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Conformational changes in the structure of Shikimate Kinase from Mycobacterium tuberculosis caused for ions and substrates.

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Short Abstract: Shikimate kinase converts shikimate in shikimate-3-phospahte. In this reaction is required ADP, magnesium and chloride. This molecules influence in the structure of shikimate kinase. Magnesium influence in the position of hydroxyl groups of shikimate. The chloride influence in the positioning of ADP. The shikimate closes of LID domain.

Long Abstract:
The shikimate pathway converts phosphoenolpyruvate and erytrose 4-phosphate in chorismate. The enzymes in this pathway are targets for the development of antimicrobial agents because it is essential for bacteria and it is absent in mammals. The shikimate kinase (SK) is the fifth enzyme of this pathway and realizes the phosphorylation of the 3-hydroxyl group of shikimate using ATP as a co-substrate. Cl- plays an important role in the affinity of SK for ATP, while the Mg2+ can be involved in the nucleophilic attack on the ATP molecule, and the shikimate seems to cause conformational changes in the SK structure. In this work, we show the structural alterations cased for Cl-, Mg2+ and shikimate in the structure of SK from Mycobacterium tuberculosis (MtSK) through of the crystallography of X-ray. The MtSK was crystallized using the diffusion vapor method in two complexes different (MtSK:ADP:Mg2+, MtSK:ADP:shikimate). The data sets for two complexes were collected at a wavelength of 1.427 Å using Synchrotron Radiation Source (Station PCr, LNLS, Campinas). The data sets were processed using the program MOSFLM and scaled with program SCALA. The crystal structures for two complexes were determined by standard molecular replacement methods using the program AMoRe. For both complexes were used as search model the structure of MtSK:ADP:Mg2+:shikimate (PDB: 1WE2). The refinement of structures was performed using the maximum likelihood-based program REFMAC5.2. The XtalView/Xfit was used for visual inspection and to add the water molecules. The water molecules also were checked with basis in the B factor. The correctness of the stereochemistry of the model was checked using program PROCHECK. The compatibility of a structure with its sequence was measured using program Verify-3D. Atomic models were superposed using the program LSQKAB from CCP4i. The PARMODEL also was used in the final analysis of structure. The MtSK:ADP:shikimate was crystallized in the space group p3221, presenting one molecule in the asymmetric unit and was solved to 1.93Å. This complex was refined to a crystallographic R-factor of 20.2% and free R-factor of 27.0%. MtSK:ADP:Mg2+ was crystallized in the space group p212121 presenting four molecules in

the asymmetric unit and was solved to 2.8Å. This structure was refined to a crystallographic R-factor of 18.3% and free R-factor of 28.0%. The structure of both complexes present good geometries, with Ramachandran indicate that the structures present a minimum 98.1% of residues in the regions allowed. The compatibility of the structures with the primary sequence also presents result enough satisfactory. The monomeric structure of MtSK present here is similar the other structures of SK described in the literature. SK is a/B protein and consists of five central parallel o-strands flanked by --helices. On the other hand the structure tetrameric obtained for complex MtSK:ADP:Mg2+ present each monomer is contact with other three, creating an intricate packing arrangement. The ADP molecule seems present an important role in the stabilization of tetramer, once the ADP molecule of the one monomer is in contact with another through of ribose group. The MtSK:ADP:shikimate was compared with the MtSK:ADP:Mg2+:shikimate deposited in the PDB. Through of this comparison is possible to observe that the absence of Mg2+ seem to cause significant effect in the position of shikimate, mainly in the 3-hidroxil groups and in some residues of its active site of MtSK, mainly Asp32 and Asp34. Furthermore, the absence of Mg2+ causes an effect in the chloride and ADP molecule. Therefore, the Mg2+ seems to be related with ordinance of 3-hidroxil groups of the shikimate. On the other hand, the Cl- seems be related the ordinance of molecule of ATP/ADP toward more next of the shikimate, or still can be occupying the position of third phosphate. In one of monomers of tetramer of the MtSK:ADP:Mg2+ complex is not observed Cl- ion and in this monomer is possible to observe that the ADP molecule is enough dislocated of the active site and posses a hydrogen-binding pattern different when compared with the other monomers or with other complexes of MtSK. This result corroborates that Cl- can be involved in the affinity of ATP/ADP toward SK. The shikimate really case a large influence in the structure of MtSK. The shikimate cause a closing of LID domain and it seems influence drastically in the structure of MtSK, once that in the absence of shikimate only was possible to crystallize the protein in a new condition and a new spatial group still describe not in the literature. The informations present here can be useful to understand the mechanism of catalysis of SK and also for design of new drugs based in structure against disease caused for microorganisms. This information also until for realization of molecular dynamics relative of to understand of movements associated with ions and substrates in the structure of shikimate kinase. Acknowledgments: Fapesp 03/12472-2.