Articles

Mapping the Active Sites of 3-Phosphoglycerate Kinase and Glycerol Kinase with Monoammine Chromium(III) ATP†

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ABSTRACT: The 12 isomers of monoammine chromium(III) ATP have been used to probe the ATP binding sites of yeast 3-phosphoglycerate kinase and glycerol kinase from *Candida mycoderma*. Inhibition studies of 3-phosphoglycerate kinase show a dramatic decrease in isomer binding only when the ammonia is in the Δ axial facial anti position. This suggests an open site architecture with only one strong contact point between the coordination sphere and the enzyme surface. These results agree well with the computer modeling studies of bidentate chromium ATP into the nucleotide site determined by X-ray crystallography [McPhillips, T., et al. (1996) *Biochemistry 35*, 4118−4127]. Both methods describe an open site strongly supporting the validity of the inhibition studies. Inhibition studies of glycerol kinase show significant decreases in binding for all the tested ammonia positions, suggesting a closed site architecture with many contacts between the coordination sphere and the surface of the enzyme. This is in good agreement with X-ray studies [Hurley, T., et al. (1993) *Science 259*, 673−677] on the *Escherichia coli* glycerol kinase. Inhibition studies of hexokinase previously reported [Rawlings, J., et al. (1993) *Biochemistry 32*, 11204−11210] more closely resemble those of 3-phosphoglycerate kinase, suggesting the surprising result that however closely hexokinase and glycerol kinase are related structurally the site around the coordination sphere in hexokinase is functionally open like that of 3-phosphoglycerate kinase.

The magnesium complex of ATP[1] is a substrate for many enzymes. When the complex is *β*-*g*-bidentate, the chiral *β* phosphorus gives rise to Λ and Δ screw sense isomers (*1*). The chirality of the MgATP substrate has been determined for a number of enzymes by the use of inert coordination isomers of tetraaquochromium(III) ATP or tetraammineco-balt(III) ATP (*2*), or by chiral sulfur-substituted ATP (*3*). However, it is not known from this information which of the water molecules in the Mg^{2+} coordination sphere are in contact with the surface of the enzyme and therefore possibly involved in binding interactions. Although X-ray crystal-lography of enzyme−MgATP complexes should be able to answer this question, such data are not always available.

An alternative to X-ray crystallography is to use ammonia-substituted inert coordination complexes of ATP. It has been previously shown (*4*, *5*) that binding of chromium ATP complexes to several kinases became weaker as the number of ammonias in the coordination sphere increased. Placing a single ammonia in the coordination sphere produced a family of isomers that allows the importance of each position of the coordination sphere to be determined, as was shown for hexokinase (*6*).

This paper employs monoammine chromium(III) ATP to evaluate the importance of each of the coordinated waters in the binding of MgATP to glycerol kinase and 3-phos-phoglycerate kinase. The NH_3CrATP inhibition studies for 3-phosphoglycerate kinase are compared to the pattern expected from examining the recently published X-ray crystal structure of a complex of the enzyme and substrate analogues (*7*).

MATERIALS AND METHODS

Reagents. ATP, NADH, 3-phosphoglycerate, methane-sulfonic acid, 2-(*N*-morpholino)ethanesulfonic acid (MES buffer), and ion-exchange resins were from Sigma. All enzymes were from Sigma: glycerol kinase (*Candida mycoderma*), 3-phosphoglycerate kinase (baker's yeast), pyruvate kinase, lactate dehydrogenase, and glyceraldehyde-3-phosphate dehydrogenase (all from rabbit muscle).

Chromatography. A Pharmacia MONO-S HR 16/10 (the generous loan of Dr. W. W. Cleland) was used at room temperature. The eluent was 1 mM H_2SO_4. Peaks were detected with a Rainin UV−visible detector set at 580 nm for monoammine chromium ATP and 600 nm for chromium ATP. Data were collected with a Rainin Dynamax integrator

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[1] Abbreviations: ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; NADH, nicotinamide adenine dinucleotide reduced form; PEP, phosphoenol pyruvate; MES, 2-(*N*-morpholino)ethane sulfonic acid; MAP, magnesium 5′-adenylyl-imidodiphosphate; NH_3CrATP, monoammine chromium(III) ATP; CrATP, chromium(III) ATP.

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connected to a Macintosh computer. For reverse phase C-18 HPLC of monoammine chromium ATP, 10 mM methanesulfonic acid (pH 2.3) was the eluent, on a Microsorb column (25 cm × 2.14 cm) from Phenomenex at room temperature. CrATP isomers were resolved with reverse phase C-18 chromatography with 10 mM methanesulfonic acid at pH 2.5, as originally described by Gruys and Schuster (*8*). Peaks were detected with a refractive index monitor (Waters) and a chart recorder.

Inhibitors. Chromium ATP (*9*) and monoammine chromium ATP (*6*) were prepared as described previously with the following modifications for monoammine chromium ATP. After synthesis, the mixture containing all isomers was chromatographed on the reverse phase column. Cycloheptaamylose chromatography was not used. Enzymatic degradation of the mixed Λ and Δ axial-meridional-syn isomers was not done. To obtain these isomers in pure form the corresponding equatorial-meridional-syn isomer was collected from reverse phase chromatography. It was determined that pure dilute isomers could be stored frozen at −80 °C prior to concentration without harmful effects. The dilute isomer was mixed with an equal volume of 1.2 M MES buffer at pH 5.5 and left at room temperature for 2 h. The resulting solution contained mainly the axial-meridional-syn isomer and unreacted equatorial-meridional-syn isomer. The isomers were concentrated by anion exchange chromatography on an AG1 × 2 (Cl^- 200−400 mesh) column and then could easily be separated by either reverse phase or MONO-S chromatography; generally the latter was employed because it also resolves the axial-meridional-syn isomers from all other isomers (except each other) and from CrATP or other likely degradation products. This method was also used to prepare other axial isomers and the Δ equatorial-facial-anti isomer on occasion. Inhibitor concentrations were determined by ultraviolet spectroscopy at 254 nm using an extinction coefficient of 17 000.

Kinetics. Kinetic measurements with glycerol kinase were based on previous studies (*10*). Pyruvate kinase and lactate dehydrogenase were used to couple the phosphorylation of glycerol to the disappearance of NADH, followed at 340 nm in a Milton Roy/SLM photodiode array UV−visible spectrometer equipped with an eight cell sample holder and a kinetic analysis software package. To minimize interconversion of the NH_3CrATP isomers, the assays were run at room temperature in pH 5.75 MES buffer and monitored for 10 min or less. Reaction conditions (final volume of 1.0 mL) were as follows: 0.1 M MES buffer, pH 5.75, 3.3 mM glycerol, 10 mM KCl, 2.5 mM $MgSO_4$ plus an amount equal to the ATP added to the reaction, ATP varied from 0.1 to 0.01 mM, 5.25 mM PEP, 0.20 mM NADH, 30 μg of pyruvate kinase, 15 μg of lactate dehydrogenase, 50 of μg glycerol kinase, and up to 0.02 mM inhibitor.

Kinetic measurements with 3-phosphoglycerate kinase were based on the assay of Bücher (*11*). Glyceraldehyde-3-phosphate dehydrogenase was used to couple the phosphorylation of 3-phosphoglyceric acid to the disappearance of NADH followed at 340 nm in a Hitachi U-2000 UV−visible spectrometer equipped with a six cell sample holder and a kinetic analysis software package. To minimize interconversion of the NH_3CrATP isomers, the assays were run at room temperature in pH 5.5 MES buffer and monitored for 10 min or less. Reaction conditions (final volume of 1.0 mL) were as follows: 0.1 M MES buffer, pH 5.5, 6.2 mM 3-phosphoglycerate, 10 mM KCl, 2.0 mM $MgSO_4$, ATP varied from 0.1 to 0.01 mM, 0.9 mM EDTA, 0.20 mM NADH, 100 μg of glyceraldehyde-3-phosphate dehydrogenase, up to 0.02 mM inhibitor, and 5 μg of 3-phosphoglycerate kinase.

Data were fitted to eq 1, which describes competitive inhibition, where V is maximum velocity, K is the Michaelis constant, K_I is the inhibition constant, A is the MgATP concentration, and I is the inhibitor concentration.

$$v = VA/[K(1 + I/K_i) + A] \quad (1)$$

The concentrations of 3-phosphoglycerate and glycerol were determined to be saturating under these conditions so that eq 1 is valid. Data analysis was done on an IBM 486 PC using a program originally written by Dr. W. W. Cleland (*12*).

Structure Visualization. The structure of the R65Q mutant of yeast 3 -phosphoglycerate kinase complexed with 3-phospho-D-glycerate and magnesium 5′-adenylylimidodiphosphate (MAP) (*7*) was obtained from the Brookhaven Protein Data Bank (identification number 1QPG) and displayed on an Indigo[2] workstation (Silicon Graphics) in the program Quanta (MSI, Burlington, MA). The structure of *Escherichia coli* glycerol kinase complexed to ADP and glycerol-3-phosphate (*13*) was also obtained (PDB identification number 1GLC).

NH_3CrATP isomer structures were constructed in Quanta using the X-ray crystal structure of chromium pyrophosphate (*14*) to establish the correct bond angles and distances in the β-γ bidentate ring. Axial isomers were constructed to form a close contact between a nonbridging oxygen of the α phosphate and the syn coordinated water molecule as described for CrATP (*9*); while the torsion angles for the α phosphorus-oxygen-β phosphorus of equatorial isomers were set to produce a more extended structure. These structures were then superimposed on the ADP or MAP structures found in the complex. Adenine, ribose, and the α phosphate positions were assumed to be the same as the MAP or ADP in the complex. None of the groups in the enzyme was moved to accommodate the inhibitor. The fit of each isomer was qualitatively evaluated, and distances to the closest groups of the enzyme were determined.

RESULTS AND DISCUSSION

Isomer Separation. The structures and names[2] of the 12 isomers of chromium monoammine ATP described by Rawlings et al. (*6*) are shown in Figure 1. The Λ and Δ axial-meridional-syn isomers are the most difficult to isolate because they are not resolved by either cation exchange chromatography or the reverse phase chromatography. Mixtures enriched in one screw sense over the other were produced from a 50/50 mixture by selective enzymatic

[2] The nomenclature of the isomers consists of four pairs of terms. The screw sense, Λ or Δ, of the ring is determined as follows: the reference axis is a line through the metal perpendicular to the chelate ring, and the bond from the chelate ring to the rest of the molecule is the skew line defining a left-hand (Λ) or right-hand (Δ) helix. The ammonia and the two coordinated phosphates can be either facial or meridional, and the ammonia can be either syn or anti with respect to the AMP. Finally, ring-puckering isomers with the AMP axial or equatorial can occur when water is syn to the AMP, allowing strong hydrogen bonds with the α phosphate.

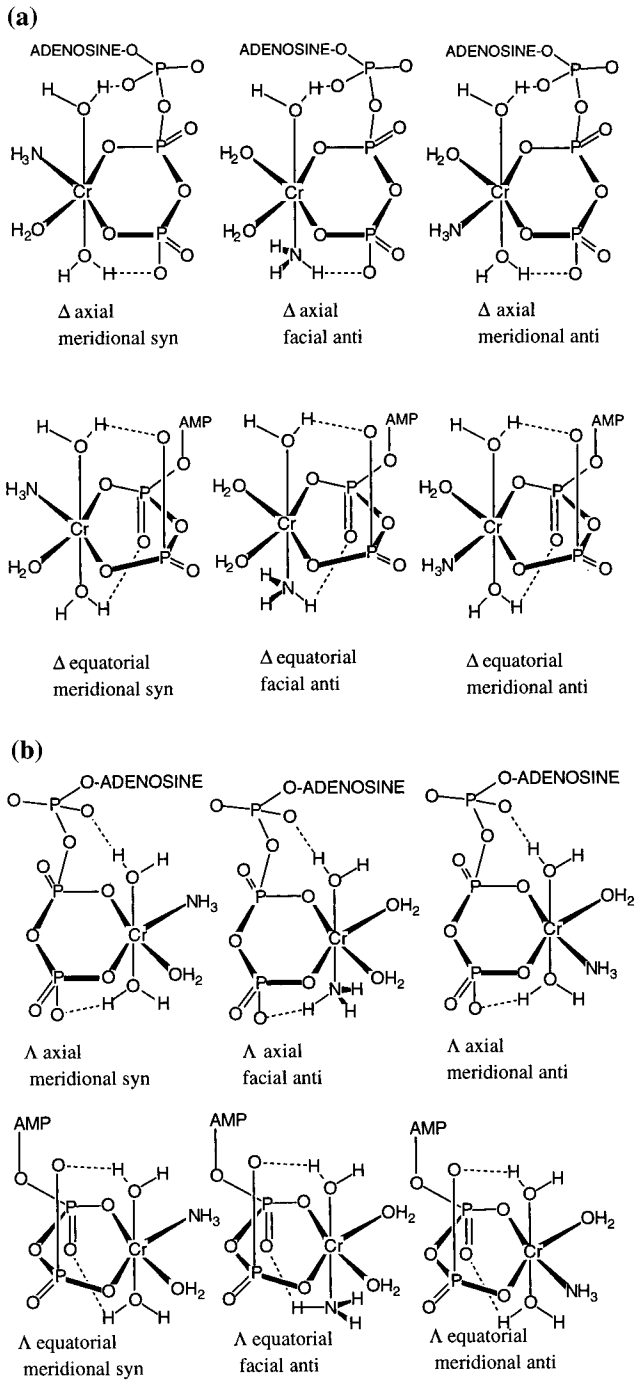

FIGURE 1: Structures of the Δ monoammine chromium(III) ATP isomers (a) and Λ monoammine chromium(III) ATP isomers (b) as determined by Rawlings et al. (*6*). Facial-syn isomers are not known.

degradation, but circular dichroism studies and kinetics both showed that significant amounts of the undesired isomer remained (6). We have developed a simpler method that produces pure samples of these isomers in amounts sufficient for kinetic studies. At pH 5.5 and room temperature, the rate of axial-equatorial isomerization is accelerated with initially little or no competing isomerization of the coordination sphere. The reactions are protected from light to minimize ammonia loss. One potentially severe side reaction is the formation of bridging complexes between one monoammine chromium ATP and another. These dimers and higher polymeric products are known to form with chromium ATP

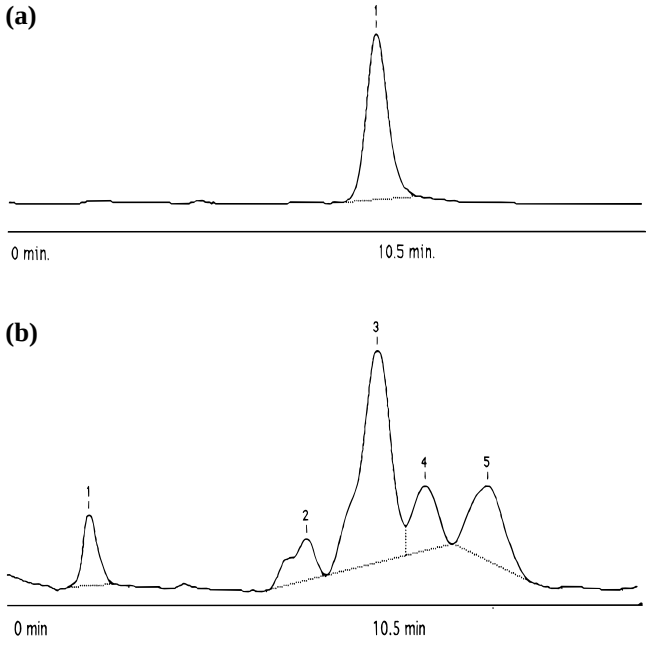

FIGURE 2: (a) Chromatograph of the purified Δ equatorial-meridional-syn isomer on the ion-exchange (MONO−S) column prior to treatment. (b): Chromatograph of the resulting mixture of Δ isomers on the Ion-exchange (MONO-S) column after treatment. Peaks are identified by elution time to be. (1) unidentified material, (2) Δ equatorial-facial-anti, with an early shoulder of Λ equatorial-facial-anti, (3) Δ equatorial-meridional-anti with an early shoulder of Δ axial facial anti, (4) Δ axial-meridional-anti and Δ equatorial-meridional-syn, and (5) the desired Δ axial-meridional-syn isomer.

(*9*) and are sometimes observed following concentration of monoammine chromium ATP isomers by Dowex-1 anion exchange chromatography if the solution being concentrated is allowed to warm. To minimize the formation of bridging complexes, the reactions were done in dilute solution. The bridging complexes are easily removed from the desired products but can severely reduce yields. Shown in Figure 2 is a MONO-S chromatogram of the starting Δ equatorial-meridional-syn isomer and the mixture of the Δ axial-meridional-syn isomer (peak 5, 19%) and unreacted Δ equatorial-meridional-syn isomer (peak 3, 52%) as well as smaller amounts of other Δ isomers following treatment. The shoulder visible on the front of peak 2 is due to the Λ equatorial facial anti isomer, showing that during the incubation only minor amounts of the Λ isomers have formed. Peak 1 is due to bridging complexes and comprises 8% of the total in this chromatogram. Structural assignments are based on elution times from the MONO-S column and from the HPLC column.

This method is not limited to the Λ and Δ axial-meridional-syn isomers, but can be used to prepare any isomer from its axial-equatorial partner. It will be most useful producing partners to those isomers easily obtained in pure form from the reverse phase HPLC column as we demonstrated for Λ and Δ axial-meridional-syn isomers. We also used this method to prepare the Λ and Δ anti-meridional-anti isomers and the Δ equatorial-facial-anti isomer.

Kinetics of 3-Phosphoglycerate Kinase. It has been shown that 3-phosphoglycerate kinase is inhibited by tridentate chromium ATP with a K_i of 28 μM and by bidentate chromium ATP with a K_i of 63 μM (*15*). The enzyme is known to be specific for the Δ screw sense isomer of

Table 1: K_i Values for Chromium ATP and Monoammine Chromium ATP Inhibiting 3-Phosphoglycerate Kinase, Glycerol Kinase, and Hexokinase[a]

ATP isomer	K_i (μM) 3-PGK	K_i (μM) GK	K_i (μM)[a] HK
	Chromium		
Δ axial	67.7 ± 19.0	27.8 ± 7.7	0.04 ± 0.005[b]
Λ axial	287 ± 59	2.85 ± 0.87	0.63 ± 0.05[b]
Λ equatorial	584 ± 149	0.619 ± 0.056	0.63 ± 0.05[b]
Δ equatorial	224 ± 61	33.5 ± 12	0.04 ± 0.005[b]
	Monoammine Chromium		
Δ axial			
facial-anti	786 ± 39	43.6 ± 4.8	4.5 ± 0.4
meridional-anti	82.0± 17.2	16.8 ± 4.2	1.8 ± 0.2
meridional-syn	70.4± 4.1	20.6 ± 3.6	0.8 ± 0.07
Λ axial			
facial-anti	105 ± 30	13.7 ± 1.3	0.45 ± 0.08
meridional-anti	452 ± 64	12.0 ± 2.5	0.05 ± 0.005
meridional-syn	232 ± 37	9.7 ± 1.0	0.06 ± 0.007
Λ equatorial			
facial-anti	1230 ± 610	20.2 ± 3.5	0.90 ± 0.09
meridional-anti	1340 ± 120	17.5 ± 3.9	0.05 ± 0.006
meridional-syn	1460 ± 700	9.45 ± 0.65	0.10 ± 0.01
Δ equatorial			
facial-anti	494 ± 130	34.8 ± 4.1	1.65 ± 0.20
meridional-anti	504 ± 90	42.3 ± 5.4	2.0 ± 0.3
meridional-syn	1200 ± 850	30.8 ± 5.6	1.7 ± 0.1

[a] Hexokinase data taken from Rawlings et al. (6). [b] Equatorial and axial chromium(III) ATP isomers were not separated.

magnesium phosphorothioate ATP (*16*). The data shown in Table 1 confirm these results. The Δ axial isomer of chromium ATP has a K_i of 67.7 μM. The enzyme shows a 4-fold preference for the Δ axial isomer over the Δ equatorial isomer. This axial preference is also seen in the Λ isomers, although it is less pronounced. The enzyme does bind the Δ isomers more tightly than the Λ, with the difference (4.2-fold) more pronounced in the tighter binding axial forms than the more weakly bound equatorial isomers (2.6-fold).

Monoammine chromium ATP Δ axial isomers show the tightest binding to the enzyme. Two of these isomers—axial-meridional-anti, K_i = 80 μM, and axial-meridional-syn, K_i =70 μM—bind as tightly as the tetraaquo Δ axial. The third isomer, axial-facial-anti, binds much more weakly with a K_i of 780 μM. This clearly suggests one position is in contact with the enzyme surface while the other two are not involved significantly in binding the isomers to the enzyme.

Monoammine chromium ATP Δ equatorial isomers bind much more weakly. All three isomers have K_i values at least twice that of the Δ equatorial chromium ATP, suggesting some contact in all three positions. One position, the equatorial-meridional-syn, is much higher (5.4-fold) than Δ equatorial chromium ATP, possibly indicating a specific interaction at this position.

Two of the monoammine chromium ATP Λ axial isomers have K_i values about the same as the Λ axial chromium ATP, or possibly lower. These two positions are likely not close to the enzyme surface. The third isomer has a K_i a little higher.

Monoammine chromium ATP Λ equatorial isomers show very weak binding for all three isomers. Although the K_i values for all three isomers are high, the difference between these K_i values and that of the Λ equatorial chromium ATP is only 2-fold. The weak binding and small differences make it difficult to interpret the binding pattern for these isomers.

Kinetics of Glycerol Kinase. It has been shown that Λ bidentate chromium ATP is a slow substrate for glycerol kinase and inhibits the magnesium ATP reaction with a K_i of 0.14 μM (*15*). Table 2 has the K_i values for the four isomers of chromium ATP and the 12 isomers of monoammine chromium ATP determined in this study. They confirm the earlier findings of (*15*), although in our hands the K_i value is somewhat higher (0.6 μM for the Λ equatorial chromium ATP isomer. In contrast to hexokinase which showed little or no preference between axial or equatorial isomers (*6*) and in contrast to 3-phosphoglycerate kinase described above which shows a preference for axial chromium ATP isomers, glycerol kinase shows a 4.6-fold tighter binding for the Λ equatorial isomer of chromium ATP compared to the Λ axial isomer. The two Δ isomers bind much more weakly as is expected from the known screw sense preference (*15*), and the preference for Λ screw sense isomers is similar to that found for hexokinase (*6*). The enzyme shows no axial-equatorial preference between the Δ isomers.

Monoammine chromium ATP Λ equatorial isomers all show a ~15−30-fold higher K_i than Λ equatorial chromium ATP. Perhaps all three positions are important in binding chromium ATP to the enzyme. Hydrogen bonds from the coordinated waters to the enzyme could help explain the much tighter binding of chromium ATP to glycerol kinase compared to that of 3-phosphoglycerate kinase. The possible effects of introducing ammonia into positions in contact with the enzyme are discussed in more detail below.

Monoammine chromium ATP Λ axial isomers show an interesting result. The K_is are all about the same, 3−5 times larger than that of Λ axial chromium ATP and about the same as or lower than the K_is of the Λ equatorial monoammine chromium ATP isomers. Ammonia substitution has overwhelmed the equatorial-axial preference displayed by chromium ATP.

Monoammine chromium ATP Δ equatorial isomers all show K_i values similar to that of Δ equatorial chromium ATP, suggesting that none of the positions is involved in favorable interactions with the enzyme.

Monoammine chromium ATP Δ axial isomers show K_i values similar to or slightly lower than Δ axial chromium ATP. Only one position, the axial-facial-anti, has a K_i value significantly higher than the others, suggesting a possible interaction point.

Modeling Studies with 3-Phosphoglycerate Kinase. The structure of a mutant yeast 3-phosphoglycerate kinase complexed with 3-phosphoglycerate and an ATP analogue has recently been published (*7*). This complex is reported to be structurally very similar to the E•S complex, despite the fact that it is much more exposed to solvent than ATP binding sites in other kinases such as hexokinase (*17*) and glycerol kinase (*13*). Table 1 shows the interactions between chromium ATP isomers and the enzyme surface. The most interesting result is the Δ axial isomer since it binds to the enzyme best and most closely mimics the natural magnesium ATP structure. As predicted by the kinetics, there is a close contact with one position of the coordination sphere and lysine 217. Figure 3 shows this interaction. The flexible lysine side chain would be expected to accommodate the isomer in a real complex, avoiding the impossibly short contact distances seen in the computer model. Substitution of ammonia for water in a coordination position close to a

Table 2: Close Contacts to Various Coodination Positions in 3-Phosphoglycerate Kinase

	distance (in angstroms) to atom-position of residue
Δ axial	
facial-anti	1.5 to N-ζ, 1.3 to C-δ, 1.1 to C-γ all of lys217
meridional-anti	2.2 to C-δ of lys217
meridional-syn	2.3 to C-δ of lys217
Λ axial	
facial-anti	none
meridional-anti	3.1 to C-β of asp216
meridional-syn	3.1 to N-ζ of lys217, 2.7 to N-δ of asn334
facial-syn	2.3 to N-ζ of lys217
Λ equatorial	gly335 and asn334 backbone atoms overlap the Cr
facial-anti	2.6 to the N of gly210
meridional-anti	2.1 to trp333
meridional-syn	2.1 and 1.7 to the amide of asn334
facial-syn	0.3 to side chain O of thr373
Δ equatorial	
facial-anti	2.6 to N-ζ of lys217, 1.6 to N-δ of asn334
meridional-anti	2.1 to N-δ of asn334, 1.2 to O of gly369
meridional-syn	1.6 to C-δ, 2.0 to O-δ, 1.6 to C-γ, 2.0 to C-β, 2.1 to C-α all of asn334, 3.1 to N-ζ of lys217
facial-syn	2.3 to O-δ of thr373, 2.2 to C-α of gly370

Possible Close Contacts to Various Coodination Positions in Glycerol Kinase

	distance (in angstroms) to atom-position of residue
Δ axial	
facial-anti	1.7 to side chain O of asp245
meridional-anti	1.5 to side chain O of asp10, 2.8 to N-α of gly12
meridional-syn	2.2 to side chain O of asp10, 2.8 to side chain N of arg17
facial-syn	1.6 to side chain N of arg17
Λ axial	
facial-anti	2.3 to side chain O of asp10, 2.5 to gly12, 3.1 to side chain N of arg17
meridional-anti	2.2 to side chain O of asp245
meridional-syn	2.1 to side chain O of asp10, 1.9 to side chain N of arg17
Λ equatorial	
facial-anti	3.4 to side chain O of asp245, 3.5 to side chain N of gln246
meridional-anti	2.6 to N of thr13, 2.6 to side chain O of thr13, 2.9 to C of gln82, 3.3 to side chain O of asp245
meridional-syn	2.6 to C-γ and 2.8 to side chain O of thr13, 3.1 to C of trp103
facial-syn	1.8 to N and 2.1 to C-α of thr13, 2.9 to side chain O of thr267
Δ equatorial	
facial-anti	2.6 to side chain O of asp245, 3.1 to C of gly12
meridional-anti	2.0 to side chain O of asp10, 3.3 to side chain O of asp245, 3.0 to O-α of gln11
meridional-syn	3.1 to side chain O of asp245, 2.8 and 2.9 to side chain O of asp10
facial-syn	1.6 to side chain N of arg17

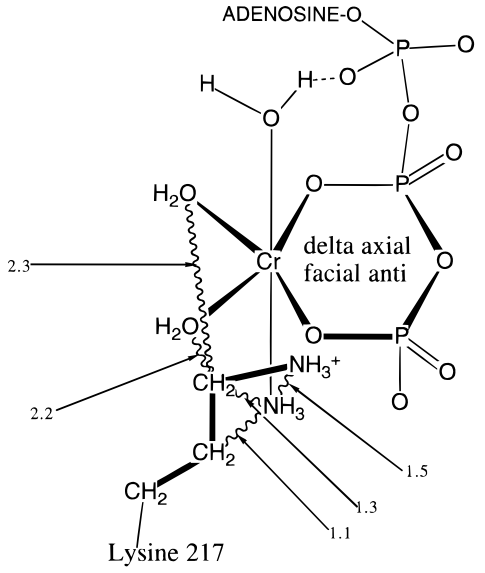

FIGURE 3: Distances (in angstroms) from the 3-PGK lys 217 side chain to various coordinated ligands on monoammine chromium ATP.

positive lysine side chain could destabilize the complex in two ways. The electron lone pair of the oxygen of a coordinated water could accept a hydrogen bond from lysine, but coordinated ammonia has no free lone pair of electrons. Coordinated ammonia has also been shown (*18*) to increase the retention time of chromium pyrophosphate complexes on cation exchange columns, suggesting that ammonia makes the positive charge more accessible to groups in the surroundings. Increasing the positive charge of the surface of the coordination complex in the vicinity of the lysine side chain will destabilize the E•I complex by electrostatic repulsion. The kinetics studies and modeling studies both suggest that the binding site for Δ axial chromium ATP is an open site with only one coordination position interacting with the surface of the enzyme.

When the Λ axial isomer is modeled, there are more potentially destabilizing contacts as shown in Table 2, consistent with the higher K_i for this chromium ATP isomer. When the ammonia substitutes in the axial-facial-anti isomer, there is no effect on K_i. This position is shown to be exposed to solvent in the model study. (Figure 4)

When Δ and Λ equatorial chromium ATP isomers are modeled they clearly do not fit as well. There are severe contacts at multiple positions with the backbone as well as the side chains for the Λ isomer. Clearly the enzyme or isomer will have to change substantially for any binding to occur. There are several potential contacts to lysine 217 and other hydrogen bond donors in this region, but it is not possible to identify contacts at this time.

Modeling Studies with Glycerol Kinase. The structure of *E. coli* glycerol kinase complexed to ADP and glycerol-3-phosphate determined by X-ray crystallography has been

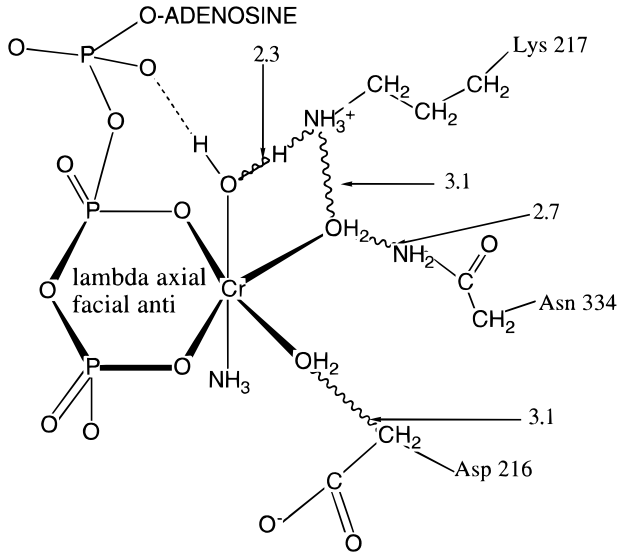

FIGURE 4: Distances (in angstroms) from the 3-PGK side chains to various coordinated ligands on monoammine chromium ATP.

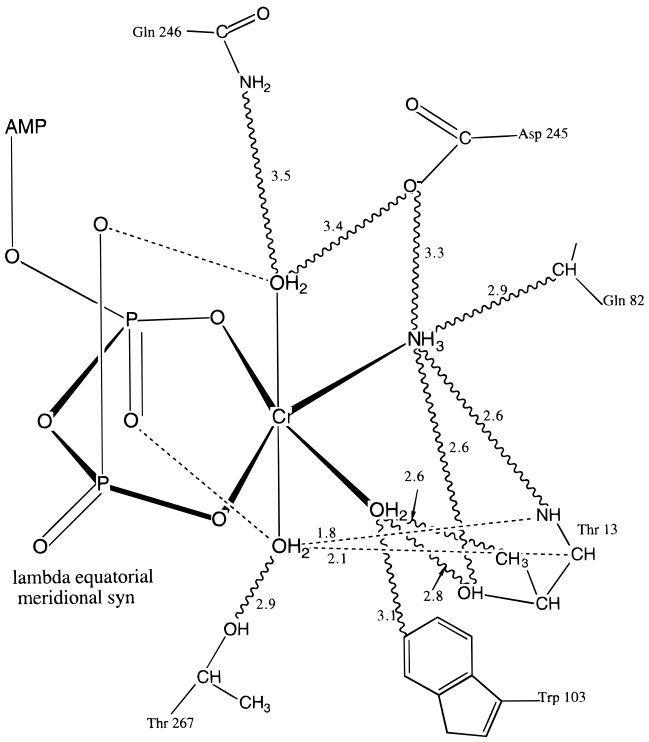

FIGURE 5: Distances (in angstroms) from glycerol kinase side chains to various coordinated ligands on Λ equatorial-meridional-syn monoammine chromium ATP.

published (*13*). In contrast to the 3-phosphoglycerate kinase structure described above, the ATP binding site is much less open and there are many more possible contacts. Since the kinetics and X-ray data are not from the same species we cannot expect exact correspondence, but it is reasonable to expect a highly conserved active site architecture. As was observed for 3-phosphoglycerate kinase the best binding isomer, Λ equatorial-meridional-syn for glycerol kinase, fits best in the active site (Figure 5). There are also no positively charged side chains closer than 3 Å to any of the positions for this isomer. Several polar side chains, asp 245, gln 246, thr13, thr267, are good candidates for interactions with the coordinated waters (Table 2). Since all three Λ equatorial monoammine chromium ATP isomers have K_i values significantly higher than Λ equatorial chromium ATP and there are polar side chains close to all three positions, it appears likely that they are all involved in binding interactions. Aspartate 245 is possibly able to coordinate directly to magnesium in the labile magnesium ATP complex.

When Λ axial isomers are placed in the active site, there are several polar side chains close to the coordinated waters. They are asp245, asp10, and arg17. Also close by is the polar main chain nitrogen of gly12.

Both Δ equatorial and Δ axial monoammine chromium ATP isomers bind about as strongly as Δ chromium ATP. Modeling studies show a severe contact to arg17 in all these isomers which may explain the Λ preference.

Comparison of Monoammine Chromium ATP and 5′-Adenylylimidodiphosphate Complexed to 3-Phosphoglycerate Kinase. The crystal structure of 5′-adenylylimidodiphosphate (MAP) complexed to 3-phosphoglycerate kinase (*7*) showed the MAP to be tridentate with nonbridging oxygens from all three of the phosphate atoms coordinated to cis positions in the octahedral magnesium coordination sphere. This is in agreement with finding that tridentate chromium ATP is a better inhibitor of this enzyme than bidentate chromium ATP (*15*). The results reported here for bidentate chromium ATP and monoammine chromium ATP also support this finding. Axial bidentate chromium complexes more closely mimic the compact tridentate structure due to the strong hydrogen bond from the nonbridging oxygen of the α phosphate to the coordinated syn water of the coordination sphere. These axial isomers are found to bind to 3-phosphoglycerate kinase 3−6 times more tightly than the equatorial isomers. MAP is bound in an open site with only one strong interaction to the coordination sphere at the position opposite the coordinated γ phosphate. Monoammine chromium ATP isomers are also bound in a site that displays only one strong contact to the coordination sphere. Possibly due to the differences between a tridentate and bidentate complex, the strong contacts are not in the same position and the modeling studies suggest lys217 rather than asp372 is the enzyme side chain closest to the monoammine chromium ATP. Overall, the agreement between the kinetics and the crystal structure is very good, strongly supporting the validity of probing kinase active sites with monoammine chromium ATP.

Comparison of ADP and Monoammine Chromium ATP in Glycerol Kinase. The kinetics results with glycerol kinase suggest a very different environment for the coordinated metal than seen for 3-phosphoglycerate kinase. All three positions seem to be in close contact with the surface of the enzyme. The modeling with the *E. coli* glycerol kinase−ADP−3-phosphoglycerol complex shows many close polar groups that might make hydrogen bonds to the coordinated waters more effectively than to coordinated ammonias.

Comparison of Monoammine Chromium ATP in Glycerol Kinase and in Hexokinase. One surprising result was the difference between the kinetic inhibition patterns of monoammine chromium ATP in glycerol kinase and hexokinase. For hexokinase, Rawlings et al. (*6*) found only one coordination position, the facial anti, to have a significantly higher K_i when ammonia was present. Hexokinase is thought to have a nucleotide binding site structurally much more like glycerol kinase than 3-phosphoglycerate kinase (*13*). The monoam-

mine chromium ATP inhibition results suggest that despite the structural similarities the surface contacts to the coordinated waters are quite different and that the hexokinase site around the coordination sphere may be functionally more like the 3-phosphoglycerate kinase site.

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