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Glucose Dehydrogenase Activity of Yeast Glucose 6-Phosphate Dehydrogenase. I. Selective Stimulation by Bicarbonate, Phosphate, and Sulfate*

Wayne B. Anderson and Robert C. Nordlie

ABSTRACT: Glucose dehydrogenase (β-D-glucose:NADP oxidoreductase) activity associated with commercial highly purified yeast glucose 6-phosphate dehydrogenase has been studied. The anions bicarbonate, phosphate, and sulfate were found to exert a marked, selective stimulatory effect on glucose dehydrogenase, although they inhibited activity with glucose 6-phosphate as substrate; bicarbonate > sulfate > phosphate in effectiveness as activators of glucose dehydrogenase. Stimulatory concentrations of anions also lowered the pH optimum of glucose dehydrogenase. For example, maximal activity was observed at pH 9.4 without bicarbonate, at pH 8.8 in the presence of 10 mM bicarbonate, and at pH 8.2 when 50 mM bicarbonate was present. In the absence of bicarbonate, glucose dehydrogenase activity at pH 8.4 was <1% of that observed with glucose 6-phosphate as substrate; however, as assayed in the presence of 50 mM sodium bicarbonate, the former activity was increased to 20% of that observed with the sugar phosphate ester as substrate. These observations suggest the possibility of a significant metabolic role for glucose dehydrogenase activity, and point up the need for caution when glucose 6-phosphate dehydrogenase is used as an analytical tool for the measurement of glucose 6-phosphate production in assay mixtures containing high concentrations of glucose and certain anions.

During the past 6 years, the catalytic properties of two multifunctional enzymes, microsomal glucose 6-phosphatase (see, for example, Nordlie and Arion, 1964; Nordlie and Soodsma, 1966) and bacterial alkaline phosphatase (Anderson and Nordlie, 1967), have been extensively studied in this laboratory. Commercial yeast glucose 6-phosphate dehydrogenase (D-glucose 6-phosphate:NADP[1] oxidoreductase, EC 1. 1. 1. 49) has been used routinely as an analytical tool for the assay of glucose 6-phosphate in these investigations. This dehydrogenase previously had been shown by Colowick and Goldberg (1963) to exhibit glucose dehydrogenase activity. Similar observations also have been made by Kuby and Noltmann (1966) and by Salas *et al.* (1965). During the course of our studies of inorganic pyrophosphate–glucose phosphotransferase activity of alkaline phosphatase, we also noted that a glucose-dependent reduction of NADP was catalyzed by yeast glucose 6-phosphate dehydrogenase preparations when the concentration of glucose was very high (Anderson and Nordlie, 1967). A variety of factors were observed to affect this activity which was detected with phosphotransferase control assay mixtures upon the addition of the dehydrogenase preparation. Because of our previous interest in carbohydrate metabolism and in multifunctional enzyme systems, we initiated detailed studies of factors affecting both glucose dehydrogenase (β-D-glucose:NADP oxidoreductase) and glucose 6-phosphate dehydrogenase activities of yeast glucose 6-phosphate dehydrogenase. This paper describes the selective stimulation of glucose dehydrogenase activity associated with the enzyme by the anions bicarbonate, sulfate, and phosphate.

Materials and Methods

Purified yeast glucose 6-phosphate dehydrogenase (140 units/mg of protein) was obtained from Boehringer Mannheim Corp., New York. The sodium salts of glucose-6-P and NADP, and also α- and β-D-glucoses, and crystalline bovine serum albumin were obtained from

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

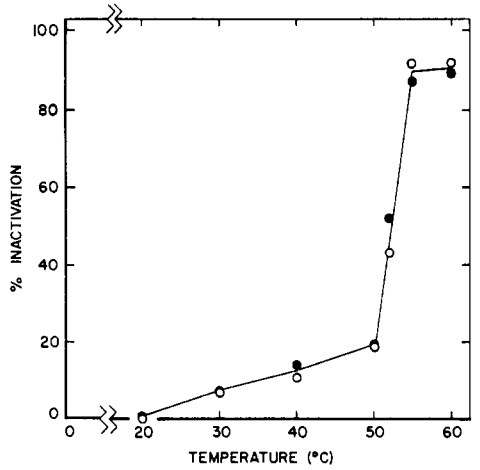

FIGURE 1: Thermal inactivation of glucose-6-P dehydrogenase (○) and glucose dehydrogenase (●) activities. Enzyme preparations were preincubated for 5 min at the indicated temperatures and then quickly cooled and assayed for activity. Assay mixtures (pH 8.0) contained, in 3.0 ml, 50 mM Tris-Cl buffer, 0.18 mM sodium NADP, and either 0.25 mM sodium glucose-6-P plus 0.063 μg of enzyme protein (glucose-6-P dehydrogenase) or 0.75 M equilibrated glucose plus 2.5 μg of enzyme protein (glucose dehydrogenase). Per cent inactivation = 100 × ((activity with unheated preparations − activity with heated preparations)/activity with unheated preparations).

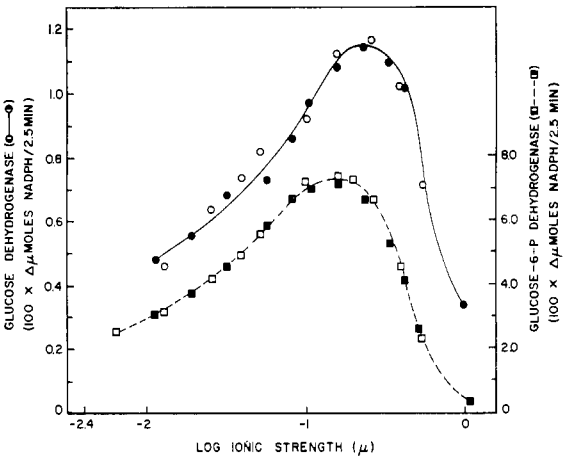

FIGURE 2: Influence of ionic strength on glucose dehydrogenase (circles) and glucose-6-P dehydrogenase (squares) activities. Basic assay mixtures (pH 8.0) contained, in 3.0 ml, 8.3 mM Tris-Cl buffer (μ 6.4 × 10^{-3}), 0.18 mM sodium NADP, and 0.25 mM sodium glucose-6-P plus 0.125 μg of purified enzyme protein (glucose-6-P dehydrogenase), or 0.5 M equilibrated glucose plus 5 μg of enzyme protein (glucose dehydrogenase). Varied amounts of NaCl (closed symbols) or Tris-Cl (pH 8.0, open symbols) were added to alter the ionic strength as indicated.

Sigma Chemical Co., St. Louis, Mo. Na_2HPO_4 and $NaHCO_3$ were products of Mallinckrodt Chemical Works, New York, and Na_2SO_4 was purchased from J. T. Baker Chemical Co., Phillipsburg, N. J. NaCl was obtained from Fisher Scientific Co., Fair Lawn, N. J. The latter four compounds were of analytical reagent grade.

The commercial ammonium sulfate suspension of glucose 6-phosphate dehydrogenase was diluted with deionized water to contain 50 μg of protein/ml. Bovine serum albumin (10 mg) was added/ml of this preparation to stabilize the diluted enzyme. This diluted preparation was subsequently dialyzed at 4° for at least 6 hr against four changes of deionized water to remove the high concentration of ammonium sulfate initially present.

Glucose dehydrogenase activity of this enzyme previously has been shown to be specific for β-D-glucose (Colowick and Goldberg, 1963; Salas *et al.*, 1965). However, in all our studies, except those specifically noted, an equilibrated D-glucose solution (specific rotation +52°) was used, due to the rapidity with which β-D-glucose mutarotates, especially at the more alkaline pH levels studied.

Dehydrogenase activities were measured spectrophotometrically by following the initial rate of change in absorbance at 340 mμ with a Beckman Model DU spectrophotometer adapted for scale expansion (Gilford attachment) and continuous recording (Brown recorder).[2] The temperature was maintained at 30 ± 0.1° by circulating water from a constant-temperature bath through thermospacers incorporated into the cuvet compartment of the spectrophotometer. Ionic strength of assay mixtures was routinely maintained constant in each experimental series by the addition of NaCl, as described in detail below. The sodium salts of all anionic effectors were utilized in order to negate any possible selective effects on activities due to cations. Reactions were initiated by the addition of enzyme to otherwise complete, thermally equilibrated assay mixtures, and activity routinely was measured for a period of 2.5 min. The pH of each reaction mixture was measured with a Beckman expanded-scale meter immediately following activity assays. Control mixtures, in which anionic effector salts were replaced by sodium chloride, were routinely assayed. Additional details of individual experiments are given in the Results section and in legends to individual figures and tables.

The CO_2 concentration of certain reaction mixtures in which the effects of bicarbonate were studied was determined by the manometric methods of Van Slyke and Cullen (see Hepler, 1949), or, at pH values <7.7, with a pCO_2 electrode. Values obtained by these means agreed well with concentration values calculated on the basis of the amounts of bicarbonate added.

Results

Highly purified commercial preparations of yeast glucose-6-P dehydrogenase were employed in all studies reported here. Previous investigations (Colowick and Goldberg, 1963; Kuby and Noltmann, 1966) have indicated that this enzyme exhibits a small amount of dehydrogenase activity when glucose is employed as the oxidizable substrate. A four-times-recrystallized preparation was employed in some of these studies (see

[2] A reference molar absorbance index of 6.22 × 10^3 M^{-1} cm^{-1} (Horecker and Kornberg, 1948; Pabst Laboratories Circular OR-17 (1961)) was employed in conversion of A_{340} readings into molar concentrations.

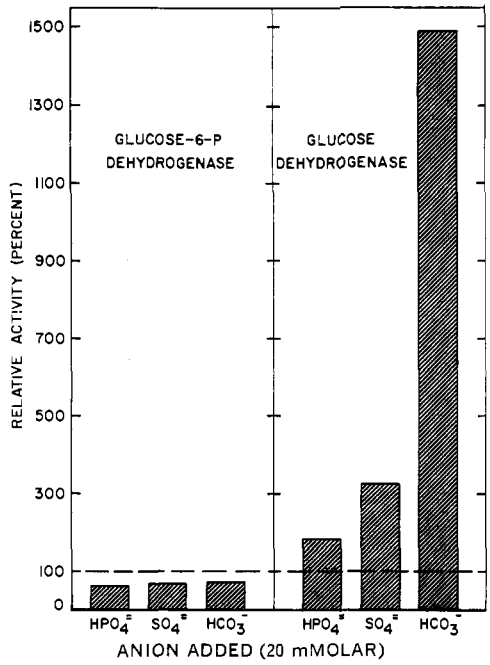

FIGURE 3: Selective effects of various anions on glucose-6-P dehydrogenase and glucose dehydrogenase activities. Assay mixtures (pH 8.2) contained, in 3.0 ml, 8.3 mM Tris-Cl buffer, 0.1 mM sodium NADP, 20 mM indicated anionic effector, and 0.1 mM sodium glucose-6-P plus 0.2 μg of enzyme protein (glucose-6-P dehydrogenase) or 0.5 M equilibrated glucose plus 5.0 μg of enzyme protein (glucose dehydrogenase). Relative activity = 100 × (activity observed in the presence of indicated anion/activity observed with control assay mixtures). The ionic strengths of control assay mixtures were adjusted to those containing the bicarbonate, sulfate, and phosphate salts by the addition of sodium chloride.

Kuby and Noltmann, 1966). Also consistent with the common identity of glucose dehydrogenase and glucose-6-P dehydrogenase activities are our findings, presented in Figure 1, that both activities were inactivated in an exactly parallel manner by heating enzyme preparations at 20, 30, 40, 52, 55, and 60° for 5 min in the absence of substrates.

Influence of Ionic Strength on Dehydrogenase Activities. Previous investigators have demonstrated a marked effect of variation of ionic strength on glucose-6-P dehydrogenase activity of yeast (Rutter, 1957; Glaser and Brown, 1955) and erythrocytes (Pinto *et al.*, 1966). The results of experiments in which the effect of ionic strength on both glucose and glucose-6-P dehydrogenase activities were studied are presented in Figure 2. Identical results were obtained whether NaCl or Tris-Cl was employed to alter ionic strength. Both glucose dehydrogenase and glucose-6-P dehydrogenase activities were stimulated in a similar manner as ionic strength was increased, although maximal activation of the former occurred at μ 0.230 while the latter activity was stimulated maximally at μ 0.155 in the experiment described. This relatively slight variation may be ascribed to a difference in enzyme concentration required for assay of the two activities under these conditions. Slight variations in the effects of ionic strength also were noted as diluted enzyme preparations aged at 4°.

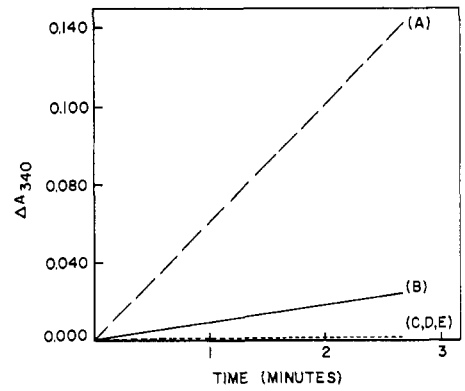

FIGURE 4: Dependence of the bicarbonate stimulatory effect on NADP, glucose, and yeast enzyme. Complete assay mixtures (A), pH 8.9, contained, in 3.0 ml, 8.3 mM Tris-Cl buffer, 0.18 mM sodium NADP, 0.5 M equilibrated glucose, 10 mM sodium bicarbonate, and 5.0 μg of enzyme protein. Results obtained when (B) bicarbonate, (C) NADP, (D) glucose, or (E) enzyme was omitted from assay mixtures also are depicted. Data are presented as spectrophotometer tracings of A_{340} as a function of incubation time observed with each assay mixture.

On the basis of the results of these experiments, buffer concentrations were kept low (8.3 mM) in succeeding experiments, and NaCl was routinely employed to adjust total ionic strength of assay mixtures to constant values in the various series of studies of the effects of anions on enzymic activities. Kinetic studies (W. B. Anderson and R. C. Nordlie, unpublished observations)[3] indicated that this salt was without effect on K_m values for NADP, glucose, and glucose-6-P over the concentration range utilized in studies described in this paper.

Differential Effects of Anions on Glucose Dehydrogenase and Glucose-6-P Dehydrogenase Activities. The inhibition of yeast glucose-6-P dehydrogenase activity by phosphate and sulfate is well documented (Glaser and Brown, 1955; Kornberg and Horecker, 1955; Lowry *et al.*, 1961; Passonneau *et al.*, 1966; Avigad, 1966), as is the inhibition of the mammary enzyme by bicarbonate when NADP (but not NAD) is employed as nucleotide substrate (Levy, 1963). As indicated in Figure 3, similar inhibitions of glucose-6-P dehydrogenase activity by 20 mM concentrations of all three of these anions also were observed with the yeast enzyme in this laboratory. However, quite interestingly, glucose dehydrogenase activity of the enzyme was markedly *stimulated* by this same concentration of all three anions (Figure 3). Phosphate and sulfate, which were most effective as inhibitors of the activity with glucose-6-P, increased the glucose dehydrogenase activity to 200 and 300% of control values, respectively, while bicarbonate, a relatively poor inhibitor of glucose-6-P dehydrogenase activity, caused a 14-fold activation of glucose dehydrogenase activity.

The dependence of the bicarbonate-effected stimula-

[3] Kinetic analyses of the effects of sodium chloride and various anions on both glucose dehydrogenase and glucose-6-P dehydrogenase activities will be the subject of a future communication.

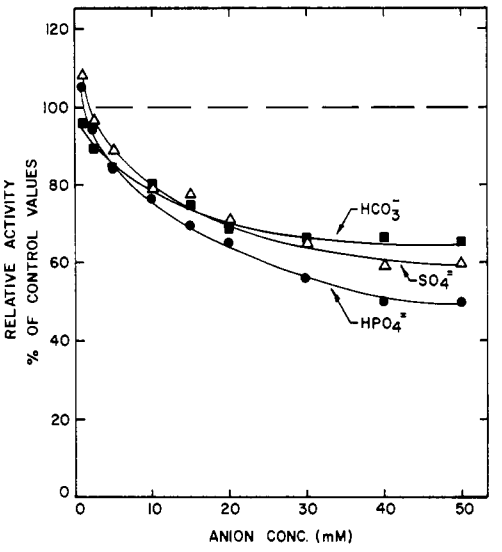

FIGURE 5: Effects of varying concentrations of bicarbonate, sulfate, and phosphate on glucose-6-P dehydrogenase activity. Assay mixtures, pH 8.2, contained in 3.0 ml, 8.3 mM Tris-Cl buffer, 0.1 mM sodium NADP, 0.1 mM sodium glucose-6-P, 0.2 μg of enzyme protein, and the indicated, varied concentrations of sodium bicarbonate (■), sodium sulfate (△), or sodium phosphate (●). Control reaction mixtures contained sodium chloride of ionic strengths corresponding to those of the salts of the experimentally studied anions. Relative activity is expressed as in Figure 3.

tion of glucose dehydrogenase activity on the simultaneous presence of enzyme, NADP, and glucose is indicated by the results of experiments described in Figure 4. A marked increase in A_{340} was noted when 10 mM bicarbonate was present in complete assay mixtures (curve A), relative to that observed in the absence of bicarbonate (curve B). No change in A_{340} was noted when NADP, glucose, or enzyme was omitted from assay mixtures containing bicarbonate (curves C–D–E, respectively). These observations rule out the possibility that increases in A_{340} routinely observed with complete assay mixtures when bicarbonate was included could arise simply from the formation of complexes between the anion and constituents of the reaction mixtures which might conceivably absorb at this wavelength.

In agreement with previous reports (Colowick and Goldberg, 1963; Salas *et al.*, 1965), glucose-6-P was definitely the preferred substrate for the enzyme (see Table I). In the absence of anions, glucose dehydrogenase activity was less than 1% that observed with glucose-6-P when sugar substrate concentrations were maintained at levels approximating their respective K_m values.[3] However, in the presence of 50 mM bicarbonate, glucose-6-P dehydrogenase activity was depressed approximately 35% while glucose dehydrogenase was markedly stimulated. Under these conditions the latter activity became relatively significant and was approximately 20% of that noted with the sugar phosphate substrate. In these experiments performed in the presence of bicarbonate, identical concentrations of enzyme were used for the assay of both types of dehydrogenase activity, thus ruling out any possibility

TABLE I: Relative Activity Levels of Glucose Dehydrogenase and Glucose 6-Phosphate Dehydrogenase Activities in the Absence and Presence of Bicarbonate.[a]

	Oxidizable Substrate	
	Glucose-6-P	Glucose
Bicarbonate absent		
Units of activity/mg of protein[b]	113.0	0.84
Relative activity (%)[c]	100.0	0.74
Bicarbonate (50 mM) present		
Units of activity/mg of protein[b]	73.6	15.4
Relative activity (%)[c]	100.0	20.9

[a] Assay mixtures (pH 8.4) contained, in 3.0 ml, 8.3 mM Tris-Cl, 0.1 mM sodium NADP, and either 0.035 mM sodium glucose-6-P or 0.33 M β-D-glucose (added directly as the powder). When 50 mM sodium bicarbonate was included, 0.5 μg of enzyme protein was employed in both glucose dehydrogenase and glucose-6-P dehydrogenase assays. When bicarbonate was absent, 5.0 μg of enzyme protein was utilized in the assay for glucose dehydrogenase while 0.05 μg of enzyme protein was employed in the assay of glucose-6-P dehydrogenase activity. [b] Units of activity are expressed in terms of μmoles of NADPH produced/2.5 min. [c] Relative activity = 100 × (activity with glucose/activity with glucose-6-P).

that the observed activity-discriminating effects of anions could be related to differences in enzyme concentrations. The observed differences likewise cannot be related to variations in K_m values for NADP, which were found identical with both glucose and glucose-6-P dehydrogenase activities, either in the presence or in the absence of bicarbonate, in supplementary studies.[3]

The effects of varied concentrations of bicarbonate, sulfate, and phosphate on both glucose dehydrogenase and glucose-6-P dehydrogenase activities are described, respectively, in Figures 5 and 6. With the exception of a slight enhancement by 1 mM phosphate and sulfate, glucose-6-P dehydrogenase activity was inhibited progressively with increasing concentrations of all three anions. At pH 7.4 (supplemental experiments), a slightly more pronounced stimulation (approximately 10%) was noted with 1–2.5 mM phosphate and sulfate, while higher concentrations of all anions also inhibited glucose-6-P dehydrogenase activity at this pH as at pH 8.2. The stimulatory and inhibitory effects exhibited by phosphate and sulfate may represent two different types of regulatory binding; Passonneau *et al.* (1966) previously have suggested that these two anions each may bind to more than one site on the enzyme. In contrast with their effects on glucose-6-P dehydrogenase activity, all three anions *activated* glucose dehydro-

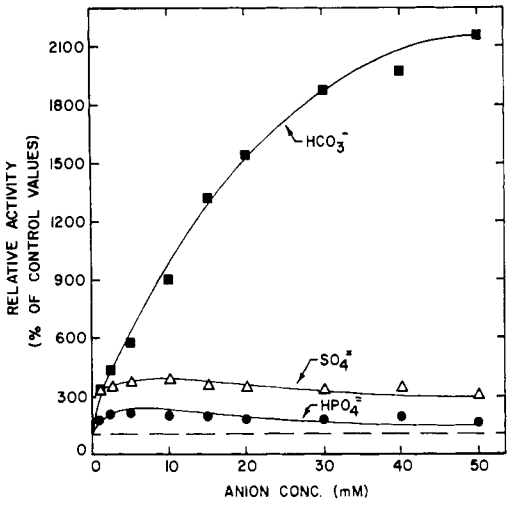

FIGURE 6: Effects of varying concentrations of bicarbonate, sulfate, and phosphate on glucose dehydrogenase activity. Assay mixtures (pH 8.2) contained, in 3.0 ml, 8.3 mM Tris-Cl buffer, 0.1 mM sodium NADP, 0.5 M equilibrated glucose, 5.0 μg of enzyme protein, and the indicated concentrations of sodium bicarbonate (■), sodium sulfate (△), or sodium phosphate (●). Experimental control reaction mixtures were prepared and assayed as in the experiment described in Figure 5. Relative activity is expressed as in Figure 3.

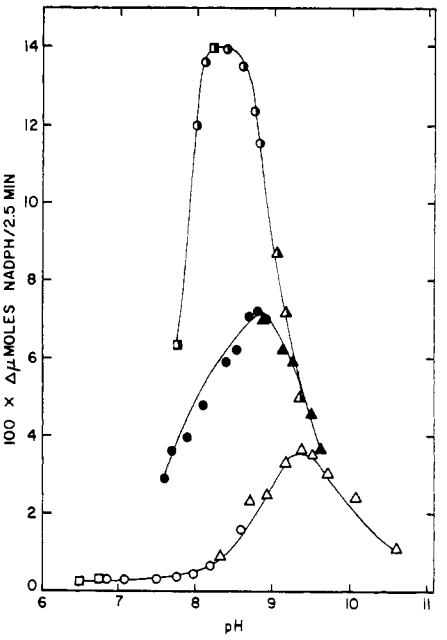

FIGURE 7: Influence of varied concentrations of bicarbonate on pH-activity profiles of glucose dehydrogenase activity. Assay mixtures contained, in 3.0 ml, 8.3 mM sodium cacodylate (squares), Tris-Cl (circles), or glycine hydroxide (triangles) buffer, 0.18 mM sodium NADP, 0.5 M equilibrated glucose, 5.0 μg of enzyme protein, and either no (open symbols), 10 mM (closed symbols), or 50 mM (half-shaded symbols) sodium bicarbonate. The pH values of all reaction mixtures were determined immediately after the 2.5-min incubation period. Ionic strength was in all cases adjusted to that in assay mixtures containing 50 mM sodium bicarbonate.

genase activity at all concentrations tested (Figure 6). Activation by bicarbonate was progressive over the entire range tested; a 20-fold stimulation was observed when the concentration of anion was 50 mM, the highest concentration studied. Maximal activation of glucose dehydrogenase activity by either phosphate or sulfate was reached at a fairly low concentration (5 mM), and thereafter the degree of stimulation decreased somewhat with increasing concentrations of anions. Similar patterns of activation also were observed at pH 7.4 in supplementary experiments.

Variation of Anion Effects with pH. Activity–pH profiles for glucose dehydrogenase activity, in the absence of bicarbonate and in the presence of 10 and 50 mM bicarbonate, are presented in Figure 7. Maximal activity was observed at pH 9.4 in the absence of bicarbonate, compared with pH 8.0 observed with glucose-6-P dehydrogenase activity in supplemental experiments. And while supplemental experiments also revealed that the pH optimum of the latter activity was unaffected by added bicarbonate, the optimum for glucose dehydrogenase activity was progressively shifted to pH 8.8 in the presence of 10 mM bicarbonate and to pH 8.2 when assayed in the presence of 50 mM bicarbonate (see Figure 7). These bicarbonate-effected shifts in pH optima also were accompanied by progressive enhancements of activity. A similar shift in pH optimum previously has been observed by Fanestil *et al.* (1963), who studied the effects of CO_2 concentrations on mitochondrial ATPase activity.

Figure 8A,B depicts the effects of 10 mM concentrations of bicarbonate and phosphate on glucose and glucose-6-P dehydrogenase activities at a variety of pH values. As indicated in these diagrams, the degree of stimulation of glucose dehydrogenase activity and inhibition of glucose-6-P dehydrogenase activity by anions was highly pH dependent. Bicarbonate produced its maximal inhibitory and stimulatory effects at pH 8.1, while phosphate exerted optimal effects on both enzymic activities at pH 7.3. It also is interesting to note that above pH 9 phosphate became an inhibitor of glucose dehydrogenase activity while its inhibitory effects on glucose-6-P dehydrogenase activity were minimal. Sulfate was found in supplementary experiments to act in a manner similar to that of phosphate.

Discussion

The effects of anions on glucose-6-P dehydrogenase activity noted in these studies agree well with the observations of earlier investigators who worked with the enzyme from yeast (Glaser and Brown, 1955; Kornberg and Horecker, 1955; Lowry *et al.*, 1961; Passoneau *et al.*, 1966; Avigad, 1966), mammary gland with NADP as substrate (Levy, 1963), and bacteria (Olive and Levy, 1967). However, the rather marked stimulation of glucose dehydrogenase activity of the yeast enzyme by bicarbonate, sulfate, and phosphate has not been described previously. Indeed, Colowick and Goldberg (1963) reported that phosphate inhibited glucose dehydrogenase activity of their yeast glucose-6-P dehydrogenase preparations. Their observations can, however, be rationalized on the basis of the high ionic strengths present in their assay mixtures. Tris buffer

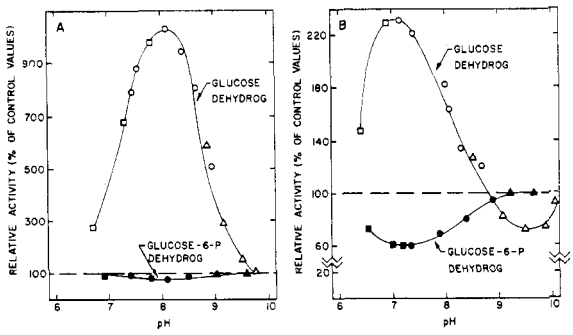

FIGURE 8: Variation of the effects of (A) sodium bicarbonate and (B) sodium phosphate on glucose dehydrogenase and glucose-6-P dehydrogenase activities as a function of pH. The composition of basic assay mixtures and determination of reaction mixture pH were as in Figure 7. Relative activity = 100 × (activity determined in the presence of 10 mM sodium bicarbonate (A) or sodium phosphate (B)/activity determined in control assay mixtures in which bicarbonate and phosphate were absent and ionic strength was adjusted with sodium chloride).

(0.2 M) was employed in their studies, and either 0.1 or 0.3 M phosphate was added to reaction mixtures. No correction was made for variations in ionic strength thus induced, however, and under these conditions elevations in ionic strength would produce significant inhibitions as our experiments described in Figure 2 indicate.

As shown by the data in Table I, glucose dehydrogenase, which is at best a minor activity relative to that catalyzed by the enzyme with glucose-6-P as substrate, can become quite significant in the presence of high concentrations (50 mM) of bicarbonate. However, it must be emphasized that the glucose concentration employed in order to obtain this effect was nearly four orders of magnitude greater than that of glucose-6-P.

Although the high K_m values for glucose make a physiological role for glucose dehydrogenase activities somewhat suspect, the rather wide distribution of such activities in nature (see, for example, Metzger *et al.*, 1964, 1965; Strecker and Korkes, 1952; Imai *et al.*, 1961; Hauge, 1966; King, 1966; Sadoff, 1966) suggests that they may have some significant metabolic function. Consistent with this idea is certain evidence in the literature, along with our observations on the activating effects of bicarbonate and other anions which are described in this paper. For example, the presence of a reaction sequence for the metabolism of glucose involving the successive action of glucose dehydrogenase, gluconolactonase (Brodie and Lipmann, 1955), and gluconate kinase (Sable and Guarino, 1952) is suggested by the fact that all three enzymes have been found in yeast preparations. Hastings and Longmore (1965) previously have emphasized the important role that bicarbonate plays in mammalian carbohydrate metabolism. Using rat liver slices, they noted a stimulation by bicarbonate of both glycogen synthesis and lipogenesis. A key gluconeogenic enzyme, phosphoenolpyruvate carboxykinase, is stimulated by the anions sulfate (Foster *et al.*, 1967) and bicarbonate (Bandurski and Lipmann, 1956), while the hydrolysis of glucose-6-P by classical glucose 6-phosphatase is inhibited by phosphate (Hass and Byrne, 1960) and by bicarbonate (Dyson and Anderson, 1968). One or more of the anions studied in the present investigation therefore have been found to stimulate glucose-6-P formation *via* accelerated gluconeogenesis, and to inhibit glucose 6-phosphatase and glucose-6-P dehydrogenase activities which catalyze removal of this hexose phosphate. Conceivably, the rate of glycogen formation might as a result of these effects be increased, since glucose-6-P serves both as a precursor of glycogen and as an activator of glycogen synthetase (Leloir *et al.*, 1959; Rothman and Cabib, 1967). Furthermore, the stimulation of glucose dehydrogenase activity by these anions may provide a mechanism for continued NADPH generation in the presence of diminished glucose-6-P dehydrogenase activity. The NADPH thus produced would then be available for continued lipogenesis and other reactions. These observations, while admittedly only suggestive of a physiologically significant regulatory rule for bicarbonate through its effects on activities of glucose-6-P dehydrogenase and other enzymes, none the less would appear to dictate further investigations of properties of glucose dehydrogenase activities possibly catalyzed by glucose-6-P dehydrogenases from sources in addition to yeast.

Finally, in view of the widespread use of yeast glucose-6-P dehydrogenase as an analytical tool in many enzymic studies, a word of caution appears to be in order concerning the accompanying glucose dehydrogenase activity described in this paper. When reactions involving large amounts of glucose and added anions such as bicarbonate or phosphate are studied, adequate experimental controls must be included to ensure that observed initial reaction rates measured by monitoring the reduction of NADP are uncomplicated by glucose dehydrogenase activity which may manifest itself (see, for example, Anderson and Nordlie, 1967).

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