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# Kinetic properties of hexokinase as assembled with a microcomputer data base

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We have constructed a relational data base containing the kinetic properties of the isoenzymes of hexokinase using the Knowledgeman data-base program on an IBM PC microcomputer. The natural subunit of this data base is the refereed publication, 165 of which were included. Reported values for the $M_r$ (approx. 97000) are in good agreement, but this agreement becomes progressively worse as one examines the $K_m$ values for glucose and ATP and the $K_i$ for glucose 6-phosphate, where the reported values are spread over three orders of magnitude. Some quantities are very thinly or unreliably determined. Experimental conditions, especially free $Mg^{2+}$ concentration, are rarely close to physiological. Reasons for the spread or uncertainty of numbers, and the distinctions that can be made between isoenzymes despite this spread, are discussed.

## INTRODUCTION

We have entered into a data base the detailed properties of the glycolytic enzymes as reported in the literature. This information is needed for a study of glycolysis in pancreatic islet $\beta$-cells, where this pathway appears to detect the glucose concentration (e.g., Matschinsky & Ellerman, 1968). Its detailed regulation is still in controversy. Careful examination of the relevant literature is necessary here, since pancreatic islets can be prepared only in small quantities by microdissection. We describe here the resulting data base, which was implemented on a laboratory microcomputer, as well as the results obtained with it from the literature for hexokinase. Our primary concern here is to characterize the enzymes phosphorylating glucose, identify which isoenzymes are present, determine their kinetic constants, and define the experimental conditions under which they were obtained.

Although the literature for hexokinase (EC 2.7.1.1) appears to contain of the order of 10000 publications (based on examination of appropriate indexes like those of Chemical Abstracts), relatively few of these publications contain useful kinetic information. We have selected and entered into the data base 165 containing the most significant and reliable kinetic information. Mammalian hexokinase has been emphasized, but important work on the yeast enzyme is also included. A MEDLINE search of publications on hexokinase kinetics was performed to check the completeness and currency of the publications used.

Hexokinase has been reported to occur as four major isoenzymes, with some minor variants. Hexokinase IV, more commonly known as glucokinase, is a distinct protein with a different kinetic mechanism. It is being treated as a separate entity. Hexokinase III occurs primarily in liver, and is inhibited by excess glucose. It has been very scantily studied; only two or three meaningful kinetic studies of this enzyme have been published. Since we are principally concerned with an organ other than liver, this particular isoenzyme has been neglected.

A preliminary report of this work has appeared (Garfinkel *et al.*, 1986*a*).

## DEFINITIONS AND CONVENTIONS

The data base contains information for hexokinase I, hexokinase II and hexokinase of unspecified isoenzyme composition, which is referred to for convenience as 'hexokinase U'. Since there are very few data from liver reported here, hexokinase U is effectively a mixture (in unspecified and probably varied proportions) of hexokinase I and II. Internally the data base refers to these isoenzymes by the EC number (2.07.01.01) for hexokinase followed by the isoenzyme number ('Unspecified' if none is given).

All concentrations are handled in $\mu$M for convenience; use of this unit permits entering data as integers, eliminating errors due to misplaced decimal points and problems with handwritten symbols. If no numerical value was specified in the original publication, a zero was entered where a numerical value is required.

Publications are internally identified by their PID (Publication IDentification number), a five-digit number, for reasons explained in the Methods section. A complete print-out of the list of publications including PID numbers is available from the authors on request.

Ratings of significance on a scale from 0 to 9 were made for each publication. Numbers (other than significance ratings) were copied from the original publications exactly as the authors calculated them. We have checked that the numbers were copied accurately. We have not yet been able to check for authors' errors, such as errors in calculation, errors resulting from the use of the Lineweaver–Burk plot or errors in the analysis underlying the calculation (e.g., Klotz & Hunston, 1984). We have generally not recalculated or refitted any of the experimental data. In several cases parameters reported in Dalziel's system of $\phi$ coefficients have been converted

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into conventional kinetic constants expressed in micromolar concentrations.

When statistical values are reported for kinetic parameters, deviant values were first deleted, as described below.

We made no attempt to alter, criticize or re-interpret the author's information (except for deleting outliers, as discussed below) in implementing the data base.

## METHODS

Enzyme kinetic data are reported almost entirely in the refereed journals. Individual papers usually contain comparable amounts of data (to within an order of magnitude), are normally devoted to one enzyme, and are in relatively similar format. Accordingly the original refereed publication is the natural unit of information for the data base, with a major division for each enzyme.

Among the common data-base types, the optimal trade-off between rigidity and flexibility on the one hand, and search time and computer power on the other, appears to be the relational data base. This type of data base permits the retrieval of information concerning a particular property or properties without printing out everything on the entries involved. In a relational data base information is handled in a number of tables related to each other by a common element (in our case, with all names encoded as numbers, including the EC number instead of the enzyme name, to speed computer searches). We used the PID of the original publication as this common element, since it is present in and unique for all entries. This is a usage close to common biochemical practice (it is analogous to obtaining a value with its associated literature reference). The PID is the common link to a table structure involving 20 full fledged tables and ten auxiliary and coding tables, which primarily define numerical identifiers. This table structure was developed after considerable experiment with a prototype data base (Garfinkel & Garfinkel, 1985); such development is common in some areas of computing. A detailed description of the reasons for choosing this program structure and how it was implemented is given elsewhere (Garfinkel *et al.*, 1986*b*).

We chose a microcomputer for our data base in order to make it available to experimenters who are likely to have microcomputers and not to use large computers. We implemented this program on an IBM PC-XT microcomputer, using one of the widely available program packages that are intended for constructing data bases but contain many other features (graphics, statistical procedures etc.) as well as the data-base program. After some trial, we selected the Knowledgeman II (Knowledgemanager) package from Micro Data Base Systems, which contains a program of the Structured Query Language/Data System (SQL/DS) type, and is standard on all sizes of computers. The principal alternative available at the time, DBASEII (since upgraded to DBASEIII), was insufficiently flexible for our purposes.

Information must be systematically extracted from the basic publications before being entered into the data base. For this purpose we use a printed form that mimics the computer's table structure. For a typical publication it takes about an hour for a graduate student to extract and write down the usable data, not counting required re-checking. The information is then encoded to represent character strings as numbers (requiring about 15 min per publication), and then entered into the data base by using an input program that asks for information as it is structured on the form (requiring 15–45 min per publication).

Data are extracted from the data base by means of queries, which are commands to retrieve data from one or more tables. We have prepared library of about 20 basic queries; these are written so that sub-string matches and complex conditions are avoided. They require only insertion of details before they are executed. Queries normally have a three-clause basic structure: a SELECT command followed by fields desired; a FROM command, followed by the identity of the tables involved; and a WHERE command, followed by applicable conditions. (The WHERE command is optional.) An example of a query is:

```
SELECT
PID, AUTHID, JOURNAL, REFEREN, YEAR
FROM PUBLICA WHERE YEAR>1980
```

## RESULTS

### Relative molecular mass

A total of 24 values for the $M_r$ of hexokinase are included in the data base. There are two badly deviant values reported, one being one-half and the other one-and-a-half times the general average, these presumably being due to different polymerization states of the protein; these were deleted. There is also one value 10% above the average, which can be considered to be due to ordinary experimental error. The remaining 22 values average out to $98000 \pm 3000$, $97000 \pm 2000$ and $97000 \pm 1000$ for hexokinases I, II and U respectively. It is noteworthy that the standard deviations given here are comparable with the error in the determination, implying both that there is good agreement on the value for the $M_r$ which is not statistically different for the various isoenzymes, and that the same entity is being measured in the several determinations. To some extent this may be considered as a form of calibration of our methods when considering the more scattered kinetic constants reported below.

### Enzymic mechanism

There are ten reports of mechanism for hexokinase I in the data base, eight rapid random equilibrium or a variant of it, and two sequential (in the nomenclature of Cleland, 1963). There are eight mechanism reports for hexokinase II; six are also rapid random equilibrium or variants thereof, one is sequential, and one is a complex mechanism. The enzymes come from a variety of sources and were studied under a variety of conditions. There appears to be fairly common agreement on a rapid random equilibrium mechanism for these two isoenzymes. For hexokinase U, there are 22 mechanism reports, only ten being rapid random equilibrium; the others report a scattered variety of mechanisms.

Conclusion. The most popular mechanism is the rapid equilibrium random type, but we are not convinced that this is indeed correct; ordered mechanisms deserve further consideration. The scatter of results for hexokinase U is much greater than for hexokinase I or II, even though it is probably a mixture of the two.

**Table 1. Average Michaelis constants for hexokinase isoenzymes**

| Substrate | $K_m$ ($\mu$M) Hexokinase I | Hexokinase II | Hexokinase U |
|---|---|---|---|
| Glucose | 49±18 | 143±47 | 96±115* |
| Fructose | 3283±884 | 2800±346 | 1497±1403 |
| Mannose | 44±32 ($n$ = 2) | None performed | 45±44 |
| ATP | 559±219 | 589±356 | 347±248† |
| MgATP | 400±230 | 635±381 | 820±589 |

* This includes values for four autopsy samples that are very high, and some values that would be reasonable for hexokinase II.

† With some outstandingly high values (over 1700) deleted. If these values were included, this mean would be 652. [This includes Mayer *et al.* (1966). Aging seems to have less effect than on the $K_i$ for glucose 6-phosphate, but examination is required.]

## Substrates and Michaelis constants

Reported values for the $K_m$ values of various substrates of hexokinase are tabulated in Table 1. Glucose is the most important substrate of hexokinase. Other sugars, sugar alcohols and analogous substances such as glucosamine may also act as substrates. For the purposes of the present paper, we are concerned primarily with glucose, fructose and mannose. These are not only physiologically the most important substrates, but they are the only ones that have been well enough studied to permit any comparison among isoenzymes.

**Glucose.** As shown in Table 1, for isoenzyme I a total of 32 values were reported for the $K_m$ for D-glucose. These average out to 49 $\mu$M with a standard deviation of 18 $\mu$M, although the distribution of the values appears not to be normally distributed or Gaussian. The range of values reported is from 20 $\mu$M to 77 $\mu$M, with one value of 120 $\mu$M, which was considered to be an outlier. Setting this range involved two cycles of deletion refinement; in the first, data for yeast, where the $K_m$ is often higher, were deleted, and then the isolated value of 120 $\mu$M was deleted. For hexokinase II, the range of reported $K_m$ values is from 70 $\mu$M to 230 $\mu$M with an average of 143±47 $\mu$M; again, reported values are not normally distributed. There are 45 reported values for hexokinase U, including one set of data obtained from human autopsy specimens that averaged out to about 400 $\mu$M. The $K_m$ including these specimens averaged out to 96 $\mu$M; without them the average $K_m$ for the remaining 41 entries averaged to 68 $\mu$M, with range from 7 $\mu$M to 267 $\mu$M. This value would be expected to be intermediate between the other two. A few values are available that permit some differentiation between the $K_m$ values for glucose of soluble, bound and particulate hexokinase I, for which the values are 72, 65 and 45 $\mu$M respectively, but in the last two cases there is only one measurement. There is also one unusually low value for the $K_m$ for ATP in a bound preparation. This is in accordance with current beliefs in the literature, but there are too few numbers to draw firm conclusions.

Conclusion. The $K_m$ values of hexokinases I and II for glucose average out to 49 and 143 $\mu$M, which are experimentally distinguishable. There is a scatter of about a factor of 3-fold for these $K_m$ values, for all isoenzymes.

**Fructose.** Relatively few values for fructose are reported both for hexokinase I (average 3.3 mM with spread from 2.4 to 5.0 mM) and for hexokinase II (average 2.8 mM). For hexokinase U, the average is 1497 $\mu$M with range 80–4000 $\mu$M. Many of the reported values are not within the range for either isoenzyme I or II, primarily because one publication has many values that are considerably lower than the mean for either isoenzyme.

Conclusion. It is generally accepted that hexokinase has less affinity for fructose than for glucose. These data bear this out. It appears that isoenzymes I and II do not really differ in affinity for fructose.

**Mannose.** Mannose stimulates the secretion of insulin by pancreatic islet $\beta$-cells. Analysis of their sugar utilization curves (Garfinkel *et al.*, 1984) by using Eadie–Hofstee plots or non-linear regression reveals a high-affinity component that is ascribed to hexokinase. The apparent $K_m$ for mannose *in vivo* is about 430 $\mu$M and the apparent $K_m$ for glucose *in vivo* is about 215 $\mu$M (Garfinkel *et al.*, 1984). The data obtained for hexokinase *in vitro* included here yield an average value of the $K_m$ for mannose of 46 $\mu$M; the average $K_m$ for glucose in the same publications was nearly equal. Where the $K_m$ values of glucose and mannose are compared for hexokinase U, the ratio $K_m$ (glucose)/$K_m$ (mannose) varies from about 1.5:1 to about 0.5:1 with an average of about 1:1. For the special case of hexokinase I, where there are two reported values for the $K_m$ for mannose and two for the $K_m$ for glucose in the same publications, the numerical value of the $K_m$ for mannose is about twice that for the $K_m$ for glucose, 44 $\mu$M as compared with 20 $\mu$M. The average values are tabulated in Table 1.

Conclusion. The average values for the $K_m$ for mannose are equal to or less than those for glucose, with sufficiently great standard deviation that they are not well determined. There appears to be appreciable discrepancy between the $K_m$ values obtained for glucose and mannose *in vitro* and those observed *in vivo* that are ascribed to hexokinase. This assignment may be incorrect: possibly this may involve or be due to an activity of glucokinase, an enzyme for which the ratio of these $K_m$ values is known to be 2:1. Alternatively, there may be a difference in conditions or some competitive inhibitor presently unknown or unappreciated. Glucose 6-phosphate, usually considered to be the most important inhibitor of hexokinase, is non-competitive against glucose; possible competitive inhibitors are other hexoses or their analogues. An activity of hexokinase II, for which there are no measurements of the $K_m$ for mannose, might also be involved; however, the strong latency of hexokinase observed in these cells suggests that their hexokinase is primarily isoenzyme I.

**Other carbohydrate-type substrates.** A wide variety of other sugars, sugar alcohols, glucosamine etc. are reported in the data base. Usually there are no more than a few values for each, and normally there is considerable disagreement where more than one value is reported; this disagreement is particularly extreme for 2-deoxyglucose. These compounds are also often competitive inhibitors

**Table 2. Average inhibition constants for hexokinase isoenzymes**

| Inhibitor | $K_i$ (μM) | | |
|---|---|---|---|
| | Hexokinase I | Hexokinase II | Hexokinase U |
| Glucose 6-phosphate | 74±80 ($n$ = 27) | 40±59 ($n$ = 12) | 148±220 ($n$ = 64) |
| Mannose 6-phosphate | 650±636 ($n$ = 2) | 5150±3040 ($n$ = 2) | – |
| ATP | 2550±1484 ($n$ = 2) | – | 900 ($n$ = 1) |
| ADP | 2400±2174 ($n$ = 8) | 3248±1804 ($n$ = 12) | 2019±1905 ($n$ = 9) |
| MgADP | 1450±1202 ($n$ = 2) | 6000±5656 ($n$ = 2) | 1000 ($n$ = 1) |
| AMP | 1853±1281 ($n$ = 8) | 2000±490 ($n$ = 5) | 5814±8041 ($n$ = 7) |
| Cyclic AMP | – | – | 20000 ($n$ = 1) |

against glucose and other sugars. There is some uncertainty with regard to *N*-acetylglucosamine, which is listed as an inhibitor but not as a substrate, although it is a substrate for other glucose-phosphorylating enzymes.

**ATP.** The actual substrate for hexokinase is $MgATP^{2-}$. It is described as either 'ATP' or 'MgATP' in comparable numbers of publications. The publications giving most of the $K_m$ values for 'ATP' as substrate were re-checked to see whether this ascription was incorrect, and it was found that $Mg^{2+}$ was actually added to the assay mixtures in the experiments reported therein, sometimes in amounts large enough to be inhibitory. Recalculation of the kinetic constants given in these publications to express them in terms of $MgATP^{2-}$ did not appear to be feasible, owing to lack of detail in the publications.

Among papers mentioning 'ATP' as substrate, there are 13 publications reporting the $K_m$ with hexokinase I. The average value is 559 μM, with range from 100 μM to 910 μM and standard deviation of 219 μM. For hexokinase II there are 13 values with a mean value of 590 μM, standard deviation 356 μM and range from 170 μM to 1.3 mM, similarly distributed. For hexokinase U, there were 39 measurements averaging 347±248 μM and ranging from 23 μM to 800 μM. These values are obtained after deletion of some outliers and values affected by the presence of glucose 6-phosphate. If the range of acceptable values is set at 100–1000 μM, the last two average values become 487 and 433 μM respectively, which is less scattered. There is considerable clustering of values within publications, i.e. some publications will have values consistently much higher than others, which are probably statistically significant. The reported numbers are considerably less sensitive to treatments such as aging of the enzyme preparations than are the $K_i$ values for glucose 6-phosphate reported in the same publication (Mayer *et al.*, 1966).

For publications giving the substrate as 'MgATP', the $K_m$ values obtained are comparable with those for 'ATP' with hexokinase I. The mean of 20 values for the $K_m$ for MgATP is 400±230 μM with range from 14 μM to 800 μM. For hexokinase II there are six values, averaging 635±381 μM with range from 170 μM up to 1.1 mM. For hexokinase U there are ten values with average 820 μM, standard deviation 589 μM and range from 280 μM to 2.1 mM.

Conclusion. The $K_m$ for ATP or MgATP is of the order of 400–600 μM, and not significantly different between the two isoenzymes. It is not well determined either numerically or semantically. The value for hexokinase U is not intermediate between those for hexokinases I and II, as would be expected.

## Inhibitors

**Glucose 6-phosphate.** Glucose 6-phosphate is usually regarded as the physiologically most significant inhibitor of hexokinase. it is usually regarded as competitive or partly competitive against ATP and non-competitive against glucose.

For hexokinase I, as shown in Table 2, there are 27 values for the $K_i$ for glucose 6-phosphate, which average out to 74 μM with standard deviation 80 μM and range 0.124–330 μM. There is very considerable clustering of the values, in the ranges 10–15 μM, 20–26 μM, 46–52 μM and 150–210 μM. This distribution is shown in Fig. 1. There are also values for isoenzyme IA of 20 and 63.5 μM and for isoenzyme IB of 42 and 44 μM. For hexokinase II there are 12 non-zero values averaging 40±59 μM and ranging from 1.4 up to 160 μM, including six values of 7 μM or below (Fig. 1). For hexokinase U, there is an average value of 148 μM with standard deviation 220 μM and range from 5 μM up to 1.3 mM (with an apparent outlier of 9 mM, which was deleted). Also included is one very substantial study (Mayer *et al.*, 1966), which gives 28 values for hexokinase U obtained under a variety of conditions, including aging of the enzyme; these values are generally distributed like the others. Considering the far-from-Gaussian character of the distribution, it is highly questionable whether ordinary statistical analysis is relevant, or even whether all the measurements reported are of the same entity. The values reported are not consistent with the hexokinase U behaving like a mixture of hexokinases I and II (i.e. they are higher than both instead of being somewhere in between). The values of the numbers reported are questionable if one wants to determine just how much inhibition will be exerted by glucose 6-phosphate under a given set of conditions.

Glucose 6-phosphate seems to conform to the usual pattern that determinations for hexokinase U have a greater spread or range than do those for isoenzyme I or II. It is expected that further investigation, including careful examination of the conditions, will be required.

**Mannose 6-phosphate.** Mannose 6-phosphate is also an inhibitor of hexokinase, similarly to glucose 6-phosphate. For hexokinase I values of 200 and 1100 μM are reported for the $K_i$ and for hexokinase II 3000 and 7300 μM. These values are in disagreement.

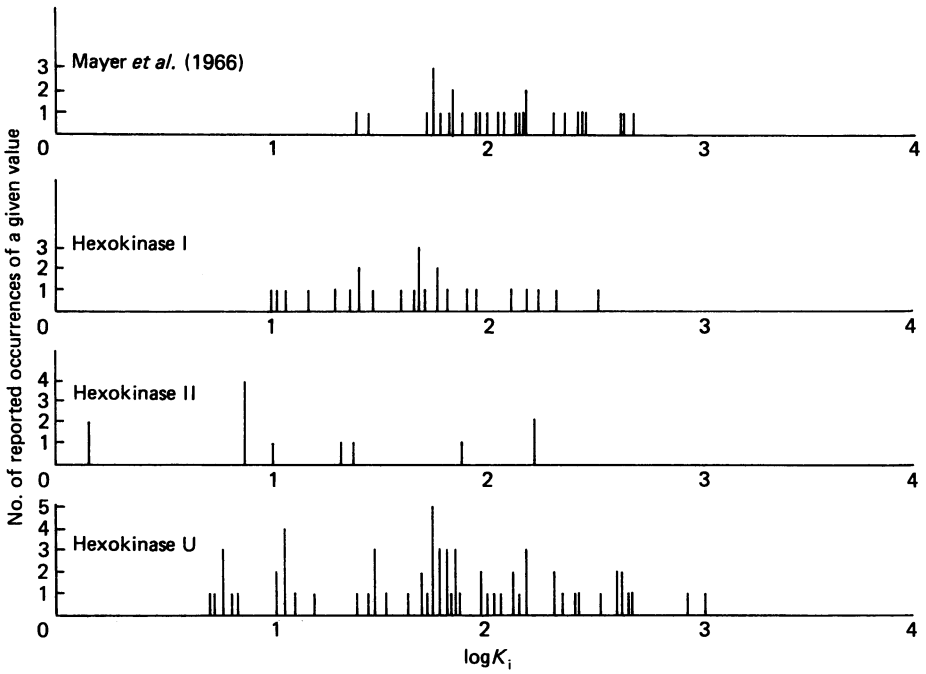


**Fig. 1. Distribution of the reported $K_i$ values of hexokinases I, II and U for glucose 6-phosphate, and of the values obtained by Mayer *et al.* (1966), which are a subset of hexokinase U [Mayer *et al.* (1966) investigated the effects of different enzyme sources, aging, particulate binding etc.]**

The abscissa shows the logarithm of the reported $K_i$. The ordinate represents the number of reported occurrences of a given value for a particular $K_i$.

**Adenosine derivatives.** Adenosine derivatives other than $MgATP^{2-}$ are inhibitors of hexokinase. $ATP^{4-}$ itself may be an inhibitor for hexokinase. Values of the order of 1 mM or greater for the $K_i$ are reported (e.g., Ning *et al.*, 1969; Kosow & Rose, 1970), but they both are widely scattered and were obtained under circumstances leading to doubt concerning their validity. However, its effect as an inhibitor of hexokinase is weak compared with the corresponding effect on phosphofructokinase (Waser *et al.*, 1983). The chelates of a number of metal ions with ATP are inhibitory, and may be involved in disease conditions (e.g. $HgATP^{2-}$ in Mimimata disease and $AlATP^{-}$ in Alzheimer's disease). Contamination of ATP by aluminium impurities may also be a cause of experimental error. Most reports indicate that adenosine derivatives are non-competitive against glucose and competitive against ATP, but there are also reports that they are non-competitive against ATP, and one report (Moore, 1968) that this depends on whether the enzyme is soluble or particulate (bound). The $K_i$ values for $ATP^{4-}$, $ADP^{3-}$, $MgADP^{-}$, AMP and cyclic AMP are listed in Table 2, and are almost all above 1 mM. The same scatter of numbers is reported as for other substrates and inhibitors. It should be noted that the binding and compartmentation properties of AMP and ADP are such that their cytoplasmic concentrations are usually well below their $K_i$ values as listed here. ADP in muscular tissues is mostly bound to the muscle proteins and metabolically inactive, and a similar situation seems to prevail in pancreatic $\beta$-cells (Garfinkel & Achs, 1986). The $K_i$ for cyclic AMP is very large compared with its cytoplasmic concentration. It must be concluded that the primary inhibition of hexokinase is exerted by glucose 6-phosphate.

## Activators

**Activation or protection of hexokinase by inorganic phosphate against inhibition by glucose 6-phosphate.** The protection of hexokinase from glucose 6-phosphate inhibition by inorganic phosphate is widely discussed in the literature and written about as if it were a well-quantified interaction. It is also reported that inorganic phosphate in the absence of glucose 6-phosphate may act as an inhibitor with a $K_i$ of 35 mM (Ellison *et al.*, 1975). However, we were able to find very few values for the activation constant in our data base: 17 and 55 $\mu$M for isoenzyme I and 800 $\mu$M for hexokinase U. There are some data available (Mayer *et al.*, 1966) from which additional values in the low millimolar range could be calculated. Since there are only three discordant values reported, this constant must be described as thinly determined to the point where its actual value is doubtful. When this activation interacts with the glucose 6-phosphate inhibition, as it does within the cell, the resultant net effect must be considered very poorly determined quantitatively. This problem is compounded by the difference between concentrations for intracellular inorganic phosphate measured by chemical analysis and the lower ones obtained by n.m.r. techniques.

## Experimental conditions

It is possible to retrieve the experimental conditions used for a given publication provided that they are sufficiently clearly stated. In general, synthetic buffers such as Tris or Hepes at a pH of 7 or above are used. There are virtually no reports of a physiological buffer (e.g. Krebs–Ringer bicarbonate buffer or Krebs–Henseleit buffer) being used. It is unusual to have $K^+$ present, and very rare to have it present in concentrations

approaching the physiological. It has been shown (Davidson & Arion, 1986) that physiological concentrations of $K^+$ are required for accurate assay of glucokinase. Rose & Warms (1982) have found that $K^+$ is one of the factors stabilizing hexokinase II in ascites tumour cells.

Thus far the scatter of the experimental conditions has been sufficient that it has not been possible to establish a trend of how any kinetic parameter changes as a function of the conditions, such as the nature and concentration of a buffer.

$Mg^{2+}$, when reported as present, is usually considerably in excess of the values now being reported for intracellular concentrations (e.g. 0.4 mM; Corkey *et al.*, 1986). It is known to be a competitive inhibitor of glucokinase, and in excess has been shown to inhibit both yeast hexokinase (Noat *et al.*, 1970) and mammalian hexokinase (Gerber *et al.*, 1974), although this appears not to be generally appreciated for the mammalian enzyme. At high concentrations $Mg^{2+}$ may effectively inhibit by converting MgATP into $Mg_2ATP$, but the work of Garner & Rosett (1973), who show an inhibition of hexokinase by excess $Mg^{2+}$, would indicate that there is some mechanism addition to this one, since their inhibitory concentrations are rather low for this particular effect. A thorough study by Bachelard (1971) indicates that $Mg^{2+}$ is an inhibitor at concentrations as low as 3 mM, and that it may be an allosteric activator under some conditions. When $Mg^{2+}$ is mentioned in the experimental conditions in the data base, it is usually high enough to exert some inhibition; the lowest fixed concentration mentioned in 1.35 mM. There is one explicit value of a $K_i$ for $Mg^{2+}$ reported, but the numerical value (30 mM) is unreasonably high. Some investigators may not even have their $Mg^{2+}$ concentrations right owing to erroneous calculations.

The difficulties with $Mg^{2+}$ in enzyme kinetics are illustrated by a quote from Knowles (1980), writing about phosphoglycerate kinase. He described the dilemmas due to it: 'What to do about the essential divalent metal ions. . . and how to treat the questions of multiple binding sites, substrate inhibition or activation, or salt effects. These elements of ill-behavior often result in nonlinear double reciprocal plots, the interpretation of which becomes more a matter of whim rather than judgement'. Learning how to handle the problems with $Mg^{2+}$ will probably be a convergent process: as we learn about what $Mg^{2+}$ concentrations in tissue are and their effects, it will be necessary to repeat experiments under more nearly physiological conditions.

### Significance ratings

As part of the incorporation of each publication into the data base, it was assigned a significance rating on a scale from 0 to 9. Publications with ratings of 4 or below were not included in the data base. These ratings appear to be more or less normally distributed (averages of large numbers of ratings range from 5.5 to 6.5). Some experiments were carried out to divide more than less significant publications. For significance rating of 7 or above, the $K_m$ values of hexokinases I, II and U for glucose were 57, 157 and 86 $\mu$M (with standard deviations comparable with those in Table 1), i.e. changed by 10% or so. Attempts at more complex calculations involving the significance ratings have not yet yielded useful results.

## CONCLUSIONS

Hexokinase I can be distinguished from hexokinase II by the $K_m$ for glucose, and probably not by the $K_m$ for ATP or MgATP. Physiologically, the glucose concentration rarely gets low enough for this difference between the properties of hexokinases I and II to be critical, and the ATP concentration is usually greater than the $K_m$ values reported for it; however, this may be effectively changed by glucose 6-phosphate inhibition, and thus requires further study.

One phenomenon important to this distinction between isoenzymes is the property of hexokinase I called 'latency': when a tissue is disrupted by sufficiently vigorous means, the hexokinase I activity increases considerably. This is an important factor in determining the isoenzyme present here: latency is clearly a property of islet-cell hexokinase and therefore it consists primarily of hexokinase I. When pancreatic islets are homogenized or sonicated, the increase in activity is 30-fold or more (assuming that glucokinase is a constant marker enzyme). Various suggestions have been made to explain latency, e.g. that the enzyme is sequestered from access to its substrate (Katzen *et al.*, 1970), or that its activity is affected by binding to particles (Katzen *et al.*, 1970), or that the enzymically active enzyme monomer dimerizes or polymerizes to an inactive form, with promotion of the dimerization by glucose 6-phosphate (Chakrabarti & Kenkare, 1974; Easterby, 1975; Vowles & Easterby, 1979). It is not clear whether this is a property of the isolated enzyme or of the enzyme *in situ* (i.e. how important is interaction with its surroundings?). The data included in this data base do not offer any definitive explanation for this phenomenon.

All the kinetic constants reported for hexokinase have a spread of values that is considerably larger than would be expected from the error of the method used to determine them. Furthermore, this spread is generally greater for the $K_m$ for ATP than for that for the hexose substrate, and still greater for the $K_i$ for glucose 6-phosphate. Velocity measurement errors and errors in adjusting concentrations will usually be 5–10% or less. The $K_m$ for fructose has perhaps the smallest spread, about a factor of 2-fold. For glucose the spread is 3-fold for hexokinases I and II, and more for hexokinase U. It is relevant that Cumme *et al.* (1973) reported in a set of determinations of equilibrium constants, from the same laboratory in the same publication, that they could not reduce their range of values below about 3-fold. It is also noteworthy that the spread of numbers increases with increasing indirectness of the determination of the number. Thus the spread for the $K_m$ for glucose (about 3-fold) is less than that for the $K_m$ for ATP, which approaches an order of magnitude. The $K_m$ for ATP is more indirectly determined because it involves calculations with $Mg^{2+}$. The spread for the $K_i$ for glucose 6-phosphate is even greater, being several orders of magnitude over the entire range of reported values. As a result of this spread, it is difficult to evaluate statistics on the reported values. Many of the entries in Table 1 have the standard deviation greater than the mean, including the entries for the $K_i$ for glucose 6-phosphate for all the isoenzymes.

For all these kinetic constants there is usually a greater range of values for hexokinase U than for hexokinase I or II. The value obtained for hexokinase U need not be

between the values for isoenzymes I and II, although hexokinase U is probably some kind of mixture of isoenzymes I and II; in some instances it may be nearly all isoenzyme I or nearly all isoenzyme II. The reason for the greater spread of results with hexokinase U is not known. It may reasonably be speculated that the purified isoenzymes are in fact purer samples of the enzyme than is the mixture of isoenzymes prevailing in hexokinase U.

Some of the variation in the kinetic parameters of hexokinase may be due to differences in the properties of different batches of the enzyme. When different batches of the same enzyme are presumably prepared approximately the same way in the same laboratory, their properties are constant only to within 10% (B. Cooperman, personal communication, on the basis of his experience with pyrophosphatase). Contamination by $Al^{3+}$ or even some vitamins could introduce further changes. Enzymes may become degraded with time and need to be re-assayed periodically. They may also undergo hysteretic effects; the history of a batch may be important. Such effects would help explain the different results reported from different laboratories. Furthermore, different batches of enzymes may have been damaged to different extents during isolation, by partial degradation of peptide chains through proteolysis. Such degradation may cause some of the observed spread for the $K_i$ for glucose 6-phosphate. Mayer *et al.* (1966) have shown that aging of hexokinase during isolation causes the kinetic properties to change. The large range of values that they reported (see Fig. 1) may be partly due to their apparent omission of dithiothreitol or other thiol-protective agents. This type of degradation, which may break peptide chains without actually releasing substantial fragments from the protein molecule, is not easily observed in common tests for identity and heterogeneity of protein, such as gel electrophoresis.

The apparent irreversibility of mammalian hexokinases and the resulting lack of information concerning the kinetic constants of the reverse reaction may be partly responsible for the reported spread in the kinetic constants. Whatever information has been reported on the kinetic properties of the reverse reaction is fairly recent (Solheim & Fromm, 1981; Ureta *et al.*, 1985). Therefore information available for more reversible enzymes, e.g. radioactivity exchange at equilibrium, the kinetic constants of the reverse reaction and whether the complete set of kinetic constants fit the relevant Haldane relations, have not been available.

It would appear that except for the $M_r$ all of the numbers tabulated here have substantial uncertainties associated with them, and they should be treated accordingly. This uncertainty appears not to be appreciated by some enzyme kineticists.

In practice, working values for these kinetic constants are usually chosen on the basis of some subjective expert judgement of reliability, such as proper matching of conditions and preparations etc. Despite the scatter of values for these constants, such expedients may nevertheless work much of the time. As an example, when we needed a model of hexokinase I under conditions where the glucose 6-phosphate concentration is unknown but relatively low (Garfinkel *et al.*, 1984), but that of glucose is important, we used the values of Grossbard & Schimke (1966), whose value for the $K_m$ for glucose (45 $\mu$M) is reasonably close to the averages described above.

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