changed by this substitution. Thus the stability of the polymer to mechanical stress is not enhanced by the energy of dephosphorylation. This conclusion is not surprising since the literature contains no results which imply that F-actin obtained from G-actin·ADP is different from that obtained from G-actin·ATP. However, the sonication data also show that the stability of the polymer is unchanged when the nucleotide remains unhydrolyzed.

In summary, the present studies show that neither the rate of actin polymerization nor the stability of the resulting polymer is coupled to the dephosphorylation of the actin nucleotide that occurs during the polymerization process. We were unable to detect any difference in the properties of F-actin when the bound ADP was substituted by AMP-PNP. Thus, although the nucleotide dephosphorylation occurs during polymerization, there now seems to be no evidence that it plays any active role in regulating or facilitating the process of the polymerization or in effecting the properties of the resulting polymer. The data do not, unfortunately, point to a possible role for the actin nucleotide, and further studies are underway to investigate the interaction of F-actin·AMP-PNP with myosin.

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Kinetics and Thermodynamics of the Formation of Glucose Arsenate. Reaction of Glucose Arsenate with Phosphoglucomutase†

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ABSTRACT: The reaction of glucose and arsenate in aqueous solution at neutral pH and room temperature produces an equilibrium mixture containing small amounts of glucose 6-arsenate and a smaller amount of the 1-arsenate as well. Under these conditions the thermodynamic stability of the 6-arsenate is essentially the same as that of glucose 6-phosphate, although the approach to equilibrium is more rapid by several orders of magnitude: $k_{form} = 1.4 \times 10^{-6}$ M^{-1} sec^{-1}; $k_{hydrol} = 4.2 \times 10^{-4}$ sec^{-1}. K_{form} thus is about 3.4×10^{-3} M. The glucose arsenates are measured by means of their rapid stoichiometric reaction with ^{32}P-labeled phosphoglucomutase to produce glucose [^{32}P]phosphates. The criteria for selection of enzyme systems to assay other arsenate esters *in situ* are discussed.

Arsenate is isosteric and isoelectronic with phosphate (Sisler, 1956) and can enter into enzyme-catalyzed reactions in its place (Braunstein, 1931; Harden, 1932; Warburg and Christian, 1939; Doudoroff *et al.*, 1947; Katz *et al.*, 1948). Although *both* acyl and glycosyl phosphates and arsenates are thermodynamically unstable at neutral pH and room temperature, the arsenates appear to differ from their phosphate analogs in that they hydrolyze much more rapidly. Hence, in enzymatic reactions which involve acyl or glycosyl transfer to inorganic phosphate, substitution of arsenate for phosphate can give rise to abortive hydrolysis products. Transient formation of acyl and glycosyl arsenates in such enzymatic reactions has been demonstrated by ^{18}O tracer studies (Slocum and Varner, 1960) but no information has been available on the rate and thermodynamics of their hydrolysis or formation.

Lagunas and Sols (1968) first proposed that the hydroxymethyl group of compounds such as glucose, fructose, fruc-

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tose-6-P, and dihydroxyacetone could react with arsenate in neutral aqueous solutions to form esters in the absence of an enzyme. The present study of the reaction of glucose and arsenate provides additional support for their hypothesis.

The present investigation was prompted by observations made during a study of the transfer of phosphate from ^{32}P-labeled phosphoglucomutase to glucose in the presence of arsenate (J. W. Long, J. D. Owens, and W. J. Ray, Jr., manuscript in preparation); the extent of phosphate transfer during a short assay interval was dependent on the mixing sequence and increased with the time elapsed after mixing of glucose and arsenate when the reaction was initiated by addition of this mixture to the enzyme. In addition to rationalizing these observations in terms of the nonenzymatic formation of glucose 6-arsenate, the present paper provides kinetic and thermodynamic data on its formation and hydrolysis and shows that the arsenate ester reacts rapidly and stoichiometrically with the phospho form of phosphoglucomutase (E_P)[1] to give the dephosphoenzyme (E_D), inorganic arsenate, and a mixture of glucose 1- and 6-phosphates. The analytical procedure used here is rather restrictive but in certain selected cases should be applicable to studies of other organic arsenates.

Experimental Procedures

Carrier-free [^{32}P]phosphoric acid was obtained from Schwarz-Mann. Sucrose phosphorylase was the kind gift of Dr. R. H. Ables, Brandeis University. All other reagents were reagent quality or better.

Glucose 1-[^{32}P]phosphate was prepared by a modification (J. W. Long and W. J. Ray, Jr., unpublished results, available on request from W. J. R.) of the procedure of Ray and Koshland (1963).

Phosphoglucomutase was prepared by a modification of the procedure described by Ray and Koshland (1962); details are available on request. A molecular weight of 62,000 (Filmer and Koshland, 1963) and an optical density (OD) of 0.77 for a solution of 1 mg/ml (Najjar, 1955) were used in calculating enzyme concentrations. [^{32}P]Phosphoglucomutase was prepared by equilibration with [^{32}P]glucose-1-P according to Ray and Koshland (1963), except that the labeled enzyme was freed of low molecular weight [^{32}P]phosphates by absorption on CM-Sephadex and thorough washing (E. J. Peck, J. W. Long, L. Ng, and W. J. Ray, Jr., manuscript in preparation). The specific radioactivity of the labeled product ranged from 0.1 to 3 mCi/μmol while specific enzymatic activities were at least 850 IU/mg. Over 98% of the radioactivity in these preparations was precipitable by 5% trichloroacetic acid; all of the precipitable radioactivity was exchangeable with glucose-1-P (see below). The enzyme was routinely activated (Ray and Roscelli, 1966) prior to use by treatment for 10 min with 2 mM Mg^{2+}–1 mM EDTA in the presence of 0.1 M imidazole and 20 mM Tris-chloride (pH 7.5) containing 0.01 mg/ml of bovine serum albumin (Pentex).

The fraction of the total radioactivity in samples of [^{32}P]-phosphoglucomutase that was soluble in 5% trichloroacetic acid (f) was determined by: (a) mixing 0.1 ml of enzyme solution (or reaction mixture containing enzyme), 0.1 ml of 1% bovine serum albumin, and 0.1 ml of 15% trichloroacetic acid and immediately plating 0.1 ml (for total radioactivity) on ringed aluminum planchets (Sigma); (b) centrifuging the remaining solution after allowing it to stand for 5 min or longer in ice and plating 0.1 ml of the supernatant (for soluble radioactivity). Samples were counted to a statistical error of <1% with a Nuclear-Chicago low-background Geiger counter. A small, zero-time blank (usually less than 0.02 of the total radioactivity), which represents the fraction of acid-soluble radioactivity initially present in the enzyme sample, was determined for each sample used and subtracted from *all* f values. Organic [^{32}P]phosphate, both acid stable and acid labile, was assayed as previously described (Peck *et al.*, 1968) on supernatants from an acid precipitation step in which 3 N $HClO_4$ was substituted for 15% trichloroacetic acid (see above).

Assay for Glucose Arsenate. The glucose arsenate present in solutions containing glucose plus arsenate was measured in a "burst" assay by (a) adding an aliquot (usually 0.1 ml) of the solution to be tested to an equal volume of [^{32}P]phosphoglucomutase (room temperature) in a "mini-beaker" (diameter ~1 cm); the enzyme, which was always in excess, was rapidly stirred with a magnetic stirrer during the addition; (b) removing and quenching aliquots of the mixture into trichloroacetic acid at 15-sec intervals; and (c) measuring f values for each of these aliquots (see above). Semilog plots of $(1 - f)$ *vs.* t were extrapolated to $t = 0$ to assess the burst, f_b; the burst does not include the small zero-time blank noted above. The burst was taken as a measure of the glucose arsenate present when the reaction was initiated; hence, the amount of glucose arsenate in any solution was calculated by multiplying f_b by the amount of enzyme used in the assay, $[E]_T$. Semilog plots were used in the extrapolation process because glucose also reacts with the phosphoenzyme in the presence of arsenate to effect phosphate transfer in a process that is first order with respect to the enzyme (see the Discussion).

Analysis of the Burst Products in the Reaction of Glucose Arsenate with [^{32}P]Phosphoglucomutase. A solution containing 0.5 M sodium arsenate (pH 7.5) and 5 mM glucose was incubated at 30° in a sealed vial until no further increase in glucose arsenate concentration was observed by means of the above assay. Aliquots of this solution (0.1 ml) were mixed with 0.005 ml of a [^{32}P]phosphoglucomutase solution (0.71 mM for limiting enzyme and 0.071 mM for limiting arsenate) and after various times (see below) 0.1 ml of 3 N $HClO_4$ and 0.1 ml of 1% bovine serum albumin were added to some of the reaction mixtures. After centrifugation, the supernatant was assayed for acid-stable radioactivity (see above). The time between mixing of the glucose plus arsenate and enzyme solutions and quenching with $HClO_4$ was varied from 1 sec to 1 min. Aliquots from other reaction mixtures were diluted and assayed for dephosphoenzyme by means of a procedure analogous to that of Passonneau *et al.* (1969). An Aminco fluorimeter equipped with a recorder was used for this assay.

Results

Reaction of Aged Solutions of Glucose Plus Arsenate with [^{32}P]Phosphoglucomutase. When aliquots of a mixture of glucose and arsenate, aged to equilibrium (see below), were assayed for glucose arsenate (see Experimental Section) by reaction with excess [^{32}P]phosphoglucomutase a burst of acid-soluble radioactivity, f_b, was observed (Figure 1), followed by a much slower release of radioactivity; in plots of log $(1 - f)$ against time, the latter phase of the reaction was linear for at

[1] Abbreviations used are: E_P, phosphoenzyme; E_A, arsenoenzyme; E_D, dephosphoenzyme; A_i, inorganic arsenate; glucose-6-A or Glc-6-A, the 6-arsenate ester of glucose; glucose-1-A or Glc-1-A, the 1-arsenate ester of glucose, presumably the α ester only (see Results); glucose-A or Glc-A, a mixture of the above arsenate esters; glucose-6-P, glucose 6-phosphate.

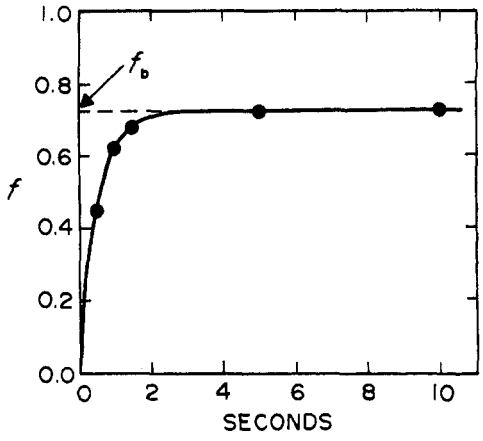

FIGURE 1: The rapid phase of the reaction of an aged solution of glucose plus arsenate with [^{32}P]phosphoglucomutase. Equal volumes of [^{32}P]phosphoglucomutase (0.49 μM) and an aged solution containing 0.1 M sodium arsenate (adjusted to pH 7.5) plus 1 mM glucose were mixed and quenched at various times, and the fraction of the total radioactivity soluble in trichloroacetic acid, f, was determined (see Experimental Procedures). Extrapolation of the linear portion of the curve to $t = 0$ gives f_b, as indicated.

least 80% release of the remaining enzyme-bound radioactivity (not shown). Under the conditions of Figure 1, the burst phase of the reaction is more than half-complete in less than 0.5 sec.

The following observations delineate the process that occurs during the burst phase of the reaction. (a) The size of the burst, f_b, is directly proportional to a component of the glucose plus arsenate mixture. Thus, Figure 2 shows that, at constant total enzyme, f_b is a linear function of the amount of the aged glucose plus arsenate solution used in the reaction, at least up to an f_b value of 0.8. This indicates that the reaction of phosphoenzyme and a minor component (see below) of the glucose plus arsenate solution goes essentially to completion and that f_b values can be used to assess the amount of the

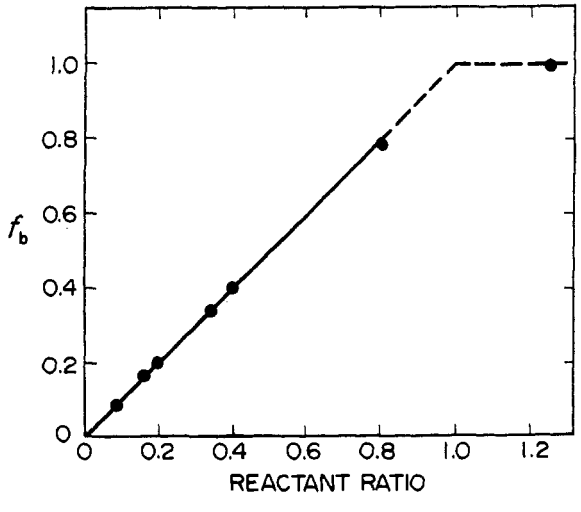

FIGURE 2: Reaction of [^{32}P]phosphoglucomutase with aged solution of glucose plus arsenate. Aliquots of an aged solution of glucose (1 mM) plus arsenate (0.1 M, pH 7.5) were mixed with 0.1 ml of a solution containing 0.34 μM [^{32}P]phosphoglucomutase in a buffer solution (50 mM Tris, 100 mM imidazole, 1 mM EDTA, and 2 mM $MgCl_2$ (pH 7.5)) and f_b values determined as described under Experimental Procedures. The reactant ratio is the volume of the solution of glucose plus arsenate added per volume of enzyme solution.

TABLE I: Effect of Varying Concentrations of Glucose and Arsenate on f_b.[a]

Arsenate (M)	Glucose (mM)	f_b[b]
0.05	2.0	0.19
0.10	1.0	0.20
0.20	0.5	0.19

[a] Solutions of glucose plus arsenate at the concentrations shown were allowed to stand for 1 week at 30°, pH 7.5, in sealed vials. Aliquots of these solutions were mixed with equal volumes of [^{32}P]phosphoglucomutase solution (1.72 μM) and the fraction of total radioactivity rapidly solubilized, f_b, was determined (see Experimental Procedures). [b] The values of f_b indicate that the concentration of the reactive component of the mixture was 0.34 μM.

latter material in a mixture of glucose and arsenate, provided an excess of phosphoenzyme is used. (b) The component in the aged mixture that gives rise to the burst is present in minute amounts, relative to glucose and arsenate, as indicated by its low apparent concentration in the assay, f_b $[E]_T$. (c) The concentration of the reactive component of the mixtures is proportional to the *product* of the concentrations of glucose and arsenate in aged mixtures (Table I).[2] (d) The radioactive product from treatments of [^{32}P]phosphoglucomutase with an excess of aged solutions of glucose plus arsenate (so that all of the enzyme-bound radioactivity was rapidly solubilized; for conditions see Experimental Procedures) was organic phosphate; approximately 15% of this organic phosphate was stable to a 10-min treatment with 0.1 N $HClO_4$ at 100°. Since glucose-6-P is stable under these conditions, while glucose-1-P is readily hydrolyzed, the reaction products (after dilution and spontaneous hydrolysis of possible arsenate esters) appear to be about 85% [^{32}P]glucose-1-P and 15% [^{32}P]glucose-6-P.

All of the above observations are consistent with the existence of low concentrations of arsenate esters of glucose in aged mixtures of glucose and arsenate according to eq 1.

$$\text{glucose} + A_i \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{glucose-A} \qquad (1)$$

The formation of ^{32}P-labeled glucose-6-P as well as labeled glucose-1-P in the reaction of aged glucose plus arsenate with ^{32}P-labeled enzyme further suggests that both the 1 and 6 isomers of glucose arsenate are present so that glucose-A in eq 1 represents a mixture of isomers (see below). The reaction with glucose-1-A or glucose-6-A could follow either or both of the two possible pathways illustrated in eq 2 for the 6 isomer.

$$\text{glucose-6-A} + E_P \begin{cases} \xrightarrow{k_{2a}} \text{glucose-1-P} + E_A \\ \xrightarrow{k_{2b}} \text{glucose-6-A-1-P} + E_D \end{cases} \qquad (2)$$

Here E_A is the arseno analog of the phosphoenzyme. Unfortunately, the present results allow no conclusions to be drawn about the relative importance of these two pathways. However, there is no substantial accumulation of E_A since an assay for the dephosphoenzyme (Passonneau *et al.*, 1969)

[2] The data in Table I actually show only that the concentration of the reactive component is proportional to $[Glc]^n[A_i]^n$; however, other data (not shown) demonstrate that $n = 1$.

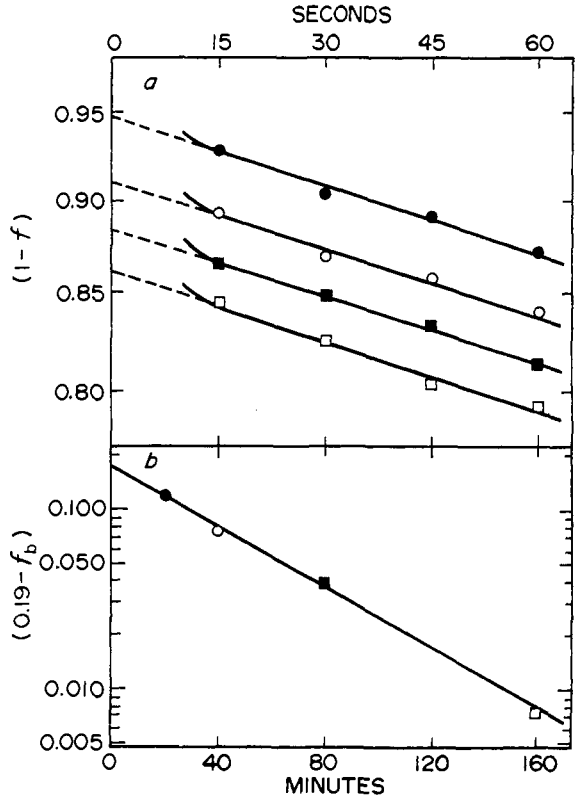

FIGURE 3: Formation of glucose 6-arsenate in a solution of 0.1 M sodium arsenate and 0.001 M glucose at 30° and pH 7.5. (a) Determination of f_b values. Equal volumes of [^{32}P]phosphoglucomutase solution and the reaction mixture were combined at various reaction times after the glucose plus arsenate reaction was initiated. Plots of f *vs.* assay time are shown for reaction times of 20 min (●), 40 min (○), 80 min (■), and 160 min (□). (b) The rate of appearance of glucose-6-A in solutions of glucose plus arsenate. Values for f_b from part a are plotted according to eq 3 by using a value of 0.19 for $(f_b)_\infty$. The intercept value at zero reaction time is 0.173.

that should not respond to E_A[3] shows that the dephosphoenzyme accumulates at a rate comparable with the rate at which the ^{32}P label is lost from the phosphoenzyme (data not shown).

For very short reaction times (prior to completion of the burst) with a limiting amount of the glucose plus arsenate solution (so that the maximum loss of the ^{32}P label from the enzyme was 20%, see Experimental Procedures),[4] a smaller percentage of the released radioactivity was stable to acid than the 15% observed with excess arsenate solution (see above). A linear extrapolation of the ratios observed *prior to completion of the burst* (12% at the shortest time interval to 19% after the longest interval) to zero reaction time gave a limiting value of about 11% [^{32}P]glucose-6-P. This indicates that the 6-arsenate ester predominates over the 1-ester by about eightfold.

Although by analogy with the glucose phosphate reaction (*cf.* Ray and Peck, 1972), we suggest that only the α anomer of glucose-1-A reacts with phosphoglucomutase, and that only this anomer is detected in the present study, it is not certain whether both the α and β anomers of glucose-6-A are detected in the above reaction. Presumably only the α anomer reacts *directly* with the enzyme, but in a long-term assay both should react because of anomerization of the β isomer. However, since the results obtained prior to completion of the burst were extrapolated to $t = 0$, it is possible that the extrapolation in effect eliminates that component of the reaction due to the β anomer, unless the anomerization is faster than the enzymatic reaction. In the case of the corresponding phosphate ester, the half-time for anomerization is about 20 sec, as calculated from the data of Lowry and Passonneau (1969). In such a case, the extrapolation used would eliminate essentially all of the effect that would be produced by the β isomer. However, 20 sec may be much too long a half-time for anomerization under the present conditions. Thus, the time course for reactions of glucose plus arsenate solutions with *excess* enzyme (*cf.* Figures 1 and 3a) produced no evidence for an anomerization process with a time constant in this range. Actually, the presence of inorganic arsenate in these reaction mixtures may make the time constant for the anomerization of the 6-arsenate ester much less than 20 sec just as inorganic phosphate increases the mutarotation rate of glucose (*e.g.*, see Messer and Dahlquist, 1966); alternatively, the anomerization rate of the arsenate ester may be intrinsically faster than that of the corresponding phosphate ester. (The mutarotation rate for the phosphate ester is some 100-fold faster than the corresponding rate for glucose, for reasons that are not well understood (Salas *et al.*, 1965).) In any case, it appears that both the α and β isomers are normally detected in burst assays in which the first assay point is taken at 15 sec, *i.e.*, *after completion of the burst*, see Figure 3a.[5] Whether both are detected in the above study, where the ratio of acid-labile to acid-stable phosphate in the products obtained *prior to completion of the burst* is extrapolated to $t = 0$, is problematical. If both are detected in the latter extrapolation, the ratio ($\alpha + \beta$)-glucose-6-P/α-glucose-1-A would be only 8, whereas the same ratio of the phosphate esters is about 17 (*cf.* Ray and Peck, 1972). If only the α isomer is detected in the latter extrapolation, the observed value of 8 represents the ratio, α-glucose-6-A/α-glucose-1-A, and is quite close to the same ratio for the corresponding phosphate esters, about 7 (Lowry and Passonneau, 1969), which seems reasonable. In such a case the ratio, ($\alpha + \beta$)-glucose-6-A/α-glucose-1-A, would be about 20, if the ratio of α to β isomers of the arsenate ester is the same as that for glucose-6-P, 0.7 (Lowry and Passonneau, 1969).

It should be pointed out that although the rate of transfer of label to glucose arsenate in Figure 1 appears to be relatively slow with respect to the transfer rate to glucose phosphate under normal assay conditions, the sluggishness of the reaction

[3] The assay depends on the reaction of E_D with glucose-1,6-P_2 to produce glucose-6-P (in addition to E_P) which further reacts with NADP plus glucose-6-P dehydrogenase to give NADPH. It should be noted that in the present study this assay can be used only if the reaction mixture (see Experimental Section) is diluted several-fold prior to the assay since glucose plus arsenate also reacts with glucose-6-P dehydrogenase at an appreciable rate (Lagunas and Sols, 1968).

[4] At the concentration of reactants used for these experiments, the rate during the burst phase of the reaction was about one-third of that in Figure 1; this sluggishness presumably reflects the increased inhibition of the enzyme at the higher arsenate concentrations used (see below).

[5] In burst assays with a fivefold excess of enzyme, the rate of loss of the ^{32}P label from phosphoglucomutase subsequent to the burst, *i.e.*, after 15 sec, was the same as the rate of loss in an identical reaction mixture in which the glucose and arsenate were not premixed. This identity argues against a slow conversion of β-glucose-6-A into α-glucose-6-A (with a subsequent rapid reaction with the enzyme) unless (a) the β isomer is present initially in much smaller amounts than the α isomer or (b) unless mutarotation of β-glucose-6-A is much slower than that of glucose (which is only 10^{-2} that of glucose-6-P—see above). Neither of these possibilities is attractive; hence, we assume that under the conditions used, mutarotation of β-glucose-6-A is somewhat faster than that of β-glucose-6-P, and that the burst includes reaction with both the α and β anomers of glucose-6-A.

under these conditions undoubtedly is partly caused by the relatively high concentration of inorganic arsenate (50 mM), which is a competitive inhibitor of the enzyme ($K_I \sim 2$ mM, J. W. Long and W. J. Ray, Jr., unpublished results), and by the very low levels of glucose arsenate present (0.17 μM, initially). Unless glucose-6-A binds much more tenaciously to the enzyme than does glucose-6-P, the catalytic efficiency in the transfer process involving these two esters must be similar in magnitude.

Rates and Equilibrium for the Nonenzymatic Formation of Glucose 6-Arsenate. Under the condition of a large excess of arsenate over glucose the appearance of glucose-6-A[6] should be pseudo first order. Equation 3 gives the expected time course of the reaction in terms of f_b values, which provide a measure of the glucose 6-arsenate formed (see above).

$$\log [(f_b)_\infty - (f_b)_t] = -k_{obsd}t/2.3 + \log [(f_b)_\infty - (f_b)_0] \quad (3)$$

Here the subscripts 0, t, and ∞ refer to the reaction time, *i.e.*, the time after mixing of glucose and inorganic arsenate. Figure 3b shows a plot of the left-hand side of eq 3 *vs.* time, using f_b values obtained from Figure 3a, for a reaction conducted at pH 7.5 and 30°.[7] Values of K_{form} and k_{obsd} for glucose-6-A (α and β isomers; see above) can be obtained, respectively, from the infinity point and the slope value plus initial reactant concentrations: $K_{form} = (3.4 \pm 0.1) \times 10^{-3}$ M^{-1} (standard deviation for four determinations) and $k_{obsd} = (4.2 \pm 0.8) \times 10^{-4}$ sec^{-1} (standard deviation for two determinations). Since $k_{obsd} = k_1[A_i] + k_{-1}$ (see eq 1), values of about 1.4×10^{-6} M^{-1} sec^{-1} for k_1 and 4.2×10^{-4} sec^{-1} for k_{-1} can be obtained. Experiments identical with those above in which the sodium arsenate solutions used were adjusted to pH 6.5 and 8.5 before being mixed with glucose indicated that k_{obsd} increases substantially at lower pH values while K_{eq} is changed only slightly. In a very concentrated, aged solution of 2 M glucose and 2 M sodium arsenate (pH 7.5), the concentration of glucose-6-A was about 17 mM (measured after a 100-fold dilution).

Discussion

Although the existence of arsenate esters has been accepted for years, no precise information regarding their thermodynamic or kinetic stability has been obtained. Generally, only an indirect proof of existence by means of ^{18}O tracer studies has been reported. This paucity of information is of course a consequence of the marked lability of such compounds.

The method used in this study to obtain detailed information on the stability of glucose-6-A circumvents some of the problems associated with the lability of organic arsenates because it does not require their isolation. The procedure is rather specific but probably could be used to obtain similar information for other organic arsenates if appropriate enzyme systems could be found. The primary requirements for such a system are (a) that the enzyme react rapidly and irreversibly with the organic arsenate and (b) that it react slowly or not at all with the hydroxyl compound plus arsenate. For example, the formation of fructose-1-A-6-P in mixtures of fructose-6-P and inorganic arsenate might well be studied by using fructose-1,6-P_2 aldolase coupled with glyceraldehyde-3-phosphate dehydrogenase (G3PDH)

$$\text{Fru-6-P} + HAsO_4^{2-} \rightleftharpoons \text{Fru-1-A-6-P} \quad (4)$$

$$\text{Fru-1-A-6-P} \overset{\text{aldolase}}{\rightleftharpoons} \text{dihydroxyacetone-A} + \text{glycerate-3-P} \quad (5)$$

$$NAD^+ + \text{glyceraldehyde-3-P} \xrightarrow{\text{G3PDH, } A_i} \text{glycerate-3-P} + NADH \quad (6)$$

The presence of arsenate in the assay mixture would assure irreversibility of the coupled reactions and the use of high levels of enzymes should ensure rapid reaction.

In the present study the equilibrium constant for formation of glucose-6-A from glucose and arsenate (0.0034 M^{-1} at 30°, pH 7.5; see Results) indicates a Gibbs energy of hydrolysis under these conditions ($\Delta G°'$) of -3.4 kcal/mol. A value of $\Delta G°'$ for glucose-6-P hydrolysis of -3.3 kcal/mol at 25°, pH 7.0, has been reported (Atkinson *et al.*, 1961).[8] The similarity of these $\Delta G°'$ values indicates that the origin of the apparent instability of glucose-6-A relative to glucose-6-P is primarily kinetic and not thermodynamic; *i.e.*, although the arsenate analog is hydrolyzed much more rapidly, it is essentially as stable thermodynamically as the phosphate ester. This in turn suggests that $\Delta G°'$ values for most arsenate esters will be about the same as those for the corresponding phosphate esters and that the effect of pH on their stability in the neutral range will be similar for both types of compounds (Carpenter, 1960) since pK_2 values for inorganic phosphate and inorganic arsenate are about the same (Sisler, 1956).

Just how much more rapidly glucose-6-A is hydrolyzed at neutral pH than glucose-6-P can be estimated by correcting the measured rate of hydrolysis of the latter ester at pH 7.4 and 100° to 30° using the measured value for E_a (Bunton and Chaimovich, 1966); this gives a rate constant of 4×10^{-9} sec^{-1}. Since the rate of hydrolysis of glucose-6-A under similar conditions is 4×10^{-4} (see Results), the rate of hydrolysis of glucose-6-A must be about 10^5-fold greater than that of glucose-6-P. This rate inequality indicates a minimum difference of 7 kcal/mol between the Gibbs free energies of activation for hydrolysis, as well as formation of these esters since their equilibrium constants are nearly identical. Thus, in 1 M phosphate (pH 7.4, 30°) the half-time for conversion of glucose to glucose-6-P would be about 6 years. (Experiments to test this prediction are not in progress!) Whether this difference in reactivity between these two types of esters is a consequence of a basically different mechanism or is caused only by a more "open" structure in compounds of third-row elements, as opposed to the corresponding second-row elements, is not

[6] Product analysis (see above) indicates that glucose-1-A accounts for between 5 and 11% of the total glucose-A present in aged solutions of glucose plus arsenate that reacts with phosphoglucomutase. Hence, the parameters calculated in this section for glucose-6-A by ignoring glucose-1-A must be considered only approximate.

[7] Note that the ordinate–intercept in Figure 3b should be equal to $(f_b)_\infty$, but is slightly smaller than this value (see Figure 3 legend). This suggests that a small but detectable fraction of the reaction occurs substantially faster than the remaining reaction. We have no ready explanation for this, except to note that the ratio of the anomaly relative to $(f_b)_\infty$, ~ 0.1, is equal to the relative amount of Glc-1-A presumed to be present in Glc-A mixtures (see above).

[8] The dependence of $\Delta G°'$ on pH from 4 to 12 should be governed by the difference in pK_2 values for glucose-6-P (Meyerhof and Lohmann, 1927) and orthophosphate (Pitzer, 1937). This means that $\Delta G°'$ should be only nominally affected by pH in this range; thus an increase in $\Delta G°'$ of only 0.2 kcal/mol is expected on raising the pH from 7.0 to 7.5. The very small pH dependence of K_{eq} observed for glucose-6-A (see Results) is in agreement with this expectation.

known. However, a difference in mechanism is indicated by the observation that increasing pH appears to decrease the rate of hydrolysis of glucose-6-A (see Results) much as it does for methyl phosphate (Bunton *et al.*, 1958) but increases that observed for glucose-6-P (Bunton and Chaimovich, 1966).

Arsenate actually enhances the reactivity of glucose toward the phosphate group of phosphoglucomutase in two different ways: (1) through formation of glucose-6-A, which is an aresenate-specific effect (present study) and (2) by increasing the rate of reaction of the enzyme with glucose by binding to the enzyme at the site normally occupied by the phosphate moiety of glucose phosphate (J. W. Long, W. J. Ray, Jr., and J. D. Owens, manuscript in preparation). This latter type of activation is not arsenate specific since both inorganic arsenate and inorganic phosphate activate phosphoglucomutase equally well. Hence, type 2 activation can easily be differentiated from type 1 activation. Figure 3a, for example, shows the effects of both types of activation: type 1 activation gives rise to the burst phase, and type 2 activation produces the much slower reaction following the burst phase, which, nevertheless, is considerably faster than the corresponding reaction involving glucose alone (manscript in preparation, see above).

The demonstration that glucose-6-A is formed spontaneously in solutions of glucose and arsenate at room temperature and near-neutral pH strongly suggests that the arsenate-specific reactivities of enzymes with nonphosphorylated substrate analogs observed by Lagunas and Sols (1968) do indeed result from nonenzymatic formation of arsenate esters, as suggested by these workers. In addition, the arsenate-dependent activity of phosphoglycerate mutase (Grisolia and Cascales, 1966) in the absence of its cofactor, glycerate 2,3-bisphosphate, can similarly be rationalized in terms of the nonenzymatic reaction of 3-phosphoglycerate with inorganic arsenate to give the mixed arsenate–phosphate derivative which presumably can substitute for the normal cofactor. In such a case, the activity produced should be a function of the time of exposure of 3-phosphoglycerate to arsenate prior to initiation of the assay, although this was not studied by the original workers.

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