Role of Arginyl Residues in Yeast Hexokinase PII†

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ABSTRACT: Yeast hexokinase PII is rapidly inactivated (assayed at pH 8.0) by either butanedione in borate buffer or phenylglyoxal, reagents which are highly selective for the modification of arginyl residues. MgATP alone offers no protection against inactivation, consistent with low affinity of hexokinase for this nucleotide in the absence of sugar. Glucose provides slight protection against inactivation, while the combined presence of glucose and MgATP gives significant protection, suggesting that modified arginyl residues may lie at the active site, possibly serving to bind the anionic polyphosphate of the nucleotide in the ternary enzyme:sugar:nucleotide complex. Extrapolation to complete inactivation suggests that inactivation by butanedione correlates with the modification of 4.2 arginyl residues per subunit, and complete protection against inactivation by the combined presence of glucose and MgATP correlates with the protection of 2 to 3 arginyl residues per subunit. When the modified enzyme is assayed at pH 6.5, significant activity remains. However, modification by butanedione in borate buffer abolishes the burst-type slow transient process, observed when the enzyme is assayed at pH 6.5, to such an extent that after extensive modification the kinetic assays are characterized by a lag-type slow transient process. But even after extensive modification, hexokinase PII still demonstrates negative cooperativity with MgATP and is still strongly activated by citrate when assayed at pH 6.5.

Hexokinase (EC 2.7.1.1) is the first enzyme of glycolysis from glucose, and the enzyme from yeast has been the subject of much recent study. Yeast hexokinase is now known to exist as two separable isozymes, PI and PII (Schulze & Colowick, 1969; Lazarus et al., 1966; Rustum et al., 1971). Each enzyme exists in a monomer–dimer self-association equilibrium, with a dimer molecular weight of about 100 000. Dissociation is promoted by increases in pH, ionic strength, and temperature, and by decreases in enzyme concentration (Schulze & Colowick, 1969; Derechin et al., 1972; Shill et al., 1974). Yeast hexokinase PII exhibits regulatory properties at physiological pH values (below pH 7) which include negative cooperativity with ATP, activation by citrate and other physiological anions (Kosow & Rose, 1971; Shill & Neet, 1975), and a substrate-induced slow transient during catalysis (Shill & Neet, 1971, 1975).

Previous studies have attempted to clarify the role of various amino acid residues in the catalytic and regulatory properties of yeast hexokinases. One glutamyl residue per subunit is proposed to be involved in catalytic transphosphorylation (Pho et al., 1977), and specific phosphorylation of a single serine residue per subunit by an abortive hexokinase:D-xylose:MgATP ternary complex leads to complete inactivation (Menezes & Pudles, 1977). Modification of a single tyrosyl residue per subunit causes a dramatic loss of enzymatic activity, although no direct involvement of this tyrosine in catalysis is indicated (Grouselle & Pudles, 1977; Coffe & Pudles, 1977), and one cysteinyl residue per subunit has been alkylated by a substrate-like reagent under appropriate conditions with concomitant loss of enzymatic activity (Otieno et al., 1977). In addition, lysyl residues have been implicated in the dissociation of yeast hexokinase into catalytically active monomers (Rossi et al., 1975). X-ray crystallographic studies (Steitz et al., 1976) have provided the conformation of hexokinase PII at a resolution of 3.5 Å for the dimer and 2.7 Å for the monomer, but the location of individual amino acid side chains is not known with any degree of certainty.

Two recent reports have strongly suggested that positively charged arginyl residues are likely to play a prominent role in the binding of anionic ATP to the kinase class of enzymes, and preliminary evidence was presented showing that yeast hexokinase in fact contains essential arginyl residues (Borders & Riordan, 1975; Riordan et al., 1977). This conclusion was arrived at from chemical modification studies with butanedione–borate, a system known to be highly selective for the modification of arginine (Riordan, 1973; Borders & Riordan, 1975). This paper presents a more detailed study on the chemical modification of arginyl residues in yeast hexokinase PII.

Materials and Methods

Yeast hexokinase PII was obtained from Worthington (HKP2, lots N4E534 and 56C482) and was further purified and stored as previously described (Peters & Neet, 1976). The specific activities of the enzyme preparations used in this report ranged from 410 to 720 units/mg under the conditions of the assay at pH 8.0. ATP, NADP, Tris,[1] Bicine, Hepes, Sephadex resins, and glucose-6-phosphate dehydrogenase (type V, citrate-free) were obtained from Sigma. Glucose 6-phosphate was a product of Boehringer Mannheim, while phenylglyoxal monohydrate and 2,3-butanedione were products of Aldrich. All other chemicals were the best grade commercially available.

Enzyme assays were performed spectrophotometrically at

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[1] Abbreviations used: Tris, tris(hydroxymethyl)aminomethane; Bicine, *N,N*-bis(2-hydroxyethyl)glycine; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

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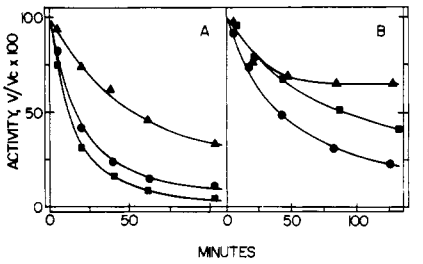

FIGURE 1: (A) Changes in the activity of yeast hexokinase PII, 40 μg/mL, in 50 mM borate, pH 8.3, vs. time as a function of butanedione concentration, 1 mM (▲), 3 mM (●), and 5 mM (■). The control retains full activity over this period of time. The enzyme was assayed at pH 8.0 as described in the text. (B) Changes in the activity of yeast hexokinase PII, 10 μg/mL, vs. time on modification with 1.5 mM butanedione in 50 mM borate, 1 mM dithiothreitol, pH 8.3. The modification was followed by assaying at pH 8.0 (●), at pH 6.5 in the presence of citrate (■), or at pH 6.5 in the absence of citrate (▲).

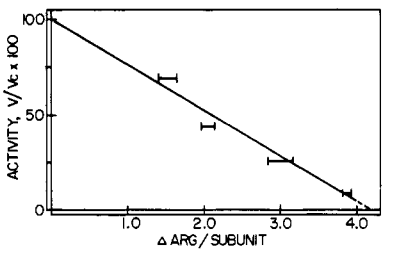

FIGURE 2: Correlation of inactivation of yeast hexokinase PII with arginine modification by butanedione. The enzyme, 1.6 mg/mL, was incubated with 3 mM butanedione, 50 mM borate, pH 8.3, and aliquots were withdrawn at approximately 10, 30, 50, and 120 min and subjected to gel filtration, assayed at pH 8.0, and further analyzed as described in the text. The number of arginines modified was determined by duplicate analysis of single samples at the various levels of enzyme activity and are given per enzyme subunit. The horizontal bars indicate the range of the duplicate analyses.

25 °C by coupling the reactions with glucose-6-phosphate dehydrogenase to follow the reduction of NADP at 340 nm. All assays contained in 1.00 mL the following concentrations of materials: 20 mM glucose, 8 mM $MgCl_2$, 0.5 mM NADP, and 3–5 units of glucose-6-phosphate dehydrogenase. Assays at pH 8.0 also contained 100 mM Tris-Cl and 2.0 mM MgATP, while those at pH 6.5 contained 100 mM Tris(hydroxyethyl)ammonium chloride and 1.0 mM MgATP. When citrate activation at pH 6.5 was measured, 0.5 mM citrate was included in the assay mixture. All reactions were initiated by the addition of hexokinase.

Chemical modifications of yeast hexokinase PII were carried out under conditions given in the figure and table legends. All modifications were carried out at 25 °C and were initiated by addition of an aliquot of a freshly prepared stock solution of modifying reagent in the appropriate buffer to a solution of the enzyme, in some cases together with substrates, in the same buffer. Activity is expressed as the ratio of the activity of the modified enzyme, V, to that of the control subjected to the same conditions but in the absence of modifying agent, V_c, multiplied by 100. In some experiments modification was monitored by assaying at pH 6.5, both in the presence and absence of 0.5 mM citrate, to determine the effect of modification on the substrate-induced slow transient process (Shill & Neet, 1971, 1975) and activation by citrate (Kosow & Rose, 1971; Shill & Neet, 1975). Activities at pH 6.5 were determined from the steady-state portion of the progress curves.

The modification of specific amino acid residues of hexokinase PII by butanedione, both in the absence and presence of substrates, was determined by amino acid analysis after acid hydrolysis. Aliquots of the reaction mixtures were separated from excess reagent, and substrates where present, by gel filtration on a Sephadex G-25 column (0.9 × 20 cm) equilibrated with 50 mM borate, pH 8.3. The hexokinase PII concentration of fractions containing protein was determined from the absorbance at 280 nm, using $A_{1\ cm}^{1\%} = 9.47$ (Schmidt & Colowick, 1973), and these fractions were immediately assayed for specific enzymatic activity. Aliquots of each were then hydrolyzed in evacuated, sealed ampoules with 6 N HCl at 110 °C for 18 h, and amino acid analyses were performed on a Beckman 120C amino acid analyzer.

Ultracentrifugal analysis of yeast hexokinase PII was carried out on a Beckman-Spinco Model E centrifuge (52 000 or 60 000 rpm, 20 °C) under conditions similar to those used for butanedione modification; i.e., 0.06 or 0.7 mg/mL enzyme, 50 mM borate, pH 8.3, in the presence or absence of substrates.

Results

Inactivation by Butanedione. The time course for the inactivation of hexokinase PII by butanedione in 50 mM borate, pH 8.3, depends on the concentration of reagent used (Figure 1A). The rate of inactivation is pseudo-first-order in butanedione, as reagent concentrations of 1, 3, and 5 mM result in half-lives for inactivation of 57, 20, and 13 min, respectively.

One feature of the modification of essential arginyl residues in enzymes by butanedione is that the modification is enhanced by borate, while a second is that the modification is reversible on removal of excess butanedione and borate (Riordan, 1973; Borders & Riordan, 1975; Borders & Wilson, 1976). The rate of inactivation of hexokinase PII is markedly reduced if modification is carried out in the absence of borate. Under conditions where the activity is reduced to 10% by 3 mM butanedione in 50 mM borate, 55% activity remains if the borate is replaced by Hepes. Inactivation of hexokinase PII by butanedione-borate is also reversible. If the enzyme is modified to 5% of the control activity by butanedione-borate and then gel filtered through a Sephadex G-25 column equilibrated with 50 mM Tris-Cl, pH 8.3, the activity returns to 70% of the control after six hours. If gel filtration is performed in borate, however, no reactivation is observed. These data strongly suggest that inactivation of hexokinase PII by butanedione is due to the modification of essential arginyl residues.

Protection against Butanedione Inactivation. Neither MgATP, 5 mM, nor glucose 6-phosphate, 5 mM, provides any protection against inactivation of hexokinase PII by 3 mM butanedione in 50 mM borate, pH 8.3, at 25 °C. Some protection is provided by 20 mM glucose, for, after 80 min modification, 35% of the original activity remains in the presence of glucose compared with only 11% activity in its absence. Significant protection against inactivation is observed when an equilibrium mixture of substrates is present, for when both glucose and MgATP are added to hexokinase PII before butanedione modification, 81% of the original activity remains after 80 min modification.

Correlation of Inactivation by Butanedione with Arginine Modification. Hexokinase PII is a dimer of apparently identical monomers and contains 18 arginyl residues per subunit (Schmidt & Colowick, 1973). Amino acid analysis was performed to identify the residues modified by treatment with butanedione-borate. The content of arginine decreases progressively with increasing inactivation. All other amino acid residues are unaffected. Extrapolation to complete inactivation indicates that loss of activity correlates with the modification of 4.2 arginyl residues per subunit (Figure 2).

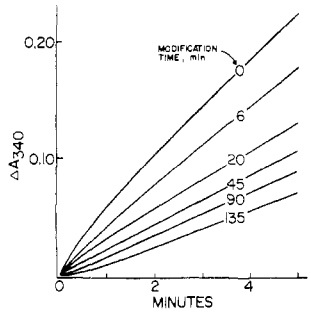

FIGURE 3: Progress curves for yeast hexokinase PII which is inactivated by 1.5 mM butanedione in 50 mM borate, 1 mM dithiothreitol, pH 8.3, for the indicated times. The assays were carried out at pH 6.5 in the absence of citrate as described in the text.

TABLE I: Correlation of Hexokinase PII Inactivation by Butanedione with Loss of Arginine, and Protection by Substrates.[a]

Enzyme	V/V_c × 100	Arg per subunit	Arg modified per subunit
Control	100	18.0	
+ Butanedione	35	14.7	3.3
+ Butanedione + MgATP	34	14.6	3.4
+ Butanedione + MgATP + glucose	80	15.9	2.1

[a] Hexokinase PII, 1 mg/mL, was incubated with 3 mM butanedione, 50 mM borate, pH 8.3, 25 °C, and then analyzed and assayed at pH 8.0 as described in the text.

In a separate experiment, hexokinase was treated with 3 mM butanedione for 30 min, both in the absence and presence of substrates, and amino acid analysis was performed as described above. In the absence of substrates, enzymatic activity is reduced to 35% of the control and approximately 3.3 arginyl residues per subunit are modified (Table I). The presence of 5 mM MgATP again provides no protection against inactivation and no protection against arginine modification. However, the simultaneous presence of both 5 mM MgATP and 20 mM glucose affords significant protection against both inactivation and arginine modification, for under these conditions the enzyme retains 80% of the native activity and only 2.1 arginyl residues per subunit are modified.

Effect of Butanedione on the Regulatory Properties of Hexokinase. When hexokinase PII is reacted with 1.5 mM butanedione, and the modification is followed by assaying at pH 8.0 and at pH 6.5 in the presence and absence of 0.5 mM citrate, some very interesting results are observed. Activity is lost at different rates when the same modification is followed by the three different assays (Figure 1B). This is most obvious after approximately 2 h of modification where the butanedione-treated enzyme shows 23% of the control activity when assayed at pH 8.0, 44% of the control activity when assayed at pH 6.5 in the presence of citrate, and has leveled off at 65% of the control activity when assayed at pH 6.5 in the absence of citrate.

Table II shows the specific activities of hexokinase PII at various times of modification by 1.5 mM butanedione, as assayed under the above conditions. The ratio of the activity at pH 8.0 to that at pH 6.5 in the absence of citrate decreases from an initial value of 10.8 to a value of 4.4 after 2 h of modification. Although the activity measured at pH 6.5 levels off with time of modification (Figure 1B), the specific activity is still much lower (4.4-fold less) than that measured at pH 8. Significantly, the citrate activation, when assays are carried out at pH 6.5, is still pronounced after extensive modification. The activation by citrate is initially 6.3-fold and is only reduced to a value of 5.1 after 2 h modification. Another interesting observation is that after 90 min modification by butanedione, the hexokinase shows a higher specific activity when assayed at pH 6.5 in the presence of citrate than it does when assayed at pH 8.0.

Modification by butanedione alters the kinetic slow transient process observed when the enzyme is assayed at pH 6.5 in the absence of citrate (Figure 3). The native enzyme shows an initial burst of activity which decreases to a slower steady-state activity after several minutes (Shill & Neet, 1971, 1975). As hexokinase is modified by 1.5 mM butanedione, the magnitude of the burst gradually decreases until after 45 min modification the assay is a linear process. At longer modification times the assay progress curves are characterized by a lag which increases to a steady state process after several minutes.

TABLE II: Inactivation of Hexokinase PII by Butanedione as Assayed Under Different Conditions.[a]

Modification time (min)	Spec act. (U/mg)			Activity ratio		
	8.0[b]	6.5[c]	6.5C[d]	8.0/6.5	8.0/6.5C	6.5C/6.5
0	492	45.5	285	10.8	1.73	6.3
30	263	27.8	187	9.5	1.41	6.7
60	159	19.9	144	7.3	1.10	6.6
90	104	19.0	109	5.5	0.95	5.7
120	76	17.2	88	4.4	0.86	5.1

[a] Hexokinase PII was incubated with 1.5 mM butanedione, 50 mM borate, 1 mM dithiothreitol, pH 8.3, 25 °C. [b] Assay mixture at pH 8.0 and 25 °C contained the following concentrations of reagents: 20 mM glucose, 2 mM MgATP, 8 mM $MgCl_2$, 0.5 mM NADP, 100 mM Tris-Cl, and 3 units of glucose-6-phosphate dehydrogenase. [c] Assay mixture at pH 6.5 and 25 °C contained the following concentrations of reagents: 20 mM glucose, 1 mM MgATP, 8 mM $MgCl_2$, 0.5 mM NADP, 100 mM tri(hydroxyethyl)ammonium chloride, and 5 units of glucose-6-phosphate dehydrogenase. [d] Assay mixture identical to that in footnote *c*, but in addition contained 0.5 mM citrate.

Hexokinase which had been modified in a manner comparable to that shown in Figure 3 was examined to determine if the negative cooperativity with ATP was retained. The enzyme was treated with 3 mM butanedione for 1 h during which time it was inactivated to 25% of the control activity as assayed at pH 8.0 and 78% of the control activity as assayed at pH 6.5. The modified enzyme was passed through a Sephadex G-25 column in 50 mM borate, pH 8.3, at 4 °C to remove reagents and was found to be stable over the time period of the ensuing kinetic analyses. Control enzyme was treated similarly except that the butanedione was omitted from the incubation. The double-reciprocal plot of ATP for the steady-state velocity of the modified was nonlinear and the Hill coefficient was determined to be 0.42 as compared with 0.15 for the control enzyme in the experiment. K_M values extrapolated either from the low or from the high ATP concentration regions were similar for the modified and native enzymes. Modified enzyme showed a lag transient comparable to that of Figure 3 at all ATP concentrations. These results indicate that hexokinase with 3–4 arginines modified by butanedione still possesses negative cooperativity with respect to ATP, even though the activity as measured at pH 8.0 is only 25% of the control and even though the enzyme has been entirely converted from a burst-type behavior to a lag transient as determined by assay at pH 6.5 (cf. Figure 3).

Inactivation by Phenylglyoxal. Hexokinase PII is very sensitive to inactivation by phenylglyoxal in 50 mM Bicine, pH 8.3, with the rate of inactivation dependent on the concentration of phenylglyoxal used (Figure 4). A phenylglyoxal concentration of 2 mM reduces the activity by 50% in 17 min, and after 1 h the activity is reduced to 20% of the control.

The effects of the presence of substrates on the inactivation of hexokinase pII by 2 mM phenylglyoxal are very similar to those obtained on butanedione modification. Magnesium ATP provides very little protection, while glucose affords significant protection, for in its presence 45% of the native activity remains after 90 min of modification while only 11% activity remains in its absence. When both MgATP and glucose are present very little inactivation by phenylglyoxal is observed, as 75% activity remains after 90 min of modification.

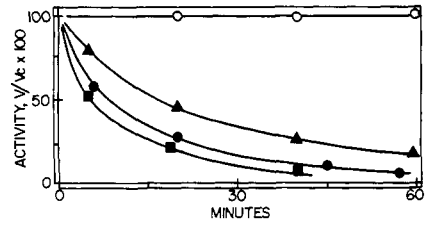

FIGURE 4: Changes in the activity of yeast hexokinase PII, 40 μg/mL, in 50 mM Bicine, pH 8.3, vs. time as a function of phenylglyoxal concentration: 2 mM (▲), 3 mM (●), and 4 mM (■). The control (○) retains full activity over this period of time. The enzyme was assayed at pH 8.0 as described in the text.

Discussion

Only very recently has information become available about residues involved in the active site of yeast hexokinase. It is now known that one glutamyl residue per subunit is near the active site (Pho et al., 1977), as are one serine (Menezes & Pudles, 1977), one tyrosine (Coffe & Pudles, 1977; Grouselle & Pudles, 1977), and one cysteinyl residue per subunit (Otieno et al., 1977). Lysyl residues have been implicated in monomer–dimer association, but are not thought to be involved at the active site (Rossi et al., 1975). Histidine residues have been ruled out as being at the active center (Grouselle et al., 1973). The present study sheds additional light on the types and role of residues at the active site.

Butanedione is highly selective for the modification of arginyl residues in proteins. The modification is enhanced by borate buffer, which likely stabilizes the adduct between the guanidino group and butanedione, and the modification is reversible on removal of excess butanedione and borate (Riordan, 1973; Borders & Riordan, 1975). Phenylglyoxal is another α-dicarbonyl reagent which has also been shown to be highly selective for the modification of arginyl residues in proteins (Takahashi, 1968). Hexokinase PII is rapidly inactivated by butanedione in borate buffer (Figure 1A) and by phenylglyoxal (Figure 4). When these data are coupled to the observations that inactivation by butanedione is augmented by borate and reversible on the complete removal of borate, they strongly suggest that the modification of essential arginyl residues is responsible for inactivation. Amino acid analysis of hexokinase PII inactivated by butanedione–borate indicates that indeed only arginine residues are lost, and that complete inactivation correlates with the modification of approximately 4.2 of the 18 arginyl residues per subunit (Figure 2).

It has been well substantiated that positively charged arginyl residues play a very general role in the functional binding of anionic substrates and cofactors to enzymes (Lange et al., 1974; Riordan et al., 1977). Along this line, it has been suggested that arginyl residues are probably involved in the binding of ATP to kinases and other classes of ATP-utilizing enzymes (Borders & Riordan, 1975), a contention which is substantiated by the findings that arginine is important in binding ATP in creatine kinase (Borders & Riordan, 1975), glutamine synthetase, carbamoyl phosphate synthetase (Powers & Riordan, 1975), adenylate kinase (Berghäuser, 1975), mitochondrial ATPase (Marcus et al., 1976), and phosphoglycerate kinase (Rogers & Weber, 1977). Thus it is somewhat surprising that MgATP alone offers no protection against inactivation of hexokinase PII by either butanedione–borate or phenylglyoxal. However, although the kinetic mechanism is random, the dissociation constant for MgATP from the enzyme–nucleotide complex is greater than 5 mM, whereas it is about 25-fold lower for the ternary complex (enzyme:glucose:MgATP) (Danenberg & Cleland, 1975). Thus, in the absence of glucose, very little binding of MgATP to hexokinase occurs and no protection against inactivation or arginine modification by butanedione or phenylglyoxal is observed. The protection against inactivation afforded by glucose is likely due to either a shielding of the active site arginyl residue(s) upon binding of sugar or an induced conformational change which makes these arginine(s) less accessible to reagent. The latter possibility is likely since it has been reported that glucose causes marked conformational changes in solution (De la Fuente et al., 1970; Roustan et al., 1974; Peters & Neet, unpublished results) and in the crystallographic structure (Fletterick et al., 1975).

Glucose is also known to promote dissociation of hexokinase (Schulze & Colowick, 1969), but protection by glucose is unlikely to result from a shift in the monomer–dimer equilibrium. Ultracentrifugal analysis under the conditions (low protein concentration) of butanedione modification indicates that hexokinase is already a monomer in the absence of glucose ($s_{20,w}$ = 3.6 S at 60 or 80 μg/mL). The possibility that glucose protection against butanedione inactivation may be due to its interaction with borate can be ruled out by the observation that glucose also provides significant protection against inactivation by phenylglyoxal in a system lacking borate entirely.

The markedly increased protection afforded by MgATP in the presence of glucose is likely due to greatly increased binding of nucleotide, subsequent to the binding of glucose, and the shielding of essential arginyl residues involved in binding the anionic polyphosphate portion of ATP. If this interpretation is correct, then the experiments involving amino acid analysis indicate that the combined presence of glucose and MgATP protects 1.2 arginyl residues per subunit from modification (2.1 vs. 3.3) coincident with the protection of approximately half the enzymatic activity (80% vs. 35%) (Table I). Extrapolated to 100% protection, this would indicate that 2 to 3 arginyl residues per subunit are protected from modification on the binding of both hexokinase substrates. Under the conditions used for the determination of the number of modified arginyl residues (1 mg/mL hexokinase in Table I), ultracentrifugal analysis indicates that the enzyme exists mainly as the dimer (at 0.7 mg of enzyme/mL) either in the presence ($s_{20,w}$ = 5.7 S) or absence ($s_{20,w}$ = 5.5 S) of 20 mM glucose plus 5 mM MgATP.

It is apparent that arginyl residues play a role in the substrate-induced slow transient process of yeast hexokinase PII (Shill & Neet, 1971, 1975). The burst-type kinetic assay, seen when the native enzyme is assayed at pH 6.5, decreases progressively with increased time of modification (Figure 3). After 45 min of modification by 1.5 mM butanedione (at which time the enzyme retains 48% of the control activity when assayed at pH 8.0), the pH 6.5 assay is linear. At longer modification

times, the pH 6.5 assay is characterized by a lag followed by a more rapid steady-state process. The lag is not due to reactivation of the modified enzyme by reversal of the butanedione modification, because, when the modification is followed by assaying at pH 8.0, linear assays are observed through the entire course of modification, and reactivation of the modified enzyme by removal of excess butanedione and borate is faster at pH 8.0 than at pH 6.5 (C. L. Borders, Jr., unpublished results). The shift from the normal burst to the lag suggests that the modification of the arginine residues shifts the initial population of enzyme to a less active conformation. Despite this shift of the initial form, the negative cooperativity of ATP in the steady state is retained. Thus, the cooperativity appears to be more closely related to the distribution of the enzyme in the steady state, which is unperturbed by the arginine modification, and less related to the initial state or the mode of transition from the initial to the steady state. It is unlikely that the arginyl residue(s) responsible for this alteration are the same ones that are related to the marked decrease in activity at pH 8.0.

It is significant that, while butanedione modification alters the slow burst-type transient of hexokinase PII (Figure 3), it has very little effect on citrate activation (Table II). If anionic citrate activates by binding to a specific regulatory site in the enzyme, these results suggest that either arginyl residues are not involved in citrate binding or, if arginyl residues are involved, they are modified much more slowly than residues at the catalytic site or at the site controlling negative cooperativity with ATP under the conditions of the experiment. Thus, although arginine modification affects the initial distribution of enzyme forms, the addition of citrate is still capable of shifting the steady state distribution of enzyme back toward more active forms. It has been suggested that citrate activation, negative cooperativity with ATP, and the kinetic slow transient are coupled regulatory properties of yeast hexokinase PII (Peters & Neet, 1977), but the results of butanedione modification indicate that these processes can be partially decoupled by arginine modification. These results are similar in many ways to the results reported for nitration of tyrosine residues of hexokinase (Coffe & Pudles, 1977), i.e., an effect on the slow transition of the enzyme but not on the citrate activation or negative cooperativity. A major difference is that arginine modification produces a lag transient whereas the tyrosine modification produces a nearly linear progress curve. These similarities and differences in the results from two different chemical modifications raise interesting implications for the molecular basis of the structural transitions that occur in yeast hexokinase.

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