Modeling the role of positively charged moieties in hydrolysis of nucleoside triphosphates

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Nucleoside triphosphatases (NTPases) hydrolyze such nucleoside triphosphates (NTPs) as ATP or GTP. Among NTPases, the most widespread is the family of so-called P-loop NTPases; its representatives, in different organisms, comprise from 10 to 18% of all products of protein-coding genes, this family contains both ATPases and GTPases [1,2,3].

The previous experimental and theoretical analyses of P-loop GTPases [4-8] have revealed sub-families of GTPases that are functionally dependent on K^+ ions. Surprisingly, only in one known case GTPases could be activated also by Na^+ ions [7]. Comparative structural analyses of particular homologous enzymes revealed that in some NTPases that are functionally independent of monovalent cations the position of a K^+ ion can be occupied by a side chain amino group of an Arg or Lys residue [4,6,7], see Fig. 1A, 1B. Since K^+ and Na^+ ions are chemically very similar, it remains obscure, why enzyme machinery has not switched its specificity from K^+ to Na^+ ions, more abundant in nature, at least in case of marine organisms. The demand of many NTPases for K^+ ions might be a reason, why all cells invest up to 50% of their energy resources into maintaing a [K+]/[Na+] ratio > 1 in the cytoplasm [9].

The more efficient catalysis of NTP hydrolysis by K^+ ions, as compared to Na^+ ions, must have some fundamental physico-chemical reason since this phenomenon was observed even upon studies of the non-enzymatic transphosphorylation reaction [10].

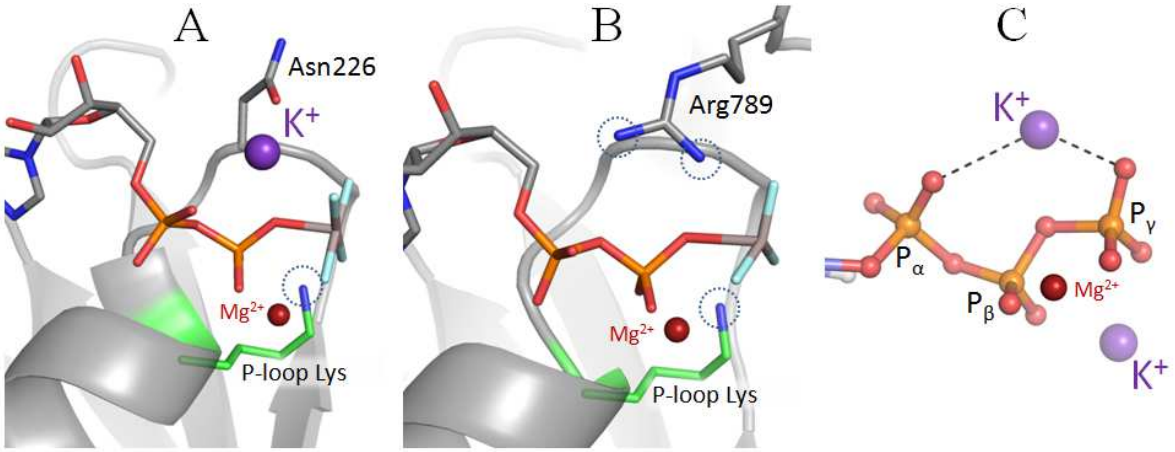

Figure 1. Location of positively charged moieties around the phosphate chains of the NTP molecules.
A. Crystal structure of a K^+-dependent GTPase MnmE [PDB:2GJ8]. B. Crystal structure of a Ras-RasGAP complex [PDB:1WQ1]; the Ras GTPase is activated upon interaction with RasGAP protein, which provides Arg residue to the active site. C. Conformation of an ATP-Mg^{2+} complex, as observed during the MD simulations.

We have addressed this problem by classical molecular dynamics (MD) simulations of an ATP-Mg^{2+} complex in a water solution. Simulations were held in the presence either of sodium or potassium ions; for comparison, MD simulations were also performed in a water solution without monovalent cations.

For simulations we used CGenFF v.2b8 - an extension of the CHARMM force field designed for drug-like molecules, which covers a wide range of chemical groups present in drug-like molecules, including a large number of heterocyclic scaffolds [11]. The resulting model of ATP-Mg^{2+} complex in solution is very accurate, but requires more computational resources to obtain a long enough trajectory to observe an ensemble of ATP-Mg^{2+} complex conformations. In our calculations we used Gromacs v.4.5.5 software with MPI implementation at the supercomputer SKIF “Chebyshev” of the Computation Center, M.V. Lomonosov Moscow State University.

The MD simulations revealed two binding sites for monovalent cations in the ATP-Mg^{2+} complex: one between the oxygen atoms of the β and γ phosphate residues (the βγ site), and another one between the oxygen atoms of the α and γ phosphate residues (the αγ site), see Fig. 1C. The binding of monovalent cations to said sites altered the conformation of the ATP phosphate chain, albeit differently for K^+ and Na^+ ions.

We compared the conformations that were obtained by MD simulations with the available crystal structures of various P-loop NTPases in complex with non-hydrolysable analogs of NTPs or transition state analogs. The conformations of the NTP-Mg^{2+} complexes in protein structures were similar to the structure of the ATP-Mg^{2+} complex as obtained by MD simulations in the presence of K^+ ions (Fig. 1). In the protein structures, the binding sites of K^+ ions could be occupied by metal ions or positively charged amino acid side chains of lysine or arginine residues (Fig. 1). In different lineages, diverse mechanisms of providing Lys or Arg residue to the active sites are used, such as di- and oligomerisation, conformational changes or interaction with other proteins. This pattern is consistent with phylogenomic analysis, as performed for P-loop GTPases, which has indicated that their primordial form was dependent on K^+ and that the ability to utilize amino acid chains as catalytic "fingers" seemed to develop later in several lineages independently [6].

Based on the data obtained, we speculate that the catalytic site of the primordial P-loop NTPase, which developed later into the most widespread catalytic site in nature, may have been shaped in K^+-rich environments. Gradually, in different lineages, K^+ ions could have been replaced by positively charged moieties of lysine and arginine residues. We consider our observation as a further evidence for the origin of the first life forms in K^+-rich environments, supposedly, at anoxic geothermal fields of the primordial Earth [6].

Acknowledgements: We appreciate very useful discussions with Drs. D.V. Dibrova, E.V. Koonin, and M.Y. Galperin. The research was supported by the Grant of the Russian Science Foundation (14-50-00029) and by the Development Program of the M.V. Lomonosov Moscow State University (the supercomputer facility).

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