with respect to nicotinamide–adenine dinucleotide phosphate in both reactions, and were competitive with respect to glucose 6-phosphate and glucose. K_i values for each inhibitor, evaluated with both glucose 6-phosphate and glucose dehydrogenase activities, were in good agreement. Competitive inhibition of glucose dehydrogenase by adenosine 5′-triphosphate also was noted with respect to HCO_3^- and HPO_4^{2-}, which markedly activate this activity of the enzyme. These same smaller anions, which previously have been shown to lower activity of the enzyme with glucose 6-phosphate as oxidizable substrate, inhibited glucose 6-phosphate dehydrogenase in a "common site" manner when present in various combinations with adenosine 5′-triphosphate. With respect to effectiveness as inhibitors, nucleoside triphosphates > corresponding nucleoside diphosphates > corresponding nucleoside monophosphates. Purine nucleoside tri-, di-, and monophosphates were more effective inhibitors than corresponding pyrimidine nucleotides. Ribose 5-phosphate was a relatively weak inhibitor, and no inhibition was noted with adenosine and adenine. The results of these studies with both activities of the enzyme support the competitive interaction of the various nucleoside tri-, di-, and monophosphates with an enzymic site also involved in the binding of smaller anionic effectors and the phosphoryl group of hexose phosphate substrate, and appear to merit the inclusion of nucleoside triphosphates and diphosphates, along with the smaller anions bicarbonate, orthophosphate and sulfate, in the mechanistic scheme for regulation described in the preceding paper in this series. The ability of these compounds to inhibit, and of smaller anions to activate, glucose dehydrogenase when bound to this same site is considered mechanistically, and the possible physiological significance of these observations is suggested.

A relatively slow oxidation of glucose by yeast glucose 6-phosphate dehydrogenase (D-glucose 6-phosphate:nicotinamide–adenine dinucleotide phosphate oxidoreductase, EC 1.1.1.49) has been described by Colowick and Goldberg (1963), Salas *et al.* (1965), and Kuby and Noltmann (1966). In two recent reports from this laboratory (Anderson and Nordlie, 1968; Anderson *et al.*, 1968), we presented further evidence for the common identity of these two dehydrogenase activities and reported that the anions orthophosphate, bicarbonate, and sulfate, which inhibit glucose-6-P[1] dehydrogenase activity, all stimulate quite markedly (15- to 20-fold) the associated glucose dehydrogenase activity. On the basis of kinetic studies, a mechanism was proposed involving a single common binding site for anions functioning both as activators of glucose dehydrogenase and as inhibitors of glucose-6-P dehydrogenase activity (Anderson *et al.*, 1968).

Inhibition by ATP of yeast glucose-6-P dehydrogenase activity has been described briefly by Passonneau *et al.* (1966) and in greater detail by Avigad (1966). In view of the selective responses of the two activities of the enzyme to inorganic anions noted in our laboratory (Anderson and Nordlie, 1968; Anderson *et al.*, 1968), we found it of interest to carry out comparative studies on the effects of ATP and other nucleoside 5′-triphosphates, diphosphates, monophosphates, and related compounds on both glucose and glucose-6-P dehydrogenase activities of the enzyme. Results of some of these experiments, including investigations of the nature of the interaction of both nucleotide and inorganic anions with these activities, are described in this paper.

Materials and Methods

The highly purified brewers' yeast glucose-6-P dehydrogenase from Boehringer Mannheim Corp. (lot control no. 6018592, 6067475, 6098195, 6127278, and 6229405) and reagents used, as well as the enzymic activity assays, were as previously described (Anderson and Nordlie, 1968; Anderson *et al.*, 1968). Solutions of reagents were adjusted to the

* From the Guy and Bertha Ireland Research Laboratory, Department of Biochemistry, University of North Dakota Medical School, Grand Forks, North Dakota 58201. *Received June 24, 1969.* This work was supported in part by a grant from the Hill Family Foundation and by Research Grants B6-0823R from the National Science Foundation and AM-07141 from the National Institutes of Health, United States Public Health Service. Presented in part at the 69th Annual Meeting of the American Society for Microbiology, Miami Beach, Fla., May 4–9, 1969 (Nordlie *et al.*, 1969).

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[1] Abbreviations used are listed in *Biochemistry 5*, 1445 (1966).

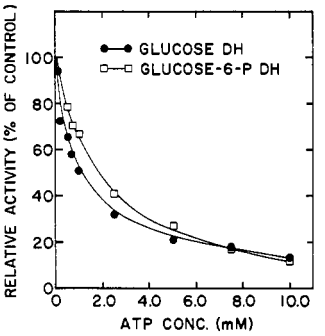

FIGURE 1: Effects of varied concentrations of ATP on glucose dehydrogenase (Glucose DH) and glucose-6-P dehydrogenase (Glucose-6-P DH). Assay mixtures (pH 7.5) contained, in 3.0 ml, 8.3 mM Tris-Cl buffer, 0.1 mM sodium NADP, indicated concentrations of disodium ATP, and either 0.5 M equilibrated D-glucose and 12.5 μg of enzyme protein (glucose dehydrogenase), or 1×10^{-5} M sodium glucose-6-P and 0.125 μg of enzyme protein (glucose-6-P dehydrogenase); $\mu = 0.100$ in all instances. *Relative activity (% of control)* = 100 × (activity noted in presence of ATP)/(activity noted in absence of ATP).

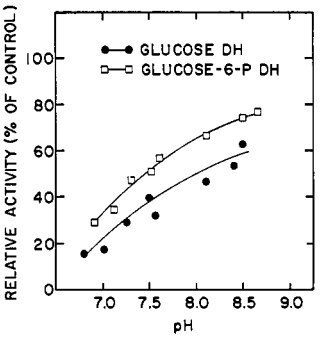

FIGURE 2: Influence of assay mixture pH on inhibition by ATP of glucose dehydrogenase and glucose-6-P dehydrogenase activities. Assay mixtures and other details were identical with those in Figure 1, except that pH was varied as indicated, and inhibition by 2.5 mM ATP was studied. The pH of all assay mixtures, measured with a Beckman expanded scale meter before and immediately after 2.5-min incubation, was found unchanged.

desired pH with dilute NaOH in all instances. Nucleotides were obtained from Sigma Chemical Co., St. Louis, Mo. Ionic strength was maintained constant at 0.100 in all experiments by the addition of appropriate supplemental amounts of NaCl to assay mixtures. Further details of individual experiments are given in the legends to figures and tables. Activity in all instances was measured under conditions such that initial reaction velocities were determined. The results of kinetic studies are presented as conventional double-reciprocal plots (Lineweaver and Burk, 1934). Michaelis constants[2] were calculated as negative reciprocals of x-axis intercepts of extrapolations of such plots (see Dixon and Webb, 1964a). Inhibitor constant values were calculated by the method described by Dixon and Webb (1964b) (competitive inhibition), or by the method described by Nordlie and Lygre (1966) when inhibition was noncompetitive. Nucleotide solutions were assayed spectrophotometrically.[3]

Results

Inhibition by ATP. That both glucose-6-P dehydrogenase and glucose dehydrogenase activities of the enzyme are inhibited by ATP is apparent from the experiments presented in Figure 1. Both activities were progressively inhibited as ATP concentrations were increased from 0.1 to 10 mM.

In order to gain further insight relative to the nature of inhibition by ATP of these two activities of the enzyme, and of the effects of various anions (see Anderson and Nordlie, 1968; Anderson *et al.*, 1968) in combination with the nucleotide,[4] a series of kinetic studies was carried out. Variations in the extent of inhibition by 2.5 mM ATP of glucose dehydrogenase and glucose-6-P dehydrogenase activities as a function of assay mixture pH are described in Figure 2. Nearly parallel increases in the extents of inhibition of both activities were noted as pH was lowered from approximately 8.6 to 6.6. A similar effect of pH on ATP-effected inhibition of glucose-6-P dehydrogenase activity previously was noted by Avigad (1966). Subsequent studies described below were carried out at pH 7.5 or 7.8 where significant, but not maximal, amounts of inhibition are exerted (see Figure 2).

Kinetics of Inhibition by ATP of Glucose-6-P Dehydrogenase Activity. The results of experiments in which inhibition by ATP was studied with respect to varied concentrations of glucose-6-P are presented as conventional double-reciprocal plots in Figure 3. As indicated by the common point of intersection of extrapolations of experimental plots on the y axis, kinetically competitive inhibition was produced by both 0.9 and 2.7 mM ATP. These observations confirm

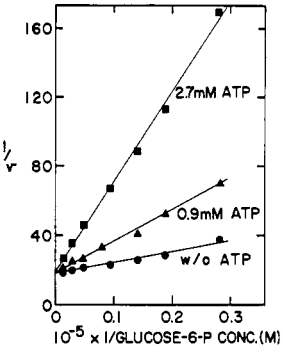

FIGURE 3: Kinetics of inhibition by ATP of glucose-6-P dehydrogenase activity studied with respect to varied glucose-6-P concentrations. Assay mixtures (pH 7.5) contained, in 3.0 ml, 8.3 mM Tris-Cl buffer, 0.1 mM sodium NADP, 0.2 μg of enzyme protein, and indicated concentrations of sodium glucose-6-P and disodium ATP (w/o ATP indicates that no ATP was present); $\mu = 0.100$ in all cases. Velocity, v, is indicated as micromoles of sodium NADPH formed per 2.5-min incubation. K_m for glucose-6-P was 3.2×10^{-5} M, while K_i values for ATP, calculated as described by Dixon and Webb (1964b), were 4×10^{-4} M with both concentrations of ATP tested.

[2] As discussed previously (Anderson *et al.*, 1968), true K_m values for this system, where the affinity of enzyme for each substrate is independent of concentration of second substrate, may be determined directly, as in Figures 3–5, 7, and 8, without the use of secondary plots.

[3] Pabst Circular OR-7, pp 5–30.

[4] To avoid possible ambiguity the term "nucleotide," as employed in this paper, refers only to inhibitory nucleoside 5′-triphosphates, diphosphates, and monophosphates, and *not* to NADP substrate.

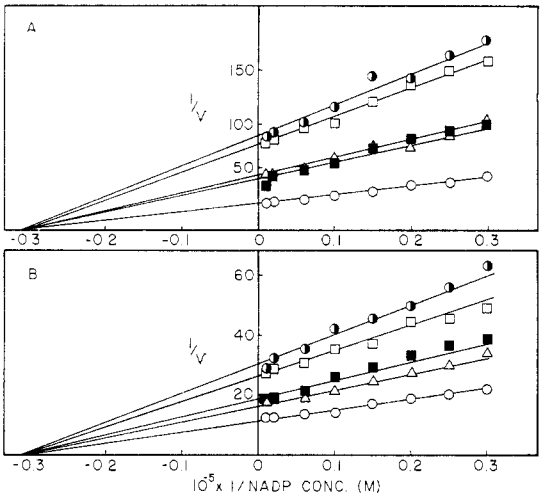

FIGURE 4: Kinetics of inhibition by nucleotides of (A) glucose dehydrogenase and (B) glucose-6-P dehydrogenase activities studied with respect to varied concentrations of NADP. Assay mixtures (pH 7.5) contained, in 3.0 ml, 8.3 mM Tris-Cl buffer, either 0.83 M equilibrated D-glucose and 10 μg of enzyme protein (A) or 0.1 mM sodium glucose -6-P and 0.20 μg of enzyme protein (B), indicated varied concentrations of sodium NADP, and 2.5 mM GTP (◑), 2.5 mM ATP (□), 2.5 mM ADP (■), 1.0 mM ATP (△), or no supplemental nucleotide (○); μ = 0.100 in all cases. Activity, v, is expressed as in Figure 3. K_m for NADP = 3.2×10^{-5} M.

TABLE I: Inhibitions of Glucose-6-P Dehydrogenase Activity by Combinations of ATP and Either Bicarbonate or Phosphate.[a]

Inhibitor Concn (mM)			Inhibition (%)	
Phosphate	Bicarbonate	ATP	Exptl	Theoretical
60			74.6	
	60		43.0	
		1	40.0	
		3	68.0	
20		3	72.8	75.3
60		1	78.4	78.3
60		3	81.2	83.4
	20	3	69.2	69.9
	60	1	56.0	59.0
	60	3	72.0	74.0

[a] Assay mixtures (pH 8.0) contained, in 3.0 ml, 8.3 mM Tris-Cl buffer, 0.1 mM sodium NADP, 0.1 mM sodium glucose-6-P, 0.125 μg of enzyme protein, and the indicated concentrations of inhibitors. "Per cent inhibition" values were calculated on the basis of activity noted in the absence and presence of inhibitor. Further details on the calculation of "theoretical inhibition" values are given in the text.

the findings of Avigad (1966), who studied the kinetics of inhibition with respect to sugar phosphate substrate, but not with respect to NADP.

The kinetics of inhibition by 1.0 and 2.5 mM ATP of glucose-6-P dehydrogenase were similarly studied with respect to NADP, which was varied from 0.033 to 1.0 mM in the presence of constant (0.1 mM) glucose-6-P in the experiments presented in Figure 4B. Studied in this fashion, inhibition was found to be kinetically of the classical noncompetitive type, as indicated by the intersection of extrapolations of all experimental plots at a common point on the x axis.

Results of studies in which inhibition by ATP in combination with various concentrations of the anions HCO_3^- and HPO_4^{2-}, which previously have been shown to inhibit potently glucose-6-P dehydrogenase activity (see Anderson and Nordlie, 1968), are given in Table I. Inhibitions were measured with each inhibitor individually and in the indicated combinations. Theoretical inhibitions listed in the table for various combinations of ATP and anions were calculated, with the aid of eq 1, on the basis of the assumption of the competitive interaction of all inhibitors at a common site on the enzyme (Webb, 1963), as described in detail in a previous paper in this series (Anderson *et al.*, 1968). The good agreement between experimentally determined inhibitions and theoretical values calculated in this manner supports the involvement of a common binding site for ATP and the various anions functioning as inhibitors of glucose-6-P dehydrogenase activity.

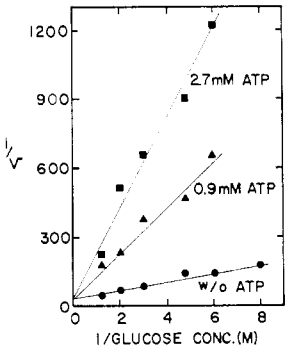

FIGURE 5: Kinetics of inhibition by ATP of glucose dehydrogenase activity studied with respect to glucose. Assay mixtures (pH 7.5) contained, in 3.0 ml, 8.3 mM Tris-Cl buffer, 0.1 mM sodium NADP, 20 μg of enzyme protein, and indicated varied concentrations of equilibrated D-glucose and disodium ATP (w/o ATP indicates that no ATP was present); μ = 0.100 in all cases. Velocity, v, is expressed as in Figure 3. K_m for D-glucose = 0.60 M, while K_i for ATP = 4×10^{-4} M with both ATP concentrations.

$$i = \frac{\frac{(I_1)}{K_i^1} + \frac{(I_2)}{K_i^2} + \cdots}{1 + \frac{(S)}{K_m} + \frac{(I_1)}{K_i^1} + \frac{(I_2)}{K_i^2} + \cdots} \quad (1)$$

where i = fractional inhibition in the presence of two (or more) competitive inhibitors acting at a common enzyme site; (I_1), (I_2), etc., indicate molar concentrations of individual inhibitors; K_i^1, K_i^2, etc., are inhibitor constants for I_1, I_2, etc.; (S) is substrate concentration; and K_m is Michaelis constant for the substrate S toward which competitive inhibition by compounds I_1, I_2, etc., is manifest.

Kinetics of Inhibition by ATP of Glucose Dehydrogenase Activity. The kinetics of ATP-effected inhibition of glucose

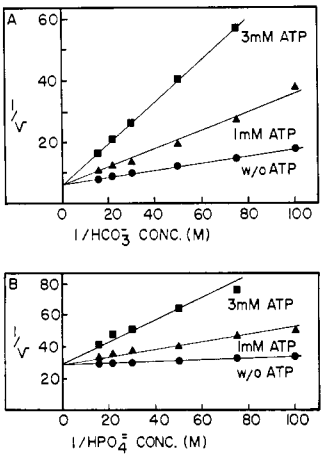

FIGURE 6: Kinetics of inhibition by ATP of glucose dehydrogenase activity studies with respect to the activators (A) bicarbonate and (B) orthophosphate. Assay mixtures, pH 7.5 (A) or 7.8 (B), contained, in 3.0 ml, 8.3 mM Tris-Cl buffer, 0.1 mM sodium NADP, 0.5 M equilibrated D-glucose, 20 μg of enzyme protein, and the indicated concentrations of disodium ATP and sodium bicarbonate (A) or disodium hydrogen phosphate (B). (w/o ATP indicates that no ATP is present); μ = 0.100 in all instances. Velocity, v, is as in Figure 3. K_i values for ATP were calculated as follows, with 1 and 3 mM ATP, respectively: (A) 5.4×10^{-4} M and 5.3×10^{-4} M, and (B) 3.4×10^{-4} M and 3.0×10^{-4} M.

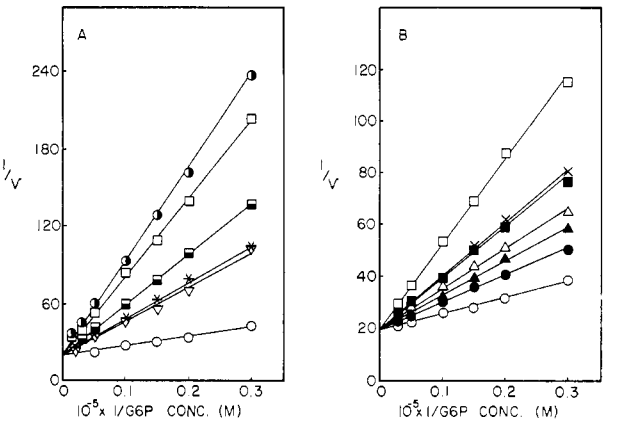

FIGURE 7: Kinetics of inhibition of glucose-6-P dehydrogenase by a variety of nucleoside 5'-triphosphate (A) and by ATP and related compounds (B), studied with respect to varied concentrations of glucose-6-P. Activities were measured in the absence of nucleotide or related compounds (○), and in the presence of 2.5 mM GTP (◑), 2.5 mM ATP (□), 2.5 mM ITP (▭), 2.5 mM UTP (*), 2.5 mM CTP (▽), 2.5 mM P_i (×), 2.5 mM ADP (■), 10 mM ribose-5-P (△), 2.5 mM PP_i (▲), or 2.5 mM AMP (●). Activities observed in the presence of 2.5 mM adenine or adenosine were identical with those noted in the absence of supplementation (○). Basic assay mixture composition and other details are as in Figure 3.

dehydrogenase activity of the enzyme was studied with respect to the substrates NADP (Figure 4A) and glucose (Figure 5), as well as the activators bicarbonate (Figure 6A) and orthophosphate (Figure 6B). In the experiment described in Figure 4A, glucose concentration was maintained constant at 0.83 M and activity was measured as a function of varied NADP concentrations in the absence of nucleotide and in the presence of 1 and 2.5 mM ATP. Figure 5 depicts an experiment in which activities without and with 0.90 and 2.7 mM ATP were assessed as a function of varied glucose concentrations with NADP constant at 0.1 mM. In the experiments in Figure 6A, B, NADP (0.1 mM) and glucose (0.5 M) were constant, and activities in the presence of 0, 1, and 3 mM ATP were assessed as a function of varied concentrations of the activitors HCO_3^- (Figure 6A) or HPO_4^{2-} (Figure 6B). Data obtained are in all cases recorded as double-reciprocal plots (Lineweaver and Burk, 1934).

As with glucose-6-P dehydrogenase activity, inhibition was noncompetitive with respect to NADP in the glucose dehydrogenase reaction (see Figure 4A). Inhibition by ATP competitive with respect to the activators of glucose dehydrogenase HCO_3^- (Figure 6A) and HPO_4^{2-} (Figure 6B) was observed. Finally, competitive inhibition was found to prevail with respect to glucose, elevation of concentrations of which reversed inhibition by the nucleotide (see Figure 5).

Inhibition by Other Nucleotides and Related Compounds. Studies of inhibitor specificity are described in Figures 7 and 8 and Table II. Results of experiments in which inhibition of glucose-6-P dehydrogenase activity by a variety of nucleoside triphosphates, studied with respect to the substrate glucose-6-P, are presented as conventional double-reciprocal plots (Lineweaver and Burk, 1934) in Figure 7A. The results of similar studies in which the relative inhibitory effects of certain compounds comprising fragments of the complete ATP molecule—ADP, AMP, adenosine, adenine, ribose-5-P, PP_i, and P_i—are compared, are described in Figure 7B. In all cases, inhibitions where observed were of the classical competitive type with respect to hexose phosphate substrate, as indicated by convergence of all experimental plots at a common point on the axis of ordinates. Likewise, inhibitions by these compounds were found competitive with respect to glucose in the glucose dehydrogenase reaction (see some representative results in Figure 8). K_i values, calculated from

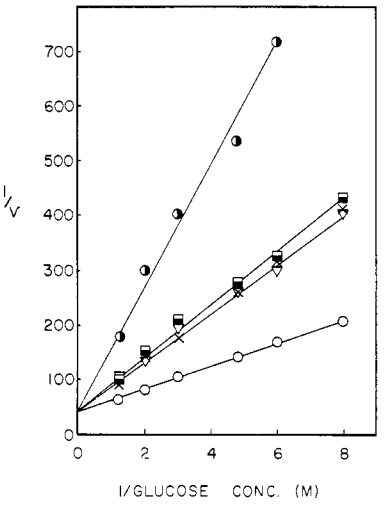

FIGURE 8: Kinetics of inhibition by nucleotides of glucose dehydrogenase activity, studies with respect to varied glucose concentration. Basic assay mixture composition was as described in Figure 5, to which was added no nucleotide (○), 2.5 mM CTP (▽), 2.5 mM UTP (×), 2.5 mM ITP (▭), and 2.5 mM GTP (◑). Other details are as in Figure 5. K_i values calculated from these and similar experiments are given in Table II.

TABLE II: Inhibitor Constant Values for Various Nucleoside 5′-Triphosphates, -Diphosphates, -Monophosphates, and Related Compounds, Determined for Glucose Dehydrogenase and Glucose-6-P Dehydrogenase Activities.[a]

Compound Tested	$K_i \times 10^4$ M	
	Glucose-6-P Dehydrogenase	Glucose Dehydrogenase
ATP	3.9	3.9
ADP	12.5	13.4
AMP	33	30
Adenosine	*b*	*b*
Adenine	*b*	*b*
Ribose-5-P	71	50
PP_i	25	27
GTP	3.1	3.1
GDP	8.5	8.1
GMP	22.5	17.6
ITP	5.0	7.0
IDP	14.6	13.4
IMP	60	54
UTP	7.6	8.0
UDP	41	42
UMP	*b*	*b*
CTP	8.3	8.0
CDP	22.5	24.0
CMP	*b*	*b*

[a] All values were calculated, at pH 7.5 and $\mu = 0.100$, on the basis of experiments described in Figures 3, 5, 7, and 8 and similar studies. [b] $K_i > 100 \times 10^{-4}$ M.

these and similar studies by the method described by Dixon and Webb (1964b), are compiled in Table II. Good agreement between values calculated for both glucose-6-P and glucose dehydrogenase activities were obtained in all instances.

Inhibitions by selected, representative nucleotides—GTP and ADP—were found to be noncompetitive with respect to NADP in both glucose dehydrogenase and glucose-6-P dehydrogenase reactions, in agreement with the similar effects noted with ATP (see Figure 4A,B).

Discussion

Inhibitor Specificity. It is apparent from the data presented above that a rather broad specificity of inhibition of glucose dehydrogenase and glucose-6-P dehydrogenase activities exists with respect to nucleoside 5′-triphosphates and nucleoside 5′-diphosphates (see Table II). Nucleoside triphosphates were uniformly more effective inhibitors than were corresponding nucleoside diphosphates. The purine nucleoside triphosphates tested—ATP, GTP, and ITP—were somewhat more potent inhibitors than were the pyrimidines CTP and UTP. This same general relationship also prevailed with respect to nucleoside diphosphates. Nucleoside 5′-monophosphate esters were relatively ineffective in comparison with their di- and triphosphate counterparts, as was ribose-5-P. No significant inhibition was observed with either adenine or adenosine. It thus is apparent that the presence of the complete nucleoside 5′-triphosphate molecule is necessary for maximal inhibition.

Mechanism of Inhibition. Previous kinetic studies in this laboratory (Anderson *et al.*, 1968) have led to the conclusion that the anions HCO_3^-, HPO_4^{2-}, and SO_4^{2-} exert effects on glucose-6-P dehydrogenase activity (inhibition) and glucose dehydrogenase activity of the enzyme (marked activation) through their binding to an enzymic site to which the phosphoryl group of the substrate glucose-6-P attaches in the absence of inhibitor, as indicated in I–VII in Figure 9 and as described in detail by Anderson *et al.* (1968). Essential features of this previously proposed mechanism of action of inorganic anions and bicarbonate include competition between glucose-6-P and such smaller anions for enzyme as depicted in II → V and II → III in Figure 9, and *activation* of glucose dehydrogenase activity through the "flexation" of the enzyme molecule accompanying binding of such small anions (compare II → IV with II → V → VII in Figure 9). Patterns of inhibition of glucose-6-P dehydrogenase activity by ATP and other nucleoside triphosphates and diphosphates noted in the present studies—competitive inhibition against glucose-6-P (Figures 3 and 7) and noncompetitive inhibition with respect to NADP (Figure 4B)—are identical with those previously observed with HCO_3^-, HPO_4^{2-}, and SO_4^{2-} (Anderson *et al.*, 1968), and are consistent with the binding of the terminal phosphoryl group of the nucleoside triphosphate or diphosphate to this same enzymic site. This competition between substrate glucose-6-P and inhibitory nucleotides for the enzyme is indicated schematically by diagrams II → III and II → VIII in Figure 9. Also strongly supporting this concept are the results of "combined inhibitor" studies (Table I) with glucose-6-P dehydrogenase activity which indicate a common binding site for ATP, HCO_3^-, and HPO_4^{2-}.

Results of studies of the effects of ATP and other nucleoside triphosphates and diphosphates on glucose dehydrogenase activity also are consistent with this single mode of interaction of such compounds with the multifunctional dehydrogenase. Nucleoside 5′-triphosphates and diphosphates were without effect on the affinity of the enzyme for NADP in this reaction (Figure 4A) as in the glucose-6-P dehydrogenase reaction (Figure 4B), and K_m values for NADP in the two reactions were identical (3×10^{-5} M). K_i values for ATP and other nucleoside triphosphates and diphosphates determined with the two types of activity were in good agreement in each instance (see Table II).

The effects of nucleoside triphosphates and diphosphates on glucose dehydrogenase activity of the enzyme differed from those previously noted with the smaller anions (see Anderson *et al.*, 1968) in that the former did not activate glucose dehydrogenase as did the latter, but rather, inhibited this reaction when tested in the absence of other anions (Figures 1, 2, 4A, and 5) and reversed competitively the activation of this activity by bicarbonate (Figure 6A) and orthophosphate (Figure 6B). The latter observations support a competition between inhibitor nucleotides and small anions for a common binding site as shown in diagrams II → V → VII and II → VIII in Figure 9, as do the data obtained with glucose-6-P dehydrogenase activity (see Table I and discussion above). Thus, the presence of either small anions (II → V → VI) or inhibitor nucleotides (II → VIII → IX) on the enzyme molecule preclude the binding of glucose-6-P while only the

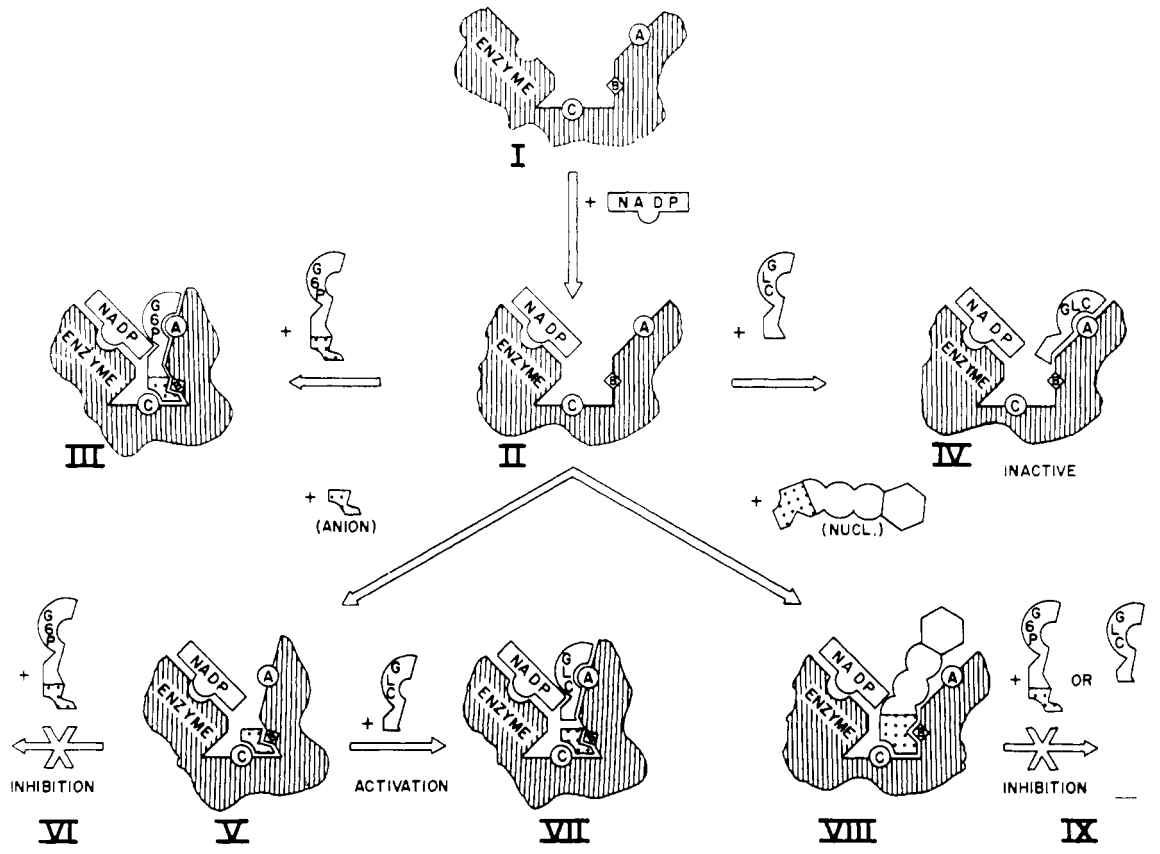

FIGURE 9: Postulated mechanism of action of nucleoside 5′-triphosphates or diphosphates and inorganic anions in selectively controlling yeast glucose dehydrogenase and glucose-6-P dehydrogenase activities. I–VII depict posulated differential effects of the smaller anions HCO_3^-, HPO_4^{2-}, and SO_4^{2-} on enzymic activities; precise details relating to this portion of the diagram are given in Anderson *et al.* (1968), to which the reader is directed.[5] A, B, and C represent binding sites for glucose-6-P (G6P). The shaded portion of the glucose-6-P molecule represents the compound's phosphoryl group. Nucleoside 5′-triphosphates and diphosphates (NUCL.) (see II → VIII) are hypothesized to compete with inorganic anions (II → V) and glucose-6-P (II → III) for enzyme (II). Neither glucose (GLC) nor glucose-6-P can bind to the nucleotide-inhibited enzyme (see II → VIII → IX), while the enzyme–inorganic anion complex (V) is active with glucose (V → VII) but not with glucose-6-P (V → VI). Further details are given in the Discussion.

former inhibit activity with unphosphorylated glucose as substrate (compare VIII → IX with V → VII). Thus, also, inhibition of glucose dehydrogenase activity by nucleoside triphosphates or diphosphates (II → VIII → IX) can be reversed through the binding of smaller activating anions to the active site in a manner competitive with these nucleotides (II → V → VII).

The competitive nature of the inhibition by nucleoside triphosphates and diphosphates observed with respect to glucose (Figures 5 and 8) may be explained either (a) on the basis of an interaction of nucleotides in some manner possibly involving either the ribose moiety, or oxygen atoms of the β- or γ-phosphoryl groups, of such inhibitory nucleotides with the enzymic binding site for hexose (A), or (b) as simply a reflection of the inability, due to steric factors, of the relatively bulky nucleoside compounds and hexose substrate to be present simultaneously on the enzyme molecule. These two alteratives do not appear to be kinetically interdistinguishable; postulate b has been incorporated arbitrarily into Figure 9, VIII.

Possible Physiological Significance. Levels of ATP, ADP, and other nucleoside triphosphates and diphosphates found effective as inhibitors are within normal physiological ranges of these compounds present in yeast cells (see Avigad, 1966; Polakis and Bartley, 1966; Schmitz, 1954). Inhibitions by nucleoside triphosphates and diphosphates, as well as by smaller anions, may constitute an effective mechanism for limiting glucose-6-P removal *via* direct oxidation, thus possibly providing for the maintenance of glucose-6-P for glycogen synthesis. Further, it would seem reasonable from the present studies that in the presence of high concentrations of glucose, reversal by certain small anions of these nucleotide-effected inhibitions of glucose dehydrogenase, and reinforcement by these same anions (HCO_3^-, HPO_4^{2-}, and SO_4^{2-}) of the inhibition by nucleotides of glucose-6-P dehydrogenase activity, could provide a mechanism for continued direct NADPH generation even under metabolic conditions in which glucose-6-P oxidation is rigorously controlled.

[5] Roman numerals I–VII were inadvertently omitted from Figure 10 in Anderson *et al.* (1968). Figure 9, above, is numbered to be compatible with the detailed mechanistic discussion of differential anion effects on the two dehydrogenase activities given in that reference.

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