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The role of the active site residues in human galactokinase: Implications for the mechanisms of GHMP kinases

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ABSTRACT

Galactokinase catalyses the phosphorylation of galactose at the expense of ATP. Like other members of the GHMP family of kinases it is postulated to function through an active site base mechanism in which Asp-186 abstracts a proton from galactose. This asparate residue was altered to alanine and to asparagine by site-directed mutagenesis of the corresponding gene. This resulted in variant enzyme with no detectable galactokinase activity. Alteration of Arg-37, which lies adjacent to Asp-186 and is postulated to assist the catalytic base, to lysine resulted in an active enzyme. However, alteration of this residue to glutamate abolished activity. All the variant enzymes, except the arginine to lysine substitution, were structurally unstable (as judged by native gel electrophoresis in the presence of urea) compared to the wild type. This suggests that the lack of activity results from this structural instability, in addition to any direct effects on the catalytic mechanism. Computational estimations of the pK_a values of the arginine and aspartate residues, suggest that Arg-37 remains protonated throughout the catalytic cycle whereas Asp-186 has an abnormally high pK_a value (7.18). Quantum mechanics/molecular mechanics (QM/MM) calculations suggest that Asp-186 moves closer to the galactose molecule during catalysis. The experimental and theoretical studies presented here argue for a mechanism in which the C_1–OH bond in the sugar is weakened by the presence of Asp-186 thus facilitating nucleophilic attack by the oxygen atom on the γ-phosphorus of ATP.

1. Introduction

The GHMP kinases are a group of structurally related small kinases which are mostly involved in intermediary metabolism [1,2]. The group is named after four members – galactokinase, homserine kinase, mevalonate kinase and phosphomevalonate kinase. It also includes 4-(cytidine 5′-diphospho)-2-C-methyl-D-erythritol kinase, isopentenyl monophosphate kinase, mevalonate diphosphate decarboxylase, archaeal shikimate kinases, N-acetyl galactosamine kinase, L-threonine kinase, glucuronokinase and galacturonic acid kinase [3–10]. In addition to these enzymes, two proteins without catalytic activity – the *Saccharomyces cerevisiae* transcriptional regulator, Gal3p and the *Caenorhabditis elegans* sex fate determining protein XOL-1 have similar folds [11–14]. The kinases phosphorylate small molecules at the expense of ATP. In most cases, the mechanism of this reaction is believed to require an aspartate or glutamate residue in the active site acting as a base and abstracting a proton from the substrate [2,15]. The resulting negatively charged substrate ion then nucleophilically attacks the γ-phosphate of the ATP, breaking the bond between the γ- and β-phosphates and thus transferring a phosphate to the substrate [16]. Release of the products enables regeneration of the active site base by water.

There is structural evidence in favour of this mechanism. In mevalonate kinase, an asparate residue is correctly positioned to participate in this mechanism. Furthermore, this asparate is close to a positively charged lysine residue which may assist in stabilisation of the negative charge on the aspartate and/or the intermediate ion [16,17]. Similar spatial arrangements are present in galactokinase and N-acetyl galactosamine kinase [14,18–21]. However, at least one member of the family cannot use this mechanism. Homoserine kinase does not have an appropriately positioned carboxylate side chain and, therefore, is believed to effect catalysis by stabilisation of the transition state [22,23]. Some authors have questioned the active site base mechanism for other members of the group, suggesting that this transition state stabilisation is more important that acid/base catalysis in all GHMP kinases [21].

Galactokinase catalyses the conversion of α-D-galactose to α-D-galactose 1-phosphate [24,25]. This is the first committed step of the Leloir pathway of galactose catabolism [15,26,27]. This reaction has attracted interest because mutations in the gene encoding the

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mammalian enzyme cause the genetic disease type II galactosemia [15,28,29]. Reduced, or zero, activity resulting from decreased catalytic activity and/or misfolding of the enzyme cause a build-up of galactose in the blood and in blood-derived fluids, for example those in the eye. In this organ, galactose which is taken up by the lens cells is reduced to the sugar alcohol galactitol (dulcitol) which cannot be transported back out of the cells. High concentrations of this substance result in cataracts, the main symptom of type II galactosemia [30]. However, the symptoms of type II galactosemia are mild compared to those seen in patients with types I and III. In these forms of the disease, irreversible liver kidney and brain damage is often seen [31]. The accumulation of galactose 1-phosphate is believed to be one cause of this greater severity. Therefore, there is interest in the specific inhibition of human galactokinase in patients suffering from types I and III galactosemia. This would, in effect, convert the more severe forms into the milder type II, the symptoms of which can be resolved by cataract surgery [32]. Recently a set of promising lead compounds has been identified by high throughput screening [33,34]. There is also biotechnological interest in galactokinases from a variety of microbial sources. Successful attempts have been made to alter their specificity by random and rational mutagenesis so that potentially useful sugar 1-phosphates can be synthesised [35–38].

The structure of galactokinase from several species, including humans, has been solved [14,18–20]. In all cases, the active site contains appropriately positioned negative and positive side chains – in the case of the human enzyme these are Asp-186 and Arg-37 respectively (Fig. 1). Enzyme mechanisms can be probed through alteration of putative catalytic residues by site-directed mutagenesis of the corresponding gene. However, these methods are not absolute and care should be taken to ensure that any loss of catalytic activity is not due to misfolding of the enzyme's tertiary structure. Here, we probe the mechanism of human galactokinase by site directed mutagenesis of Arg-37 and Asp-186 and discuss the results in terms of the mechanistic implications for GHMP kinases.

2. Materials and methods

2.1. Protein expression and purification

Human hexahistidine-tagged galactokinase was expressed in, and purified from, *Escherichia coli* cells. Competent *E. coli* HMS174(DE3) cells were transformed with a plasmid which directs the expression of this enzyme [39]. A single colony resulting from this transformation was picked and grown shaking overnight at 37 °C in 5 ml of Luria–Bertani (LB) medium supplemented with 100 μg ml^{-1} ampicillin. This culture was diluted into 1 l of the same medium and grown until the cells reached mid-log phase (estimated by an A_{600nm} value between 0.6 and 1.0). The culture was induced by the addition of IPTG to a final concentration of 2 mM and grown for a further 2–3 h. After this time, the cells were collected by centrifugation at 4200*g* for 15 min, resuspended in 20 ml buffer R (50 mM Hepes-OH, pH 7.5, 150 mM NaCl, 10% (v/v) glycerol) and frozen at −80 °C.

These cell pellets were thawed, disrupted by sonication (three 30 s pulses at 100 W with 30 s gaps for cooling) and the insoluble material removed by centrifugation (24,000 g for 15 min). The supernatant was applied to a 1 ml ice jacketed nickel agarose column (His-Select, Sigma, Poole, UK) which had been pre-equilibrated in buffer A (50 mM Hepes-OH, pH 7.5, 500 mM NaCl, 10% (v/v) glycerol) and allowed to pass through under gravity. The column was then washed with 20 ml of buffer A and the recombinant human galactokinase eluted by the application of three 2 ml washes of buffer B (buffer A supplemented with 250 mM imidazole). Fractions containing galactokinase were identified by 10% SDS–PAGE and dialysed overnight against 1 l of buffer D (buffer R supplemented with 2 mM dithiothreitol) at 4 °C. Protein concentrations were determined by the method of Bradford [40] using bovine serum albumin as a control. The purified protein was stored in 50–100 μl aliquots at −80 °C.

Variant forms of human galactokinase were generated by mutating the human galactokinase expression plasmid using the QuikChange site-directed mutagenesis protocol [41]. Mutated DNA sequences were verified (MWG Biotech, Eberburg, Germany) and the variant proteins expressed and purified using the same procedure as the wild type.

2.2. Kinetic analysis of variant forms of human galactokinase

The initial rate of reaction was measured by coupling the production of ADP to the reactions catalysed by pyruvate kinase (PK) and lactate dehydrogenase (LDH). In this system the rate of NADH utilisation (which can be measured by monitoring the absorbance at 340 nm) equals the rate of production of ADP [39,42]. Reactions (900 μl) contained 50 mM Hepes-OH, pH 7.5, 150 mM NaCl, 10% (v/v) glycerol, 1 mM dithiothreitol, 1 mM phospho*enol*pyruvate, 5 mM $MgCl_2$, 3.4 units of PK and 5.0 units of LDH. The reactants were mixed in a cuvette and equilibrated to 37 °C before the reaction was initiated by the addition of wild type (0.20 μM) or variant (0.15–1.0 μM) galactokinase. The A_{340nm} was monitored at 37 °C for 10 min in a Cary 100 spectrophotometer. The initial, linear portion of the progress curve was identified by visual inspection and fitted to a straight line to give the initial rate. These rates were converted to molar units using the extinction coefficient of NADH (6220 l mol^{-1} cm^{-1} [43]).

Since human galactokinase catalyses a two substrate reaction by an ordered, ternary complex mechanism [42], initial rates were determined over a range of subsaturating galactose and ATP concentrations. These values were selected so that they formed a "grid" with galactose concentrations varying along one axis and ATP along the other. The rates along each row and column were fitted to the equation $v = V_{max,app}[S]/(K_{m,app} + [S])$, where $V_{max,app}$ and $K_{m,app}$ are the apparent values of V_{max} and K_m and [S] is the concentration of the variable substrate, using non-linear curve fitting [44] as implemented in GraphPad Prism 3.0 (GraphPad Software, CA, USA). The appropriate values of $V_{max,app}$ were plotted against the concentration of galactose and these data fitted to the equation $V_{max,app} = V_{max}[galactose]/K_{m,gal} + [galactose]$ where $K_{m,gal}$ is the K_m for galactose. A similar procedure was used to obtain $K_{m,ATP}$, the K_m for ATP [42,45].

2.3. Protein stability assay

Aliquots (10 μl) of wild type and variant galactokinase (final concentration, 7.5 μM) were incubated at 37 °C for 30 min in the presence of native gel loading buffer (125 mM Tris–HCl, pH 8.8; 20% (v/v) glycerol; 1% (w/v) DTT; 0.002% (w/v) bromophenol blue) and increasing concentrations of urea (0–2 M). After this time, the samples were loaded directly onto 8% native polyacrylamide gels (made up in 375 mM Tris–HCl, pH 8.8) and electrophoresed in 25 mM Tris–HCl, pH 8.8, 250 mM glycine for approximately 40 min at 0.03 mA constant current). Following electrophoresis, gels were stained in .25% (w/v) Coomassie Brilliant Blue R250 (Sigma) (dissolved in 10% (v/v) acetic acid/45% (v/v) ethanol) and destained in 5% (v/v) ethanol; 7.5% (v/v) acetic acid.

2.4. Computational estimate of pK_a values of active site residues

The Protein Data Bank (PDB) entry for human galactokinase (1WUU) contains two polypeptide chains. To simplify the analysis, only the first of these was considered. This structure contains three

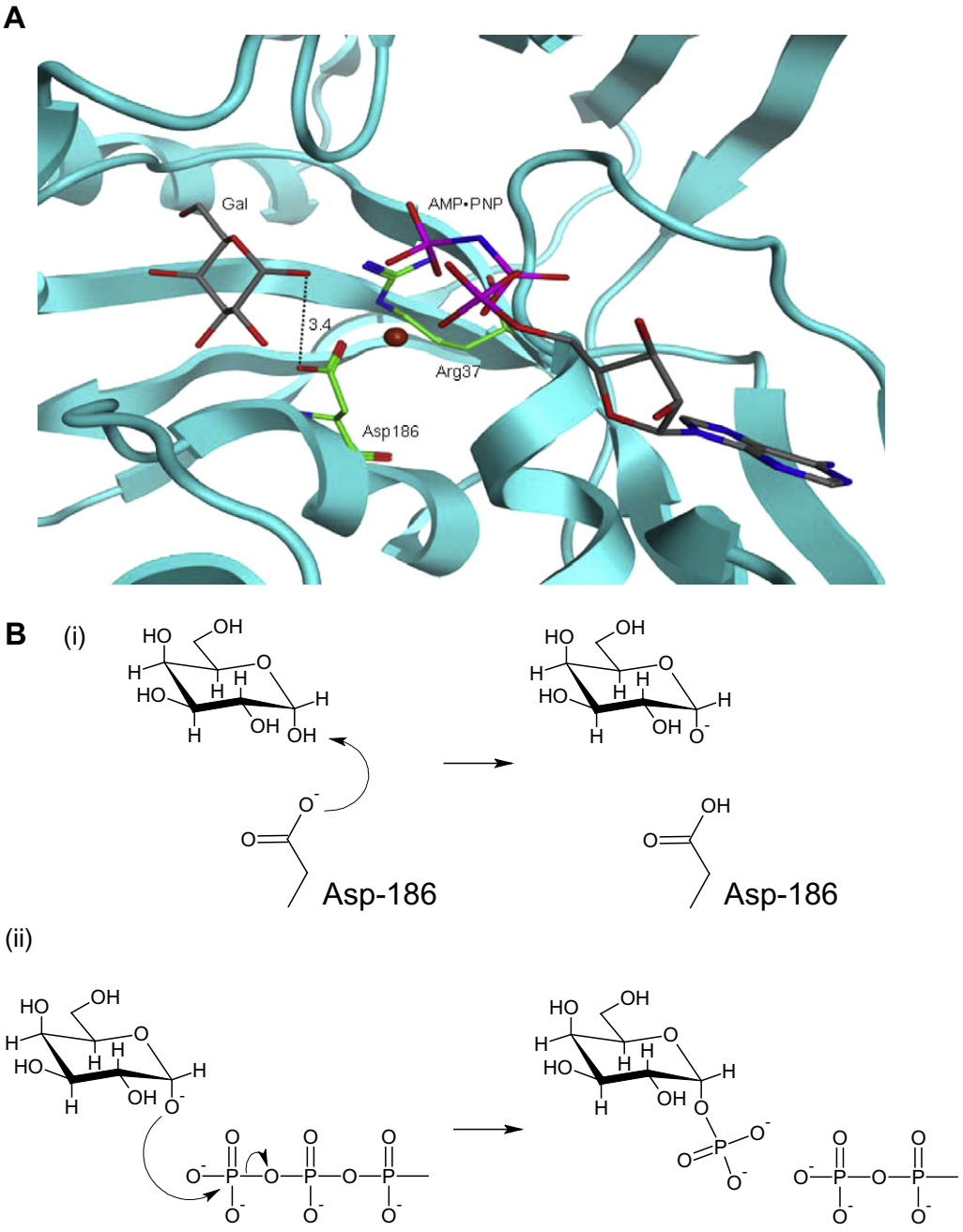

Fig. 1. (A) Structure of the active site of human galactokinase (PDB: 1WUU [19]). Arg-37, Asp-186, galactose (Gal) and AMP.PNP are indicated. The red sphere represents the bound magnesium ion and the distance between the carboxylate group of Asp-186 and C_1–OH of galactose is shown in Angstroms next to a dotted line linking these groups. (B) A potential active site base mechanism for galactokinase. Asp-186 is stabilised in its anionic form by the neighbouring Arg-37. The aspartate residue abstracts a proton from C_1–OH of the sugar. The resulting, highly nucleophilic, alkoxide ion attacks the γ-phosphorus of the ATP resulting in transfer of the phosphate group to the sugar. Once the products have diffused away from the active site, the protonation state of Asp-186 can be restored by reaction with water. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

bound ligands, galactose, a magnesium ion and the ATP analogue AMP.PNP [19]. Ser-230 and Leu-231, which are missing in the experimentally derived structure, were built into the model using the loop refinement tools in DS. Selenomethionine residues were altered to methionine and the bound AMP.PNP altered to ATP by substitution of the nitrogen atom bridging the β- and γ-phosphates with an oxygen atom. The resulting model, including bound ATP, galactose and Mg^{2+}, was then minimised using the Charmm force field (2000 steps SD followed by 2000 steps CONJ). The pK_a values were then calculated using the method of Spassov and Yan [46] as implemented in DS 2.5 (Accelrys Inc, San Diego, CA, USA).

2.5. Quantum mechanics/molecular mechanics (QM/MM) calculations

TIP3 waters were added in a box with a radius of 8 Å. The system was minimized by the following procedure using the AMBER99 force field: first, the protein complex was constrained and the waters were minimized by 100 steps of SD minimization followed by 100 steps of CONJ minimization; then the backbone atoms of the protein were constrained and water and side-chain atoms of the protein were minimized by 100 steps of SD minimization followed by 100 steps of CONJ minimization; finally, all the constraints were removed and the entire system was subjected

to 100 steps of SD minimization followed by 100 steps of CONJ minimization.

After minimization, the water molecules which were further than 6 Å from any atoms in the protein system were removed. Atoms which were further than 6 Å from any atoms in the core part (magnesium ion, ATP and galactose) of the resulting system were kept fixed and rest of the atoms are subjected to QM/MM optimization.

The QM/MM calculation was performed using two-layered Onium as implemented in the Gaussian03 package. Truncated Asp-186, Arg-37, Ser-142 and ATP as well as galactose, the magnesium ion and two structural water molecules were treated as a high level layer by QM (Fig. S1) using the PM3 method and rest of the atoms were treated as a low level layer by MM using the AMBER99 force field.

3. Results

3.1. Expression and purification of variant forms of human galactokinase

Four variants were studied – D186N, D186A, R37K and R37E. All of these could be expressed in, and purified from *E. coli* (Fig. 2). However, R37E was produced in lower yields that the other proteins and was more susceptible to degradation. This suggests that this variant may be more structurally unstable than the other variants and the wild type.

3.2. Kinetic consequences of active site mutations in human galactokinase

The R37K variant had detectable galactokinase activity ($K_{m,gal}$ = 135 ± 43 μM; $K_{m,ATP}$ = 13 ± 7 μM and k_{cat} = 9.3 ± 0.6 s^{-1}). These values are consistent are similar to those obtained with the wild type under identical conditions ($K_{m,gal}$ = 130 ± 50 μM; $K_{m,ATP}$ = 5.0 ± 3.6 μM and k_{cat} = 2.9 ± 0.3 s^{-1}) and with recently published data on this variant [33]. They demonstrate that a lysine residue can, partially, substitute for an arginine one at position 37. However, reversal of the positive charge at this position did not support catalysis; there was no activity detected with the R37E variant up to concentrations of 1.0 μM. Substitution of Asp-186 for either alanine or asparagine resulted in a protein with no detectable galactokinase activity (up to concentrations of 1.0 μM). This is consistent with previous data on the human and rat enzymes [33,47].

3.3. Effects of active site mutations on the stability of human galactokinase

It has been shown previously that point mutations in human galactokinase can dramatically affect the folding of the enzyme. Several disease-associated and active site variants result in proteins which are insoluble following expression in *E. coli* [42,45]. Although all the variants studied here were present in the soluble fraction following disruption of the *E. coli* cells, the low yield and increased degradation of R37E suggests that some of them may be destabilised compared to the wild type. To investigate this possibility the electrophoretic mobility of the variants was compared in increasing concentrations of the denaturant urea. The wild type protein migrated as a discrete band in native gel electrophoresis in the absence of urea. As the urea concentration increased this band broadened and an increasing quantity of the protein did not migrate beyond the top of the gel (Fig. 3). This is consistent with the unfolding of a compact, globular protein to a more mobile, extended form. Similar results were observed for the R37K variant. The R37E and D186A variants gave rise to a number of bands

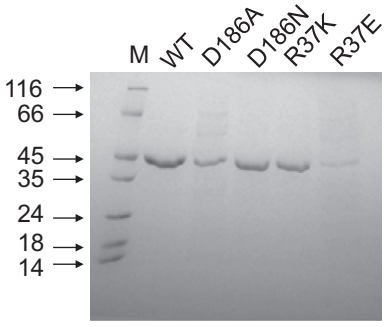

Fig. 2. Purified wild type and variant human galactokinase. Each protein (50 pmol) was resolved by 10% SDS–PAGE and visualized by staining with Coomassie blue. The masses of the molecular mass markers (*M*) are shown to the left of the gel in kilodaltons.

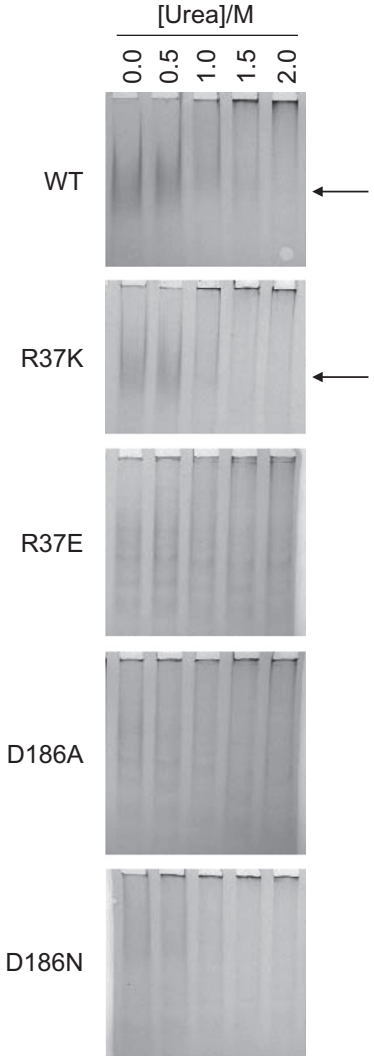

Fig. 3. The stability of the wild type and variant galactokinases. Each variant was resolved by native gel electrophoresis (as described in Section 2.3) in the presence of increasing concentrations of urea (indicated above the gels). The wild type and R37K variant migrate as relatively discrete bands (indicated by an arrow) in the absence of urea. As the concentration of denaturant increases, the majority of the protein fails to leave the wells. The remainder runs as a smear. The R37E and D186A variants all run as a mixture of bands and smears with some material remaining in the wells, even in the absence of urea. The D186N variant runs as a smear with some material remaining in the well at all concentrations of the denaturant.

against the background of a smear. This suggests a mixture of conformations and some unfolded material. D186N did not show discrete bands following electrophoresis under the same conditions even in the absence of urea (Fig. 3). These results suggest that these three variants are partially unfolded in solution.

3.4. Estimation of the pK_a of key residues within the active site of human galactokinase

Given that the lack of activity observed in the R37E, D186A and D186N variants may result from conformational instability, the pK_a values of the residues at positions 37 and 186 in the presence of substrates were estimated for the wild type and variant proteins (Table 1a). Largely similar results were obtained for the wild type enzyme when a Generalized Born model was used instead of the vacuum environment in the minimization step (Table 1b). These predictions show that the pK_a of Arg-37 is high, and that this is not affected by the nature of the side chain at position 186. This suggests that this arginine residue remains protonated throughout the catalytic cycle of the enzyme. The close proximity of this residue to the negatively charged γ-phosphate group probably helps maintain this residue in the protonated state. In addition, the model predicts an overall net charge on the protein of −3 which would favour retention of a positive charge on the arginine. If Arg-37 is substituted for a lysine, this residue also has a very high pK_a value (Tables 1a and 1b). The pK_a of Asp-186 is displaced upwards from its random coil value of 4.0 [48] (Tables 1a and 1b). A third pK_a calculation using a Poisson–Boltzmann continuum model using Charmm 22 force field. (Charmm version c35b4) was carried out for the wild type galactokinase and the pK_a for Asp-186 was estimated to be 6.14. The pK_a of this residue is affected by the nature of the residue at position 37. Mutation of Arg-37 to lysine or, especially, glutamate reduces the effect on Asp-186 (Tables 1a and 1b). The consequences of the R37E variant are less clear. The vacuum model suggests that a glutamate at position 37 will have a pK_a value raised by over five units, whereas the Generalized Born solvation model suggests a slightly depressed value (Tables 1a and 1b).

Table 1a
Computational estimation of pK_a values of key active site residues. In each case the pK_a values were estimated using the method of Spassov and Yan [46] in the presence of bound substrates and Mg^{2+}.

Protein	Position 37		Position 186	
	Residue	pK_a	Residue	pK_a
Wild type	Arg	14.00	Asp	7.18
R37K	Lys	14.00	Asp	6.28
R37E	Glu	9.53	Asp	6.17
D186A	Arg	14.00	Ala	–
D186N	Arg	14.00	Asn	–

Table 1b
All parameters were the same as for the results reported in (a), except that Generalized Born model was used instead of the vacuum environment in the minimization step.

Protein	Position 37		Position 186	
	Residue	pK_a	Residue	pK_a
Wild type	Arg	14.00	Asp	5.37
R37K	Lys	14.00	Asp	4.15
R37E	Glu	6.66	Asp	3.33
D186A	Arg	14.00	Ala	–
D186N	Arg	14.00	Asn	–

Note: – Indicates no ionization in the physiological range.

3.5. QM/MM prediction of the reaction mechanism

In the crystal structure of human galactokinase, no structural waters were resolved coordinating the magnesium ion in the active site [19]. However, their positions can be deduced by superimposing the structures of human galactokinase and N-acetylgalactosamine kinase [21] (Fig. 4). The carboxylate group of Asp-186 appears to help stabilise one of these two structural waters.

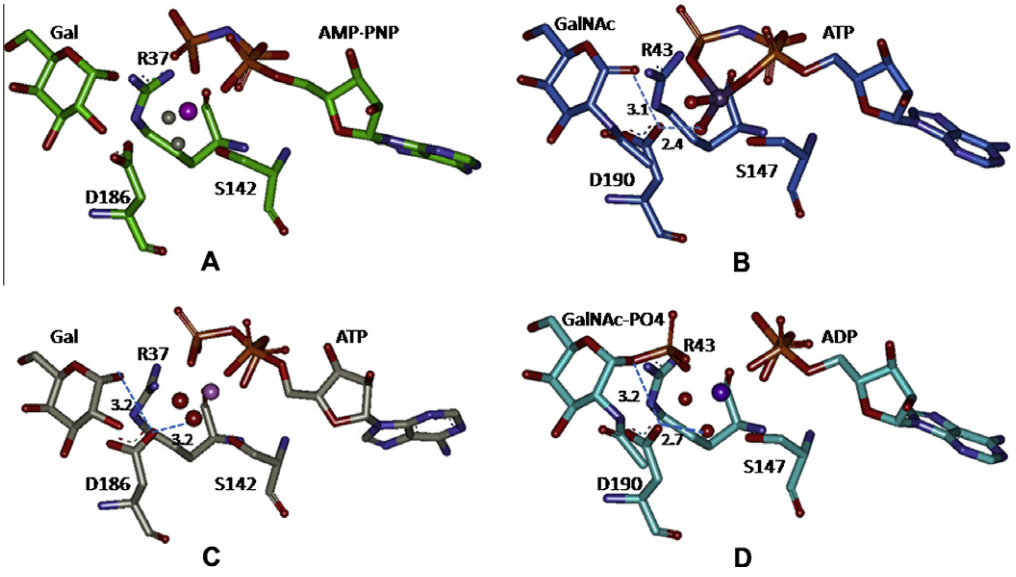

Fig. 4. Postulated phosphorylation mechanism of galactokinase (A) The active site of human galactokinase (PDB code: 1WUU [19]). The magnesium ion is shown as a large sphere, approximately in the middle of the structure (in pink online). Two waters which are coordinated to the magnesium ion were modelled based on the coordinated waters in the structurally related N-acetylgalactosamine kinase and are shown as smaller spheres (grey online) to the left of the magnesium ion. (B) Reactants in the N-acetylgalactosamine kinase active site (PDB code: 2A2D [21]). The magnesium ion is shown as a large sphere (purple online). (C) Optimized structure of human galactokinase derived from the QM/MM method. The magnesium ion is shown in as a large sphere (pink online). Two optimized waters oxygen atoms are shown to the left of the ion (red online). The carboxylate oxygen of Asp-186 moves towards the C_1–OH oxygen, with the distance decreased from 3.4 Å to 3.2 Å. (D) Products in the N-acetylgalactosamine kinase active site (PDB code: 2A2C [21]). The manganese ion is shown as a large sphere (purple online). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Our QM/MM calculations demonstrate that the hydroxyl oxygen on C_1 of the galactose molecule directly attacks the γ-phosphate of ATP (Fig. 4). Compared to the crystal structure, the distance between Asp-186 and the C_1 oxygen shortens (by 0.2 Å) in a model in which the two structural waters are included (Fig. 4C). Here, the distance becomes similar to that of C_1–O distance in N-acetylgalactosamine kinase (Fig. 4B), suggesting that phosphorylation occurs with a similar mechanism to N-acetylgalactosamine kinase where Asp-186 functions to stabilize, and polarize, the structural water (Fig. 4B and D).

4. Discussion

These results clearly demonstrate that the active site residues Arg-37 and Asp-186 influence the overall activity of human galactokinase. This is consistent with previous work on the human and rat enzymes [33,47]. However, the native gel electrophoresis experiments show that all the variant forms, except R37K, are structurally destabilized with respect to the wild type protein. This suggests that their effect on the enzyme's activity most likely results from this general destabilization as well as any specific effects on the catalytic mechanism.

Similar experiments have been carried out with other GHMP kinase family members. Substitution of Lys-13 (equivalent to Arg-37 in human galactokinase) for methionine in rat mevalonate kinase reduced the activity of the enzyme, but not to zero [49]. Replacement of Asp-204 (equivalent to Asp-186) in human mevalonate kinase resulted in a 10^4-fold reduction in V_{max}. This variant retained the ability to bind to a fluorescent substrate analogues, suggesting that it retained essentially the same structure as the wild type [50]. Similar results were seen when the equivalent lysine and aspartate residues were substituted in yeast mevalonate diphosphate decarboxylase [51]. The results obtained with these two enzymes provide support for key roles for these active site lysine and asparate residues. The dramatic reductions observed in V_{max} provide evidence for a role for the aspartate as active site bases; however the retention of some activity means that other explanations for the data cannot be ruled out [50]. Furthermore, it is possible that the presence of the fluorescent ATP analogues may help stabilize the overall structure in the direct binding experiments. Studies on the effects of pH on the enzymatic activity of GHMP kinases have provided contradictory results. *S. cerevisae* galactokinase (Gal1p) showed little variation in activity over the pH range 6.0–9.5 [42]. (This range could not be extended as the protein precipitates at higher and lower pH values [52].) In contrast, N-acetyl galactosamine kinase, mevalonate kinase and homoserine kinase activities all show a clear dependence on pH [53–57]. This is particularly interesting as homoserine kinase does not have a carboxylate containing side-chain in a suitable position to act as an active site base [22,23] and suggests that the pH effects may arise from effects other than direct interference with the catalytic mechanism. Such studies of pH effects on the steady state enzyme kinetic parameters are notoriously difficult to interpret. In addition to denaturation and precipitation effects, it is also important to take account of potential ionisation of the substrates and the possibility of inducing local conformational changes to active site. Consequently it can be difficult to assign pH effects to specific residues in the protein [58].

In order to abstract a proton from the sugar, Asp-186 needs to be thermodynamically able to accept a proton under the conditions under which the enzyme operates. The computational estimate of the pK_a for this residue (Tables 1a and 1b) suggests that this is likely to be so. However, the distance between the carboxylate group in this residue and C_1–OH is 3.4 Å; both the C_2–OH and C_3–OH are closer. However, the QM/MM calculations suggest that this distance shortens during the catalytic cycle, by 0.2 Å, falling into hydrogen bonding range, as observed in N-acetylgalactosamine kinase. Therefore, we propose a mechanism in which the C_1–OH bond in the sugar is polarised by Asp-186. This increases the electron density around the oxygen on the sugar and weakens the O–H bond, facilitating nucleophilic attack on the γ-phosphorus of the ATP. Whether this O–H bond breaks before or after the nucleophilic attack is an unresolved question. In this mechanism, one role of Arg-37 is to help raise the pK_a of Asp-186. The positive charge on Arg-37 will also help bind negatively charged species in the active site. Indeed, given its proximity to the substrates, it is possible that this residue plays a more direct role in the overall mechanism, as has been recently proposed for Lys-169 in human glucokinase [59]. Both Arg-37 and Asp-186 also play key roles in maintaining the overall structure of the protein. We further propose that similar mechanisms may operate in other GHMP kinases, although the apparently anomalous position of homoserine kinase still needs to be resolved.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bioorg.2011.03.001.

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