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Original scientific paper

The inhibition of adenylate kinase by 2,4-thiazolidinedione evaluated by protein-ligand docking

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Abstract

Due to its crucial role in nucleotide metabolism, adenylate kinase deserves a special attention in screening of potential inhibitors. Herein, we report the assessment of the relative orientation of the ligand 2,4-thiazolidinedione to adenylate kinase crystallized in closed conformation. Protein-ligand docking was performed to estimate the binding energy and inhibition constant of 2,4-thiazolidinedione to the adenylate kinases' active sites from different organisms. Our results revealed the best orientation of 2,4-thiazolidinedione is with Gram-positive and acid fast bacteria adenylate kinase – K_i = 0.76±0.1 mM and binding energy -4.26±0.08 kcal/mol. Human adenylate kinases display unfavourable interactions, the binding affinity fluctuating among K_i=0.84 mM and 8.8 mM (3.88±3.51); the energy binding -3.56±0.57. From the three human adenylate kinases analysed, only isoenzyme 2 shows a binding conformation similar to its counterpart from E. coli. Adenylate kinase - this small enzyme needed for survival of every organisms - interacts differently with 2,4-thiazolidinedione, this selectivity being the most important evidence of the present study.

Keywords

molecular docking; 2, 4-thiazolidinedione

Introduction

Bacterial resistance to antibiotics is an evolving problem of medical practice [1,2]. Discovery of new antibacterial agents and/or reevaluating of different targets are impetuously needed [3], [4]. These kinds of studies are usually constructed by comparison of well characterized pathogens. *Escherichia coli* (*E. coli*) is an opportunistic Gram-negative pathogen, widely isolated from nosocomial infections [5]. *Streptococcus pneumoniae* (*S. pneumoniae*) – a Gram-positive cocci – is an extracellular organism responsible for pneumoniae, otitis media, meningitis and even sepsis in very young children and adults above 65 years [6,7]. Tuberculosis treatment remains one of the most challenging problems for clinicians; multidrug-resistant *Mycobacterium tuberculosis* (*M. tuberculosis*) strains considerably diminish treatment options [8].

Adenylate kinase (AK) – ATP: AMP phosphotransferase; EC 2.7.3.4 – belong to the nucleotide kinase super family which catalyzes conversion between adenylate nucleotide by the following reaction: Mg^{2+} ATP + AMP ↔ Mg^{2+} ADP + ADP. AK is widely studied due to its implication in nucleotide metabolism and to its ubiquities – it is described in Archaea, prokaryotes and eukaryotes. Some AKs are very well characterized,

many of them have the crystal structures solved and biochemical parameters established. AK is a flexible protein, which can adopt different conformations during catalytic process [9,10].

AK is, due to its universality and its flexibility, an attractive target for screening of new inhibitors. Thiazolidinediones (TZDs) derivates – synthetic agonists for the peroxisome proliferator-activating receptor-gamma receptor – were demonstrated as potential inhibitors of *S. pneumoniae* out-growth [11]. As we are interested in inhibition of AK by thiazolidine derivates [12], the present study is focused on 2,4-thiazolidinedione interaction with the active site of bacterial and human AKs.

Materials and Methods

Data Collection and Design

The sequences and annotations for the proteins examined were collected from NCBI Protein Database (http://www.ncbi.nlm.nih.gov/protein). AKs X-ray 3D structures were downloaded from the Protein Data Bank (http://www.rcsb.org/pdb). AKs co-crystallized with the substrates or substrates analogs like the inhibitor P1,P5-di(adenosine-5')pentaphosphate (Ap5A) ($C_{20}H_{29}N_{10}O_{22}P_5$), were stored for further procedures. The structure of the ligand 2,4-thiazolidinedione ($C_3H_3NO_2S$) was retrieved from the Open Chemistry Database – National Center for Biotechnology Information PubChem Compound Database; CID=5437, https://pubchem.ncbi.nlm.nih.gov/compound/5437. This open database provided chemicals as SDF files.

Protein-ligand docking

To analyze the binding of 2,4-thiazolidinedione, we performed protein-ligand docking by AutoDock4, a widely used non-commercial program, which provided useful instruments for accurate prediction of binding interaction [13–15]. The AutoDockTools (http://mgltools.scripps.edu/downloads) offer an excellent interface for non-bioinformaticians. Interpreting the docking data was possible by employing of free 3D molecular visualization tools – BIOVIA Discovery Studio Visualizer 2016 (DS Visualizer 2016); (http://accelrys.com/products/discovery-studio/visualization-download.php).

AKs included in the present study were presented in the Table 1. Firstly, from the 3D structure, substrate or substrate analogs (Ap5A) and all solvent molecules were removed and a closed conformation was obtained, which allows an algorithm based on a rigid protein structure. In our case, this docking algorithm was sufficient to obtain reliable data. The Lamarckian Genetic Algorithm (LGA), with a maximum of 2,500,000 energy evaluations, was choose as searching parameter [16].

Multiple sequence alignment

Multiple sequence alignment was performed using Clustal Omega program (http://www.ebi.ac.uk/Tools/msa/clustalo), an open access tool provided by the European Bioinformatics Institute – EMBL-EBI [21,22].

Statistical evaluations*:* Data were expressed as mean ± SD.

Inclusion criteria: AKs from any microorganism or from human origin with crystal structure determined by X-ray crystallography co-crystallized with substrate or substrate analogs, so a closed conformation is provided.

Exclusion criteria: AKs with no crystal structure recorded in protein databases or 3D structure in open conformation.

Table 1 *The main characteristics of the AKs used in protein-ligand docking*

ACCESSION NUMBER	Source	co-crystallized with substrate or substrate analogs	No. of residues	References
Bacterial source				
3HPQ	*E. coli*	Ap5A	214	[17]
1ANK	*E. coli*	AMP, AMPPNP	214	[18]
4W5J	*S. pneumoniae*	Ap5A, Mg^{2+}	217	[19]
1ZIP	*B. stearothermophilus*	Ap5A, Mn^{2+}, and Mg^{2+}	217	[9]
2CDN	*M. tuberculosis*	two ADP molecules and Mg^{2+}	201	[20]
2RGX	*Aquifex aeolicus*	Ap5A, Zn^{2+}	206	[10]
Homo sapiens				
2C95	(Chain A – AK1)	Bis(Adenosine)-5'-Tetraphosphate; malonate ion	196	Unpublished
2C9Y	(Chain A – AK2)	Bis(Adenosine)-5'-Tetraphosphate; Ethylene Glycol	242	Unpublished
2BWJ	(Chain A – AK5)	AMP, Cl^-, SO_4^{2-}	199	Unpublished

Results and Discussion

Protein-ligand docking

AKs from different sources were subjected to docking with 2,4-thiazolidinedione. It is already established that AK is a natural flexible protein, binding and releasing of its substrates are followed by motions of the AMP-binding domain and LID domain. As a result of sophisticated conformational transitions a so-called closed conformation results during catalytic process [23,24]. Starting from these premises, rigid protein structures were considered for further docking algorithm. For each AK, ten conformations were obtained as a result of docking process. The conformation with the smallest binding energy (Δ*G*) and the smallest inhibition constant (K_i) was considered the best. As it is shown in the Table 2, regarding AKs from Gram-positive and acid fast species, Δ*G* values are almost the same with a mean of -4.26±0.08 kcal/mol, and K_i are clustered around the mean 0.76±0.1 mM. On the other hand, the human counterpart AKs exhibit more diverse values. Human AK isoenzyme 2 (2C9Y) – mitochondrial origin – exhibits values similar to enzymes of bacterial sources.

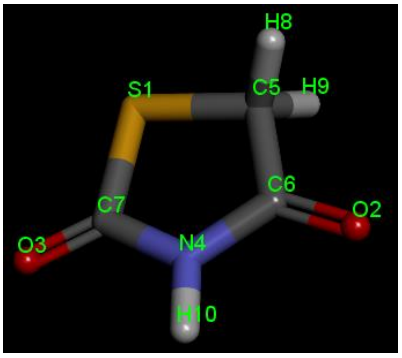
Active sites residues which interact with the ligand 2,4-thiazolidinedione are shown in the Table 3. The most striking observation is related to AKs of human origin, as it is illustrated in the Figures 1 and 2. The AK5 (2BWJ) – expressed exclusively in the brain [25] – is clearly a poor target, none of the residues from the active site being involved in 2,4-thiazolidinedione binding. Further, in the case of AK2 (2C9Y), the ligand forms standard hydrogen bonds with two nonpolar residues from AMP-binding region (Gly100 and Phe101) and with Lys28 from ATP-binding domain. Besides, from the AKs examined in the present study, AK2 and one AK from E. coli 3HPQ are the only ones which bind 2,4-thiazolidinedione with P-loop domain residues (Lys28 and Lys13, respectively). Notably, the same oxygen atom (O2) (Scheme 1) of the ligand forms hydrogen bond with lysine but different atoms are involved – NZ regarding 2C9Y and CE for 3HPQ (Figures 3 and 4). On the contrary, apart from the residues involved in AMP-binding, AK1 shows only a glycine from NMP-binding domain which binds the ligand. This might be a reasonable explanation of almost twofold value K_i of AK1 (1.99 mM) compared with AK2 (0.84 mM).

A unique detail is observed in the case of Gram-positive and acid fast AKs – a favorable interaction between Pi-sulfur of the ligand and the nonpolar residue Phe35 from NMP-binding domain (Figures 5 and

6) might play an important role in stabilization of AK-2,4-thiazolidinedione complex [26]. Multiple sequence alignment exhibits a conservative replacement of Phe35 with leucine on other AKs. Moreover, in A. aeolicus' AK, van der Waals' forces between Leu35 and the only sulfur atom of the ligand are noticed.

Table 2. Energy binding and inhibition constant (Ki) of the best conformation of the complex AK-2, 4-thiazolidinedione

AK	Δ*G*, Kcal/mol	K_i, mM
Gram-negative bacteria		
3HPQ	-4.0	1.17
1ANK	**-4.18**	**0.86**
2RGX	-4.22	0.81
Mean ± SD	-4.13±0.09	0.94±0.16
Gram-positive and acid fast bacteria		
1ZIP	-4.36	0.64
4W5J	-4.27	0.77
2CDN	-4.16	0.89
Mean ± SD	-4.26±0.08	0.76±0.1
Homo sapiens		
2C95	-3.68	1.99
2C9Y	**-4.19**	**0.84**
2BWJ	-2.8	8.8
Mean ± SD	-3.56±0.57	3.88±3.51
Total		
Mean ± SD	-3.98 ± 0.46	1.86 ± 2.48

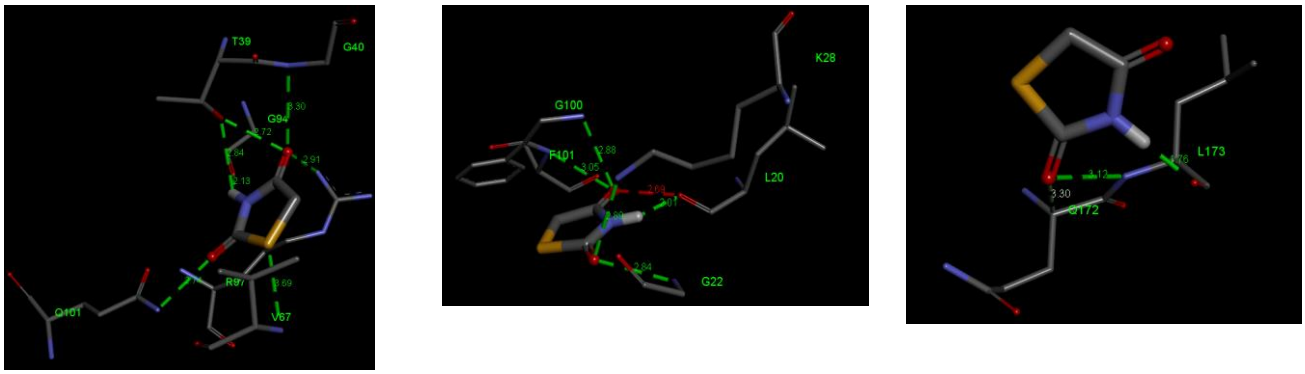


Scheme 1. The numbering of 2,4-thiazolidinedione atoms

Table 3. Residues of substrate binding domains, according to Protein Database (www.rcsb.org/pdb/home/home.do) details Code color for interactions with 2,4-thiazolidinedione: in green are shown conventional hydrogen bonds; blue – van der Waals' forces, brown – carbon hydrogen bonds; orange – Pi-sulfur, red – unfavorable acceptor-acceptor

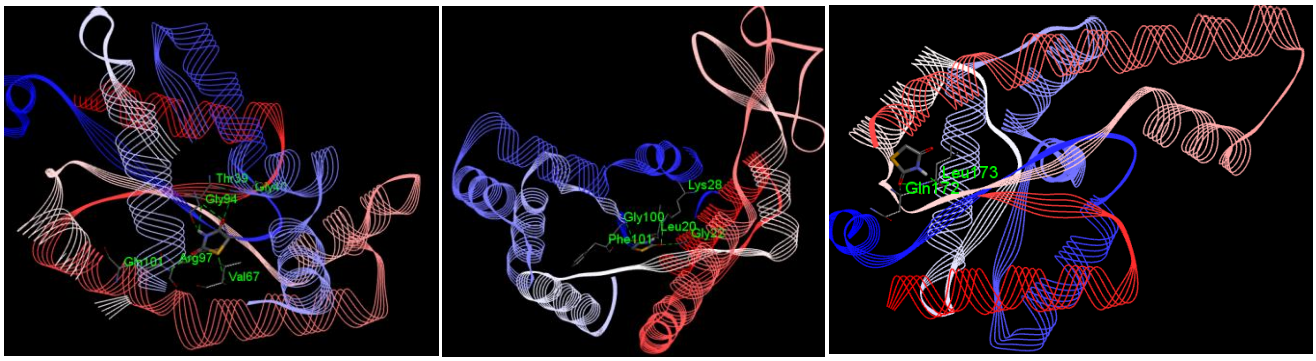
AK	NMP-binding domain	AMP binding	LID domain	ATP binding (P-loop)
3HPQ	Ser30-Val60	Lys57-Val59, Gly85-(Phe86, Pro87)-Arg88, Thr31, Arg36, Gln92, Arg167	Gly122-Asp159	Gly10-(Lys13)-Thr15, Val132-Tyr133, Arg119, Arg123, Lys200
1ANK	Ser30-(Leu58)- Val59	Lys57-(Leu58)-Val59, Gly85-(Phe86)-Arg88, Thr31, Arg36, Gln92, Thr156, Arg167	Gly122-Asp159	Gly10-Thr15, Val132-Tyr133, Arg119, Arg123, Lys200
2RGX	Ser30-(Thr31/Thr31, Leu35, Leu58)-Val59	Glu57-Val59, Gly82, Phe83, Pro84, Arg85, Thr31/Thr31, Gln89, Arg131	Gly123-Asp153	Gly10-Thr15, Val133-Tyr134, Arg120, Arg124, Lys189
1ZIP	Ser30-(Thr31, Phe35, Leu58)-Val59	Asp57-(Leu58)-Val59, Gly85-Arg88, Ser30, Arg36, Gln92, Gln159, Arg171	Gly126-Asp163	Gly10-Thr15, Thr136-Tyr137, Arg127, Gln199
4W5J	Ser30-(Phe35)- Val59	Glu57-(Leu58)-Val59, Gly85-(Gly86, Tyr87)-Arg88, Thr31, Arg36, Gln93, Arg156, Arg167	Gly127-Asp159	Gly10-Thr15, Thr137-Phe138, Arg128, Gln195
2CDN	Ser30-(Phe35, Leu58)-Val59	Asp57-(Leu58)-Val59, Gly85-(Tyr86)-Arg88, Thr31, Arg36, Gln92, Arg129, Arg140	Gly126-Asp132	Gly10-Thr15, Arg127, Gly166
2C95	Ser38-(Gly40)- Val67	Gln65-Val67, Gly94-Arg97, Thr39, Arg44, Gln101, Arg138, Arg149	Lys131-Asp141	Gly18-Thr23, Arg132, Gly177
2C9Y	Ala45-Val74	Lys72-Val74, Gly100, Phe101, Pro102, Arg103, Thr46, Arg51, Gln107, Arg175, Arg186	Gly141-Asp178	Gly25-(Lys28)-Thr30, Ser151-Tyr152, Arg138, Arg142, Gln214
2BWJ	Ser41-Val70	Asp68-Val70, Gly97-Arg100, Thr42, Arg47, Arg104, Arg152	Gln136-Asp144	Gly21-Thr26, Arg135, Gly180

The interactions of 2,4-thiazolidinedione with AKs of Gram-negative bacteria A. aeolicus – a hyperthermophilic species – and two structures from E. coli are shown in the Figures 3 and 4. In the latter cases, even though the aminoacid sequences and the structures are identical, different ligand orientations were observed.



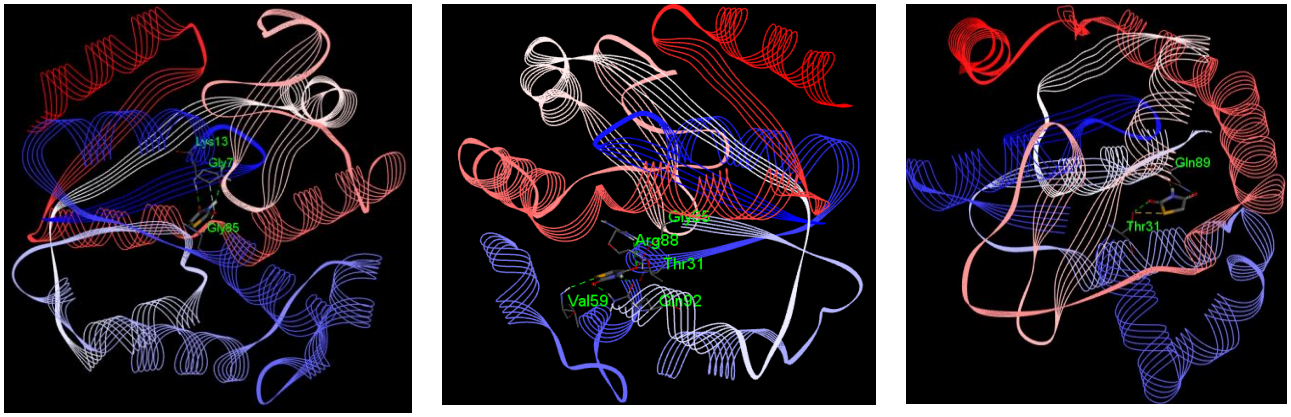
2C95 **2C9Y** **2BWJ**

Figure 1. Human AKs docked with 2,4-thiazolidinedione. 2C95-AK1; 2C9Y-AK2; 2BWJ-AK5 Code color for interactions: in green are shown conventional hydrogen bonds; yellow – carbon hydrogen bonds; red – unfavorable bump



2C95 **2C9Y** **2BWJ**

Figure 2. Interaction 2,4-thiazolidinedione with of human AKs



3HPQ **1ANK** **2RGX**

Figure 3. Interaction 2,4-thiazolidinedione with of AKs from Gram-negative microorganisms

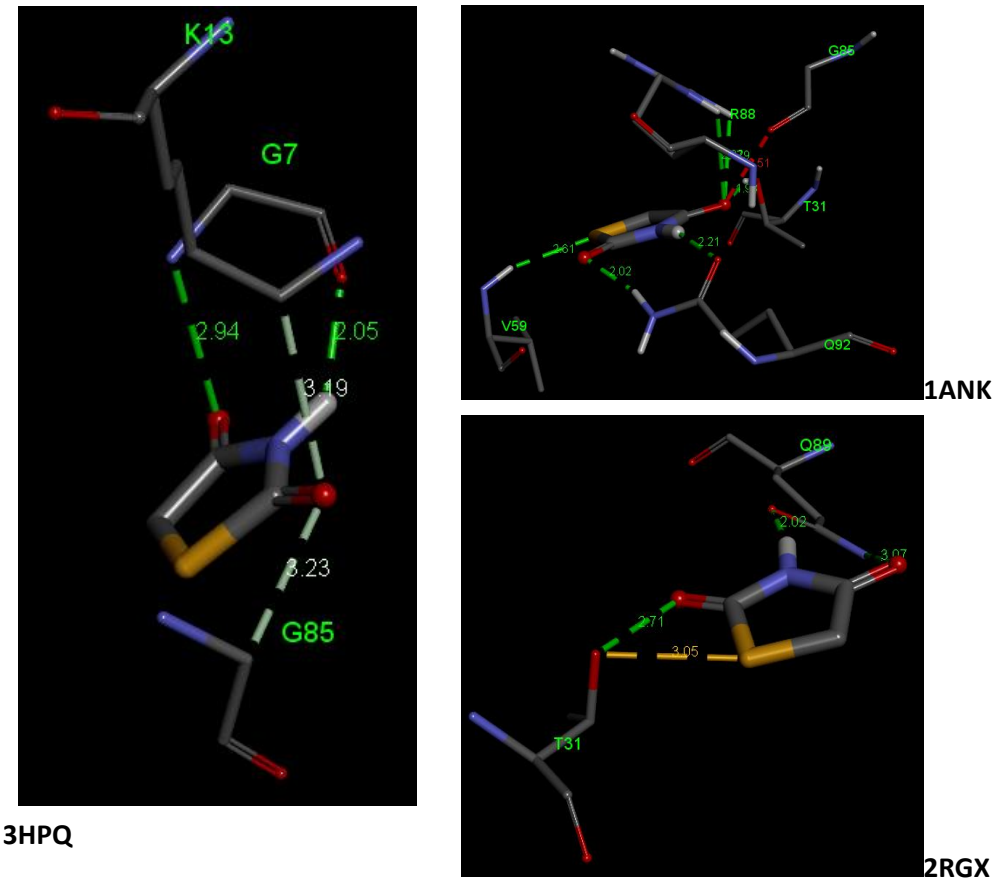
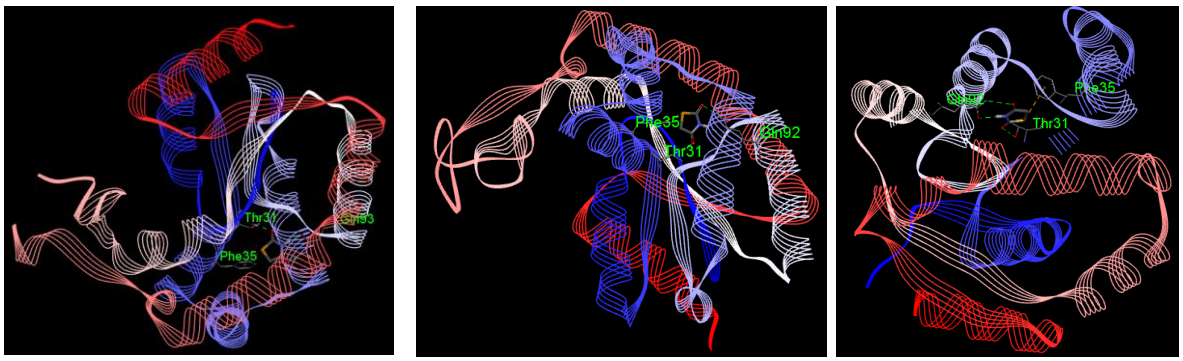


Figure 4. AKs from Gram-negative microorganisms docked with 2,4-thiazolidinedione. 3HPQ-E. coli; 1ANK- E. coli; 2RGX- A. aeolicus. Code color for interactions: in green are shown conventional hydrogen bonds; yellow – carbon hydrogen bonds; orange – Sulfur-X; red – unfavorable acceptor-acceptor

One explanation could reside on the fact that the two enzymes being co-crystallized with different substrate analogs (Table 1). It is well known that AK is a flexible enzyme, the LID domain and NMP-binding domain being highly dynamic. Therefore, the transition between open and closed states is characterized by important conformational fluctuations (dynamics). Studies performed with AK mutated in the residues which do not alter its structure, demonstrated that the binding interactions of different ligands affect the dynamics of AK, without accompanying structural changes of the enzyme [23,27,28].

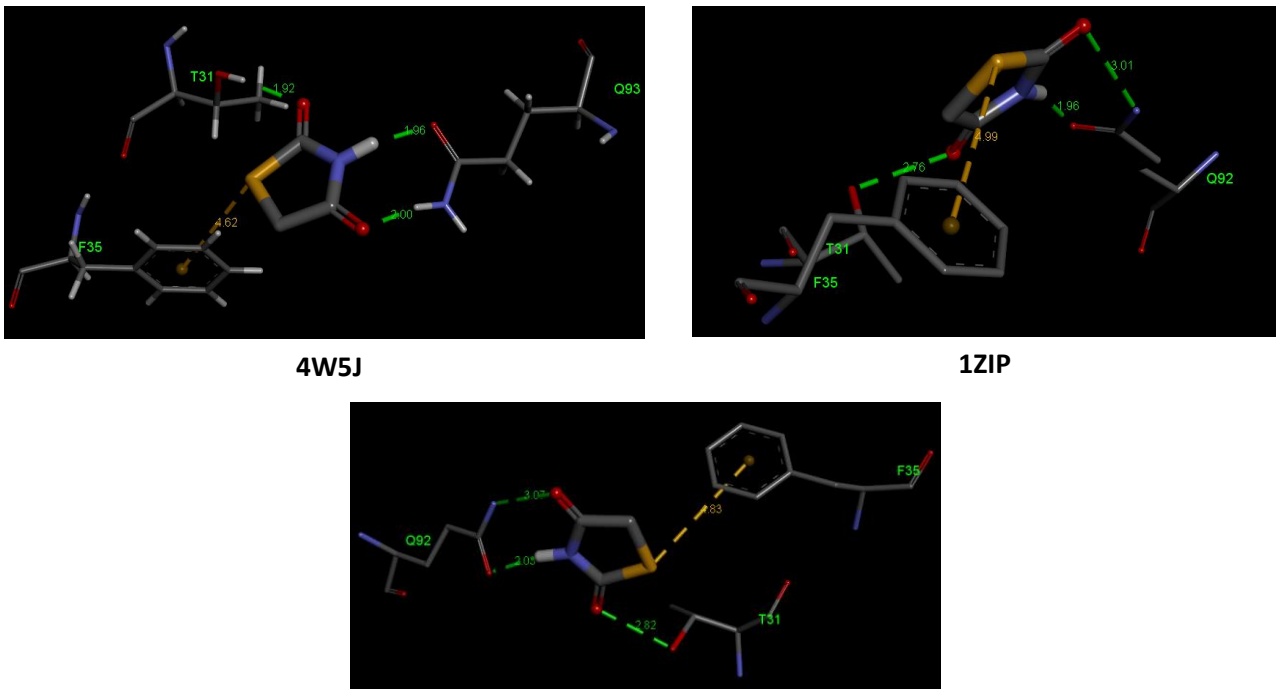
A. aeolicus's AK (2RGX) shows particularly comparable docking with *S. pneumoniae* (4W5J) and *M. tuberculosis* (2CDN) counterparts. The exception is Phe35 residue which is not present in Gram-negative AKs. On the contrary, 2RGK binds the sulfur from 2,4-thiazolidinedione structure to Thr31 residue.

Moreover, glutamine, a polar residue strictly conserved in AKs – the last residue of the consensus sequence motif [LIVMFYWCA] – [LIVMFYW] (2)–D–G–[FY]–P–R–X(3) –[NQ] – promotes hydrogen bonds with O2 / O3 in *A. aeolicus*, *E. coli* (1ANK), AK1, *M. tuberculosis,* and Gram-positive AKs. On these six AKs, sulfur atom contributes to protein-ligand complex stabilization also. Contrary to AKs of Gram-positive and acid fast bacteria where Pi-sulfur interactions were observed, hydrogen bonds were noticed for the rest. Regarding 1ANK and AK1, the valine residue Val59, respectively Val67, strictly conserved in AKs, participates in hydrogen bonding with sulfur atom.



4W5J **1ZIP** **2CDN**

Figure 5. Interaction 2,4-thiazolidinedione with of AKs from Gram-positive and acid fast microorganisms



4W5J **1ZIP**

2CDN

Figure 6. AKs from Gram-positive and acid fast microorganisms docked with 2,4-thiazolidinedione. 4W5J- S. pneumoniae; 1ZIP- B. stearothermophilus; 2CDN- M. tuberculosis. Code color for interactions: in green are shown conventional hydrogen bonds; yellow – carbon hydrogen bonds; orange – Pi-sulfur

In Gram-negative microorganisms, the ligand 2,4-thiazolidinedione binds different residues. Looking at E. coli's AKs, we noticed that Gly85, another residue strictly conserved from the consensus sequence motif, promotes a hydrogen bonding with the sulfur in the instance of 3HPQ, but failed to establish a valid interaction in 1ANK. On the contrary, in human AK2, this residue (Gly100) interacts with the O2 atom from the 2,4-thiazolidinedione structure.

As it is demonstrated in the Figures 5 and 6 and in multiple sequence alignment (Figure 7), AKs from Gram-positive and acid fast microorganisms, bind almost in the same manner the residues involved in AMP-binding (Thr31, Gln93, and Phe35), a notable difference is for AK from *B. stearothermophilus* In this latter instance, the smallest binding affinity is noticed – K_i = 0.64 mM. One can speculate other Gram-positive pathogens AKs' have the same inhibition pattern.

AKs of human origin display unfavorable interactions, the binding affinity fluctuating among 0.84 mM

and 8.8 mM. Mitochondrial isoenzyme 2 of AK behaves in the same manner as one *E. coli*'s AK - 3HPQ. As demonstrated in the Figure 7, they bind the same residues and have comparable binding affinity, K_i = 0.84 mM and 0.86 mM (Table 2). On the contrary, even though AK2 has energy binding and K_i comparable to Gram-positive microorganisms, 2,4-thiazolidinedione binds AMP-binding pocket and ATP-binding domain simultaneously. Considering these different binding patterns of human AKs the selectivity of 2,4-thiazolidinedione is an advantage in the context of antimicrobial treatment.

Multiple sequence alignment

Primary structure offers useful details; comparisons of even small identities could be exploited for finding interesting patterns. It would be of interest to speculate that enzymes with similar primary structure, behave the same with otherwise well characterized enzyme. Sometimes, observing minute details on primary structure has a major impact in decipher intriguing mechanism of a particular protein. The ligand chooses for the present work, 2,4-thiazolidinedione, has proven to bind important residues involved in AMP-binding.

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Homo sapiens AK 5            --------SMGGFMEDLRKCKIIFIIGGPGSGKGTQCEKLVEKYGFTHLSTGELLREELA
Homo sapiens AK 1            ----------SMEEKLKKTNIIFVVGGPGSGKGTQCEKIVQKYGYTHLSTGDLLRSEVS
Mycobacterium tuberculosis   MGSSHHHHHHSSGLVPRGSHMRVLLLGPPGAGKGTQAVKLAEKLGIPQISTGELFRRNIE
Bacillus stearothermophilus  --------------------MNLVLMGLPGAGKGTQAEKIVAAYGIPHISTGDMFRAAMK
Streptococcus pneumoniae     ---------------GAMGSMNLLIMGLPGAGKGTQAAKIVEQFHVAHISTGDMFRAAMA
Aquifex aeolicus             --------------------MILVFLGPPGAGKGTQAKRLAKEKGFVHISTGDILREAVQ
Escherichia coli/1ANK        --------------------MRIILLGAPGAGKGTQAQFIMEKYGIPQISTGDMLRAAVK
Escherichia coli/3HRQ        --------------------MRIILLGAPGAGKGTQAQFIMEKYGIPQISTGDMLRAAVK
Homo sapiens AK 2            ----SMAPSVPAAEPEYPKGIRAVLLGPPGAGKGTQAPRLAENFCVCHLATGDMLRAMVA
                                                  ..:* **:*****.  :       :::**:::*  :

Homo sapiens AK 5            SESERSKLIRDIMERGDLVPSGIVLELLKEAMVASLGDTRGFLIDGYPREVKQGEEFGRR
Homo sapiens AK 1            SGSARGKKLSEIMEKGQLVPLETVLDMLRDAMVAKVNTSKGFLIDGYPREVQQGEEFERR
Mycobacterium tuberculosis   EGTKLGVEAKRYLDAGDLVPSDLTNELVDDRLNNPDA-ANGFILDGYPRSVEQAKALHEM
Bacillus stearothermophilus  EGTPLGLQAKQYMDRGDLVPDEVTIGIVRERLSKDDC-QNGFLLDGFPRTVAQAEALETM
Streptococcus pneumoniae     NQTEMGVLAKSYIDKGELVPDEVTNGIVKERLSQDDIKETGFLLDGYPRTIEQAHALDKT
Aquifex aeolicus             KGTPLGKKAKEYMERGELVPDDLIIALIEEVFPK----HGNVIFDGFPRTVKQAEALDEM
Escherichia coli/1ANK        SGSELGKQAKDIMDAGKLVTDELVIALVKERIAQEDC-RNGFLLDGFPRTIPQADAM---
Escherichia coli/3HRQ        SGSELGKQAKDIMDAGKLVTDELVIALVKERIAQEDC-RNGFLLDGFPRTIPQADAM---
Homo sapiens AK 2            SGSELGKKLKATMDAGKLVSDEMVVELIEKNLETPLC-KNGFLLDGFPRTVRQAEMLDDL
                             . :  .      :: *.**       :: . :         .::**:** : *.. :

Homo sapiens AK 5            IG----DPQLVICMDCSADTMTNRLLQMS--------------------------RSSL
Homo sapiens AK 1            IG----QPTLLLYVDAGPETMTQRLLKRG--------------------------ETSG
Mycobacterium tuberculosis   LERRGTDIDAVLEFRVSEEVLLERLKGRG------------------------------
Bacillus stearothermophilus  LADIGRKLDYVIHIDVRQDVLMERLTGRRICRNCGATYHLIFHPPAKPGVCDKCGGELYQ
Streptococcus pneumoniae     LAELGIELEGIINIEVNPDSLLERLSGRIIHRVTGETFHKVFNPPVDY-----KEEDYYQ
Aquifex aeolicus             LEKKGLKVDHVLLFEVPDEVVIERLSGRRINPETGEVYHVKYNPPPP-------GVKVIQ
Escherichia coli/1ANK        -KEAGINVDYVLEFDVPDELIVDRIVGRRVHAPSGRVYHVKFNPPKVEGKDDVTGEELTT
Escherichia coli/3HRQ        -KEAGINVDYVLEFDVPDELIVDRIVGRRVHAPSGRVYHVKFNPPKVEGKDDVTGEELTT
Homo sapiens AK 2            MEKRKEKLDSVIEFSIPDSLLIRRITGRLIHPKSGRSYHEEFNPPKEPMKDDITGEPLIR
                                   .   :: .    . :  *:

Homo sapiens AK 5            PVDDTTKTIAKRLEAYYRASIPVIAYYETKTQLHKINAEGTPED-VFLQLCTAIDSIFL-
Homo sapiens AK 1            RVDDNEETIKKRLETYYKATEPVIAFYEKRGIVRKVNAEGSVDS-VFSQVCTHLDALLN-
Mycobacterium tuberculosis   RADDTDDVILNRMKVYRDETAPLLEYYRDQL--KTVDAVGTMDE-VFARALRALGK----
Bacillus stearothermophilus  RADDNEATVANRLEVNMKQMKPLVDFYEQKGYLRNINGEQDMEK-VFADIRELLGGLAR-
Streptococcus pneumoniae     REDDKPETVKRRLDVNIAQGEPIIAHYRAKGLVHDIEGNQDIND-VFSDIEKVLTNLK--
Aquifex aeolicus             REDDKPEVIKKRLEVYREQTAPLIEYYKKKGILRIIDASKPVEE-VYRQVLEVIGDGN--
Escherichia coli/1ANK        RKDDQEETVRKRLVEYHQMTAPLIGYYSKEAEAGNTKYAKVDGTKPVAEVRADLEKI---
Escherichia coli/3HRQ        RKDDQEETVRKRLVEYHQMTAPLIGYYSKEAEAGNTKYAKVDGTKPVAEVRADLEKI---
Homo sapiens AK 2            RSDDNEKALKIRLQAYHTQTTPLIEYYRKRGIHSAIDASQTPDV-VFASILAAFSKATCK
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Figure 7. Multiple sequence alignment. In gray background is marked ATP-binding domain – the Walker A motif GXXXXGKGT/S; in blue background are shown leucine and valine residues, very well conserved in AKs family; in green background is highlighted the consensus sequence [LIVMFYWCA] – [LIVMFYW] (2)–D–G–[FY]–P–R–X(3)–[NQ], were aspartic acid and arginine, strictly conserved in AKs family, are bolded; in red are colored the residues which interact with 2,4-thiazolidinedione

The main limitation of the present work is the availability of AKs' crystal structures deposited in the public databases. Even though some databases have some weaknesses – some of them being redundant – they remain a powerful tool in fundamental research. Unfortunately, kinetic experiments were not

performed, which was another limit of the present study. But the biochemical characterization of a particular enzyme, not always deciphers all capabilities of the protein of interest.

To put these findings in perspective, two approaches could be taken in consideration. Either the screening of library protein molecules [28] to find receptors to the highest affinity to 2,4-thiazolidinedione, or design novel TZDs derivates with better binding affinity. Inevitably, inhibitors used for bacterial infections could interact with human counterpart's target. Although these evidences are not strong enough to persuade us that 2,4-thiazolidinedione is selective inhibitors for bacterial AKs, a novel perspective of AK potentiality is revealed. Results obtained in this study lead us to the conclusion that refining the structure of 2,4-thazolidinedione could be a viable idea in developing potential inhibitors for vital enzymes like AK.

Conclusions

This study demonstrated an effort to reveal interactions of AK of bacterial / human origin with 2,4-thiazolidinedione. The most noteworthy evidences were achieved referring to Gram-positive and acid fast bacteria's AKs; 2,4-thiazolidinedione interacts with the same residues belonging to NMP-binding region. Further, the complex comprising AKs of *S. pneumoniae* and *M. tuberculosis* exhibit the same ligand-protein pattern. Human AKs interactions with 2,4-thiazolidinedione are divergent; a single notable connection being observed in case of mitochondrial isoenzyme 2 and its counterpart from *E. coli*. AK, a small enzyme needed for survival, could be an attractive target for TZDs derivates, at least in case of *M. tuberculosis* and Gram-positive microorganisms.

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