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Mechanistic origin of the sigmoidal rate behaviour of glucokinase

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Model studies are presented which demonstrate that reactions proceeding by a random ternary-complex mechanism may exhibit most pronounced deviations from Michaelis–Menten kinetics even if the reaction is effectively ordered with respect to net reaction flow. In particular, the kinetic properties and reaction flow characteristics of glucokinase can be accounted for in such terms. It is concluded that insufficient evidence has been presented to support the idea that glucokinase operates by a 'mnemonical' type of mechanism involving glucose binding to distinct conformational states of free enzyme. The sigmoidal rate behaviour of glucokinase can presently be more simply explained in terms of glucose binding to differently ligated states of the enzyme.

INTRODUCTION

The hexokinase occurring in the liver of vertebrates appears to be of major importance for regulation of the concentration of glucose in blood and is usually classified as a glucokinase (ATP:D-glucose 6-phosphotransferase, EC 2.7.1.2). The kinetic and physicochemical properties of the glucokinase isolated from rat liver have been particularly well characterized (for a review see Pollard-Knight & Cornish-Bowden, 1982). Initial-velocity studies of the latter enzyme have established that the catalytic reaction proceeds by a ternary-complex mechanism at a rate conforming to Michaelis–Menten kinetics with respect to MgATP concentration, but showing a sigmoidal dependence on the concentration of glucose (Niemeyer *et al.*, 1975; Storer & Cornish-Bowden, 1976). The possibility that the observed deviations from Michaelis–Menten kinetics may reflect glucose binding to differently ligated forms of the enzyme (Ferdinand, 1966; Griffin & Brand, 1968) has been considered difficult to reconcile with the detailed rate behaviour of the system (Storer & Cornish-Bowden, 1977; Cárdenas *et al.*, 1979). Velocity data for glucokinase, therefore, have been interpreted in the light of the 'mnemonical' model discussed by Ricard *et al.* (1974), which is based on the fact that substrate binding to different conformational states of free enzyme under certain conditions may contribute higher-degree terms to the rate equation (Rabin, 1967).

The 'mnemonical' mechanism for glucokinase catalysis has been most strongly advocated by Cornish-Bowden and co-workers. They found from inhibition studies and isotope-exchange measurements that the productive ternary complex is formed by an effectively ordered mechanism, with glucose combining first to the enzyme (Storer & Cornish-Bowden, 1977; Gregoriou *et al.*, 1981). Evidence for the existence of an alternative pathway involving the binary enzyme–MgATP complex was presented, but, since this pathway appeared to make little contribution to the net reaction, it was concluded that the observed deviations from Michaelis–Menten kinetics cannot be attributed to random addition of the substrates.

There is strong reason to question the validity of the latter argument, because theoretical analyses have failed to reveal any intuitively obvious relationship between the rate behaviour of and partitioning of reaction flow in random ternary-complex systems (Pettersson, 1969; Andersson *et al.*, 1984). The present paper draws attention to this interpretational complication and shows that the basic kinetic properties of glucokinase can be accounted for without the assumption that glucose interacts with distinct conformational states of free enzyme.

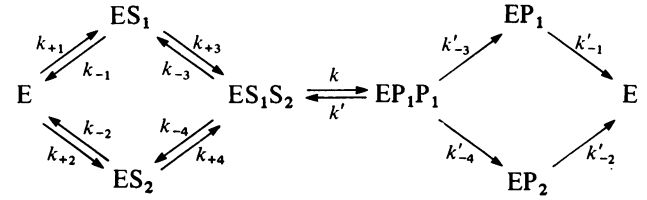
RESULTS

Theory

According to Gregoriou *et al.* (1981), the glucokinase-catalysed phosphorylation of glucose (S_1) by MgATP (S_2) proceeds minimally by a random ternary-complex mechanism (Scheme 1), with preferential utilization of the pathway involving intermediate formation of the ES_1 complex. The steady-state rate equation corresponding to Scheme 1 is given by:

$$\frac{v}{c_E} = \frac{(\alpha_{11}+\alpha_{21}[S_1]+\alpha_{12}[S_2])[S_1][S_2]}{\beta_{00}+\beta_{01}[S_2]+\beta_{02}[S_2]^2+(\beta_{10}+\beta_{11}[S_2]+\beta_{12}[S_2]^2)[S_1]+(\beta_{20}+\beta_{21}[S_2])[S_1]^2} \quad (1)$$

where coefficients α_{ij} and β_{ij} are related to rate constants in the mechanism as indicated in Table 1 (Dalziel, 1958); c_E denotes the total concentration of enzyme. The partitioning of reaction flow between the alternative



Scheme 1. The random ternary-complex mechanism

Table 1. Relationships between coefficients in eqn. (1) and rate constants in Scheme 1

$$\alpha_{21} = k_{+1}k_{+3}k_{+4}$$
$$\alpha_{12} = k_{+2}k_{+3}k_{+4}$$
$$\alpha_{11} = k_{+1}k_{-2}k_{+3} + k_{-1}k_{+2}k_{+4}$$
$$\beta_{21} = k_{+1}k_{+3}k_{+4}(A+B)$$
$$\beta_{20} = k_{+1}k_{+4}(1+k_{-3}A)$$
$$\beta_{12} = k_{+2}k_{+3}k_{+4}(A+B)$$
$$\beta_{11} = k_{+3}k_{+4} + (k_{+1}k_{-2}k_{+3} + k_{-1}k_{+2}k_{+4})(A+B) + (k_{+1}k_{+3}k_{-4} + k_{+2}k_{-3}k_{+4})A$$
$$\beta_{10} = k_{-1}k_{+4}(1+k_{-3}A) + k_{+1}k_{-2}(1+k_{-3}A+k_{-4}A)$$
$$\beta_{02} = k_{+2}k_{+3}(1+k_{-4}A)$$
$$\beta_{01} = k_{-1}k_{+2}(1+k_{-3}A+k_{-4}A) + k_{-2}k_{+3}(1+k_{-4}A)$$
$$\beta_{00} = k_{-1}k_{-2}(1+k_{-3}A+k_{-4}A)$$
$$A = (K' + k'_{-3} + k'_{-4})/k(k'_{-3} + k'_{-})$$
$$B = (k'_{-1}k'_{-2} + k'_{-2}k'_{-3} + k'_{-1}k'_{-4})/k'_{-1}k'_{-2}(k'_{-3} + k'_{-4})$$

pathways for formation of the ternary ES_1S_2 complex can be described in terms of the flow quotient Q defined as:

$$Q = \frac{\text{Flow via } ES_1}{\text{Flow via } ES_2} \quad (2)$$

At steady state we have:

$$Q = \frac{k_{+1}k_{+3}(k_{-2} + k_{+4}[S_1])}{k_{+2}k_{+4}(k_{-1} + k_{+3}[S_2])} \quad (3)$$

and the steady-state concentration of species ES_2 can be expressed as:

$$[ES_2] = \frac{v}{[S_1]} \cdot \frac{k_{-1}k_{+2}(1+k_{-3}A+k_{-4}A) + k_{+1}k_{+3}k_{-4}A[S_1] + \beta_{02}[S_2]}{\alpha_{11} + \alpha_{21}[S_1] + \alpha_{12}[S_2]} \quad (4)$$

with A defined as in Table 1.

When:

$$k_{-1} \gg k_{+3}[S_2] \quad (5)$$

coefficients α_{12}, β_{12} and β_{02} in eqn. (1) become of insignificant magnitude (Dalziel, 1958), and the reaction will conform approximately to Michaelis–Menten kinetics with respect to S_2. Eqn. (3) then reduces to:

$$Q \approx \frac{k_{+1}k_{+3}(k_{-2} + k_{+4}[S_1])}{k_{+2}k_{+4}k_{-1}} > \frac{k_{+1}k_{+3}k_{-2}}{k_{+2}k_{+4}k_{-1}} = \frac{k_{-3}}{k_{-4}} \quad (6)$$

showing that reaction flow via ES_1 will predominate as soon as $k_{-3} \gg k_{-4}$.

Introducing the ratio:

$$R = \frac{k_{-4}}{k_{-3}} \quad (7)$$

as a parameter, eqn. (6) can be expressed as:

$$Q > \frac{1}{R} \quad (8)$$

It follows that the relative contribution to net reaction flow provided by the pathway via ES_2 will be less than R when eqn. (5) applies.

Model studies

Eqn. (1), relationships in Table 1 and rate constant values given in Table 2 define a kinetic model which utilizes the parameter R as a variable, and which can be considered to satisfy the condition in eqn. (5) for

Table 2. Kinetic model considered in the paper

Rate-constant values refer to the reaction in Scheme 1, and R denotes a positive constant.

Rate constant	Value	Unit
k_{+1}	3000	$s^{-1} \cdot mM^{-1}$
k_{+2}	49.5 R	$s^{-1} \cdot mM^{-1}$
k_{+3}	330	$s^{-1} \cdot mM^{-1}$
k_{+4}	5 R	$s^{-1} \cdot mM^{-1}$
k_{-1}	20000	s^{-1}
k_{-2}	5 R	s^{-1}
k_{-3}	30	s^{-1}
k_{-4}	30 R	s^{-1}
k	10000	s^{-1}
k'	100	s^{-1}
k'_{-1}	10000	s^{-1}
k'_{-2}	10	s^{-1}
k'_{-3}	145	s^{-1}
k'_{-4}	1	s^{-1}

$[S_2] < 5$ mM. Certain values in Table 2 have been chosen more or less arbitrarily, but others have been carefully selected to render the model suitable for simulation of the initial-velocity data reported for the glucokinase reaction by Storer & Cornish-Bowden (1976). The plots of $[S_1]/v$ versus $[S_1]$ in Fig. 1 establish that the model does provide a most satisfactory fit to the latter data when $R = 0.05$, i.e. when the ES_2 pathway contributes less than 5% to the net reaction. For this (and any lower) value of R, the model will account for the observed qualitative flow characteristics of the glucokinase reaction as indicated by the isotope-exchange measurements reported by Gregoriou *et al.* (1981); note that values of primed constants in Table 2 have been chosen such that the model is consistent also with a non-limiting rate of the phosphotransfer step and with preferential utilization of the enzyme–MgADP pathway for product dissociation.

The interesting property of the above kinetic model from a theoretical point of view is that reaction rates calculated for $R = 0.05$ differ insignificantly (less than 5% over the concentration ranges considered in Fig. 1) from those obtained for R values tending towards zero. In other words, the simulated set of curves in Fig. 1 will look essentially the same for any vlaue of $R \leqslant 0.05$, and the pronounced deviations from Michaelis–Menten kinetics predicted by the model (Fig. 1) will persist however minute a contribution to net reaction flow the ES_2 pathway is assumed to provide. This confirms the existence of the interpretational complication mentioned in the Introduction: observations indicating that one of the alternative pathways for ternary-complex formation in Scheme 1 is of little significance with respect to net reaction flow cannot be taken to exclude that this pathway may contribute most significantly to the higher-degree rate behaviour of the system.

The variation with substrate concentrations of the steady-state concentration of species ES_2 in Scheme 1 was calculated from eqn. (4) for the kinetic model and experimental conditions considered in Fig. 1. The results in Fig. 2 show that the concentration of ES_2 increases drastically with decreasing values of $[S_1]$ for any fixed value of $[S_2]$. This indicates that the pronounced deviations from Michaelis–Menten kinetics exhibited by

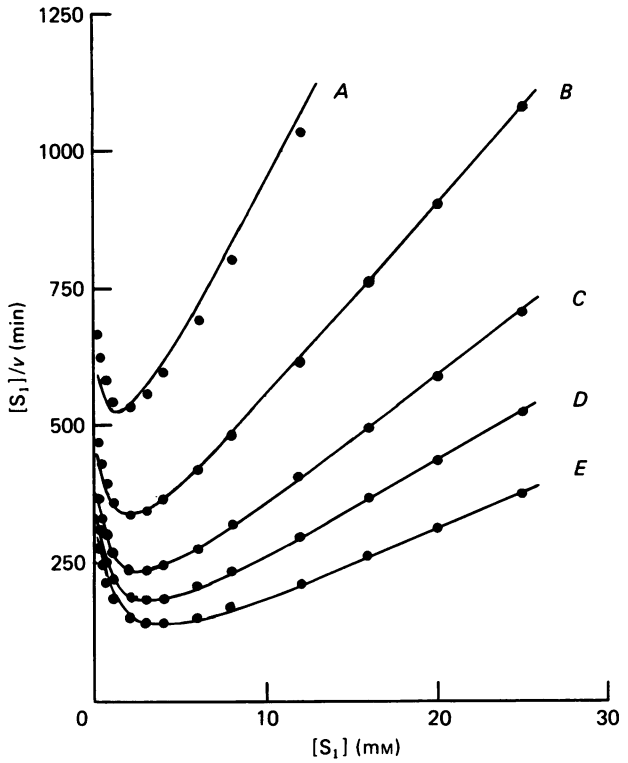


Fig. 1. Dependence on substrate concentrations of the initial velocity for the glucokinase reaction

Experimental points (○) were calculated from the empirically establised rate equation (Storer & Cornish-Bowden, 1977) for glucose (S_1) concentrations in the range 0.2–25 mM at fixed MgATP concentrations of 0.018 mM (curve *A*), 0.215 mM (curve *B*), 0.43 mM (curve *C*), 0.86 mM (curve *D*) and 4.3 mM (curve *E*). Curves drawn show the rate behaviour predicted by Scheme 1 for the model in Table 2 when $R = 0.05$ and $c_E = 0.01$ μM.

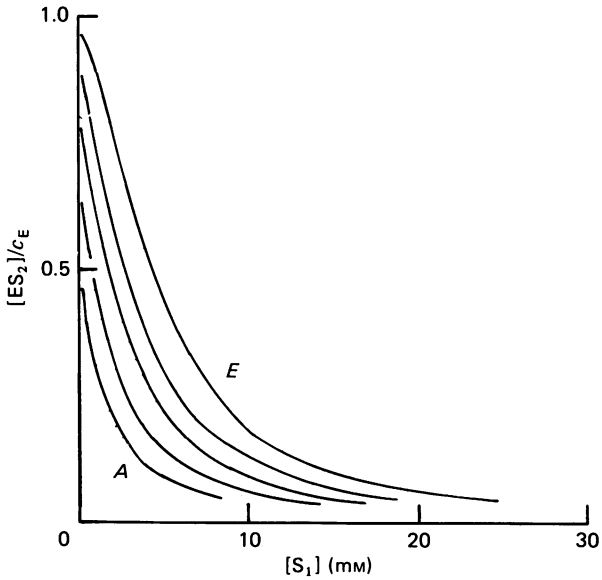


Fig. 2. Dependence on substrate concentrations of the concentration of species ES_2

Curves drawn were calculated from eqn. (4) for the model reaction and experimental conditions considered in Fig. 1.

the model reaction in Fig. 1 can be attributed to accumulation of an enzymic intermediate (ES_2) that is not on the main catalytic pathway. The fact that accumulation of such an intermediate may take place explains why the reaction kinetics can be strongly influenced by a pathway that does not contribute significantly to net reaction flow.

DISCUSSION

Results presented in this paper establish that enzymes operating by the random ternary-complex mechanism in Scheme 1 may exhibit most pronounced deviations from Michaelis–Menten kinetics even if one of the alternative pathways for ternary-complex formation contributes negligibly to net reaction flow. The model reaction simulated in Figs. 1 and 2 may be regarded as effectively ordered in the sense that the pathway involving the binary ES_1 complex accounts for more than 95% of the catalytic reaction flow. Over the concentration ranges considered, the model reaction will appear to be ordered also according to the criteria discussed by Gregoriou *et al.* (1981) for flux-ratio determinations by isotope-exchange techniques, and attempts to detect the ES_2 complex by such techniques will fail for the simple reason that the latter intermediate does not contribute significantly to net reaction flow. Nevertheless, the rate behaviour of the system will be strongly affected by the existence of the minor pathway and show deviations from Michaelis–Menten kinetics reflecting accumulation of the ES_2 complex.

In particular, model studies reported in Fig. 1 demonstrate that the random mechanism in Scheme 1 may account for the basic kinetic properties of rat liver glucokinase. This does not mean that the likely mechanistic origin of the sigmoidal rate behaviour of the enzyme now has been established. There is evidence indicating that the glucokinase reaction leads to formation of an 'abortive' enzyme–MgADP–glucose complex (Storer & Cornish-Bowden, 1977), and formation of such a complex could provide a reasonable alternative explanation for the observed higher-degree rate-dependence on glucose concentration. The 'mnemonical' mechanism advocated by Cornish-Bowden and co-workers points to another possible source of higher-degree glucose concentration terms in the rate equation. It would seem obvious from the present results, however, that a discrimination between these mechanistic alternatives cannot be made by qualitative arguments relating to the reaction flow characteristics of the system. The observation that glucokinase operates by a basically random ternary-complex mechanism (Gregoriou *et al.*, 1981) implies that the rate equation minimally includes higher-degree terms deriving from random substrate binding. The magnitude and kinetic significance of these terms can be estimated only on the basis of quantitative information about the absolute or relative magnitude of critical rate constants in the mechanism, and such information presently appears to be sparse. No transient-state kinetic or equilibrium data for substrate binding to the enzyme are available, and the partially informative flux-ratio determinations reported by Gregoriou *et al.* (1981) refer to a pH distinct from that at which the rate behaviour of the enzyme has been experimentally detailed.

Similar considerations apply for the observation that glucose most probably forms an 'abortive' ternary

complex with the enzyme–MgADP species (Storer & Cornish-Bowden, 1977). Such complex-formation, also, must be assumed to contribute higher-degree terms to the rate equation, and the magnitude of these terms has to be quantitatively assessed before any reliable conclusions can be drawn as to their kinetic significance.

On the other hand, no experimental results have been reported that would indicate that glucokinase exists in distinct conformational states showing differential reactivity towards glucose. It would seem reasonable to conclude, therefore, that the available evidence lends little support to the proposals that glucokinase operates by a 'mnemonical' type of mechanism (Cárdenas *et al.*, 1979; Gregoriou *et al.*, 1981). The rate behaviour of glucokinase can presently be more simply explained in terms of glucose binding to differently ligated states of the enzyme.

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