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Problem-based learning

The kinetic properties of liver glucokinase and its function in glucose physiology as a model for the comprehensive study of enzymes' kinetic parameters and reversible inhibitors

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Abstract

Glucokinase is a key enzyme in carbohydrate metabolism in mammals. Particularly relevant is the involvement of the liver enzyme for the maintenance of blood glucose concentrations. In playing this key role, the occurrence of normal kinetic parameters of liver glucokinase is critical. Modification of K_m and/or V_{max} results in pathological situations occurring with hypo- or hyperglycemia. Based on this, an exercise is proposed in which different mice cell lines with abnormalities in glucose utilization are analyzed to determine the metabolic alteration at a molecular level. The student must determine the cause of abnormality by processing the kinetic data. Also, the characterization of different compounds that are reversible inhibitors of the enzyme from kinetic data is required to identify a drug that can correct the pathology in each case. One major objective of the problem is to learn about the meaning of kinetic parameters of enzymes, as well as the action of different types of reversible inhibitors.

It is frequently difficult to introduce to the students concepts related to enzymes. For example, we have previously pointed out the importance of key concepts in enzymology for pharmacy courses and an improved way of teaching the significance of enzyme activity and the experimental ways of measuring it [1]. Another relevant subject in enzymology is related to the meaning of the kinetic parameters, K_m and V_{max}, of one enzyme and the way that reversible inhibitors produce an effect on the enzyme by modifying these parameters.

We developed a problem section in which different mouse cell lines exhibit distinctive pathological clinical situations, produced by changes occurring in K_m and/or V_{max} of a key enzyme involved in carbohydrate metabolism. Kinetic data are presented in the problem, in order that students can determine which change modifies the enzyme's kinetic properties and causes the metabolic alteration. A second step in the problem is to find out, after processing kinetic data, the effect of different compounds that are reversible inhibitors of the enzyme under study. Once students find the type of inhibition each compound produces, they must determine the compounds that can be used to rectify the metabolic anomaly in each mouse line.

Glucokinase is thought to be the principal determinant of both hepatic and islet glucose utilization [2,3]. In the liver, there is a system of two enzymes catalyzing phosphorylation of glucose to glucose-6P: hexokinase and glucokinase. The latter enzyme is relevant to the functioning of the tissue in buffering concentrations of glucose in blood, maintaining it at about 5 mM [4]. Hexokinase has a low K_m for glucose, $\leqslant 0.1$ mM, and is strongly inhibited by glucose-6P the product of the reaction. The main function of this enzyme is to operate in tissues such as brain, which require a continuous supply of glucose that can be incorporated into the cell and phosphorylated even when blood glucose is extremely low. Product inhibition of hexokinase is relevant to avoid phosphate depletion of the cell, because of the high affinity of the enzyme for their substrates [4].

In contrast, glucokinase has a much higher K_m for glucose (~ 10 mM) but is insensitive to product inhibition. Glucose concentration within the liver reflects that of the blood, since the hexose equilibrates across the plasma membrane. Because the K_m of glucokinase for glucose is about twice the normal blood glucose level,

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any increase in the latter leads to a proportional increase in glucose phosphorylation by the enzyme. Thus, the system allows the liver to take up glucose at significant rates only when its concentration in blood increases. Hence the action of glucokinase makes it possible to maintain blood glucose at normal levels (for a more detailed information see [4]). For the adequate functioning of this buffer system, both the affinity and the activity of glucokinase are essential, since they maintain blood glucose concentration within narrow limits. It has been demonstrated that under certain circumstances, diminished levels of glucokinase may cause diabetes. However, hypoglycemic conditions are found when the enzyme activity is significantly increased over the normal value [2,3].

In our student exercise, we suppose the existence of seven different mouse cell lines which can be utilized in the study of possible clinical (or sub-clinical) abnormalities. The pathology observed in the different cell lines are centered on the inability to maintain blood glucose levels at normal values (even under situations of hypo- or hyperglycemia) which is presumably caused by an alteration in the kinetic properties of liver glucokinase. These alterations may become critical under certain stressful conditions. The student is challenged to identify the kinetic causes of the problem in each case, as well as to find an adequate drug that can alleviate each specific pathological situation.

Problem

Glucokinase activity is determined (an assay is carried out at saturating levels of glucose and ATP) in crude extracts obtained from liver homogenates of each mouse cell line, including a control corresponding to a liver with normal enzyme. The measurement of glucokinase activity in crude extracts has the complexity of the presence of hexokinase, which catalyzes the same reaction. To solve this problem, a method has been developed in which the assay is performed at two different glucose concentrations, 0.5 and 100 mM [5]. The activity measured at 0.5 mM glucose indicate only that of hexokinase. Indeed, the subtraction of the activity measured at 0.5 mM glucose from the activity determined at 100 mM glucose represents the glucokinase activity in the extract [5]. The results in Table 1 show the glucokinase activity thus determined in the extracts from the liver of the different mouse cell lines.

In a second step, hepatic glucokinase from each of the mouse cell lines, (including normal mice) is purified to electrophoretic homogeneity. Then, the kinetic parameters K_m and V_{max} are determined for the different pure enzymes by assaying their activity at saturating levels of ATP and variable concentrations of glucose. Experimental data are given in Table 2.

Table 1
Glucokinase activity measured in crude extracts obtained from the normal mouse line (control) or from the different mouse lines affected by pathological situations related to altered glucose blood levels

Case	Glucokinase (units/mg h.t.[a])
Control, ML-*N*[b]	0.0039
ML-1	0.0195
ML-2	0.0036
ML-3	0.0200
ML-4	0.0041
ML-5	0.0137
ML-6	0.0252
ML-7	0.0108

[a]mg h.t., milligrams of hepathic tissue.
[b]ML, mouse line; *N*, normal.

Table 2
Activity values for glucokinase purified from liver cell cultures of the normal mouse line (control) or from the mouse lines exhibiting different alterations in the maintenance of glucose blood levels. Enzyme activity was assayed at the different specified glucose concentrations in the respective meda

Glucose (mM)	Glucokinase activity (units/mg)							
	Control, ML-*N*	ML-1	ML-2	ML-3	ML-4	ML-5	ML-6	ML-7
1.00	13.3	66.7	46.5	15.7	40.0	35.3	36.3	6.2
2.00	24.0	119.9	66.5	30.6	72.0	53.4	69.6	12.3
3.60	38.0	190.0	82.8	49.5	114.0	70.1	112.5	21.2
5.40	48.0	240.0	93.2	75.2	144.0	78.9	177.4	32.2
10.80	67.0	335.0	101.2	132.0	201.0	90.2	277.2	56.8
14.85	76.0	380.0	103.5	172.3	228.0	95.6	335.8	76.0
20.25	85.0	425.0	106.95	214.3	255.0	97.8	388.4	96.5
27.00	92.0	460.0	109.25	260.7	276.0	102.8	439.6	117.1
32.40	97.0	485.0	112.0	284.9	291.0	103.2	487.0	132.2
36.45	98.0	490.0	112.8	297.0	294.0	103.9	527.5	142.0
38.25	98.5	492.5	113.27	298.1	295.5	104.2	538.3	146.7

After characterizing the enzyme alteration producing different metabolic anomalies in each of the mouse lines, a screening of different compounds was carried out to identify those that could be used as drugs to solve the pathology in each clinical case under study. Four compounds A–D were found to be reversible inhibitors of liver glucokinase (with a very minor effect on hexokinase) purified from the normal line, affecting the enzyme activity as given in Table 3.

Questions

On the basis of the above studies:

(a) Indicate the cause for the metabolic alteration found in each mouse cell line.

Table 3
Activity of glucokinase purified from the normal mouse line assayed at different concentrations of the substrate glucose and in the presence of a fixed amount of compounds A, B, C or D

Glucose (mM)	Compound A (units/mg)	Compound B (units/mg)	Compound C (units/mg)	Compound D (units/mg)
1.00	12.0	6.5	5.0	5.7
2.00	19.0	11.7	9.6	10.4
3.60	28.0	18.1	17.2	16.0
5.40	34.0	23.5	25.1	21.0
10.80	44.0	33.5	40.9	30.0
14.85	46.0	37.9	50.2	35.0
20.25	48.0	41.7	54.0	39.0
27.00	49.0	44.9	58.6	44.0
32.40	50.0	46.7	61.2	47.0
36.45	50.5	47.7	63.7	48.0
38.25	50.7	48.1	66.8	48.5

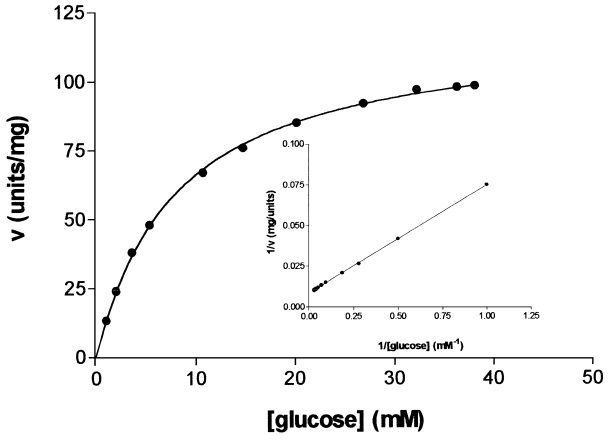

Fig. 1. Saturation kinetics for glucose corresponding to glucokinase purified from ML-*N*. The main figure illustrates enzyme activity versus glucose concentration as reported in Table 2. Inset: double reciprocal plot of the same data.

(b) What anomaly in the blood glucose level would you expect to find in each case?
(c) Indicate the compound that could be used in each case to normalize the blood glucose concentration.

Answers

From Table 1 it is observed that the level of glucokinase activity is increased compared to the control in the liver of mice lines 1, 3, 5, 6, and 7. In such cases, at least one cause of the problem may be attributed to the high level of enzyme activity. The data in Table 2 can be used to determine the kinetic parameters of the purified enzymes. This can be done by using non-linear regression analysis of the Michaelis–Menten plots [6] or by obtaining linear double reciprocal plots [7,8], as illustrated in Fig. 1 for the normal situation. By doing this, the values of K_m and V_{max} given in Table 4 are obtained for the different cases. With this information it is possible to answer Questions (a) and (b). As indicated in Table 5, kinetic causes for the metabolic alteration (a) and levels of blood glucose (b) in each mouse cell line are:

ML-1. (a) The higher V_{max} of glucokinase (Table 4) is related to the increase of enzyme activity in the liver (Table 1). (b) The increased capacity of the liver to convert glucose into glucose-6P will produce hypoglycemia. In addition, there will be a tendency toward cellular Pi exhaustion.

ML-2. (a) The level of glucokinase in the liver extract is normal (Table 1), which correlates with unaltered V_{max} of the enzyme (Table 4). However, the affinity of the enzyme toward glucose is increased (Table 4). (b) The higher affinity of glucokinase will result in hypoglycemia and a tendency toward cellular Pi exhaustion.

ML-3. (a) Both the, K_m and V_{max} values of glucokinase are higher (Table 4), the latter resulting in a higher enzyme activity in the liver (Table 1). (b) In this case it is more difficult to determine the possible blood glucose levels, since the higher K_m will produce an opposite effect of the higher V_{max}. In Table 2, it is seen that at glucose concentrations usually found in the blood (4–8 mM), values of enzyme activity are higher, which indicate a marked tendency toward hypoglycemia.

Table 4
Kinetic parameters for the different mouse lines under study, calculated from data shown in Table 2

Mouse line	K_m (mM)	V_{max} (units/mg)
ML-*N*	8.1	119.7
ML-1	7.9	593.7
ML-2	1.5	116.4
ML-3	37.6	606.3
ML-4	7.9	356.5
ML-5	2.1	109.5
ML-6	21.7	824.9
ML-7	56.7	363.0

ML-4. (a) Glucokinase activity in liver extracts is normal (Table 1), even when its V_{max} is higher (Table 4). Probably, the increase in V_{max} is compensated by a lower expression of glucokinase in the liver tissue. Consequently, in this case glucokinase is not causing a pathological situation. If the latter occurs, the cause may have a different origin. Thus, it is not possible to answer (b) this case.

ML-5. (a) Levels of glucokinase activity are increased in the liver (Table 1), although the pure enzyme exhibits a normal V_{max} (Table 4). This suggests that the expression of hepatic glucokinase is enhanced. In addition, the enzyme exhibits a lower K_m (Table 4). (b) From the latter, conditions of hypoglycemia and a tendency toward cellular Pi exhaustion are expected.

Table 5
Answers to the kinetic cause for the metabolic alteration, the level of blood glucose found in each mouse cell line, and the compound to be used to normalize the respective anomaly

Mouse line	Answer to question		
	a (alteration in glucokinase)	b (blood glucose level)	c (compound)
ML-1	Higher V_{max} (5.0-fold)	Hypoglycemia	B
ML-2	Lower K_m (5.4-fold)	Hypoglycemia	C
ML-3	Higher V_{max} (5.1-fold) and higher K_m (4.6-fold)	Hypoglycemia	A
ML-4	Higher V_{max} (3.0-fold), although lower expression.	?	—
ML-5	Lower K_m (3.9-fold) and higher level of expression	Hypoglycemia	D
ML-6	Higher V_{max} (6.9-fold) and higher K_m (2.7-fold)	Hypoglycemia	A, B, better A + B
ML-7	Higher V_{max} (3.0-fold) and higher K_m (7.0-fold)	Hyperglycemia	B, but in addition to an activator

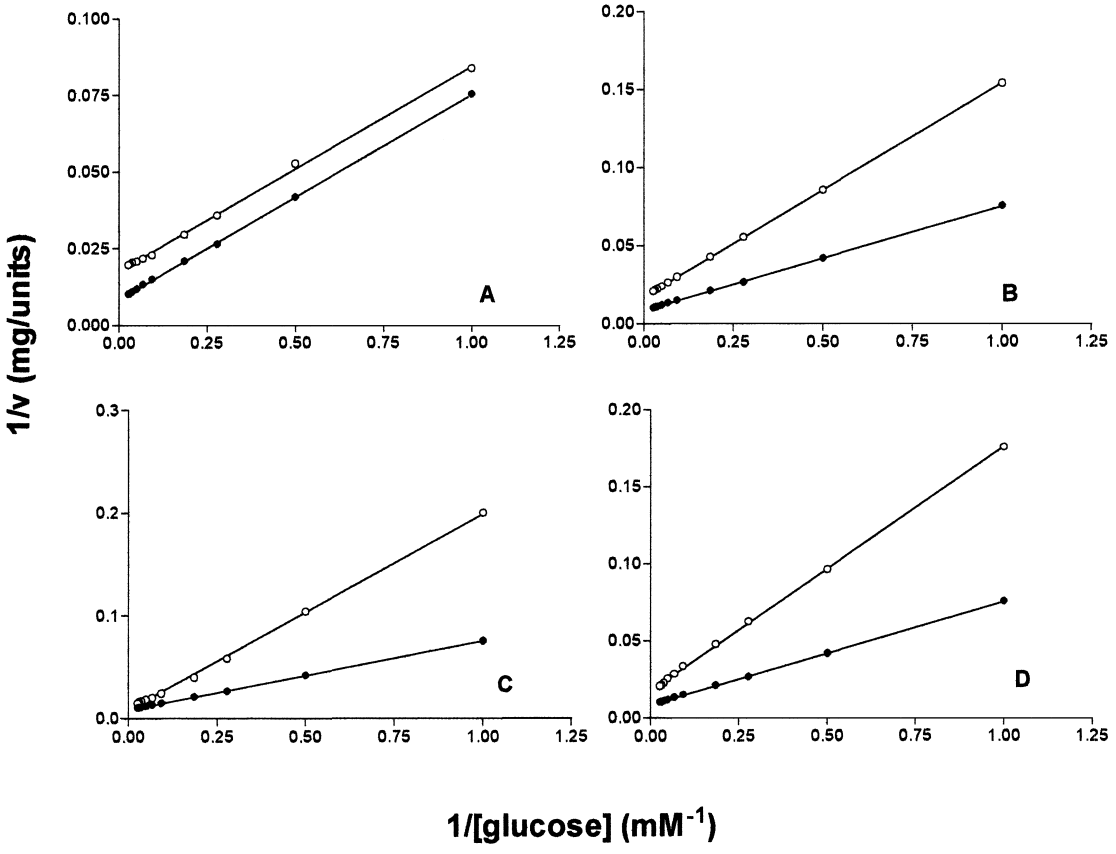

Fig. 2. Double reciprocal plots to determine the reversible inhibitory effect produced by compounds A–D on glucokinase activity with respect to glucose. In each case, data obtained with enzyme purified from liver of ML-*N* assayed at different glucose concentrations in the absence (•, data in Table 2) or in the presence (◦, data in Table 3) of a fixed amount of inhibitor A–D.

ML-6. (a) Glucokinase activity in liver extracts is higher (Table 1) in agreement with the higher V_{max} of the enzyme (Table 4). Additionally, the K_m for glucose is higher (Table 4). (b). The relatively higher increase in V_{max} (seven-fold) than in K_m (three-fold) results in a higher activity at almost all glucose concentrations (Table 4). Consequently, hypoglycemia will occur.

ML-7. (a) Both kinetic parameters for glucokinase are increased (Table 4), as is the enzyme activity in liver extracts (Table 1). (b) Here, the increase in K_m (seven-fold) is higher than in V_{max} (three-fold) which produces enzyme activities lower than the control (ML-*N*) in the physiological range of glucose concentration (4–8 mM, see Table 2). Consequently, a tendency toward hyperglycemia is expected.

From double reciprocal plots (Fig. 2) of velocity versus glucose concentration determined in the absence (Table 2) or in the presence (Table 3) of compounds A–D, their

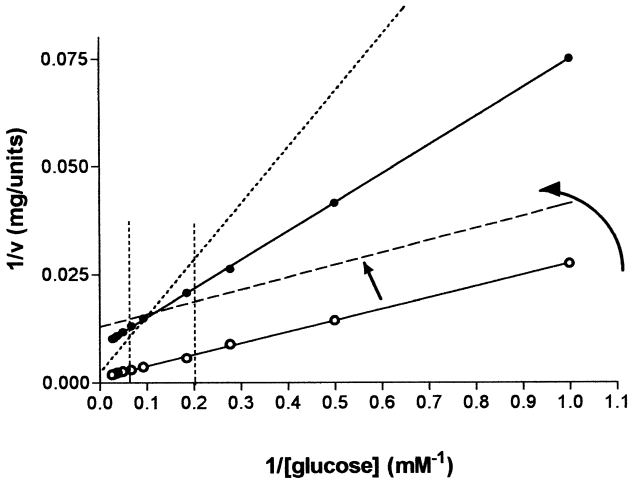

Fig. 3. Double reciprocal plots of glucokinase activity versus glucose concentration, obtained using the data in Table 2, corresponding to ML-*N* (•) or ML-6 (◦). Vertical dashed lines show the range of physiological glucose concentrations. The effects of the non-competitive inhibitor A (—-) or the non-competitive inhibitor B (⋯⋯) on the kinetics of ML-6 show the way that each compound can contribute to correct the anomalous situation. Small and large arrows show the way that A and B affect the enzyme kinetics of ML-6.

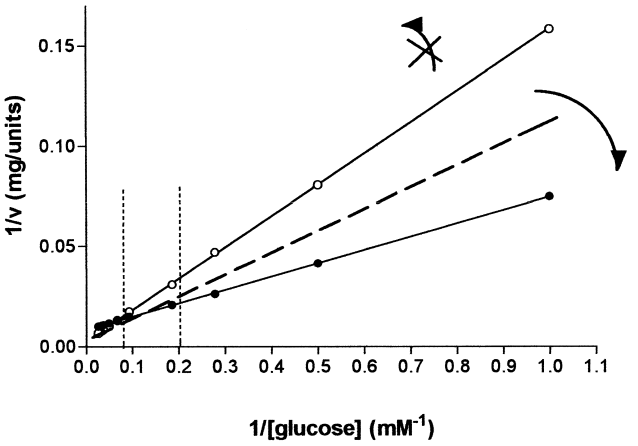

Fig. 4. Double reciprocal plots of glucokinase activity versus glucose concentration, obtained from data in Table 2, corresponding to ML-*N* (•) or ML-7 (◦). Vertical lines show the range of physiological glucose concentrations. The crossed arrow shows the way that a reversible inhibitor alters the kinetics for ML-7, which is not useful to correct the anomaly. The effect of an activator modifying (decreasing) K_m is shown (—-, clock-wise arrow) as servicable to reverse the altered situation.

inhibitory effects on glucokinase activity are characterized [7,8] with respect to glucose, these compounds are reversible inhibitors of the type [7,8]: A, uncompetitive; B, non-competitive; C, competitive; and D, mixed. Thus, the, answer to Question (c) for each pathological case can be proposed as in Table 5:

ML-1. Compound B will decrease the V_{max} of glucokinase.

ML-2. Compound C will increase the K_m of the enzyme.

ML-3. Compound A will increase both parameters, K_m and V_{max}, of glucokinase.

ML-4. As for b, it is not possible to answer c in this case.

ML-5. Compound D will affect glucokinase by increasing K_m and decreasing V_{max}. Although the V_{max} of the enzyme is unaltered, D is necessary to reduce the glucokinase activity in the cell in this case.

ML-6. The ideal compound to be used should decrease V_{max} and K_m, although to a greater extent the former. Fig. 3 shows kinetics of glucokinase from ML-*N* and ML-6, as well as how the latter is affected by an uncompetitive (compound A) and a non-competitive (compound B) inhibitor. As shown, compound A affects the enzyme from ML-6 yielding parallel lines in Fig. 3, in a way that at a certain concentration of A, the enzyme activity at physiological glucose concentrations can be brought very close to the activity in ML-*N*. Fig. 3 also shows the effect of the non-competitive inhibitor B on the enzyme from ML-6, which partially corrects the abnormality (at physiological glucose levels) by decreasing V_{max}. A more satisfactory correction could be achieved by using a mixture of compounds A and B.

ML-7. Fig. 4 illustrates the change in the kinetics for this situation with respect to ML-*N*. In this case, the use of inhibitors will not be effective in decreasing K_m to a higher extent than V_{max}. In fact, it is best to use an enzyme activator that decreases K_m without altering V_{max} [7]. In this way the enzyme kinetics will be more accurately corrected, rendering activity values very close to those normally found at physiological glucose concentrations (Fig. 4). A combination of an activator of the type that decreases K_m and increases V_{max} [7], together with the non-competitive inhibitor B (to decrease V_{max} modified by the activator) would also be effective.

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