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Regulation and Kinetics of Glucose-6-phosphate Dehydrogenase from *Candida utilis*†

Adeyinka Afolayan

ABSTRACT: Torula yeast (*Candida utilis*) glucose-6-phosphate dehydrogenase catalyzes only the NADP-dependent oxidation of glucose-6-P. When $NADP^+$ is the variable substrate, a hyperbolic rate–concentration response curve was obtained below 1 mM of the coenzyme. At higher $NADP^+$ concentration, a marked substrate inhibition and/or inactivation was observed. NADPH is an allosteric effector as well as a competitive inhibitor. The response curves at various ($NADP^+$):(NADPH) ratios at two different total pyridine nucleotide concentrations of 50 and 200 μM, respectively, were found to be sigmoidal. It is not unlikely that both the absolute concentration of $NADP^+$ and NADPH and the ratio of their levels might be important for regulation. While ATP severely inhibited the enzyme, neither AMP nor 6-phosphogluconate had any effect on the enzyme. Both spermidine and 3′,5′-cyclic AMP are activators. The extent of activation by cyclic AMP depends on the concentration of the cyclic nucleotide itself as well as on $NADP^+$ concentration. Thus, cyclic AMP relieves torula yeast glucose-6-P dehydrogenase of substrate inhibition observed at high $NADP^+$ concentration. The physiological implications of these results are discussed. The nature of reaction mechanism inferred from the kinetic data is also presented and discussed. A simple ordered sequential mechanism with $NADP^+$ binding first to the enzyme has been proposed.

Glucose-6-phosphate dehydrogenase (glucose 6-phosphate:NADP oxidoreductase, EC 1.1.1.49)[1] is an enzyme which has been highly purified and extensively studied from many sources including microorganisms and mammalian tissues (Glaser and Brown, 1955; Noltmann *et al.*, 1961; Marks *et al.*, 1961; Kirkman and Hendrickson, 1962; Chung and Langdon 1963; Yoshida, 1966; Luzzatto and Afolayan, 1968, 1971; Afolayan, 1969; Afolayan and Luzzatto, 1971; Sanwal, 1970; Olive *et al.*, 1971). In addition, one noteworthy development is a growing interest in the regulation and control of the activity of this enzyme by some metabolites, cations and nucleotides. However, the mechanism of regulation and control is not clearly understood.

Since glucose-6-P dehydrogenase is the initial enzyme of the pentose phosphate pathway, a branching sequence from the glycolytic pathway and which produces NADPH and pentose, the existence of sophisticated mechanism for the control and regulation of its activity is not surprising. Moreover, the reduced coenzyme is the major source of metabolic hydrogen in aerobic organisms and is necessary for the formation of glutamate, lipids, deoxyribonucleotides, cell walls, and other cellular components. Recently, Luzzatto (1967), Afolayan and Luzzatto (1971), and Luzzatto and Afolayan (1971) showed that both the absolute amount of NADP and NADPH concentrations as well as their ratio are crucial in

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[1] Abbreviations used are: $NADP^+$, oxidized nicotinamide–adenine dinucleotide phosphate; NADPH, reduced nicotinamide–adenine dinucleotide phosphate; NAD^+ and NADH, oxidized and reduced nicotinamide–adenine dinucleotide.

the regulation of activities of genetic variants of this enzyme from human red cells. Glucose-6-phosphate dehydrogenase from *Escherichia coli* has also been suggested to be regulated by NADH (Sanwal, 1970).

Most of the enzymes employed so far in the regulation studies were only partially purified. It is thus desirable to extend these studies to, and see if these regulatory and control models would hold for, crystalline glucose-6-phosphate dehydrogenase. In this respect, torula yeast (*Candida utilis*) glucose-6-P dehydrogenase which has been highly purified to a crystalline stage and is readily available has been chosen for study. This communication is therefore a report of kinetic investigations of torula yeast glucose-6-P dehydrogenase with a view to throwing more light on to the nature of regulation and control of its activity by its substrates and various metabolites. Furthermore, the nature of the reaction mechanism inferred from kinetic data is also presented.

Materials and Methods

Highly purified commercial torula yeast (*C. utilis*) glucose-6-phosphate dehydrogenase (lot no. 108B-700-1 of specific activity 200 units/mg obtained from Sigma Chemical Co., St. Louis, Mo.) was used; glucose 6-phosphate (sodium salt), 6-phosphogluconic acid (trisodium salt), oxidized and reduced nicotinamide–adenine dinucleotide phosphate, reduced nicotinamide–adenine dinucleotide, and adenosine triphosphate were also obtained from the same company. Spermidine and 3′,5′-cyclic AMP Grade A were obtained from Calbiochem, Switzerland. Tham (trishydroxymethylaminomethane) and boric acid, all ACS grades, were from Fisher Scientific Co. All other reagents used were of analytical grade. Details of individual experiments are given in the legends to the figures. Assays were carried out at room temperature using either Perkin-Elmer fluorescence spectrophotometer Model 203 (particularly for low concentrations of $NADP^+$) or Unicam SP-500 Series No. 2 spectrophotometer set at wavelength of 340 mμ to monitor the production of NADPH. The Perkin-Elmer fluorescence spectrophotometer used was set at 340 mμ (excitation) and NADPH production was assayed by its emission at 470 mμ. The instrument was set at zero with 0.1 N H_2SO_4 and 100% setting was with 0.2 ppm of quinine sulfate in 0.1 N H_2SO_4. Activities are expressed either as change in fluorescence intensity in arbitrary units per minute or change in absorbance per minute. In a preliminary experiment, a linear relationship was found between concentration of NADPH (dissolved in 0.05 M Tris–borate, pH 7.5) and fluorescence intensity. All assays were carried out in 0.05 M Tris–borate buffer (pH 7.5), unless otherwise stated, and at room temperature of 25°. The enzyme stock was dissolved in this buffer containing 10^{-7} M NADP and 10^{-4} M EDTA and dialyzed against 1 l. of the same buffer for 3 hr and changed two times. The enzyme solution was then cleared by spinning at 3000 rpm on an International equipment centrifuge (IEC) at 4° for 30 min. The supernatant was carefully pipetted out while the debris was discarded. A relatively slow oxidation of glucose by brewer's yeast glucose-6-phosphate dehydrogenase at pH above 8 had been reported in recent time by the laboratory of Anderson (Anderson and Nordlie, 1968; Anderson *et al.*, 1968; Horne *et al.*, 1970). However, we did not detect any glucose dehydrogenase activity in torula yeast (*C. utilis*) glucose-6-phosphate dehydrogenase under the conditions of our experiments. The results of kinetic studies are presented as conventional double reciprocal plots. Michaelis constants were determined as negative intercepts on the x axis by extrapolation of such plots (see Dixon and Webb, 1964).

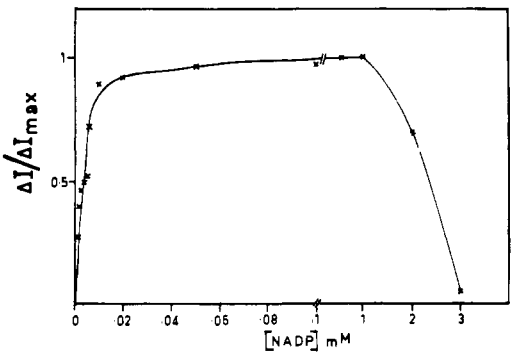

FIGURE 1: Relative reaction velocity of yeast glucose-6-phosphate dehydrogenese (100 μg/ml final concentration) as a function of $NADP^+$ concentration. The rate, ΔI, expressed as change in fluorescence intensity (arbitrary units) per minute using a Perkin-Elmer fluorescence spectrophotometer Model 203 (see Methods). ΔI_{max} is the rate at saturating concentration of $NADP^+$. Assays were carried out in 0.05 M Tris–borate buffer (pH 7.5) and glucose 6-phosphate was saturating at 100-μM final concentration. Total volume of assay mixture was 3 ml in a cuvet of 1-cm light path.

Results

Effect of $NADP^+$. When the concentration of $NADP^+$ was varied between 1 μM and 3 mM at constant concentration of glucose-6-P (0.1 mM), the rate–substrate concentration response curve obtained was of typical Michaelis–Menten response type (Figure 1). However, at higher $NADP^+$ concentration there was drastic inhibition of the enzyme. The absence of sigmoidal response at low $NADP^+$ concentration contrary to what had been obtained for other enzymes such as some genetic variants of red cell glucose-6-phosphate dehydrogenase (Luzzatto, 1967; Afolayan and Luzzatto, 1971) is significant. We are not aware of any report about inhibition or inactivation of any glucose-6-phosphate dehydrogenase by excess $NADP^+$ concentration. The inhibition observed at high $NADP^+$ concentration was found to be independent of pH values tested (Table I). There are many reports in the literature about the dual response of glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* (Demoss *et al.*, 1953) and *Pseudomonas aeruginosa* (Lessie and Neidhart, 1967) to $NADP^+$ and NAD^+. However we were unable to

TABLE I: Effect of High Concentration of $NADP^+$ on Torula Yeast Glucose-6-phosphate Dehydrogenase.[a]

$NADP^+$ (mM)	ΔOD_{340}/min at pH			
	7	7.5	8.0	8.5
0.5	0.02	0.095	0.112	0.113
3	0.003	0.016	0.03	0.031

[a] Assays were carried out in 0.05 M Tris–borate buffer of different pH values as indicated. Final concentrations of $NADP^+$ were as indicated while glucose 6-phosphate concentration was fixed at 100 μM (final). The total volume of the reaction mixture was 3 ml.

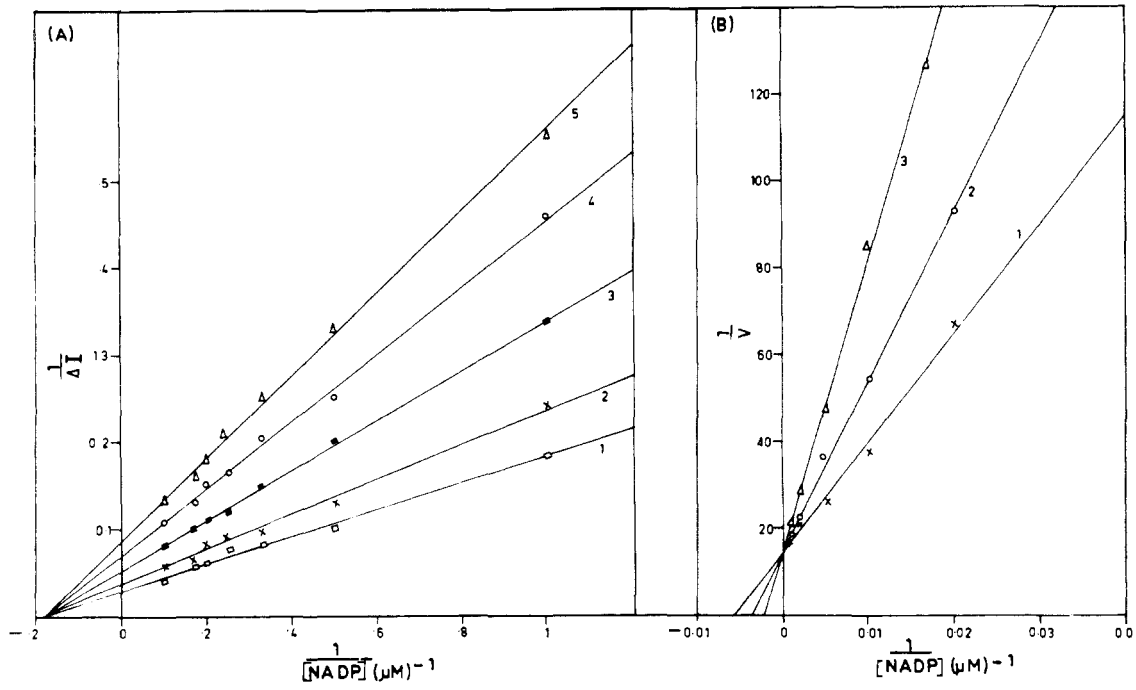

FIGURE 2: (A) Double-reciprocal plots of the rate (ΔI/min, see legend to Figure 1) *vs.* variable concentration of $NADP^+$ at fixed concentration of glucose 6-phosphate as follows: (1) 100, (2) 80, (3) 60, (4) 40, and (5) 20 μM. Assays were carried out in a total volume of 3 ml in 0.05 M Tris–borate buffer (pH 7.5), and enzyme concentration was 100 μg/ml. The points at which inhibition occurs at high $NADP^+$ concentration were not plotted. (B) Lineweaver–Burk plots of effects of NADPH. Assays were carried out in 0.05 M Tris–borate buffer (pH 7.5) with $NADP^+$ concentration varied as indicated on the abscissa and 100 μM glucose 6-phosphate using using Unicam SP-500 series no. 2 spectrophotometer. Velocity was expressed in ΔOD_{340}/min. NADPH concentration was as follows: (1) 0, (2) 25, (3) 50 μM. The total reaction mixture was 3 ml in a cuvet of 1-cm light path.

detect any activity of the enzyme at any concentration of NAD^+ when this nucleotide was substituted for $NADP^+$ (A. Afolayan, unpublished data). This corroborates the work of Domagk *et al.* (1969).

When the concentration of $NADP^+$ was varied at different constant concentration of glucose 6-phosphate, the plots of the double reciprocals ($1/v$ *vs.* $1/S$) produced lines that intersect on the abscissa (Figure 2A). This indicates that the apparent affinity of the enzyme for the coenzyme is independent of glucose 6-phosphate. The data are also consistent with compulsory-order mechanism for the $NADP^+$-linked reaction (Cleland, 1963) and also corroborate the data obtained for *L. mesenteroides* glucose-6-phosphate dehydrogenase (Olive *et al.*, 1971) (see Discussion). The replots of the slopes and the intercepts were linear.

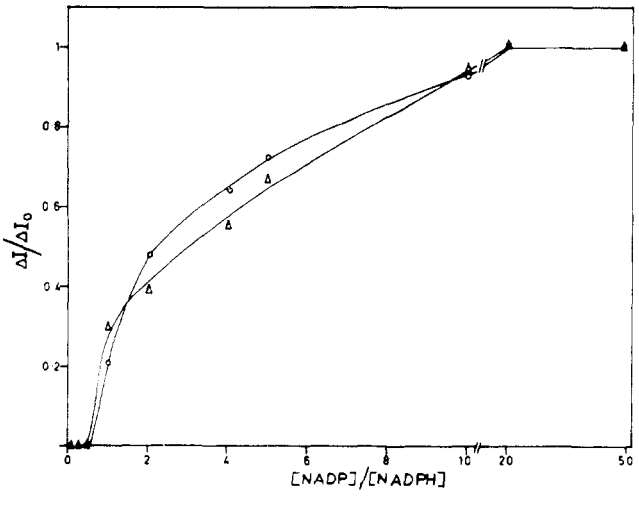

FIGURE 3: Effect of varying ratio of $[NADP^+]$:[NADPH] on the yeast glucose-6-phosphate dehydrogenase. The total concentration of $NADP^+$ and NADPH was maintained at final value of (○) 50 μM and (△) 200 μM. Assays were carried out in 0.05 M Tris–borate buffer (pH 7.5) and contained 100 μM glucose 6-phosphate all in a total volume of 3 ml. The rate is expressed as change in fluorescence intensity (arbitrary units) per minute (see legend to Figure 1). ΔI_0 is the rate corresponding to $[NADP^+]$:[NADPH] of 50.

The K_m for $NADP^+$ is 5.8 μM while that for glucose 6-phosphate is 76 μM. These values are in agreement with the data for *L. mesenteroides* glucose-6-phosphate dehydrogenase (Olive *et al.*, 1971).

Product Inhibition. Product inhibition studies were carried out with NADPH. When $NADP^+$ was the varied substrate, linear competitive inhibition was obtained (Figure 2B). When glucose 6-phosphate was the varied substrate, the inhibition was linear noncompetitive (data not shown). We found that 6-phosphogluconate had no effect on the activity of the enzyme. The dissociation constant for NADPH was calculated from Figure 2B data according to Webb (1963) and is 32 μM which is in agreement with the value (37.6 μM) for *L. mesenteroides* glucose-6-phosphate dehydrogenase (Olive *et al.*, 1971).

Effect of $NADP^+$:NADPH. When the effect of $NADP^+$:NADPH ratio (the sum total concentration of the nucleotides being held constant at 50 and 200 μM, respectively) on the activity of the enzyme was conducted, sigmoidal response curves were obtained for the two different total pyridine nucleotide concentrations (Figure 3). Similarly a sigmoidal response curve was obtained when the total nucleotide concentration was held constant at 25 μM (data not shown). This type of response curve is not surprising in view of the

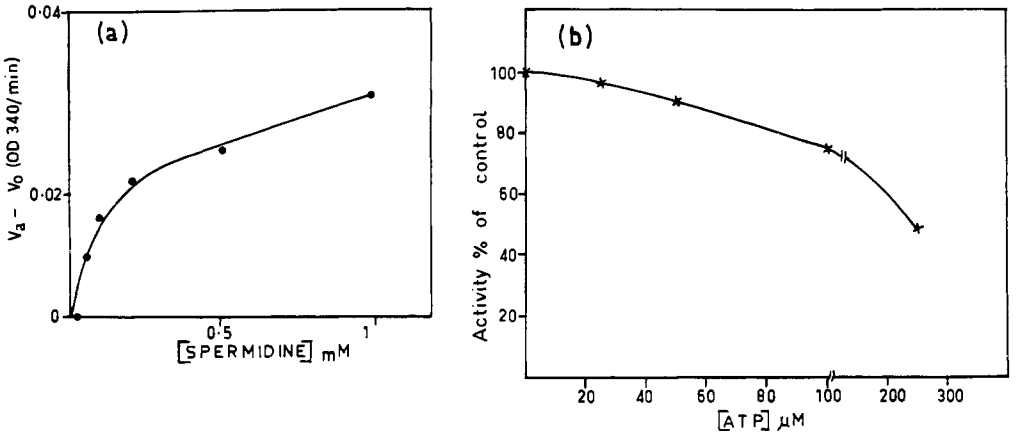

FIGURE 4: (a) Effect of spermidine on yeast glucose-6-phosphate dehydrogenase. Assays were carried out in 0.05 M Tris–borate buffer (pH 7.5). The reaction mixture contained, in final concentration, NADP, 500 μM; glucose 6-phosphate, 100 μM; and variable spermidine as indicated on the abscissa. V_a = velocity in the presence of spermidine. V_0 = velocity in the absence of spermidine. V_0 is 0.028 OD_{340}/min. (b) Effect of ATP. Assays were carried out in 0.05 M Tris–borate buffer (pH 7.5) and reaction mixtures contained in final concentration 500 μM $NADP^+$; 100 μM glucose 6-phosphate and variable ATP as indicated. Velocity was expressed in ΔOD_{340}/min. The per cent activity of the control was plotted.

competitive inhibition data (see Figure 2b) obtained for NADPH. At low NADP:NADPH ratio, the enzyme is severely inhibited. The enzyme however displays low sensitivity to NADPH concentration at high $NADP^+$ concentration.

Effect of NADH. Sanwal (1970) suggested that NADH might play the role of an allosteric effector for *E. coli* glucose-6-phosphate dehydrogenase. For the torula yeast enzyme, NADH turned out to be an activator (Table II). The apparent affinity of the enzyme for the $NADP^+$ was increasing as a function of increase in NADH concentration. This is different from its role as a noncompetitive inhibitor obtained for the *E. coli* glucose-6-phosphate dehydrogenase (Sanwal, 1970). Product inhibition by NADH may be unnecessary in view of the fact that torula yeast glucose-6-phosphate dehydrogenase does not reduce NAD (A. Afolayan, unpublished data). However, we do not know the physiological significance of the NADH activation observed in our experiments.

Effects of Spermidine, ATP, AMP, and 3′,5′-Cyclic AMP. It is well known that glucose-6-phosphate dehydrogenase from many sources including yeast (Kornberg, 1950) is activated by magnesium ions even though this cation is not essential for activity. In the activation of many enzymes however, metal ions can be replaced by spermidine and other polycations. As indicated in Figure 4a, torula yeast is activated by spermidine. The apparent K_m for spermidine calculated by Dixon and Webb (1964) plot was 25.6 μM. This value is much lower than 0.2 mM obtained for *E. coli* glucose-6-P dehydrogenase by Sanwal (1970).

The inhibition of torula yeast glucose-6-P dehydrogenase by ATP (Figure 4b) is similar to inhibition by the same compound observed with brewer's yeast (Anderson *et al.*, 1968) and red cell enzymes (Marks *et al.*, 1961). The K_i for ATP is 1.7×10^{-4} M, unlike the value of 4×10^{-4} M for brewer's yeast glucose-6-P dehydrogenase (Horne *et al.*, 1970). We found, however, that AMP has neither inhibitory nor activating effect on torula yeast glucose-6-P dehydrogenase, compared with inhibitory effect on brewer's yeast enzyme reported by Anderson's group.

TABLE II: Effect of NADH on the Affinity of Torula Yeast Glucose-6-phosphate Dehydrogenase for $NADP^+$.[a]

NADH (μM)	App K_m for $NADP^+$ (M)
None	2×10^{-4}
25	1.08×10^{-4}
50	0.77×10^{-4}

[a] Assays were carried out in 0.05 M Tris–borate buffer (pH 7.5) and monitored with Unicam SP-500 spectrophotometer (see Methods). Glucose-6-P concentration was fixed at 100 μM. NADP concentration was varied between 50 and 500 μM. The total volume of the reaction mixture was 3 ml.

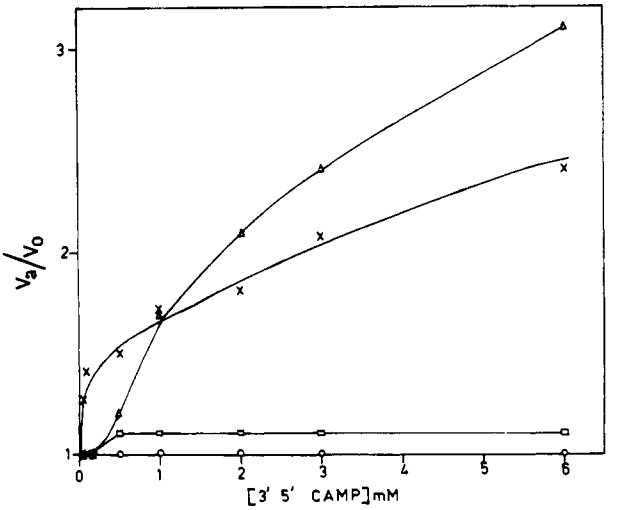

FIGURE 5: Effect of 3′,5′-cyclic AMP on yeast glucose-6-phosphate dehydrogenase. Assays were carried out in 0.05 M Tris–borate buffer (pH 7.5), and the total reaction mixture volume was 3 ml in a cuvet of 1-cm light path. Glucose-6-phosphate final concentration was 100 μM. Velocity was expressed as ΔOD_{340}/min. V_0 = velocity in the absence of 3′,5′-cyclic AMP and V_a = velocity at various concentrations of 3′,5′-cyclic AMP. The final concentration of $NADP^+$ is as follows: (○) 20 μM, (□) 50 μM, (△) 500 μM, and (×) 1 mM.

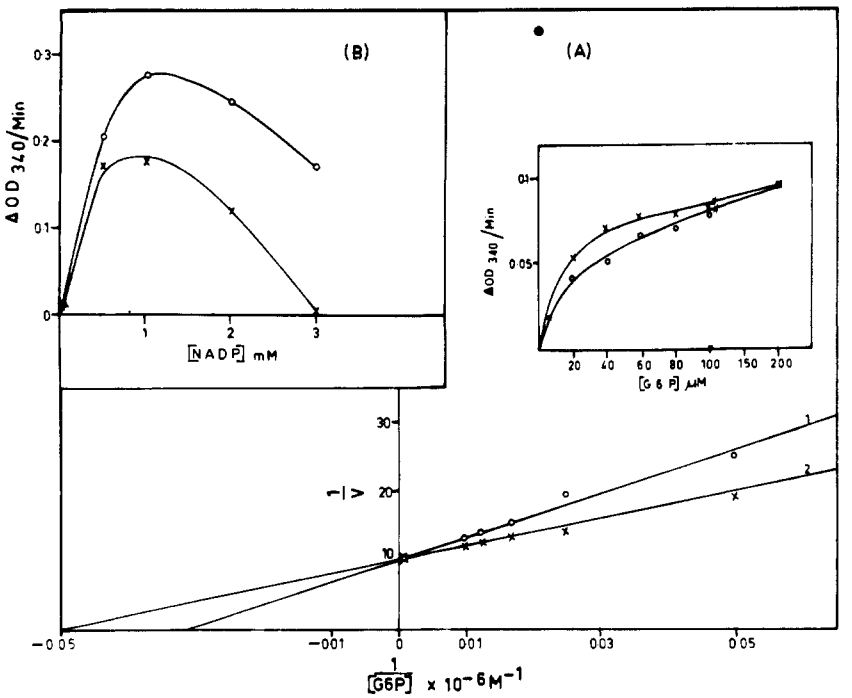

FIGURE 6: Effect of 3′,5′-cyclic AMP. (A) Double-reciprocal plots of velocity (ΔOD_{340}/min) *vs.* variable concentration of glucose 6-phosphate without (1) or with (2) 500 μM cyclic AMP. NADP final concentration was 500 μM. Assays were carried out in 0.05 M Tris–borate buffer (pH 7.5). The inset is the Michaelis–Menten plot with (×) and without (○) 500 μM 3′,5′-cyclic AMP. (B) At high $NADP^+$ concentration. Assays were carried out in 0.05 M Tris–borate buffer (pH 7.5). The reaction mixture contained in final concentration of $NADP^+$ as indicated; glucose 6-phosphate, 100 μM: with (○) and without (×) 3 mM 3′,5′-cyclic AMP.

The activating effect of 3′,5′-cyclic AMP is of interest. This activating effect seems to depend on both cyclic AMP concentration and $NADP^+$ concentration. In Figure 5, one notes the very low sensitivity of the enzyme at 20 and 50 μM $NADP^+$, while at higher $NADP^+$ concentrations, 500 μM and 1 mM, the enzyme becomes more sensitive to the activating effect of 3′,5′-cyclic AMP. We also found that cyclic AMP slightly relieves glucose-6-P dehydrogenase of NADPH inhibition (A. Afolayan, unpublished data). In Figure 6A, we observed that cyclic AMP activates the enzyme by increasing the apparent affinity of the enzyme for glucose-6-P. However, the activating effect is less pronounced at high glucose-6-P concentration. We also noted in Figure 6B that the inhibition of the enzyme at high concentration of NADP is partially relieved by 3 mM cyclic AMP.

Discussion

Kinetic Mechanism. The kinetic mechanism for red cell glucose-6-phosphate dehydrogenase has been described as a sequential ordered mechanism by Soldin and Balinsky (1968) although from their experiments, they were unable to rule out either a rapid-equilibrium random mechanism in which NADPH acts as a dead-end inhibitor by combining with the enzyme–glucose 6-phosphate complex or a Theorell–Chance mechanism. The kinetic data of Sanwal (1970) on glucose-6-phosphate dehydrogenase from *E. coli* and those of Olive *et al.* (1971) on the same enzyme from *L. mesenteroides* are both compatible with an ordered sequential mechanism with $NADP^+$ combining with the free enzyme first and NADPH being the last product to be released. However, these studies do not rule out other mechanisms.

Our data (Figure 2A) on the initial velocity studies on torula yeast (*C. utilis*) glucose-6-phosphate dehydrogenase are consistent with a sequential mechanism. Further support for this comes from the linear competitive product inhibition studies with NADPH (Figure 2B) which are consistent with the oxidized coenzyme being bound first and the reduced coenzyme released last. Our experiments have not ruled out rapid equilibrium random mechanism. The data published by Olive *et al.* (1971) on *L. mesenteroides* glucose-6-phosphate dehydrogenase definitely ruled out a rapid equilibrium random mechanism and we think that this may likely hold for torula yeast enzyme too. However, our data on the other hand do not exclude a Theorell–Chance mechanism since no information is obtained concerning the existence of significant steady-state levels of the ternary complex.

Effect of $NADP^+$. Generally, regulatory enzymes display sigmoidal rate–substrate concentration response curves which have been interpreted as indication of more than one molecule of the ligand being bound in a cooperative manner (Monod *et al.*, 1965; Atkinson, 1966; Stadtman, 1966). These types of regulatory enzymes are referred to as modular independent (Sanwal, 1970). The data of Luzzatto (1967), Afolayan and Luzzatto (1971), and Luzzatto and Afolayan (1971) on saturation kinetics of genetic variants of red cell glucose-6-phosphate dehydrogenase are consistent with this scheme. However, not all regulatory enzymes display sigmoidal rate–concentration response curves. Some enzymes do so only in the presence of certain modulators or effectors and are therefore described as being modulator dependent. The glucose-6-phosphate dehydrogenase from *E. coli* (Sanwal, 1970) which displays sigmoidal curves only in the presence of high concentration of NADH is an example.

The lack of sigmoidal rate–concentration response curve for torula yeast glucose-6-phosphate dehydrogenase may not indicate absolute lack of interaction between enzyme subunits which is characteristic of other regulatory enzymes.

There are two possibilities—either this enzyme truly belongs to the class of modulator-dependent regulatory enzymes as postulated by Sanwal (1970) and exemplified by *E. coli* glucose-6-P dehydrogenase (Sanwal, 1970) and threonine deaminase (Maeba and Sanwal, 1966) or it displays positive cooperativity which occurs at much lower concentration of $NADP^+$ than presently employed. In a case like the latter possibility, an induced transition—an extension of induced-fit theory of Koshland (Koshland *et al.*, 1966)—takes place from a state of low affinity for $NADP^+$ to a state of high affinity for the coenzyme. This model has been put forward for the A^- variant of glucose-6-P dehydrogenase from red cell which singularly displayed hyperbolic rate–concentration curve (Afolayan and Luzzatto, 1971), compared to the sigmoid curves for other genetic variants of red cell glucose-6-P dehydrogenase. Whichever mechanistic model that accounts for this kinetic behavior, it is clear that the level of $NADP^+$ in the cell is highly critical and deserves to be controlled. This scheme therefore renders $NADP^+$ a target substrate. The level of $NADP^+$ in *Saccharomyces cerevisiae* (baker's yeast) has been estimated to be 0.1–0.3 and 0.13–0.16 mM when the organism is grown on glucose and galactose supplemented media, respectively (Polakis and Bartley, 1966). Thus, intracellular level of the coenzyme may probably not rise up to the range of 1–3 mM where substrate inhibition occurs.

As for the decrease in the rate at very high $NADP^+$ concentration (see Figure 1), our experiments cannot distinguish between three mechanisms (a) substrate inhibition (see Webb, 1963), (b) inactivation by $NADP^+$, or (c) probably combination of these phenomena. However, one explanation that can be offered is that once the active site or sites are saturated by $NADP^+$, at higher concentration, the coenzyme binds to other sites thereby causing conformational change that renders the enzyme partially or completely inactive. The partial relief of the decrease in rate caused by 3 mM 3′,5′-cyclic AMP (see Figure 6B) seems to support this contention of $NADP^+$ binding at other sites besides the active site.

Effect of NADPH and NADH. The response obtained when the effect of varying $NADP^+$:NADPH ratio was investigated (see Figure 3) might indicate that the ratio of the nucleotides as well as their absolute concentrations is very important for regulation. Polakis and Bartley (1966) estimated the level of NADPH in baker's yeast growing at constant rate to be between 50 and 180 μM depending on the source of carbon energy. It is probable that NADPH serves as an allosteric inhibitor at low concentration while at higher concentration it is a feedback inhibitor of the enzyme by competing with $NADP^+$. At the estimated internal ratio of $NADP^+$:NADPH of about 2, considering glucose grown baker's yeast (Polakis and Bartley, 1966) and assuming the same condition operates in torula yeast, the glucose-6-phosphate dehydrogenase will be working between 42 and 48% of its normal activity (see Figure 4). A much higher ratio of $NADP^+$:NADPH will thus be more beneficial for the enzyme. The level of NADH in baker's yeast is estimated to be between 0.4 and 0.7 mM depending on the source of carbon when this microorganism is growing steadily. This is far above the level of either $NADP^+$ or NADPH. Therefore if NADH should be inhibitory, the pentose phosphate pathway will hardly operate. In this respect, the activating effect observed with NADH for torula yeast glucose-6-P dehydrogenase by lowering the apparent K_m for $NADP^+$ as a function of increase in its concentration (see Table II) may not be out of place. However, we do not know the metabolic significance of the activation observed with this reduced coenzyme.

Effect of Spermidine. Glucose-6-phosphate dehydrogenase from many sources including yeast (Kornberg, 1950) is activated by Mg^{2+} ions. However, for many enzymes, the metal ions can be replaced by polyamines such as spermidine. The activating effect of spermidine observed for torula yeast (*C. utilis*) is consistent with this general characteristic. Sanwal (1970) observed the same activating effect of spermidine for *E. coli* glucose-6-P dehydrogenase. However, the affinity of torula yeast glucose-6-P dehydrogenase for spermidine seems to be higher than that of *E. coli* glucose-6-P dehydrogenase (see Results). The replacement of metal cations such as Mg^{2+} ions by polyamines is analogous to the preservation of bacterial ribosomes by either magnesium ions or polyamines. The activation by spermidine appears to be specific for glucose-6-phosphate dehydrogenase as 6-phosphogluconic dehydrogenase is not activated by spermidine (Scott and Cohen, 1953).

Effect of 3′,5′-Cyclic AMP. A considerable amount of information is now available about the regulatory role of 3′,5′-cyclic AMP in microorganisms and higher organisms. For microorganisms, this nucleotide has been implicated as a derepressor supressing catabolite repression (Ullman and Monod, 1968; Pastan and Perlman, 1968; Perlman and Pastan, 1968). In higher organisms, the consensus of opinion is that this nucleotide is involved in the hormonal regulation of metabolic processes (Robison *et al.*, 1968). Even though hormones are absent in the microorganisms, cyclic AMP has been detected in *E. coli* (Markman and Sutherland, 1965) and *Brevibacterium liquefaciens* (Okabayashi *et al.*, 1963). However, there has not been any available information about the quantitative level of 3′,5′-cyclic AMP in yeast cells although Betz and Moore (1967) put the total level of AMP in yeast cell to be between 17 and 250 μM while ATP level is between 1 and 2 mM. The level of the cyclic nucleotide in *E. coli* is related to the nutritional status of the microorganism. Markman and Sutherland (1965) showed that in glucose starvation, the concentration of the nucleotide rose as high as 10^{-4} M.

It was Sanwal and Smando (1969) that demonstrated for the first time that 3′,5′-cyclic AMP directly affects the activity of *E. coli* malic enzyme as an allosteric inhibitor. Similarly Yang and Deal (1969) found that 3′,5′-cyclic AMP is a potent inhibitor of yeast (Red Star baker's) glyceraldehyde-3-phosphate dehydrogenase. Yang and Deal found that 0.5 and 5 mM of the cyclic nucleotide caused 50 and 90% inhibition of the enzyme, respectively.

Mansour (1963) however found that the inhibition of guinea pig heart phosphofructokinase by ATP, by about 90%, was considerably decreased to 10% in the presence of 10^{-4} M 3′,5′-cyclic AMP. The activating effect of this cyclic nucleotide was observed only when there was inhibition of the enzyme caused by high level of ATP. In our own experiments, we found that 3′,5′-cyclic AMP was an allosteric activator of torula yeast glucose-6-phosphate dehydrogenase. The activating effect of the cyclic nucleotide depends on the inhibition and/or inactivation by high concentration of $NADP^+$. It is significant that the lowest concentration of cyclic AMP at which we had activation was 100 μM when $NADP^+$ concentration was at 1 mM (see Figure 5). The physiological implication of these activating effects obtained at various concentrations of cyclic AMP can have metabolic significance if $NADP^+$ level actually rises in the yeast cell to the point when inhibition and/or inactivation can occur. Furthermore, the activation of the enzyme by cyclic AMP by increasing the apparent affinity of the enzyme for glucose 6-phosphate

(see Figure 6A) is also interesting in view of the fact that glucose 6-phosphate has been implicated as a catabolite repressor in yeast (Gancedo *et al.*, 1967). The level of glucose 6-phosphate in yeast cell has been put between 0.6 and 2.7 mM (Betz and Moore, 1967).

In conclusion, our data about the activating effects of 3′,5′-cyclic AMP on torula yeast glucose-6-phosphate dehydrogenase are quite clear and sufficient to suggest a possible metabolic role for cyclic AMP in yeast cells. However, any metabolic significance to be attached to these data is only tentative. The full evaluation of the significance of these effects may be strengthened by a knowledge of the actual *in vivo* level of the cyclic nucleotide in yeast cells.

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