Mechanism of liver glucokinase

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Summary

Glucokinase is the enzyme primarily responsible for the phosphorylation of glucose in the livers of mammals and other vertebrates. It differs from the other hexokinases in being insensitive to inhibition by glucose 6-phosphate and in responding co-operatively to changes in the glucose concentration in the physiological range. These properties accord well with the presumed function of glucose phosphorylation in the liver as a means of controlling the blood-glucose concentration. Glucokinase has the unusual property for a co-operative enzyme of being a monomeric enzyme with a single active site. The co-operativity consequently requires a purely kinetic explanation and cannot be explained by analogy with subunit interactions in proteins that display co-operativity in equilibrium binding. The behaviour is consistent with a 'mnemonical' type of mechanism, i.e. one in which the co-operativity derives from the occurrence of two interconvertible forms of free enzyme that are not at equilibrium in the steady state. As co-operativity is observed only with glucose and not with the other substrate, $MgATP^{2-}$, a corollary of this interpretation is that glucose must bind predominantly or exclusively before $MgATP^{2-}$. This order of binding is supported by isotope-exchange measurements, though the alternative order also appears to be possible as a minor route of reaction. Stereochemical investigations reveal that glucokinase resembles other hexokinases in that the form of $MgATP^{2-}$ that reacts with the enzyme is the $\beta\gamma$-bidentate complex with the Λ-screw sense, and that the reaction proceeds with inversion of configuration at phosphorus.

Introduction

The existence of an enzyme with properties now known to apply to glucokinase was first suggested (1) when liver slices were shown to phosphorylate glucose at a rate that depended on the concentration of glucose in the medium. This indicated that the enzyme responsible for the reaction should have a K_m for glucose around normal blood glucose concentrations (5 mM), much higher than the K_m values for the other glucose-phosphorylating enzymes known in mammals, the hexokinases types I, II and III (2–4) (0.2–0.5 mM). This observation led to the discovery of rat-liver glucokinase (ATP: D-glucose 6-phosphotransferase, EC 2.7.1.2), or hexokinase type IV, which is half-saturated at about 5 mM-glucose and is only weakly and non-allosterically inhibited by glucose 6-phosphate (5–9).

The name glucokinase and the classification as EC 2.7.1.2 strictly implies an enzyme that is specific for glucose. The rat-liver enzyme is marginally more specific for glucose than the other mammalian hexokinases but the effect is not great and other hexoses, such as mannose, 2-deoxyglucose and fructose are also phosphorylated, though at appreciable rates only at concentrations that are far too high to have any physiological significance (9). It is arguable therefore whether glucokinase is the proper name for the liver isoenzyme, and some

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workers prefer to call it hexokinase type IV, within the classification EC 2.7.1.1. We agree that this is more appropriate, but for convenience we shall use the name glucokinase in this review, as this is by far the most commonly used name in the literature.

A major function of the liver is to regulate the concentration of glucose in the blood. This is achieved by synthesis and storage of glycogen when the blood glucose concentration rises and breakdown of glycogen when it falls (10, 11). The position of glucokinase as the first enzyme in the conversion of glucose into glycogen and its kinetic properties make it appropriate as a mediator of this regulation, and loss of control in diabetes mellitus appears to be related to a decrease in glucokinase activity in the liver (12). The recovery of control on administration of insulin is accompanied by an increase to the normal level of glucokinase activity (13, 14), and there is a parallel variation in the level of glucokinase in the liver with the intake of glucose in the diet.

Studies of the mechanism of action of glucokinase were initially hindered by the lack of an efficient purification procedure and by the lability of the enzyme. It is now possible, however, to purify it in high yield by means of a procedure involving affinity chromatography on Sepharose-N-6-aminohexanoyl-2-amino-2-deoxy-D-glucopyranose, and conditions of high stability have been determined (15–17).

Our comments in this review will refer to observations on glucokinase from rat liver unless otherwise indicated. However, similar enzymes have been identified in the livers of numerous other mammalian and amphibian species (18, 19), and the limited evidence suggests that much of the knowledge that exists about the rat-liver enzyme can be generalized and is likely to be relevant, therefore, to the role of glucokinase in human diabetes mellitus.

Properties of glucokinase

Glucokinase is the principal glucose-phosphorylating enzyme in rat liver. The true phosphoryl donor is considered (20) to be the divalent cation nucleoside complex $MgATP^{2-}$, for which the K_m value is 0.55 mM (5). $MgITP^{2-}$ can also act as phosphoryl donor but the rates of reaction are considerably lower (9).

Glucokinase is relatively insensitive to product inhibition by glucose 6-phosphate, and has an inhibition constant of about 60 mM (18). This is far too high to have any physiological significance, and contrasts with the values of 0.2–0.9 mM that have been found for the other three mammalian hexokinases (22). These values are comparable with the normal hepatic concentrations of glucose 6-phosphate of 0.1–0.3 mM (23) and indicate that the already low activities of these enzymes in the liver would be substantially decreased by inhibition. Glucokinase is therefore likely to be responsible for virtually all of the phosphorylation of glucose that occurs in the liver.

Glucokinase is the only mammalian hexokinase reported to have a molecular mass as low as 50kDa (21, 24, 25). The reported values have varied widely, in the range 48–68kDa, but with homogeneous preparations all methods give a molecular mass close to 48kDa (17), about half of the value of 96kDa reported for the other hexokinases (26–28).

Glucokinase displays Michaelis-Menten kinetics with respect to one substrate, $MgATP^{2-}$, but various anomalies were reported after the kinetics with respect to the other substrate, glucose, were first studied (9, 29). These anomalies are now known to be due to the fact that the kinetics with respect to glucose show positive co-operativity (5, 30). Purified glucokinase gives a Hill coefficient of about 1.6 at saturating $MgATP^{2-}$, and somewhat smaller values at lower concentrations of $MgATP^{2-}$, though some co-operativity is evident at the lowest $MgATP^{2-}$ concentration at which measurements can be made (5, 31). The co-operativity appears to be a general feature of liver glucokinase, as it is also found in the enzyme from various other mammals and amphibians (31). The co-operative properties of the enzyme indicate that the values of K_m for glucose reported in some earlier studies must be treated with caution. In some cases these values are meaningless; in others they correspond approximately to half-saturation concentrations.

Co-operativity of glucokinase

Deviations from Michaelis-Menten kinetics observed for glucokinase (9, 29, 30) have been of great importance in the development of an under-

standing of the mechanism of the enzyme. Plots of rate against glucose concentration are sigmoid: the corresponding Hill coefficient is about 1.6 at saturating $MgATP^{2-}$, and decreases as the $MgATP^{2-}$ concentration decreases. Although measurements cannot be made in the absence of $MgATP^{2-}$ a Hill coefficient of 1.0 (no co-operativity) at zero $MgATP^{2-}$ would be a reasonable extrapolation of the data (5, 31). Thus an adequate mechanism for glucokinase must explain why glucose co-operativity occurs only when induced by $MgATP^{2-}$, a substrate that displays no co-operativity itself.

Glucose co-operativity has also been observed for the glucokinase activity of isolated rat hepatocytes (32), but as with the purified enzyme there is no co-operativity with $MgATP^{2-}$. Various treatments such as photooxidation with methylene blue and heating the enzyme before assay do not alter the Hill coefficient (31), but assay in the presence of glycerol decreases the Hill coefficient, with a value of 1.05 observed in the presence of 30% glycerol (32). Use of 2-deoxyglucose as substrate instead of glucose also results in a loss of co-operativity (31).

Because glucose is a monomeric enzyme under all conditions of the assay (17, 34) models that explain co-operativity in terms of a reversible association of the enzyme (35, 36) must be excluded. Similarly, the quasi-equilibrium models postulating subunit interactions (37, 38) can also be excluded. However, analogous models in which two or more interacting sites exist on a monomeric enzyme could not initially be excluded with such confidence, though the absence of a precedent made them seem unlikely. The close similarity between the amino acid compositions of glucokinase and hexokinase type II suggests that the latter, though strictly speaking also a monomer, resembles a dimer of glucokinase (39): if so, any model postulating two similar sites on glucokinase would imply four similar sites on hexokinase type II; though not impossible, it is yet an extravagant hypothesis in the absence of direct evidence. Affinity labelling has now shown that glucokinase has only a single active site and so models of this kind can definitely be excluded (40).

It follows that the co-operativity of glucokinase must be explained in purely kinetic terms, because co-operativity is impossible for a single binding site at equilibrium. Several non-equilibrium models have been suggested (41–44) for kinetic co-operativity, but these have not often been considered in relation to real experimental systems. On the basis of computer fitting of data and product-inhibition experiments Storer & Cornish-Bowden (21) postulated a 'mnemonical mechanism' to account for the kinetics of glucokinase: this kind of mechanism was first worked out by Ricard et al. (45) as a development of the idea of enzyme memory first put forward by Rabin (42) and applied by them to hexokinase type L_I from wheat germ (46).

The essential feature of the mnemonical mechanism, which is illustrated in Fig. 1, is that the form of the enzyme released at the end of the catalytic cycle (E) is different from the form that is predominant in the absence of substrates (E′). Although these two forms of the free enzyme are interconvertible, the reactions between them are slow enough for them not necessarily to be close to equilibrium while the catalytic reaction is proceed-

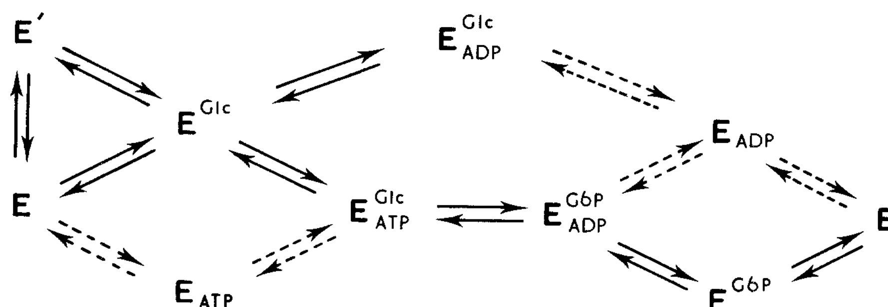

Fig. 1. Proposed mechanism for glucokinase. This mechanism includes the mnemonical mechanism as a special case (with steps shown by broken lines omitted) and also the mechanism of Cárdenas et al. (31). Abbreviations: Glc, glucose; ATP, $MgATP^{2-}$; ADP, $MgADP^{-}$; G6P, glucose 6-phosphate.

ing. The details of how this model accounts not only for glucose co-operativity but also for the lack of $MgATP^{2-}$ co-operativity and the dependence of glucose co-operativity on the concentration of $MgATP^{2-}$, are considered in the next section.

Characteristics of the mnemonical mechanism

Mathematical details of the mnemonical mechanism have been given by Ricard et al. (45). Here we shall discuss its properties in qualitative terms. For simplicity we shall only consider the reactions represented by full lines in Fig. 1; the reactions shown by broken lines will be assumed to be missing. Three sets of conditions need to be considered:

1. Low [$MgATP^{2-}$]. When the concentration of $MgATP^{2-}$ is very small the rate of any second-order reaction involving it must be very small. Consequently there is no way in which the enzyme-glucose complex can be removed fast enough to prevent establishment of equilibrium between it and the two forms of free enzyme. Thus, as a single binding site at equilibrium cannot exhibit co-operativity, the binding of glucose at equilibrium must be non-co-operative, as in the case when the concentration of $MgATP^{2-}$ is too low to perturb equilibrium appreciably.

2. High [$MgATP^{2-}$] but low [Glucose]. At high concentrations of $MgATP^{2-}$ it is possible in principle for the enzyme-glucose complex to react so fast with $MgATP^{2-}$ that glucose binding cannot be maintained at equilibrium. Under these circumstances it is necessary to consider not only the $MgATP^{2-}$ concentration but the glucose concentration as well. If this is low the rate of any second-order reaction involving glucose must be slow. Consequently the less stable form of free enzyme E released at the end of the cycle is likely to relax to the more stable form E′ before it encounters a glucose molecule. The kinetics observed at these concentrations should therefore be those characteristic of glucose reacting with E′. Now, as this is the more stable enzyme form, it must by thermodynamic considerations bind glucose less tightly than the other form E, because the same enzyme-glucose complex is produced in both cases. Consequently at high [$MgATP^{2-}$] but low [Glucose], glucose should appear to bind weakly.

3. High [$MgATP^{2-}$] and high [Glucose]. When both substrates are present at high concentrations, it is still possible for $MgATP^{2-}$ to react with the enzyme-glucose complex before it can equilibrate, but now in addition glucose can react with the less stable enzyme form E before it has time to relax to the more stable form E′. Thus under these conditions behaviour characteristic of glucose binding to E should be observed and, for the reason indicated already, this should indicate tighter binding than that observed at lower concentrations of glucose.

It follows from these considerations that at high $MgATP^{2-}$ concentrations the tightness of glucose binding should appear to increase with the glucose concentration; in other words the enzyme should show positive co-operativity with respect to glucose. On the other hand there should be no co-operativity with respect to $MgATP^{2-}$ because $MgATP^{2-}$ enters into only one step of the reaction and thus by the ordinary characteristics of steady-state rate equations (47, 48) there can be no dependence on $[MgATP^{2-}]^2$ (or higher-order terms). Finally, because the glucose co-operativity depends on the capacity of $MgATP^{2-}$ to react rapidly with the enzyme-glucose complex it should disappear at low concentrations of $MgATP^{2-}$. All of this behaviour is in fact observed with glucokinase (5).

One other feature of the mnemonical model not previously mentioned is the occurrence of a non-productive ternary complex enzyme-glucose-$MgADP^{-}$. This complex was postulated by Storer & Cornish-Bowden (21) partly to account for the non-linear inhibition behaviour observed with $MgADP^{-}$ but also, and perhaps more important, because plausibility demands it. Any active site that is capacious enough to accommodate both glucose and $MgATP^{2-}$ simultaneously must also be capacious enough to accommodate both glucose and $MgADP^{-}$ simultaneously. In our view, therefore, mechanisms postulated for kinases that do not include such non-productive ternary complexes require more explaining than mechanisms that do; it is not necessary to regard the enzyme-glucose-$MgADP^{-}$ complex as an ad hoc feature of the glucokinase mechanism introduced simply to account for otherwise inconvenient product-inhibition data.

An alternative explanation of glucokinase co-operativity

Cárdenas et al. (31) made observations on glucokinase similar to those of Storer & Cornish-Bowden, but explained them in a somewhat different way. Their model, which is also included in Fig. 1 if the steps indicated by broken lines are considered to operate, is based on the existence of alternative pathways of substrate binding. When such alternative pathways exist and substrate binding is not at equilibrium in the steady state, rate equations contain terms in the squares of both substrate concentrations, and Ferdinand (41) has shown that co-operative behaviour can be explained in this way. Cárdenas et al. found that the existence of alternative pathways of substrate binding was not in itself sufficient to account for the detailed behaviour they observed and consequently allowed for isomerization of the free enzyme, as postulated in the mnemonical model. As we shall discuss below, isotope-exchange measurements at chemical equilibrium indicate some degree of randomness of substrate binding (49) and so all of the steps shown in Fig. 1 seem to have some reality. It follows that there is little disagreement between ourselves and Cárdenas et al. about the complete mechanism, but there is some disagreement about the emphasis to be placed on the various aspects: we regard the isomerization of free enzyme as the main source of the co-operativity, whereas Cárdenas et al. regard the existence of alternative pathways of substrate binding as more important.

Isotope exchange

Isotope-exchange measurements at chemical equilibrium can be used to determine whether more than one pathway for substrate binding to an enzyme exists and if so whether both pathways contribute significantly to the rates measured under steady-state conditions. Exchange measurements (49) of this kind provide support for the mechanism proposed for glucokinase by Cárdenas et al., because neither the exchange between ATP and ADP nor that between glucose and glucose 6-phosphate is inhibited at saturating concentrations of all four reactants. This observation cannot easily be explained except by supposing that a pathway exists in which $MgATP^{2-}$ binds to the free enzyme to give a binary complex capable of reacting with glucose. In other respects the equilibrium experiments were in agreement with previous ideas about the mechanism. First, there was no exchange between glucose and glucose 6-phosphate in the absence of ATP, or between ATP and ADP in the absence of glucose; this showed that the reaction must proceed through a ternary complex and not through a substituted enzyme. In addition, exchange between ATP and ADP was much faster than between glucose and glucose 6-phosphate; this showed that interconversion between ternary complexes, the chemical step in the reaction, could not be rate-limiting, because if it were all exchanges would occur at the same rate. This characteristic was expected from the steady-state kinetics because co-operativity in a monomeric enzyme required that substrate binding should not necessarily be at equilibrium in the steady state.

Although isotope exchange at chemical equilibrium provides a sensitive test for the existence of alternative pathways it is not necessary for the pathways so detected to make a significant contribution to the rate under ordinary conditions. Further information may, however, be obtained from two kinds of exchange experiment in the steady state (49). The simpler and better known approach is the use of radioactive reactants to trap binary complexes (50). For the glucokinase reaction, if a particular complex, e.g. an enzyme-$MgADP^{-}$ complex, exists in significant concentrations when the reaction is proceeding forwards, it ought to be possible to detect it by adding labelled glucose 6-phosphate to the reaction mixture and showing that there is transfer of label to glucose or ATP. This kind of experiment did in fact show that an enzyme-glucose-6-phosphate complex existed as an intermediate in the forward reaction, and an enzyme-glucose complex as an intermediate in the reverse reaction, but did not detect any enzyme-$MgATP^{2-}$ or enzyme-$MgADP^{-}$ complexes (49). The same result was obtained with the more quantitative approach using measurements of flux ratios, which was developed by Britton (51). We have recently described the principles of this approach in simple qualitative terms elsewhere (52), and will not therefore repeat the description here.

We may summarize the results of the isotope-

exchange measurements by saying that the experiments at chemical equilibrium indicate that the alternative pathways postulated by Cárdenas et al. (31) and shown by broken lines in Fig. 1 do exist, but that the steady-state experiments suggest that they make a negligible contribution to the total rate. It is difficult, therefore, to believe that they play a major role in generating the co-operativity observed with glucose.

Product inhibition

Results of product-inhibition experiments (21) conflict to some degree with those of the flux-ratio experiments. In particular, glucose 6-phosphate displays competitive inhibition with respect to $MgATP^{2-}$ but mixed inhibition with respect to glucose, the opposite from what one would expect for a compulsory-order mechanism with glucose as first substrate and glucose 6-phosphate as second product. This is not an unusual property, however, for a hexokinase, and is seen, for example, with the brain isoenzyme (53); it is usually interpreted as evidence that glucose 6-phosphate and $MgATP^{2-}$ compete for a phosphate-specific site (53). We found this an implausible idea and instead suggested (21) that glucose 6-phosphate might be the first product to be released and that apparent competition might arise from simultaneity of this release with the binding of $MgATP^{2-}$, as in the Theorell-Chance mechanism (54). The flux-ratio experiments, however, seem to rule out this interpretation and suggest that the idea of competition for a phosphate-specific site may be correct. Nonetheless, it is clear that further work will need to be done before the inhibition characteristics of glucokinase can be regarded as properly understood.

Affinity labelling of glucokinase

Connolly and Trayer (55) showed that the alkylating agent N-bromoacetyl-glucosamine was a true affinity label for rat muscle hexokinase type II. The kinetics and pH dependence of inactivation were consistent with reaction of the reagent with a single group of pK_a 8.9, with an apparent first-order rate constant of $1.5 \times 10^{-3}\ s^{-1}$. This behaviour is typical of reactions of model thiols with alkylating agents and suggests that the reacting residue is probably a cysteine. Bovine brain hexokinase type I (56) and yeast hexokinase (57) behave similarly.

With a view to obtaining information about the residues around the active site of glucokinase, Connolly & Trayer (40) subsequently studied the reactivity of rat liver glucokinase with several alkylating reagents, some of them substrate analogues. N-bromoacetylglucosamine is a strong inhibitor of the glucokinase-catalysed reaction, competitive with respect to glucose, but this inhibition approximates to ordinary reversible inhibition and irreversible inactivation of glucokinase is very slow; this contrasts with the rapid inactivation of hexokinase type I from bovine brain (56) and hexokinase type II from rat muscle (55) by the same compound. If the bromoacetyl group is made more 'mobile' by interposing a three-carbon or longer 'spacer' group between it and the sugar ring, as in the compounds N-(N-bromoacetyl glycyl/ glucosamine and N-(N-bromoacetyl)-6-aminohexanoylglucosamine, the inhibition still approximates to reversible competitive inhibition on a short time scale, but slow time-dependent irreversible inactivation also occurs. The inactivation kinetics showed that these compounds behave as true affinity labels, binding reversibly at the active site, increasing the local concentration of bromoacetyl groups and allowing subsequent alkylation. The stoichiometry of these reactions also contrasts with the behaviour with other hexokinases, as 2 mol of label are incorporated per 1 mol of enzyme under the conditions used. However, in the presence of glucose only 1 mol of label per 1 mol enzyme is incorporated and there is no loss of activity. This result indicates that one labelling site is remote from the active site and so no revision of the previous conclusion that glucokinase contains only one glucose-binding site per molecule is required.

Glucokinase differs in its inactivation behaviour from hexokinases types I and II in the further respect that the reacting group does not ionize in the pH range 6.5–9.0. This makes it unlikely that a cysteine residue is being attacked and suggests that the group is a methionine sidechain or a carboxylate group. Carboxylation of methionine is, however, usually reversed by prolonged incubation with 2-mercaptoethanol, but this treatment does not restore activity to labelled glucokinases (58), so a carboxylate group is the most likely site of labelling.

It is not, of course, necessary for this group to be involved in the catalytic mechanism: it may simply be required for maintaining the correct conformation for catalysis, or blocking it with a bulky reagent may do no more than prevent access of substrates to the active site.

$MgATP^{2-}$ binding and the stereochemistry of phosphoryl transfer

Bock (20) showed that the true phosphoryl donor in kinase-catalysed reactions is $MgATP^{2-}$. This complex can exist in six diastereomeric states, as illustrated in Fig. 2. If the Mg^{2+} binds to the non-bridge oxygen of the β-phosphorus in the $\beta\gamma$-bidentate chelate of $MgATP^{2-}$, this phosphorus atom becomes chiral and gives rise to two diastereoisomers, with AMP above or below the plane of the six-membered ring. Similarly, the $\alpha\beta$-bidentate complex of $MgATP^{2-}$ gives rise to an additional chiral centre at the α-phosphorus, and so both the γ-phosphate and the adenosine group can independently exist above or below the plane of the ring; thus this $\alpha\beta$-complex exists as four diastereoisomers.

It is unlikely that any enzyme would accept all of these diastereoisomers indiscriminately. Isomer preference might arise either from a preference for a specific metal-nucleotide conformation, or from a direct interaction between the nucleotide and the enzyme. Darby and Trayer (59) have used the

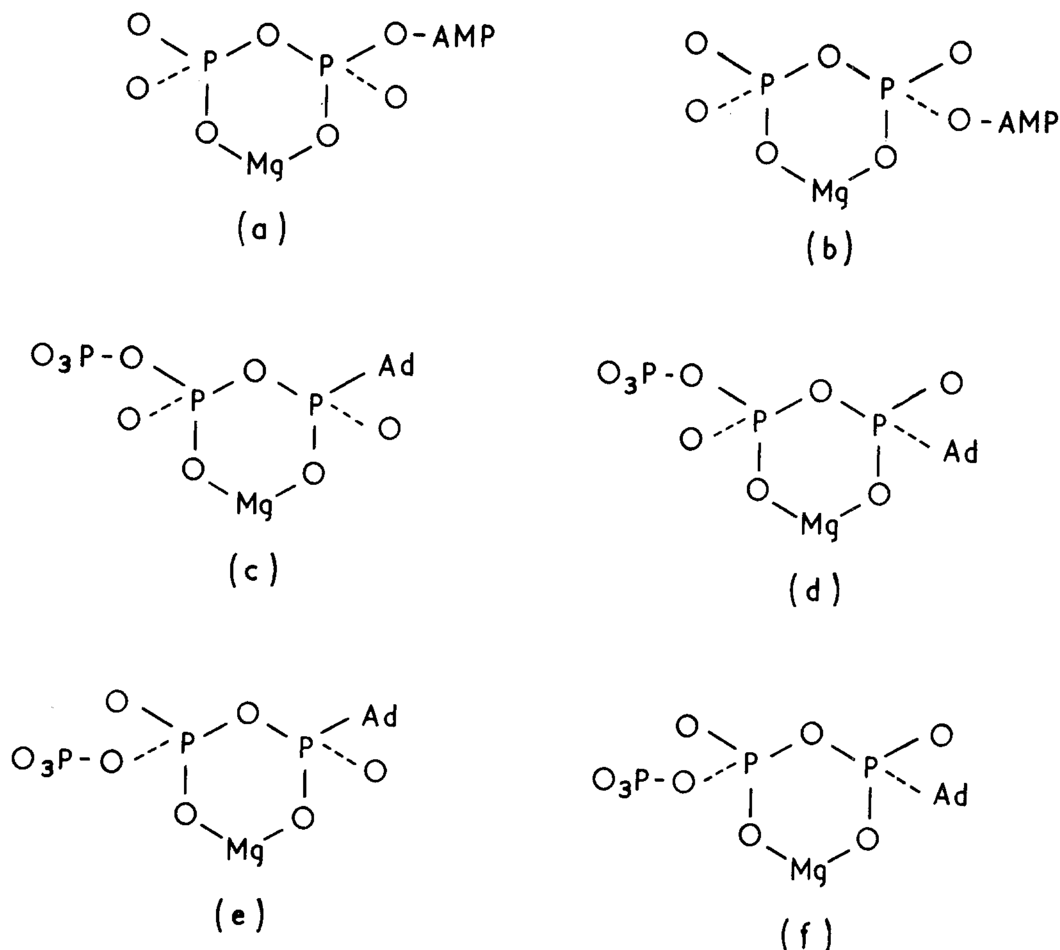

Fig. 2. Stereoisomers of $MgATP^{2-}$. The two isomers in the top row, (a) and (b), are $\beta\gamma$-bidentate complexes; the others, (c) to (e), are $\alpha\beta$-bidentate complexes. The three on the left, (a), (c) and (e) have the Λ-screw sense, the others (b), (d) and (f) the Δ-screw sense. Glucokinase is specific for isomer (a).

elegant approach developed by Eckstein and Goody (60) and by Jaffe and Cohn (61) to determine which preferences existed for glucokinase. They used the phosphorothioate analogues of ATP, in which the chirality at either the α- or the β-position is fixed by replacing a non-bridging oxygen atom with sulphur. These compounds allow one to distinguish which complex binds to the enzyme, but do not distinguish between the two ways in which the preference can arise. However, replacement of Mg^{2+}, which coordinates preferentially to O, with Cd^{2+}, which has a strong preference for S, or Mn^{2+}, Co^{2+}, or Zn^{2+}, which can coordinate to either O or S, does allow this distinction to be made. In the case of glucokinase, the reactive form of $MgATP^{2-}$ is the bidentate complex with the Λ screw sense (structure (a) in Fig. 2) and the preference arises from direct interaction between the enzyme and the non-bridging O atoms of the α-phosphate.

The catalytic step in a kinase-catalysed reaction will normally involve interconversion by phosphoryl transfer of two ternary complexes. In the case of glucokinase this would be the conversion of the enzyme-glucose-$MgATP^{2-}$ complex into the enzyme-glucose-6-phosphate-$MgADP^{-}$ complex. In principle there are four mechanisms by which such a transfer could occur (62):

1. the associative 'in line' mechanism, similar to a Walden inversion in an S_N2 reaction, in which the new bond begins to be made before the old one is broken;
2. the dissociative mechanism, in which the transferred group is dissociated (as metaphosphate) before the new bond is formed, as in an S_N1 reaction;
3. the adjacent mechanism with pseudorotation;
4. the double-displacement mechanism, in which there are two transfers, first to the enzyme and second from the enzyme to the acceptor.

The first mechanism will occur with inversion of configuration. The second is likely to occur with inversion also (unlike the corresponding S_N1 reaction in free solution, in which there is racemization) because the lifetime of the intermediate is likely to be too short to permit racemization, and the occurrence of the reaction on the template provided by the enzyme will also discourage racemization; nonetheless, some degree of racemization would be possible with this mechanism. The third and fourth mechanisms will proceed with retention of configuration (in the latter case because retention is the result of two inversions). By using adenosine 5'[γS, ^{16}O, ^{17}O, ^{18}O] triphosphate (63, 64) as the phosphate donor and a method based on ^{31}P nmr spectroscopy for determining the chirality at phosphorus of the product, D-glucose-6-[^{16}O, ^{17}O, ^{18}O]phosphate (65), Lowe and Potter (66) were able to determine the stereochemical course of the reaction catalysed by yeast hexokinase. The same technique has recently been applied to glucokinase (67). The reaction is found to proceed with inversion of configuration: this permits either the associative 'in-line' or the dissociative mechanisms. The former is much more likely, however, both because of the instability of the metaphosphate ion and because of the diastereoisomer of $MgATP^{2-}$ that binds to glucokinase (59).

Conclusions

It is becoming clear that the chemistry of the mechanism of action of glucokinase is very similar to that of the other mammalian hexokinases. Consequently, the considerable quantitative differences in kinetic behaviour are likely to arise from quantitative differences in rate constants rather than from any fundamental differences in structure or mechanism. The co-operativity of glucokinase seems most easily explained in terms of a 'mnemonical' mechanism involving two forms of free enzyme that differ in their kinetic properties and are not at equilibrium under steady-state conditions. Relatively minor adjustments to the rate constants would allow essentially the same mechanism to operate without co-operativity, in accordance with the view that the non-co-operative hexokinases are not fundamentally different.

The kinetic properties of the mammalian hexokinases appear to be ideally suited to their physiological roles. In brain and muscle, the main function of glucose phosphorylation is to satisfy the metabolic needs of the tissues, and it is appropriate, therefore, that hexokinases types I and II, the isoenzymes predominant in brain and muscle respectively, are extremely sensitive to inhibition by glucose 6-phosphate but almost unaffected by variations in glucose concentrations in the physiological range. In the liver, on the other hand, glucose phosphorylation does not occur primarily to satisfy

the metabolic needs of the liver but to buffer the concentration of glucose in the blood. It is equally appropriate, therefore, that glucokinase, the liver isoenzyme, is insensitive to glucose 6-phosphate but very sensitive to glucose.

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