# Kinetic Studies of the Brain Hexokinase Reaction. A Reinvestigation with the Solubilized Bovine Enzyme*

Martha Copley and Herbert J. Fromm

ABSTRACT: Initial rate experiments were carried out with solubilized bovine brain hexokinase prepared according to the procedure of Schwartz and Basford (Schwartz, G. P., and Basford, R. E. (1967), *Biochemistry 6*, 1070) from the glucose side of the reaction. Investigations with product inhibitors adenosine 5′-diphosphate and glucose 6-phosphate and substrate inhibitors adenosine 5′-monophosphate and mannose were undertaken. There appears to be a difference in the Michaelis constant for adenosine 5′-triphosphate and in the dissociation constant for the complex enzyme–glucose 6-phosphate between the solubilized and particulate enzymes. Some differences were also noted in the product inhibition patterns for the solubilized and particulate hexokinases; however, the results are in basic agreement with the data obtained with the mitochondrial enzyme in 1962 (Fromm, H. J., and Zewe, V. (1962a), *J. Biol. Chem. 237*, 1661).

The mechanism of action of brain hexokinase, as alluded to from initial velocity experiments, is consistent with a Ping-Pong pathway of enzyme and substrate interaction.

In 1962, we reported studies on the kinetics and mechanism of action of the enzyme hexokinase (ATP[1]: D-hexose-6-phosphotransferase, EC 2.7.1.1) from bovine brain tissue (Fromm and Zewe, 1962a). This enzyme was shown to be associated with the mitochondrial fraction of brain and was purified for the kinetic studies by the procedure of Crane and Sols (1953). A number of investigators have reported the solubilization of brain hexokinase in the last few years (Moore and Strecker, 1963; Jaganathan, 1963; Rose and Warms, 1967; Schwartz and Basford, 1967).

The mechanism of action of hexokinase from different sources, as inferred from initial rate investigations, does not appear to be the same. The yeast enzyme, for example, requires that substrates glucose and ATP be present simultaneously on the enzyme before product formation can occur (Fromm and Zewe, 1962b; Zewe *et al.*, 1964; Fromm *et al.*, 1964). On the other hand, kinetic studies of brain hexokinase (Fromm and Zewe, 1962a) and a muscle hexokinase isozyme which is believed to be of the brain type (Katzen and Schimke, 1965), appear to exhibit a Ping-Pong pathway of enzyme and substrate interaction (Hanson and Fromm, 1965).

The recent elegant investigations of Schwartz and Basford (1967) on the solubilization, purification, and kinetics of bovine brain hexokinase suggest certain differences between the solubilized and mitochondrial enzymes. Although the Michaelis constant ($K_m$) for glucose appears to be approximately the same for these enzymes, soluble hexokinase has a $K_m$ for ATP which is about ten times greater than that reported for the enzyme associated with the mitochondria (Fromm and Zewe, 1962a). Initial rate studies of the former investigators are consistent with either a Ping-Pong mechanism or a sequential mechanism for the solubilized enzyme. When glucose was varied and initial velocity was measured at different fixed concentrations of ATP, data which are in harmony with a Ping-Pong mechanism were obtained (Schwartz and Basford, 1967). On the other hand, when ATP was the varied substrate and initial rates were determined at different levels of glucose, findings which are in agreement with a sequential mechanism were reported (Schwartz and Basford, 1967). It is indeed difficult to rationalize these results with the usual types of mechanisms of enzyme action proposed by Alberty (1953) for two substrate systems. Because of these factors as well as the alteration in $K_m$ for ATP with the soluble enzyme relative to that for the particulate enzyme, a reinvestigation of the kinetics and mechanism of brain hexokinase as solubilized by Schwartz and Basford (1967) was undertaken. The results to be reported below appear to be in basic agreement with the findings published by Fromm and Zewe (1962a).

## Experimental Section

*Materials.* D-Glucose and D-mannose were obtained from Pfanstiehl Laboratories, Inc. Glucose-6-P dehydrogenase and yeast hexokinase were products of

* From the Department of Biochemistry and Biophysics, Iowa State University of Science and Technology, Ames, Iowa 50010. *Received July 31, 1967.* Journal Paper J-5561 and Project No. 1666 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. This research was supported in part by Research Grant AM-11041 from the National Institutes of Health, U. S. Public Health Service.

[1] Abbreviations used: AMP, ADP, and ATP, adenosine 5′-mono-, di-, and triphosphates; TPN, oxidized triphosphopyridine nucleotide; DPNH, reduced diphosphopyridine nucleotide; glucose-6-P, glucose 6-phosphate.

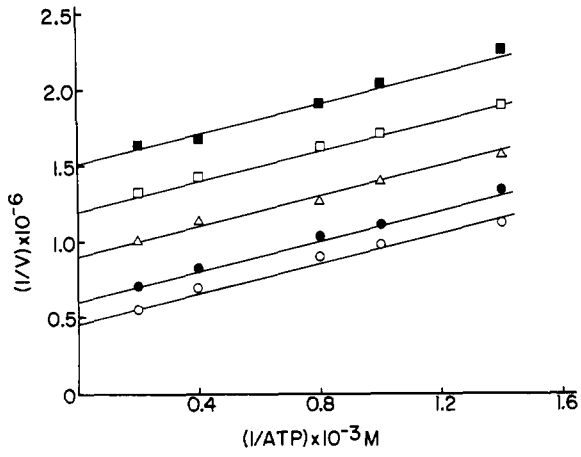


FIGURE 1: Plot of reciprocal of initial velocity ($v$) *vs.* reciprocal of molar concentration of ATP. Glucose concentrations were held constant at 100 (○), 50 (●), 25 (△), 17 (□), and 12.5 μM (■). $v$ was determined as a function of ATP concentration which was varied in the concentration range from 5.0 to 0.71 mM. Velocities are expressed as the molar concentration of glucose-6-P formed in the reaction mixture over a period of 1 min after addition of enzyme. Other details of the experimental protocol are given under Experimental Procedure.

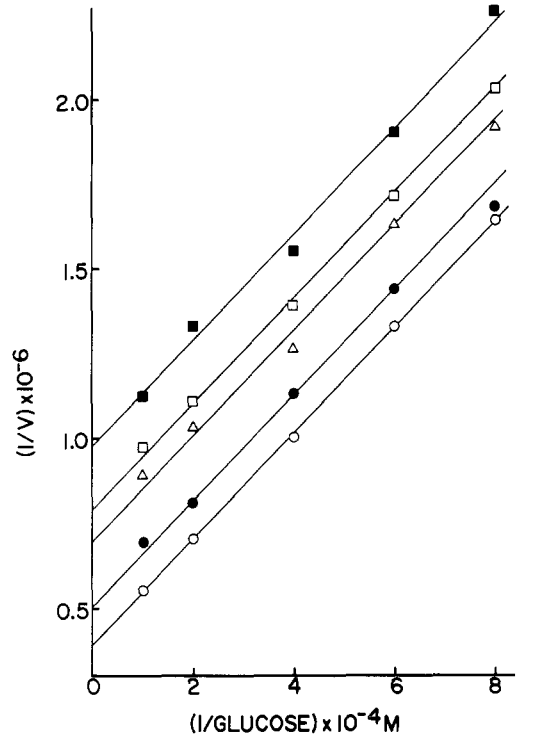


FIGURE 2: Plot of reciprocal of initial reaction velocity ($v$) *vs.* reciprocal of molar concentration of glucose. ATP concentrations were held constant at 5.0 (○), 2.5 (●), 1.25 (△), 1.0 (□), and 0.71 mM (■). $v$ was determined as a function of glucose concentration which was varied in the concentration range from 100 to 12.5 μM. Other experimental details are presented in the legend to Figure 1.

C. F. Boehringer und Soehne. ATP, ADP, AMP, TPN, and glucose-6-P were purchased from Sigma Chemical Co. Ion-low water, obtained by passing distilled water through a Rohm and Hass Amberlite MB-3 resin, was used to prepare all reagents. Bovine brain hexokinase was purified by the method of Schwartz and Basford (1967) and had a specific activity of 30. Bovine serum albumin (1 mg/ml) was added to stabilize the enzyme preparation.

*Methods.* Substrates, products, and inhibitors of the hexokinase reaction were assayed enzymatically as described elsewhere (Fromm and Zewe, 1962a,b; Zewe *et al.*, 1964; Fromm *et al.*, 1964). Initial velocity measurements were made in a Cary recording spectrophotometer, Model 15 (0–0.1 slide wire). The reaction mixture samples were incubated at 28°, and the temperature of the spectrophotometer cell housing was maintained at 28° by circulating water from a temperature-controlled bath through thermospacers. Reactions were initiated by addition of enzyme, and initial rates were determined with a coupled assay system containing glucose-6-P dehydrogenase and TPN as described elsewhere (Fromm and Zewe, 1962a). In those experiments in which glucose-6-P was used as a product inhibitor, product ADP was measured using a coupled enzymatic assay system (Wellner *et al.*, 1966).[2]

[2] The procedure for the continuous spectrophotometric assay of ADP as described by Wellner *et al.* (1966) was modified as follows. The enzymes lactate dehydrogenase and pyruvate kinase were dialyzed before use and the concentrations of phosphoenolpyruvate, DPNH, and KCl were 0.5, 0.05, and 10 mM, respectively.

Velocity in all kinetic experiments to be reported here is expressed as molarity of product formed per minute. Reactions were carried out in 0.032 M Tris buffer (pH 7.6).

The concentration of $Mg^{2+}$ (as $MgSO_4$) was adjusted in each reaction mixture so that the concentration of free $Mg^{2+}$ was essentially 1 mM. Calculations used to determine the proper $Mg^{2+}$:ATP ratio were made using a value of 20,000 for the stability constant of $MgATP^{2-}$ (O'Sullivan and Perrin, 1964). When ADP and AMP were used as inhibitors, additional $Mg^{2+}$ was added assuming stability constants of 2000 for $MgADP^{-}$ and 100 for MgAMP (Bock, 1960) so as to maintain the free $Mg^{2+}$ concentration at 1 mM.

## Results

*Initial Velocity Experiments.* In Figures 1 and 2 are presented data in the form of Lineweaver–Burk (1934) plots of reciprocal of initial reaction velocity as a function of reciprocal of substrate concentration. Examination of these data suggests that the curves are parallel. Qualitatively, very similar findings were obtained with mitochondrial hexokinase (Fromm and

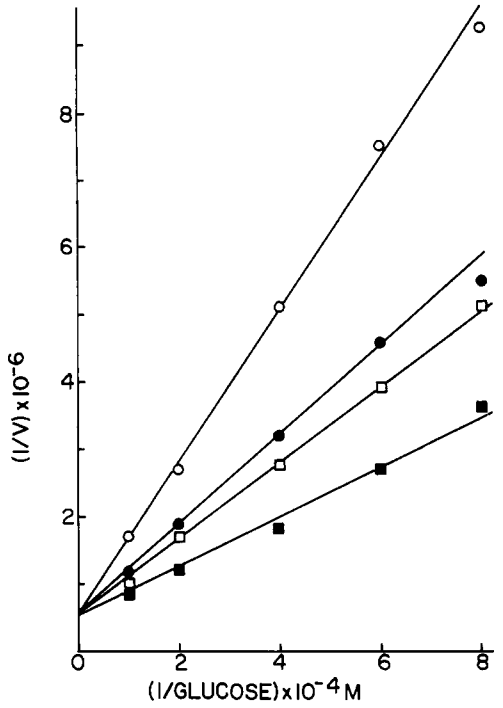


FIGURE 3: Plot of reciprocal of initial reaction velocity ($v$) *vs.* reciprocal of molar concentration of glucose in the presence and absence of mannose. ATP concentration was held constant at 1.78 mM and glucose varied in the concentration range 100–12.5 μM. Mannose concentrations are none (■), 0.16 (□), 0.24 (●), and 0.48 mM (○). Other experimental details are presented in the legend to Figure 1.

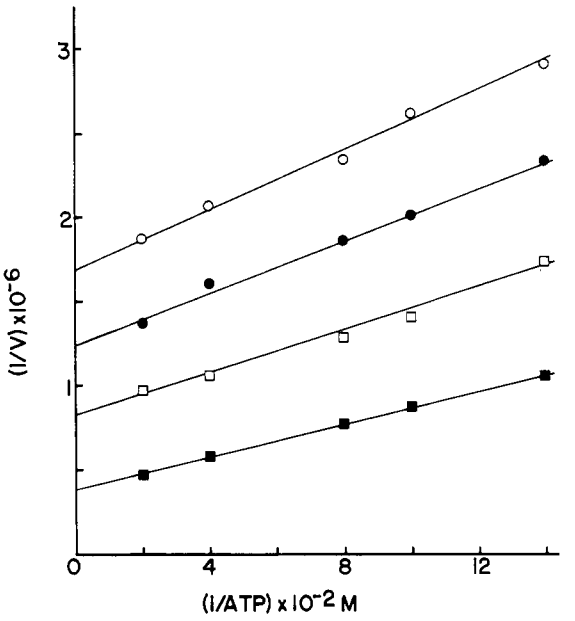


FIGURE 4: Plot of reciprocal of initial reaction velocity ($v$) *vs.* reciprocal of molar concentration of ATP in the presence and absence of mannose. Glucose concentration was held constant at 96 μM and ATP varied in the concentration range 5.0–0.71 mM. Mannose concentrations are none (■), 0.1 (□), 0.2 (●), and 0.3 mM (○). Other experimental details are presented in the legend to Figure 1.

Zewe, 1962a). When the concentration of ATP was varied in the range 0.3–1.8 mM data similar to those illustrated in Figures 1 and 2 were obtained. A replot of the results shown in the figures permitted calculation of the Michaelis constants for glucose and ATP. The values obtained were 52 μM and 1.7 mM, respectively. These values are in good agreement with the parameters reported by Schwartz and Basford (1967); however, our earlier investigations with mitochondrial hexokinase gave Michaelis constants of 64.5 μM and 0.34 mM for glucose and ATP, respectively. It appears that although the $K_m$ for glucose is not appreciably altered by the solubilization of bovine brain hexokinase, this process increases the $K_m$ for ATP by a factor of five.

The results of Figures 1 and 2 would appear to exclude sequential mechanisms for brain hexokinase. Furthermore, these data are consistent with, but certainly not proof of, a Ping-Pong pathway of enzyme and substrate interaction. The simplest mechanism for hexokinase is shown in Scheme I. The steady-state rate equation for this mechanism, when transposed into the form suggested by Dalziel (1957), is

$$\frac{E_0}{v} = \phi_0 + \frac{\phi_1}{A} + \frac{\phi_2}{B} \qquad (1)$$

where $E_0$, $v$, $A$, $B$, $\phi_0$, $\phi_1$, and $\phi_2$ represent total enzyme, initial velocity, ATP, glucose, $[(1/k_3) + (1/k_7)]$, $[(k_2 + k_3)/k_1k_3]$, and $[(k_6 + k_7)/k_5k_7]$, respectively.

For the pathway outlined in Scheme I, it is assumed that E′ is a phosphoryl-enzyme intermediate. Another possible mechanism which could account for the data, but which will not be discussed in detail at this time, is one suggested by Najjar and McCoy (1958) for yeast hexokinase. For this mechanism E and E′ represent enzyme–glucose and enzyme–glucose-6-P, respectively.

Scheme I

$$E + A \underset{k_2}{\overset{k_1}{\rightleftarrows}} EA \underset{k_4}{\overset{k_3}{\rightleftarrows}} E' + C$$

$$E' + B \underset{k_6}{\overset{k_5}{\rightleftarrows}} EB \underset{k_8}{\overset{k_7}{\rightleftarrows}} E + D$$

*Mannose Inhibition.* Studying initial rates in the presence and absence of competitive inhibitors of substrates has proven to be a useful approach in analyzing the mechanism of enzyme action (Fromm, 1964, 1967). Mannose has been reported to be a competitive inhibitor of glucose in a number of different hexokinase systems (Fromm and Zewe, 1962a,b). It was for these

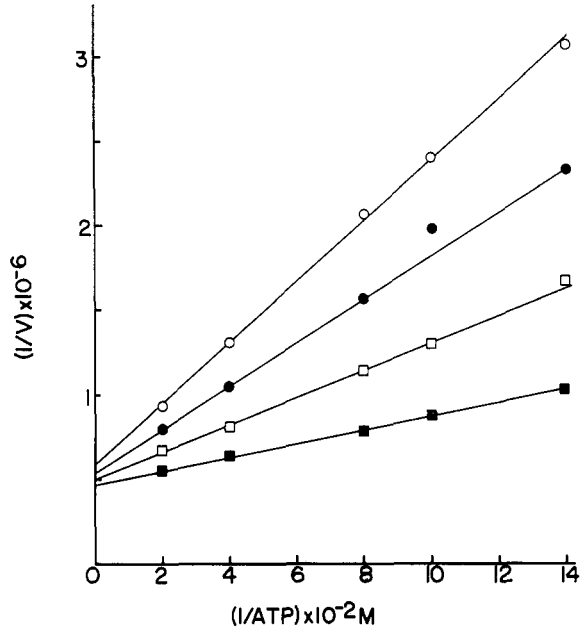


FIGURE 5: Plot of reciprocal of initial reaction velocity ($v$) *vs.* reciprocal of molar concentration of ATP in the presence and absence of AMP. Glucose concentration was held constant at 96 μM and ATP varied in the range 5.0–0.71 mM. AMP concentrations are none (■), 6 (□), 12 (●), and 18 mM (○). Other experimental details are presented in the legend to Figure 1.

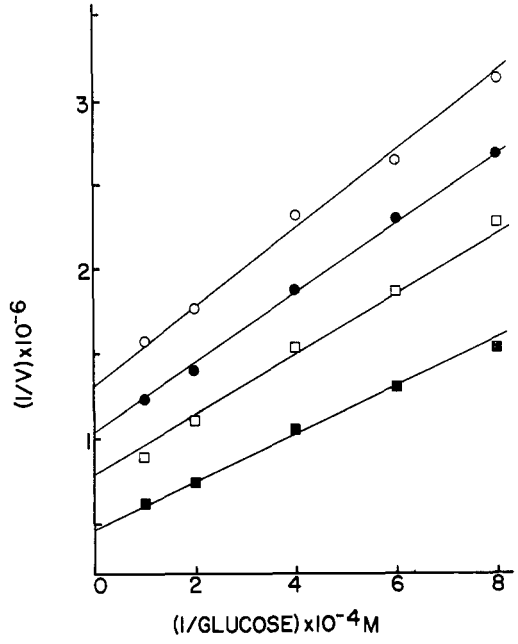


FIGURE 6: Plot of reciprocal of initial reaction velocity ($v$) *vs.* reciprocal of molar concentration of glucose in the presence and absence of AMP. ATP concentration was held constant at 2.5 mM and glucose varied in the range 100–12.5 μM. AMP concentrations are none (■), 10 (□), 20 (●), and 30 mM (○). Other experimental details are presented in the legend to Figure 1.

reasons that experiments with mannose were undertaken.

In Figures 3 and 4 are shown results of kinetic studies in which mannose was used. The data of Figure 3 indicate clearly that mannose is a competitive inhibitor of glucose with the solubilized hexokinase system when product glucose-6-P is assayed for initial rate measurements. If one extrapolates the initial velocity data of Figure 4, it can be shown that mannose inhibition relative to ATP is mixed.

These findings are readily explained by evoking the mechanism of Scheme I and by adding the step

$$E' + \text{mannose} \underset{k_{10}}{\overset{k_9}{\rightleftharpoons}} EM \underset{k_{12}}{\overset{k_{11}}{\rightleftharpoons}} E + \text{mannose-6-P}$$

Equation 2 can be derived by making the usual steady-state assumptions for the effect of mannose (M).

$$\frac{E_0}{v} = \phi_0 + \frac{\phi_1}{(\text{ATP})} + \frac{\phi_2}{(\text{glucose})}\left[1 + \frac{k_9(\text{M})}{(k_{10} + k_{11})} \times \left(1 + \frac{k_{11}}{k_3}\right)\right] + \frac{k_9 k_{11}\phi_1\phi_2(\text{M})}{(k_{10} + k_{11})(\text{ATP})(\text{glucose})} \quad (2)$$

It is possible from eq 2 and the data of Figure 4 to determine the lower limit of the dissociation constant of the complex EM. This value was calculated to be 21 μM. In the case of bovine mitochondrial hexokinase, the analogous value was approximately 36 μM (Fromm and Zewe, 1962a).

*AMP Inhibition.* AMP has been reported to be a competitive inhibitor for ATP with yeast hexokinase (Fromm and Zewe, 1962b) and a mixed inhibitor relative to ATP with the brain hexokinase isozyme in muscle tissue (Hanson and Fromm, 1965). Figures 5 and 6 depict results obtained when kinetic studies were undertaken in the presence of this nucleotide inhibitor. Data from these double-reciprocal plots suggest that AMP acts as a mixed inhibitor of both hexokinase substrates. These results are consistent with the mechanism outlined in Scheme I, if the rather reasonable assumption is made that AMP can bind to the enzyme at both the ATP and ADP sites. Under these conditions, AMP (I) can react as follows

$$E + I = EI,\ K_1;\quad E' + I = E'I,\ K_2$$

The rate expression which accounts for these effects is given by eq 3.

$$\frac{E_0}{v} = \phi_0 + \frac{\phi_1}{(\text{ATP})}\left(1 + \frac{I}{K_1}\right) + \frac{\phi_2}{(\text{glucose})}\left(1 + \frac{I}{K_2}\right) \quad (3)$$

The dissociation constants $K_1$ and $K_2$ have values of 4.0 and 52.5 mM, respectively. It is this latter value, which is relatively high, that is responsible for the

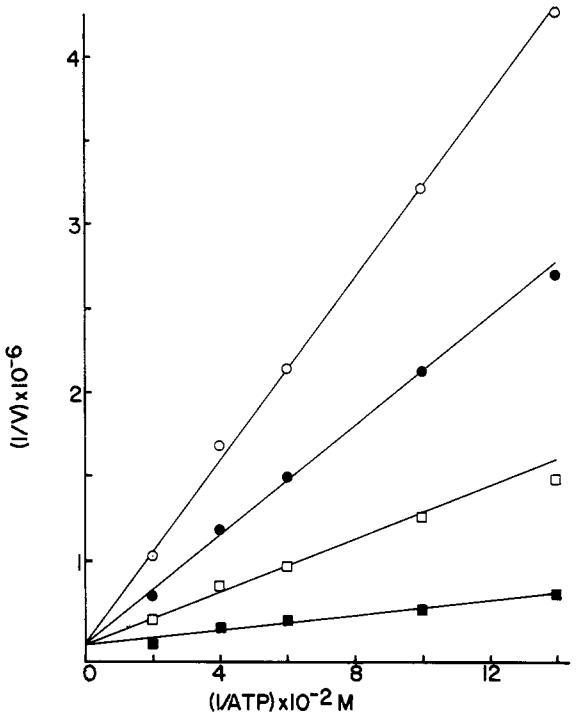


FIGURE 7: Plot of reciprocal of initial reaction velocity ($v$) *vs.* reciprocal of molar concentration of ATP in the presence and absence of glucose-6-P. Glucose concentration was held constant at 100 $\mu$M and ATP varied in the range 5.0–0.71 mM. Glucose-6-P concentrations are none (■), 22 (□), 44 (●), and 66 $\mu$M (○). Other experimental details are presented in the legend to Figure 1.

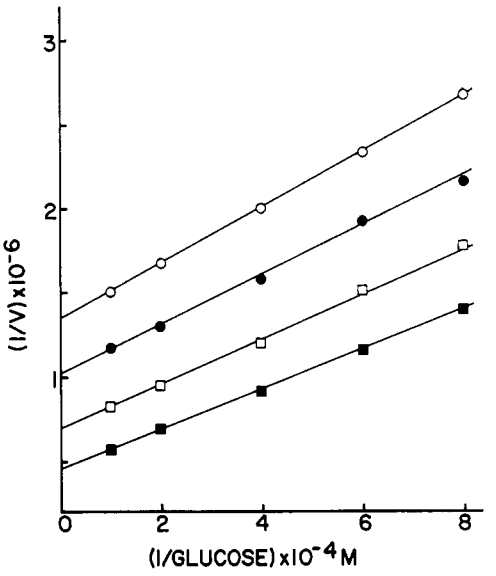


FIGURE 8: Plot of reciprocal of initial reaction velocity ($v$) *vs.* reciprocal of molar concentration of glucose in the presence and absence of glucose-6-P. ATP concentration was held constant at 2.5 mM and glucose varied in the range 100–12.5 $\mu$M. Glucose-6-P concentrations are none (■), 22 (□), 44 (●), and 66 $\mu$M (○). Other experimental details are presented in the legend to Figure 1.

small intercept increments in Figure 5 and the small slope changes in Figure 6. It is noteworthy that similar results were observed for the soluble brain-type hexokinase isozyme of rat skeletal muscle (Hanson and Fromm, 1965).

*Glucose-6-P Inhibition.* Studies of the effects of product inhibitors on the initial rates of enzyme-catalyzed reactions have proven to be a valuable method for reaching conclusions regarding reaction mechanisms (Alberty, 1958). In the reinvestigation of the kinetics of bovine brain hexokinase, experiments were undertaken with both glucose-6-P and ADP. With mitochondrial brain hexokinase, it was observed that glucose-6-P was a true competitive inhibitor of ATP and essentially an uncompetitive inhibitor of glucose (Fromm and Zewe, 1962a). Other investigators have also found that glucose-6-P acts as a competitive inhibitor of ATP with a number of mammlian hexokinases (Uyeda and Racker, 1965; Grossbard and Schimke, 1966).

In Figures 7 and 8 are depicted data on the effect of glucose-6-P as a product inhibitor of solubilized brain hexokinase. The results of Figure 7 support the view that glucose-6-P is a competitive inhibitor of ATP; however, inhibition relative to glucose appears to be mixed. If we call D in Scheme I glucose-6-P, then the steady-state rate equation for inhibition by this product is

$$\frac{E_0}{v} = \phi_0 + \frac{\phi_1}{(\text{ATP})}\left[1 + \frac{k_8(\text{D})}{k_7}\right] + \frac{\phi_2}{(\text{glucose})} + \frac{k_6 k_8 \phi_1(\text{D})}{k_5 k_7(\text{ATP})(\text{glucose})} \quad (4)$$

Cursory examination of the results of Figures 7 and 8 indicates that the data are in agreement with eq 4. The difference between the present findings and those reported for mitochondrial hexokinase (Fromm and Zewe, 1962a) appears to be largely quantitative. In the kinetic studies with mitochondrial hexokinase, glucose-6-P was reported to be an uncompetitive inhibitor of glucose (Fromm and Zewe, 1962a); however, the results of Figure 8 suggest this inhibition to be mixed with the solubilized enzyme. It is possible that the small slope changes in the presence of glucose-6-P, shown in Figure 8, were not apparent in the earlier studies because a less sensitive assay was employed at that time. However, it is also possible that the last term of eq 4 is smaller relative to other terms in the rate equation with the particulate enzyme as compared to the solubilized brain hexokinase. The value calculated for the dissociation constant of the enzyme–glucose-6-P complex ($k_7/k_8$) is 11.7 $\mu$M. This figure is lower by a factor of 5.7 than the analogous value reported for the particulate enzyme (Fromm and Zewe, 1962a).

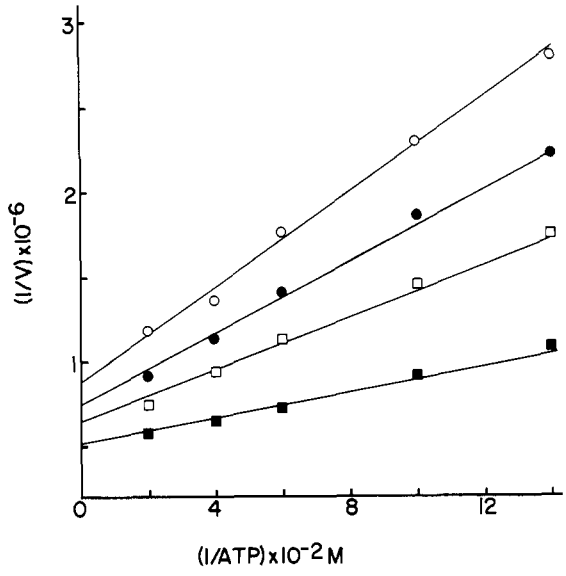


FIGURE 9: Plot of reciprocal of initial reaction velocity ($v$) *vs.* reciprocal of molar concentration of ATP in the presence and absence of ADP. Glucose concentration was held constant at 100 $\mu$M and ATP varied in the range 5.00–0.71 mM. ADP concentrations are none (■), 1.2 (□), 2.4 (●), and 3.6 mM (○). Other experimental details are presented in the legend to Figure 1.

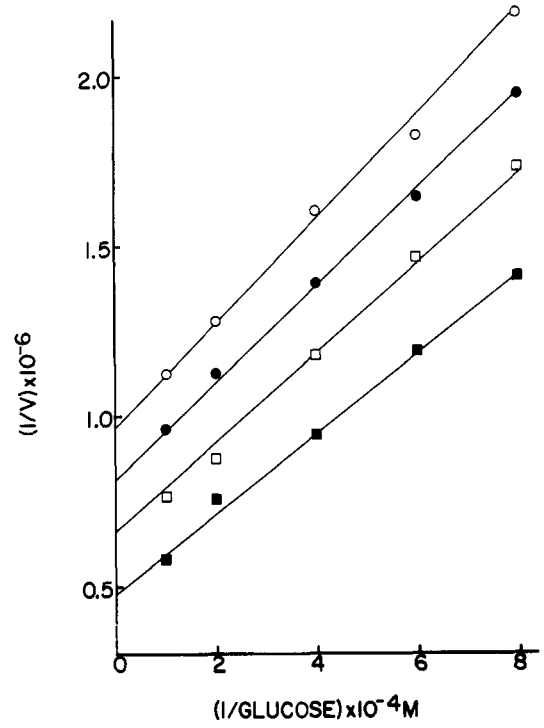


FIGURE 10: Plot of reciprocal of initial reaction velocity ($v$) *vs.* reciprocal of molar concentration of glucose in the presence and absence of ADP. ATP concentration was held constant at 2.5 mM and glucose varied in the range 100–12.5 $\mu$M. ADP concentrations are none (■), 1.2 (□), 2 4 (●), and 3.6 mM (○). Other experimental details are presented in the legend to Figure 1.

*ADP Inhibition.* Figures 9 and 10 illustrate inhibition patterns for brain hexokinase with product ADP. The double-reciprocal plots show that ADP acts as a mixed inhibitor for both substrates. These data may readily be explained on the basis of the mechanism outlined in Scheme I where ADP is taken to be product C. The rate equation for the effect of ADP on the initial velocity of the forward reaction is shown in eq 5.

$$\frac{E_0}{v} = \phi_0 + \frac{\phi_1}{(\mathrm{ATP})}\left(1 + \frac{\mathrm{C}}{K_1}\right) + \frac{\phi_2}{(\mathrm{glucose})} \times \left(1 + \frac{k_4(\mathrm{C})}{k_3}\right) + \frac{k_2 k_4 \phi_2(\mathrm{C})}{k_1 k_3(\mathrm{ATP})(\mathrm{glucose})}\left(1 + \frac{\mathrm{C}}{K_1}\right) \quad (5)$$

In the derivation of eq 5 it was assumed that ADP may bind at the ATP site on hexokinase and thus form the abortive binary complex (enzyme–ADP). The dissociation constant for this complex is taken to be $K_1$. Cursory examination of the data presented in Figures 9 and 10 suggest that they are consistent with eq 5. It was possible to calculate $k_3/k_4$ and the inhibition constant $K_1$ from the kinetic results. The values determined were 2.44 and 1.56 mM, respectively.

With mitochondrial hexokinase, ADP acted as a mixed inhibitor of ATP and as an uncompetitive inhibitor of glucose (Fromm and Zewe, 1962a). The difference between the data of the earlier study and those reported here could be due to the higher concentrations of ADP which were used in the present investigation.

## Discussion

The results of the present investigation suggest that the kinetic mechanism of action of bovine brain hexokinase is essentially unaltered when the mitochondrial enzyme is solubilized. Although there appears to be certain significant differences between the two states of hexokinase, *i.e.*, alteration in the $K_m$ for ATP and in the dissociation constant of the enzyme–glucose-6-P complex, most of the properties of the particulate enzyme are unaffected by solubilization. The question arises as to whether those changes which are observed are a reflection of removal of the enzyme from the mitochondrion *per se*, or whether they are caused by Triton X-100 and diethylaminoethylcellulose treatment. It is clear that an answer to this problem must await further investigation.

It seems probable from the present studies that hexokinase exhibits a Ping-Pong pathway of enzyme and substrate interaction; however, this contention cannot be proven from kinetic experiments of the type undertaken here. As suggested above, there are two Ping-Pong mechanisms which appear to be in harmony with the kinetic results. One of these involves the participation of an enzyme–phosphate intermediate and the other an enzyme–glucose intermediate. A

choice between these two possibilities can be made from isotope exchange half-reactions. Both mechanisms should exhibit an ADP–ATP exchange in the absence of sugar substrates; however, if enzyme–phosphate is an obligatory intermediate, there should be a glucose–glucose-6-P exchange reaction in the absence of nucleotide substrates. This latter exchange would not be expected to occur if the Najjar–McCoy (1958) mechanism is applicable.

Another mechanism which could account for the findings presented in Figures 1–10 has been considered elsewhere (Scheme 2 of Hanson and Fromm, 1967). This pathway of enzyme and substrate interaction is a modification of the Najjar–McCoy (1958) mechanism and contains the reaction E + glucose $\rightleftarrows$ E-glucose as the initial step in the sequence. If the dissociation constant for this step is very small relative to other terms in the rate equation, the kinetics will appear to be Ping-Pong (Hanson and Fromm, 1967). Derivation of rate equations for this mechanism which accounts for the effects of mannose, AMP, and glucose-6-P requires no assumptions other than those usually made in steady-state derivations. This mechanism is also consistent with the data for ADP inhibition if it is assumed that ADP acts as a product inhibition and that the abortive ternary complex enzyme–glucose–ADP can be formed. It should be possible to choose between this mechanism and the usual Ping-Pong pathways from isotope-exchange half-reactions and initial velocity studies of the reverse reaction.

The strongest evidence in support of a Ping-Pong mechanism for hexokinase is provided by the results of Figures 1 and 2. It is, of course, possible that the curves are not really parallel but merely exhibit a subtle convergence. This could occur if the (AB) term present in the rate equations for sequential mechanisms (Alberty, 1953) is very small relative to other terms in the expression. The data for hexokinase inhibition by mannose suggest that the interaction of substrates with enzyme could be sequential, in which case glucose would be the obligatory first substrate for an ordered mechanism (Fromm, 1964). Based upon the data of Figures 3 and 4 alone, the mechanism could also be random. Although the results for inhibition by AMP are difficult to reconcile with a sequential enzyme–substrate pathway, such mechanisms cannot be excluded by the findings presented in Figures 5 and 6 (Hanson and Fromm, 1967).

The basic product inhibition patterns appear to be clearly at variance with ordered sequential mechanisms (Alberty, 1958). For an ordered ternary complex mechanism, the mannose inhibition data suggest that glucose would be the first substrate to add to the enzyme; however, product inhibition experiments predict that glucose-6-P should be a competitive inhibitor of glucose and a mixed inhibitor of ATP. The data of Figures 7 and 8 are in agreement with a rapid-equilibrium random mechanism for brain hexokinase if it is assumed that the abortive ternary complex enzyme–glucose–glucose-6-P may form when glucose-6-P is present as a product inhibitor. On the other hand, the results of Figures 9 and 10 exclude this mechanism if one assumes no abortive ternary complex formation. In the unlikely event that ADP(C) may interact with hexokinase as follows: E + C = EC, $K_1$; $\overline{\text{EC}}$ + B = ECB, $K_2$; EC + A = ECA, $K_3$; EA + C = ECA, $K_4$; and EB + C = ECB, $K_5$; then a rate equation which is consistent with the ADP product inhibition patterns may be obtained; however, the formation of a complex such as enzyme–ATP–ADP, where ADP binds at the glucose site, seems to be rather unlikely. The kinetic evidence presented in this report, although hardly conclusive, suggests that a Ping-Pong mechanism is most likely for solubilized brain hexokinase.

## References


Alberty, R. A. (1953), *J. Am. Chem. Soc. 75*, 1928.

Alberty, R. A. (1958), *J. Am. Chem. Soc. 80*, 1777.

Bock, R. M. (1960), *Enzymes 2*, 3.

Crane, R. K., and Sols, A. (1953), *J. Biol. Chem. 203*, 273.

Dalziel, K. (1957), *Acta Chem. Scand. 11*, 1706.

Fromm, H. J. (1964), *Biochim. Biophys. Acta 81*, 413.

Fromm, H. J. (1967), *Biochim. Biophys. Acta 139*, 221.

Fromm, H. J., Silverstein, E., and Boyer, P. D. (1964), *J. Biol. Chem. 239*, 3645.

Fromm, H. J., and Zewe, V. (1962a), *J. Biol. Chem. 237*, 1661.

Fromm, H. J., and Zewe, V. (1962b), *J. Biol. Chem. 237*, 3027.

Grossbard, L., and Schimke, R. T. (1966), *J. Biol. Chem. 241*, 3546.

Hanson, T. L., and Fromm, H. J. (1965), *J. Biol. Chem. 240*, 4133.

Hanson, T. L., and Fromm, H. J. (1967), *J. Biol. Chem. 242*, 501.

Jaganathan, V. (1963), *Indian J. Chem. 1*, 192.

Katzen, H. M., and Schimke, R. T. (1965), *Proc. Natl. Acad. Sci. U. S. 54*, 1218.

Lineweaver, H., and Burk, D. J. (1934), *J. Am. Chem. Soc. 56*, 658.

Moore, C. L., and Strecker, H. J. (1963), *Federation Proc. 22*, 413.

Najjar, V. A., and McCoy, E. E. (1958), *Federation Proc. 17*, 1141.

O'Sullivan, W. J., and Perrin, D. D. (1964), *Biochemistry 3*, 18.

Rose, I. A., and Warms, J. V. B. (1967), *J. Biol. Chem. 242*, 1635.

Schwartz, G. P., and Basford, R. E. (1967), *Biochemistry 6*, 1070.

Uyeda, K., and Racker, E. (1965), *J. Biol. Chem. 240*, 4682.

Wellner, V. P., Zoukis, M., and Meister, A. (1966), *Biochemistry 5*, 3509.

Zewe, V., Fromm, H. J., and Fabiano, R. (1964), *J. Biol. Chem. 239*, 1625.