Structural characterization of the closed conformation of mouse guanylate kinase

Nikolina Sekulic‡, Ludmila Shuvalova‡, Oliver Spangenberg †, Manfred Konrad †, and Arnon Lavie‡

‡University of Illinois at Chicago, Department of Biochemistry and Molecular Biology,
1819 West Polk Street, Chicago, IL 60612

†Max Planck Institute for Biophysical Chemistry, Department of Molecular Genetics,
Am Fassberg 11, 37077 Göttingen, Germany

Correspondence should be addressed to A.L. email LAVIE@UIC.EDU
Tel (312) 355-5029
Fax(312) 355-4535
Summary

Guanylate kinase (GMPK) is nucleoside monophosphate kinase that catalyses the reversible phosphoryl transfer from ATP to GMP to yield ADP and GDP. In addition to phosphorylating GMP, antiviral prodrugs such as acyclovir, ganciclovir and carbovir, and anticancer prodrugs such as the thiopurines, are dependant on GMPK for their activation. Hence, structural information on mammalian GMPK could play a role in the design of improved antiviral and antineoplastic agents.

Here we present the structure of the mouse enzyme in an abortive complex with the nucleotides ADP and GMP, refined at 2.1 Å resolution with a final crystallographic R-factor of 0.20 (R_free = 0.24).

Guanylate kinase is a member of the nucleoside monophosphate (NMP)-kinase family, a family of enzymes that despite having a low primary structure identity share a similar fold, which consists of 3 structurally distinct regions termed CORE, LID and NMP-binding. Previous studies on the yeast enzyme have shown that these parts move as rigid bodies upon substrate binding. It has been proposed that consecutive binding of substrates leads to “closing” of the active site bringing the NMP-binding and LID regions closer to each other and to CORE. Our structure, which is the first of any guanylate kinase with both substrates bound, supports this hypothesis. It also reveals the binding site of ATP, and implicates arginines 44, 137, and 148 (in addition to the invariant P-loop lysine) as candidates for catalyzing the chemical step of the phosphoryl transfer.
Introduction

Guanylate kinase (GMPK, ATP:GMP phosphotransferase, EC 2.7.4.8) is a critical enzyme for the biosynthesis of GTP and dGTP by catalyzing the phosphoryl transfer from ATP to (d)GMP resulting in ADP and (d)GDP (1,2). GMPK also plays an important role in the recycling of the second messenger cGMP (3). In addition to these physiological roles, GMPK is essential for the activation of prodrugs used for the treatment of cancers and viral infections (4,5). Therefore, it is medically important to elucidate its enzymatic mechanism and the structural basis for its nucleotide specificity. Our current structural understanding of this enzyme is derived from the apo- and GMP-bound structures of the yeast GMPK (6), and from analogy to other nucleoside monophosphate (NMP) kinases (7).

It has been shown that the induced fit mechanism (8) plays an important role in NMP kinases, of which adenylate kinase (AMPK) is the best characterized (9-11). NMP kinases catalyze phosphoryl transfer by binding both donor and acceptor nucleotides to form a ternary complex. Comparison of the crystal structures of nucleotide-free AMPK to the one in which a single substrate is bound (AMP or ATP), and to the complex in which both substrates are present revealed the conformational changes that occur along the reaction coordinate – from an open unbound enzyme, via a partially-closed intermediate in which a single substrate is present, to the fully-closed form in the presence of both substrates. These substrate-induced conformational changes can be described as resulting from rigid body movements of three regions relative to each other; a CORE region, a LID region, and an NMP-binding region.
Prior to this work, a structure of GMPK in which both substrate-binding sites are occupied was not available. Due to low sequence identity among the various NMP kinases, and to known structural differences between them (e.g. the NMP binding domain of AMPK (9) is all helical while that of yeast GMPK consists of a four-stranded beta-sheet and two short helices (12)), it is difficult to interpolate with high precision from a structure of one NMP kinase to a related one. Therefore, we set out to elucidate the crystal structure of the fully closed conformation of guanylate kinase. In addition, since the medically most relevant GMPK would be the human enzyme, we commenced our work with this enzyme. Contrary to published reports that found the human GMPK (hGMPK) to be inactive when expressed in *Escherichia coli* (13), we were successful in obtaining large quantities of active hGMPK (14). Unfortunately, crystals grown with the human enzyme were not suitable for X-ray diffraction experiments. However, we were successful in obtaining crystals of the mouse GMPK in complex with GMP and ADP (mGMPK\textsubscript{GMP-ADP}). Herein, we report this structure with an emphasis on the enzyme's conformational changes that occur due to the presence of nucleotides in both substrate-binding sites. The very high sequence similarity between the mGMPK and hGMPK (88% identity, 93% homology) assures that the information we have gained on the mouse enzyme is directly transferable to the human enzyme.
Experimental procedures

**Protein expression and purification.** The mGMPK coding region (13) was cloned in the bacterial expression vector pGEX-RB (15) as a GST-GMPK fusion protein. Expression was induced by 0.5 mM IPTG and cells were grown ~14 hours after induction at 32 °C. A purification protocol was developed that included a gluthatione-sepharose column (Pharmacia), cleavage of the fusion protein with thrombin on the column, passage of the cleaved protein via a benzamidine column (to bind the thrombin) and a gel filtration column (S75, Pharmacia). Total yield of 400 mg of protein from 6 L of culture was obtained. Prior to crystallization mGMPK was dialyzed against 25 mM KCl, 25 mM Tris/HCl pH 7.5, 10 mM DTT.

Dynamic Light Scattering experiments indicated the existence of a single ~20 kD species in solution, which is consistent with the calculated molecular weight of ~22 kD. This result is in agreement with the gel filtration profile.

**Crystallization and X-ray data collection.** Crystals were grown at room temperature using hanging drops containing equal volumes of protein solution and reservoir solution. The protein solution contained 10 mg/ml of mGMPK, 2 mM GMP, 2 mM ADP and 5 mM MgCl₂, while the reservoir contained 38-46% (w/v) PEG 4K, 1M Na-citrate pH 5.6 and 0.1-0.2 M ammonium-acetate. Tetragonal crystals appeared within 4-6 days and exceeded the size of 240 μm x 200 μm x 200 μm. 100% mineral oil (light white; Sigma M3516) was used as cryoprotectant. Attempts to obtain crystals in which AppNHp replaces ADP were not successful.
Data were collected at 100 Kelvin on Rigaku RAXIS IIc detector, by using focused Cu Kα radiation from Rigaku RU-H2R rotating anode x-ray generator at a power of 50 kV, 100 mA. The crystal diffracted to 2.1 Å resolution and data were processed with XDS (16). The model used for molecular replacement was the 1.9 Å structure of yGMPK crystallized with GMP (6). Refinement was done in CNS (17) for X-ray data collected within resolution range of 30 to 2.1 Å. The final crystallographic R and Rfree are 0.20 and 0.24, respectively.
Results

Quality of structure

Mouse guanylate kinase crystallized in space group P4_1212 (a = 67.2 Å, c = 108.7 Å) with one molecule per asymmetric unit. Data to 2.1 Å resolution were collected at cryogenic temperature. The final structure consists of a single polypeptide chain (amino acids 5-194 out of 198 were modeled; weak or no density was observed for both termini), 174 water molecules, the nucleotides GMP and ADP and a potassium ion. The crystallographic R-factor is 0.20 for reflections within the resolution range of 20-2.1 Å. All main-chain dihedral angles are found in favorable and allowed region of the Ramachandran plot (18). See Table 1 for data collection and refinement statistics.

Overall structure

The overall fold of the mouse guanylate kinase is very similar to that of the yeast enzyme (6). Consisting of 198 amino acid residues, mGMPK is 11 residues longer than yGMPK - two of these amino acids are located at the N-terminus and 9 at the C-terminal part of the protein. mGMPK is built from a total of 8 α-helices and 2 β sheets that form 3 structurally and functionally distinct parts. These are the CORE (residues 4-32, 96-122, 164-193; helices α1, α4, α7 and α8 and strands β1, β7, β8 and β9), NMP-binding region (residues 36-88, helices α2 and α3 and strands β3, β4, β5 and β6) and the LID (residues 124-155; helices α5 and α6) region (Figure 1). These parts are interconnected with four hinges.

The division of mGMPK to encompass these three regions is based on the analysis of three GMPK complex structures: apo-enzyme, enzyme with one, or two
nucleotides bound. While the relative conformations of the regions (CORE, LID, and NMP-binding) are different between the three structures, comparison of the same region among the three structures reveals low RMSD (Figure 2). Our nomenclature differs from that previously established in two aspects. First, we define a bigger LID region that includes residues 126 – 156 (previously defined only as seven residues (6)). Second, we define hinges based on higher RMSDs of residues in the interface between regions, and not on B-factors as previously done. We believe that this RMSD-based classification represents a less biased approach than a one based solely on B-factor analysis, avoiding the requirement for a hinge to be mobile in a static crystal structure.

In our structure, there are two hinges between each pair of contacting domains. NMP-binding and CORE regions are connected through hinge-1 (32-36) and hinge-2 (90-96; part of helix3), while CORE and LID regions are linked by hinge-3 (124-125) and hinge-4 (157-164; part of helix6).

Of the nine additional C-terminal residues found in mGMPK in comparison to yGMPK, five residues had traceable electron density and were modeled as helix α8. The function of these additional C-terminal residues found in mammalian (13), bacterial (19), and plant (20) GMPKs is not clear.

Conformational changes induced by binding of nucleotides

The structure presented here is the first of a guanylate kinase in which both nucleotide-binding sites are occupied. The mGMPK in complex with GMP and ADP is in a closed conformation, which allows us, by comparing it with previously reported open
closed conformation of mouse guanylate kinase

(yGMPK\textsubscript{apo}) and partially-closed (yGMPK\textsubscript{GMP}) structures of yGMPK (6), to delineate the effect of each nucleotide on the enzyme’s conformation (Figure 3).

Nucleotide-induced movements in NMP kinases affect the relative orientation of rigid regions while maintaining to a large extent the overall fold of each individual region. If one uses the hand as an analogy to NMP kinase structure, the CORE region would be the palm of the hand, the LID region the thumb, and the rest of the fingers the NMP-binding region. Analogously to fingers and thumb that close over the palm of the hand to make a fist, in the presence of nucleotides the LID and NMP-binding regions move towards each other by means of “hinges” that connect the two regions to the CORE region. The high folding similarities of these relatively rigid regions – CORE, NMP-binding, and LID – between the mGMPK and yGMPK make it possible to overlay regions between the two species. Superposition of all three models (yGMPK\textsubscript{apo}, yGMPK\textsubscript{GMP}, and mGMPK\textsubscript{GMP-ADP}) based only on the CORE region clearly shows a closing of individual regions with successive binding of nucleotides. The nucleotide-free yGMPK structure is characterized by the farthest distance between the LID and NMP-binding regions, which we name the open conformation of the kinase. This conformation appears highly flexible, consistent with the presence of two molecules in the asymmetric units of the yGMPK\textsubscript{apo} structure that differ slightly in the relative conformations of the LID and NMP-binding regions (6). This higher mobility of the nucleotide-free enzyme is also manifested by a structure having higher average main chain B-factors (Figure 4).

The enzyme with only GMP bound, as compared to the apo-GMPK structure, is characterized by a significant movement of the NMP-binding region towards the LID region, with a concomitant smaller move of the LID region in the same direction (i.e. not

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towards the NMP-binding region). However, the overall effect of GMP binding is to bring these two regions closer to each other and form the partially closed enzyme conformation. A detailed analysis of the change in conformation from the apo to the GMP-bound enzyme has been presented recently (6).

In the presence of nucleotides at both binding sites, both LID and NMP-binding regions are pulled closer to each other and to the CORE yielding an even more compact conformation. This closed conformation is also characterized by a lower overall B-factor in comparison to the apo- and GMP-bound structures, with a significant reduction of apparent mobility of the LID region (Figure 4). The increased rigidity of the ternary complex structure can be accounted by the newly formed interactions between residues of the LID and the nucleotides (Figure 5e), as well as a direct interaction between the LID and NMP-binding region (salt bridge between Glu140 and Arg44).

**GMP-binding site**

The major interactions involved in binding of GMP in our mGMPK\textsubscript{GMP-ADP} ternary complex are similar to those previously reported for the yGMPK\textsubscript{GMP} structure (Figure 5b & d). Specificity between AMP and GMP is accomplished by discriminating base interactions - Ser\textsubscript{37} that interacts with the guanine carbonyl at the 6 position (an amino group in adenine), and by carboxylic acids Glu\textsubscript{72} and Asp\textsubscript{103} that interact with the guanine amino group at position 3 (not present in adenine). Notably, Glu\textsubscript{72} makes a bidentate interaction with two H-bond donors; N1, which is protonated in guanine (but unprotonated in adenine), and the amine group at position two of the base. In adenylate kinase (21,22), specificity for adenine is obtained due to the presence of a glutamine in

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the analogous position of the glutamic acid in guanylate kinase, also via a bidentate interaction with N1 (this time an H-bond acceptor) and the amine group at position six. Our structure supplies an explanation for the rather limited success in changing the substrate specificity of mGMPK to accept AMP as a substrate by replacing the glutamic acid with a glutamine (23) (see discussion).

A strong peak in the electron density map close to atom N7 of the guanine base was modeled as a potassium ion (modeling of this density with a smaller atom resulted in a very low B-factor; note that KCl was present in the crystallization buffer). Additionally, distances between this six-coordinated metal to its ligand are in agreement with the expected values for potassium (24). The importance of this interaction for GMP binding, is not clear, and was not observed in the yGMPK_{GMP} structure.

For the yeast GMPK it has been reported that dGMP is a poor substrate in comparison to GMP, with a $\sim 4$-fold higher Km and a $\sim 8$-fold reduced $k_{cat}$ (25). The residue responsible for this difference is Asp101 (conserved between mouse and yeast), which interacts with the 2'-hydroxyl group of the ribose of GMP.

Similarly to the yGMPK_{GMP} structure, in the mGMPK_{GMP-ADP} structure we observe tyrosines 53 and 81, and arginines 41 and 44 to interact with the phosphate group of GMP. However, the interaction of these arginines with GMP is strengthened in the mGMPK_{GMP-ADP} structure in comparison to the yGMPK_{GMP} structure. For example, Arg44, which corresponds to Arg41 in the yeast structure, and which makes only a weak H-bond in that structure (3.4 Å), makes a stronger (2.6 Å) interaction with phosphate of GMP in mGMPK_{GMP-ADP}. Not present in the yGMPK_{GMP} complex structure is the interaction between the LID arginine (Arg148) and the phosphate group of GMP that we
observe in the mGMPK ternary complex. Formation of the latter arginine-GMP interaction and strengthening of the former is made possible by the conformational changes induced by the binding of both substrates that bring the LID and NMP-binding region closer to each other. These facts, as well as a position that potentially enables these arginine side chains to interact with both substrates concomitantly (i.e. the GMP phosphate and the $\gamma$-phosphate of ATP), make these two residues potential candidates for taking part in catalyzing the phosphoryl transfer.

**ADP-binding**

Our mGMPK$_{\text{GMP-ADP}}$ structure represents the enzyme in an abortive complex in which a substrate (GMP) and a product (ADP) are bound. Inhibition of GMPK activity by GMP has been attributed to the formation of this complex, which slows down but does not arrest release of ADP (25). The presence of ADP provides us with important information on the enzyme’s conformation in the presence of two nucleotides and on the interactions made with the phosphoryl donor. The binding site of ADP/ATP is located between the LID and CORE regions (Figure 5e). The adenine base is held in position through the strictly conserved Arg133 (a stacking interaction), the side-chain of Asn171, and the main-chain carbonyl of Asp172 (Figure 5 a & b).

As was previously observed in other NMP-kinase structures, the ATP/ADP ribose does not directly interact with any protein atom, but rather is stabilized by several water molecules. Phosphate group binding is through the P-loop, a sequence highly conserved among ATP-binding proteins (26). The main chain atoms of this motif, localized in the loop between strand $\beta 1$ and helix $\alpha 1$, provide hydrogen-bonding interactions with the $\beta$
phosphate. Additionally, the $\beta$-phosphate interacts with the side chains of Ser18, Lys17 (both from the P-loop) and Arg137 (from the LID). The latter basic amino acids could potentially also interact with a $\gamma$-phosphate, and in this way play a role in catalyzing the chemical step of phosphoryl transfer. The $\alpha$-phosphate is within H-bond distances from the side chains of Thr19 and Arg137 (Arg137 also interacts with the $\beta$-phosphate).
Discussion

The ability of guanylate kinase to catalyze the transfer of a phosphoryl group from ATP to GMP to yield GDP is important both physiologically and medicinally. Physiologically, through being a component of the metabolic pathway of GTP and dGTP formation, GMPK plays a vital role in DNA and RNA synthesis (2). Additionally, the recycling of the second messenger cGMP and the overall concentrations of GTP, which is involved in the regulation of numerous pathways, is dependent on GMPK activity (3). The medicinal importance of GMPK is in the activation pathway of guanosine-analogue prodrugs. Examples are mercaptopurine and thioguanine used for the treatment of various types of cancers, acyclovir and ganciclovir for the treatment of herpes infections and in cancer suicide gene therapy, and carbovir against HIV (4,5,27). Despite its importance, up to now there was no structure of a mammalian GMPK, and the available structures that focused on the yeast enzyme had no, or only a single substrate present in complex with the enzyme. To better understand the active site properties of the mammalian enzyme, and to characterize the enzyme in presence of nucleotides at both substrate-binding sites, we solved the crystal structure of the mouse guanylate kinase in complex with GMP and ADP.

Role of arginines from LID and NMP-binding regions. The LID region in most NMP kinases (eukaryotic thymidylate kinases being an exception (28)) contain several arginine residues that have been shown to participate in catalyzing phosphoryl transfer by direct interactions with the \( \gamma \)-phosphate of ATP (29). In the nucleotide–free and GMP-bound yGMPK structures, this region is too far away to fulfill this catalytic role. Upon binding of ATP, a conformational change takes place that brings the LID to a position where its
arginines (Arg137 and Arg148) can directly interact with the nucleotide’s phosphates. In the mGMPK<sub>GMP-ADP</sub> structure, Arg137 interacts with the α- and β- phosphates of ADP and Arg148 interacts with the GMP phosphate. The analogous arginines in adenylate kinase have been shown by mutational analysis to be essential for catalysis (30). Additionally, structural studies performed with <i>Dictyostelium discoideum</i> uridylate kinase have also demonstrated the importance of these LID arginines (29,31). Notably, in the structure of uridylate kinase in a complex that mimics the transition state it was observed that the arginine that would correspond to Arg148 of mGMPK interacts with the transferred phosphoryl group. It is therefore likely that Arg148, which we observe only to interact with GMP, would actually interact with the γ-phosphate of ATP (we have ADP in our structure) or would potentially act as a clamp by interacting with both the phosphates of GMP and ATP. Additionally, this residue is held in its position by H-bonding with Ser13 (CORE) and Ser144 (LID) through NH1 and Nε, respectively. It is possible that the presence of ATP would favor an interaction with γ-phosphate of ATP instead of Ser13. On the other hand, Arg44, which is part of NMP-binding region, is also positioned to potentially interact with the γ-phosphate of ATP, suggesting its possible involvement in directly catalyzing the chemical step of phosphoryl transfer. This residue is additionally stabilized in our structure with the previously mentioned interaction with Glu140. Ambiguity that arises, if Arg44, Arg148, or both directly interact with the transferred phosphoryl group, cannot be resolved by our structure.

*Inherent flexibility of the enzyme.* In the analysis by Blaszczyk et al. of the conformational changes that occur in γGMPK upon GMP binding it was noticed that the temperature factors of residues in helix 3, which compose one of hinges between the
NMP-binding region and the CORE region, have increased significantly in comparison to those seen in the apo yGMPK structure (6). The authors propose that helix 3 acts like a spring in the movement of the NMP-binding region, and speculate that the ternary complex will likewise have increased mobility in this helix. Higher mobility of the analogous helix in adenylate kinase was also observed by Müller et al.(32) However, we observe below average B-factors for this helix in the mGMPK\textsubscript{GMP-ADP} structure (Figure 4). Consistent with our observation are low B-factors for the analogous helix seen in a number of uridylate kinase ternary complex structures (29,31). We conclude that the higher B-factors for this helix seen in the yGMPK\textsubscript{GMP} structure may be due to do the inherent flexibility of the enzyme when only a single substrate is present, and that the more rigid, fully-closed structure we observe in the presence of both nucleotides is a true representation of this state. Importantly, our results and those of others (29,31,32) question the proposal that helix 3 serves as a spring to prevent the ternary complex from getting trapped at an energy minimum.

**Base specificity of NMP binding site.** Our structure also serves to rationalize the limited success achieved in work in which the goal was to change the substrate specificity of mGMPK to accept AMP instead of GMP as substrate (23). This was attempted by mutating the two carboxylic acids that interact with the guanine base to uncharged residues, as are present in adenylate kinase. The E72Q/D103N double mutant, while able to functionally complement adenylate kinase temperature-sensitive bacterial strains, possessed only marginally improved kinetics with AMP. This we attribute to the inability of the introduced glutamine in position 72 of mGMPK to mimic its counterpart in adenylate kinase. While in both nucleoside monophosphate kinases there is an interaction...
between the side-chain of this residue to N1 of guanine/adenine, in mGMPK Glu72 interacts with the NH2 at position 2 while in adenylate kinase the glutamine analogous to Glu72 interacts with the NH2 at position 6. As a result of this difference (an interaction with NH2 at position 2 or 6), despite the presence of a glutamine in mGMPK instead of Glu72 in the double mutant, this residue will not be able to interact with the NH2 at position 6 of the adenine ring. The position of the Glu72 side-chain will be constrained by the proximity to Thr83, making a shift from proximity to position 2 to position 6 of the ring not possible. Using our structure we are now in a position to design mutations that would take this fact into consideration (for example, mutating Thr83 to a glycine to provide room for the introduced glutamine at position 72 to make a bidentate interaction with the adenine ring).

The closed conformation of the ternary complex shows that ADP is mostly solvent exposed, while GMP is mostly buried. This would suggest an ordered substrate binding and product release mechanism in which GMP binds first, followed by ATP, and after the chemical step, ADP leaves the enzyme prior to GDP (an ordered sequential mechanism). However, recent kinetic results are consistent with a random sequential mechanism, though not ruling out an ordered mechanism (25). To reconcile a random sequential mechanism for GMPK with our closed conformation structure of mGMPK_{ADP-GMP} we must speculate that the closed structure we observe is the most stable of the ternary complex conformations, but that the molecule undergoes opening (“breathing”) to transiently produce a more open structure. It is to this transient open structure that substrates bind in a random order, or from which products dissociate in a random order. In conflict with such a random sequential mechanism are recent results on
the herpes simplex virus thymidine kinase using isothermal titration calorimetry (ITC) that demonstrated ordered binding to this kinase (33). Planned ITC experiments with mGMPK will resolve this ambiguity.

**Binding of therapeutically important nucleotide analogs.** The purine base analogs 6-mercaptopurine and 6-thioguanine have been in clinical use for nearly 50 years (34), but it is still not possible to pinpoint a single biochemical pathway for thiopurine cytotoxicity. One mechanism would be due to the incorporation of thiopurine in DNA, which induces DNA damage, such as single strand breaks, DNA-protein cross-links, interstrand cross-links, and sister chromatid exchanges (35-39). This mechanism requires the sequential phosphorylation of thiopurine prodrugs to their activated triphosphorylated forms. Guanylate kinase is the rate-limiting enzyme in the phosphorylation pathway of thiopurines, with a Km of over 2 mM for 6-thioguanosine monophosphate (6T-MP) and a maximal velocity of 3% of that with GMP (40). We modeled 6T-MP in the active site of mGMPK to try to understand the structural reasons that make this GMP analog such a poor substrate. The sulfur atom, which substitutes for the oxygen atom found in guanine, is predicted, if bound in an identical fashion to GMP, to make H-bonds with Ser37 and Thr83. An explanation for the poor activity of GMPK with 6T-MP might be steric clash with these alcohol side-chains. To test this hypothesis, we made mutants in which the serine and the threonine, singly and in combination, were replaced by an alanine or glycine. However, kinetic analysis of these mutants showed no improved activity with 6T-MP as a substrate (unpublished data). An alternative explanation for the poor activity of 6T-MP implicates the H-bonding capability of this analog with the enzyme. GMP makes a bidentate interaction with Glu72, but since 6T-MP is predicted to exist mostly as
the tautomer in which the sulfur atom is protonated (an –SH group) and the ring nitrogen at position 1 is unprotonated, this interaction cannot be obtained with the thiopurine analog. This suggests that replacing the conserved Glu72 with a glutamine would enable such an interaction to take place. Whether such a variant of mGMPK, and in combination with mutations of Ser37 and Thr83, will efficiently phosphorylate thioguanines is currently being tested.

In summary, we present the structure of the first mammalian guanylate kinase in complex with GMP and ADP. Comparison of the structure with the available yeast guanylate kinase structures allows us to better understand the conformational changes that occur along the reaction coordinate, to explain the substrate specificity of this enzyme, and to rationalize and model mutants of this enzyme designed to modify the substrate selectivity.
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The atomic coordinates and structure factors will be deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org).

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**Figure Legends**

**Figure 1. Ribbon diagram of mGMPK\textsubscript{GMP-ADP}** The different regions of the enzyme are color-coded: CORE (cyan), NMP-binding (red) and LID (green), interconnected with 4 hinges (yellow). The nucleotides ADP and GMP (red) and the potassium ion (green sphere) are also shown. All structural figures were generated using MOLSCRIPT (41) and RASTER3D (42).

**Figure 2. Overlay of each individual region from the three GMPK structures.**
Calculations of superposition matrices were done according to residues specified in text, with largest RMSD for all three regions being between mGMPK\textsubscript{GMP-ADP} and yGMPK\textsubscript{apo}. (a) overlay of LID region. (b) overlay of NMP-binding region. (c) overlay of CORE region. Highest RMSD values were: for CORE 1.62 Å, for NMP-binding region 0.76 Å, and for LID 0.86 Å. Regions from yGMPK\textsubscript{apo} are displayed in green, yGMPK\textsubscript{GMP} in blue, and mGMPK\textsubscript{GMP-ADP} in magenta.

**Figure 3. Three conformational states of GMPK.** Overlay of yGMPK\textsubscript{apo} (green), yGMPK\textsubscript{GMP} (blue) and mGMPK\textsubscript{GMP-ADP} (magenta). The nucleotide-dependent conformational states are open in the apo-structure, partially closed in the presence of GMP, and fully closed in the presence of GMP and ADP.

**Figure 4. Main-chain B-factor plot.** Deviation from the average B-factor as a function of residue number is plotted for yGMPK\textsubscript{apo} (green), yGMPK\textsubscript{GMP} (blue) and
mGMPK$_{\text{GMP-ADP}}$ (magenta). Domains and secondary structure elements are shown at the bottom. yGMPK$_{\text{apo}}$ crystallized with two different molecules in asymmetric unit, though only one, more open, form is shown here for clarity.

**Figure 5.** Binding sites of GMP and ADP (a) Distance map showing residues involved in binding of ADP and in (b) of GMP. For clarity, backbone atoms of the P-loop are shown in green. Residues that are making interactions not previously observed in the yGMPK$_{\text{GMP}}$ structure are shown in red. For example, the Asp101 interaction with the GMP ribose observed in the mGMPK$_{\text{ADP-GMP}}$ complex is made possible by the additional closing of the structure as a result of ADP binding, and is absent in the yGMPK$_{\text{GMP}}$ structure (Asp98 in yGMPK). Distances are in angstroms. (c) Ball-and-stick representation of the ADP and in (d) of the GMP binding sites. (e) Stereoview of the active site with residues involved in phosphate interactions shown in red, the nucleotides in blue.
Table 1. Data Collection and Refinement Statistics

| Data collection |   |
|-----------------|---|
| X-ray source    | Rotating anode, λ = 1.5412 Å |
| Unit cell (Å)   | \(a=67.2\), \(c=108.83\) |
| Space group     | \(P_{4_1}2_12\) |
| No. molecules / a.u. | 1 |
| Resolution limit (Å) | 20 - 2.1 |
| Measured reflections | 105,017 |
| Unique reflections | 14,942 |
| Completeness (%; overall/last shell) | 99.9 / 98.4 |
| \(I/\sigma I\) (overall/last shell) | 12.2 / 5.7 |
| \(R_{sym}\) (overall/last shell) | 8.4 / 22.4 |

| Refinement |   |
|------------|---|
| Resolution limit (Å) | 20 - 2.1 |
| No. reflections (working/free) | 13,491 / 1,451 (9.6%) |
| \(R_{cryst}\) (overall/last shell) | 0.20 / 023 |
| \(R_{free}\) (overall/last shell) | 0.24 / 0.27 |
| No. residues/non-H atoms | 190 / 1474 |
| rmsd from ideal geometry (Å) |   |
| Bond length | 0.008 |
| Angle distances | 1.356 |
| Estimated coordinate error (Å) | 0.28 |

| Ramachandran plot statistics |   |
|-----------------------------|---|
| Residues in most favored regions | 90.5% |
| Residues in allowed regions | 9.5% |
| Residues in disallowed regions | 0.0% |

\(^{a}R_{sym} = \Sigma |I-\langle I\rangle|/\Sigma I,\)

\(^{b}R_{cryst} = \Sigma ||F_{obs}|-|F_{calc}||/\Sigma |F_{obs}|, 10\% randomly omitted reflections were used for \(R_{free}\)
closed conformation of mouse guanylate kinase

Figure 2

a. LID region

b. NMP-binding region

c. CORE region

- yGMPKapo
- yGMPKAMP
- mGMPKAMP-AMP
Figure 3

closed conformation of mouse guanylate kinase

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Figure 4
closed conformation of mouse guanylate kinase

Figure 5

a.  

b.  

c.  

d.  

e.  

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Nikolina Sekulic, Ludmila Shuvalova, Oliver Spangenberg, Manfred Konrad and Arnon Lavie

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