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TEXTBOOK ERRORS

Hexokinase and 'glucokinase' in liver metabolism

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Rat liver contains four hexokinase isoenzymes, one of which, despite often being called 'glucokinase', is no more specific for glucose than the others. However, it does differ from them in displaying a sigmoid kinetic response to glucose, requiring much higher glucose concentrations for activity, and being insensitive to physiological concentrations of glucose 6-phosphate.

'HEXOKINASE AND GLUCOKINASE are two enzymes that catalyse the phosphorylation of α-D-glucose at the 6 position. Hexokinase will accept several other hexoses as substrates, but glucokinase, which predominates in liver, is highly specific for glucose. It also differs from hexokinase in having a high Michaelis constant for glucose, about 10 mM, and in not being inhibited by the product of the reaction, glucose 6-phosphate.' This composite statement is based on what appears in nearly all modern textbooks of biochemistry[1–9], and, indeed, in the opening paragraph of *TIBS* last year[10]. While such statements are probably intended to provide a concise view of the physiological differences between the hexokinase isoenzymes, from the enzymological point of view it would be hard to find a way of packing more errors into a few words.

'Glucokinase', also known as hexokinase D (or type IV), is one of four hexokinase isoenzymes (A to D, or types I to IV) that can be clearly distinguished in the rat liver[11,12]. In most of the other 100 or so vertebrate species that have been studied, the adult liver likewise contains three or four isoenzymes[13].

The hexokinase isoenzymes can catalyse the phosphorylation of both α- and β-D-glucose, although with different kinetic constants[14,15]. Generally, there is a somewhat higher limiting rate for phosphorylation of the β-anomers of glucose and mannose, but much higher affinities for the α-anomers.

The idea that liver hexokinase D ('glucokinase') is more specific for glucose than the other isoenzymes derives from the general agreement that it shows much more activity with glucose than with fructose at sugar concentrations of 100 mM, whereas no such striking difference is apparent with the other isoenzymes. However, for hexokinases A, B and C this concentration is saturating for both hexoses, whereas for hexokinase D it approaches saturation for glucose though it is well below saturation for fructose[16]. Much earlier Walker[17], and later others[18–20], pointed out that although the name 'glucokinase' (and a

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different EC number) for this isoenzyme might be a useful guide to its physiological role it was unsatisfactory as the enzyme was not wholly specific for glucose.

Assessing specificity by kinetic measurements at a single concentration, with substrates considered one at a time, was once quite standard. However, there is now widespread acceptance of Fersht's view that discrimination between substrates that are simultaneously present provides the only meaningful measure of specificity[21]. Examination of the four hexokinase isoenzymes on this basis shows that they are similar in sugar specificity, and that if any difference exists it is that hexokinase D is less effective at discriminating between glucose and fructose than some of the other isoenzymes[16]. Thus the name 'glucokinase' is a misnomer for hexokinase D and it should be reserved for enzymes that are genuinely specific for glucose, such as those from moulds, bacteria and invertebrate animals.

Even Walker's suggestion[17] that the name 'glucokinase' offers a 'useful guide to its physiological role' is arguable: although it is probably true that glucose is the only physiologically significant substrate for hexokinase D this is no less true for the other isoenzymes, as it has long been known that fructose metabolism in liver is initiated by phosphorylation to fructose 1-phosphate, a reaction catalysed by fructokinase[22].

The Michaelis constant is a parameter of the Michaelis–Menten equation; it has no meaning when applied to an enzyme that does not obey Michaelis–Menten kinetics[23]. Kinetic data for hexokinase D published more than 20 years ago indicated easily observable deviations from Michaelis–Menten kinetics[24]. Subsequent more detailed investigations[25–29] showed that the plot of rate against glucose concentration is sigmoid, with a maximum slope at a concentration of about 2.5 mM, close enough to the 'normal' blood glucose concentration of 5 mM to suggest that the sigmoidicity has a physiologically important role. Yet none of the textbooks cited above mentions the sigmoidicity, and those that include figures purporting to illustrate the kinetics of hexokinase and 'glucokinase' show cleanly hyperbolic curves for both isoenzymes[7,9]: these are little changed from ones published in textbooks written before the sigmoidicity was well-established[30].

It might be argued that statements that 'glucokinase' has a K_m of 10 mM are not intended to imply adherence to Michaelis–Menten kinetics, but only that the enzyme is half-saturated at a concentration far higher than that needed for the other isoenzymes. This is certainly correct, but it seems to be a rather indirect and confusing way of expressing it.

The statement that hexokinase D is not inhibited by glucose 6-phosphate is correct under physiological conditions, as substantial inhibition requires concentrations above about 50 mM (Ref. 27). Nonetheless, from the point of view of enzymology the bare statement can be misleading, as it implies an anomaly that does not exist: the weak inhibition is quite consistent with the fact that not only glucose 6-phosphate, but also glucose, binds much more weakly to hexokinase D than to the other isoenzymes.

The statement with which we began contains or implies five errors, of which the most serious are the false statement of hexose specificity and the failure to recognize the sigmoid character of the response of hexokinase D to glucose. It is hard to understand the persistence with which these errors are copied from textbook to textbook, given that the facts are not controversial and that the main conclusion that their authors wish to draw from the discussion – that 'glucokinase' is better adapted than 'hexokinase' to respond to high blood-glucose concentrations – accords better with the actual kinetic behaviour of these enzymes than it does with the fictitious behaviour that is described[18].

As all of the textbooks we cite refer to the hexokinases in the context of their physiological role they may, perhaps, be forgiven for omitting all mention of what enzymologists might regard as the most interesting aspect of hexokinase D, namely that its sigmoid response to glucose is a property of a monomeric enzyme with only a single binding site for glucose. Cooperative behaviour has been widely regarded as impossible for monomeric enzymes, and, as we have discussed elsewhere[31], there are not many other examples.

We suggest an alternative textbook statement along the following lines: 'Mammals have several isoenzymes to catalyse the formation of glucose 6-phosphate from glucose. Hexokinase D (or type IV), the predominant isoenzyme in the liver, is a low-affinity hexokinase and differs significantly from the others. Its levels vary markedly with dietary and hormonal status; it requires much higher glucose concentrations (about 10 mM) for half-saturation, with a sigmoid dependence, and it is insensitive to physiological concentrations of glucose 6-phosphate. It is thus well adapted to respond to variations in blood-glucose levels. Despite its popular but misleading name of "glucokinase", its sugar specificity is similar to that of the other isoenzymes.'

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