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Deprotonated imidodiphosphate in AMPPNP-containing protein structures

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Many different proteins utilize the chemical energy provided by the cofactor adenosine triphosphate (ATP) for their proper function. A number of structures in the Protein Data Bank (PDB) contain adenosine 5′-(β,γ-imido)triphosphate (AMPPNP), a nonhydrolysable analog of ATP in which the bridging O atom between the two terminal phosphate groups is substituted by the imido function. Under mild conditions imides do not have acidic properties and thus the imide nitrogen should be protonated. However, an analysis of protein structures containing AMPPNP reveals that the imide group is deprotonated in certain complexes if the negative charges of the phosphate moieties in AMPPNP are in part neutralized by coordinating divalent metals or a guanidinium group of an arginine.

1. Introduction

Adenosine 5′-triphosphate (ATP) is a cofactor that supplies various enzymes and other proteins with chemical energy released by its hydrolysis to adenosine 5′-diphosphate (ADP) and phosphate. ATP has several ionizable groups and at neutral pH exists mostly as an ion carrying four negative charges. This is also the reason for its ability to chelate metal ions. In fact, in cells ATP prevalently exists in the form of an Mg^{2+} complex. Its nonhydrolysable analog adenosine-5′-(β,γ-imido)triphosphate (AMPPNP), in which the O atom bridging the two terminal phosphate groups is replaced by an imide moiety, is often used in various biochemical investigations of proteins that utilize ATP as a substrate or cofactor (Larsen *et al.*, 1969; Yount, Babcock *et al.*, 1971; Yount, Ojala *et al.*, 1971). The Protein Data Bank (PDB; Berman *et al.*, 2000) includes more than 320 structures of protein models containing AMPPNP; about two thirds of these are complexed with Mg^{2+}, whereas several contain Mn^{2+}.

In all protein structures that contain AMPPNP this molecule interacts through hydrogen bonds and ionic interactions with polar functions of the surrounding amino acids, as well as with solvent molecules. Whereas the N atoms of the adenine and the O atoms of the ribose may act as donors or acceptors of hydrogen bonds, the O atoms of the phosphates can only coordinate metals or accept hydrogen bonds from donors carrying protons (hydroxyl, amide or amino groups of a protein, or water molecules).

It is usually assumed that, as in typical imides, the bridging N atom in AMPPNP carries one H atom, and such a form is always assumed in all publications describing protein complexes with AMPPNP. However, a detailed inspection of such complexes deposited in the PDB suggests that this is not always the case.

2. Results and discussion

There are more than 320 crystal structures of proteins in the PDB that contain AMPPNP. Among them about 200 contain Mg^{2+} ions, whereas 26 contain Mn^{2+} ions. These ions are octahedrally coordinated by six O atoms: usually two or three from the AMPPNP phosphates, with the others being hydroxyl O atoms of serines and threonines and water molecules. Amide O atoms of asparagines or

Table 1
Selected protein structures from the PDB containing AMPPNP in which the imide N atom is deprotonated.

The atoms donating hydrogen bonds to the deprotonated imide and the atoms octahedrally coordinating the divalent metal ion are specified. There is no metal ion in the 1ank structure; its place is taken by the amine group of a lysine. O^{α}, O^{β} and O^{γ} are the O atoms of the consecutive phosphate groups of AMPPNP.

Protein	PDB code	Resolution (Å)	N_{imide} hydrogen bonds	Metal coordination	Reference
Thymidylate kinase	1nn5	1.50	Arg N	Mg: O^{β}, O^{γ}, Ser OG, 3 wat	Ostermann *et al.* (2003)
Kinesin KIF1A	1vfv	1.85	Gly N	Mg: O^{β}, O^{γ}, Ser OG, 3 wat	Nitta *et al.* (2004)
Thymidine kinase 1	2qq0	1.50	Tyr N	Mg: O^{β}, O^{γ}, Thr OG1, 3 wat	Segura-Peña *et al.* (2007)
tRNA-dependent kinase	3a4l	1.80	Gly N, Arg NH1	Mg: O^{β}, O^{γ}, Ser OG, 3 wat	Araiso *et al.* (2009)
RadA recombinase	3ewa	2.00	Gly N	Mg: O^{β}, O^{γ}, Thr OG1, 3 wat	Li *et al.* (2009)
Myosin domain	3myk	1.84	Gly N, Asn ND2	Mg: O^{β}, O^{γ}, Ser OG, Thr OG1, 2 wat	Frye *et al.* (2010)
Mre11–Rad50	3qkt	1.90	Gly N	Mg: O^{β}, O^{γ}, Ser OG, Gln OE1, 2 wat	Williams *et al.* (2011)
Topoisomerase VI-B	1mx0	2.30	Gly N, Met N	Mg: O^{α}, O^{β}, O^{γ}, Asn OD1, 2 wat	Corbett & Berger (2003)
NS3 helicase	2jlr	2.00	Gly N	Mn: O^{β}, O^{γ}, Thr OG1, Gln OE2, 2 wat	Luo *et al.* (2008)
Adenylate kinase	1ank	2.00	Gly N, Arg NH2	Lys NZ (no metal)	Berry *et al.* (1994)
EphA3 kinase	3fxx	1.70	Ala O	Mg: O^{α}, O^{β}, O^{γ}, 3 wat	Not published

glutamines sometimes also take part in metal coordination. In a great majority of these structures it is not possible to deduce whether the imide moiety is protonated or not, since it either does not make any contacts within a suitable distance limit or is involved in hydrogen-bonding contact(s) with water molecules or hydroxyl groups, which can serve equally well as donors or acceptors of a hydrogen bond. However, in many instances the chemical groups that are located in hydrogen-bonding contacts with the AMPPNP imide are capable of donating a proton but are not able to accept one; some selected representative examples are presented in Table 1 and Fig. 1. Such groups include peptide amides (atoms designated by 'N' in the PDB format) and the side-chain amides of asparagines (ND2) and glutamines (NE2), as well as the positively charged side-chain groups of lysines (amines) and arginines (guanidines). It can be safely assumed

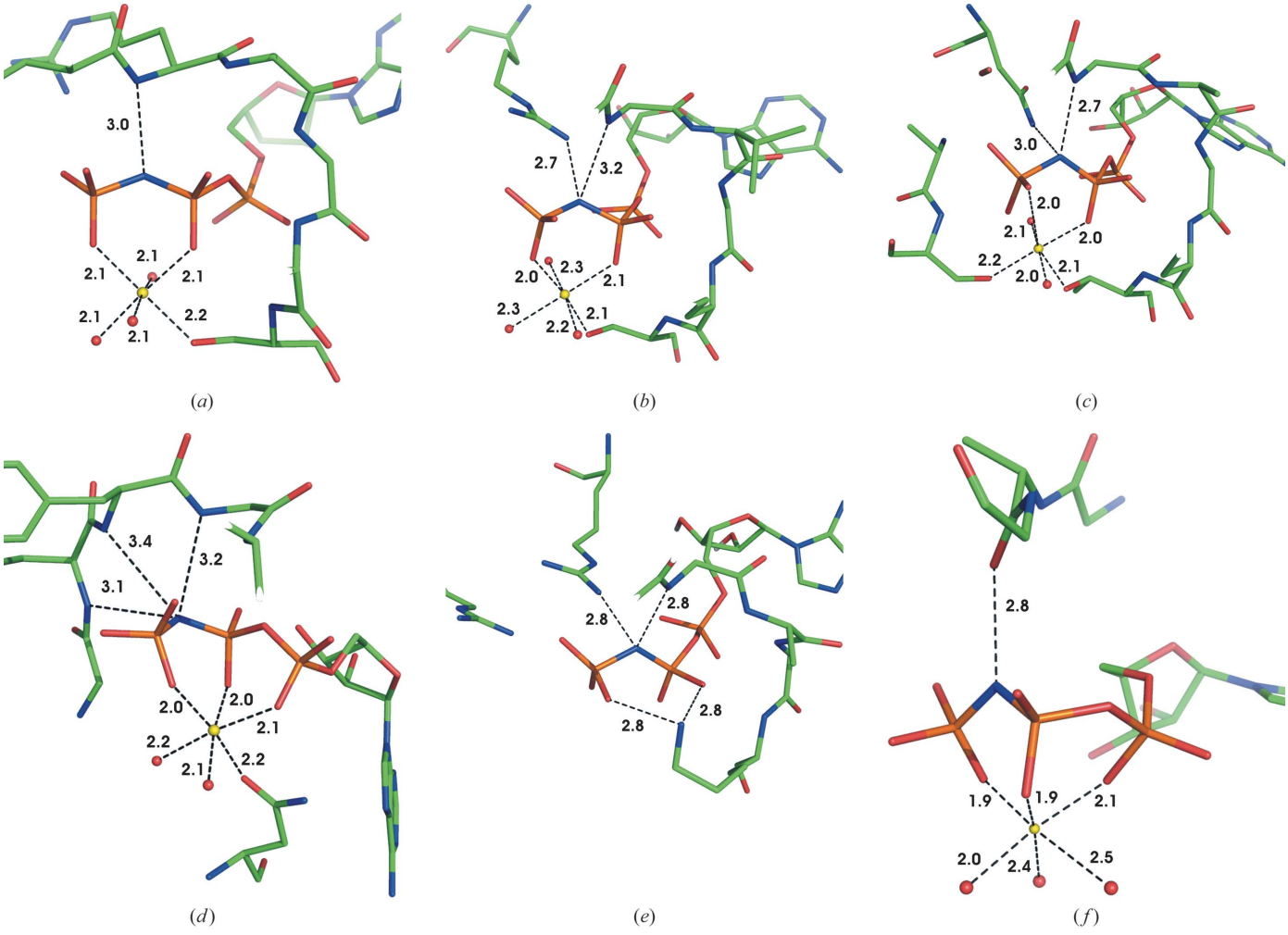

Figure 1
The molecule of AMPPNP and its environment in the following proteins: (*a*) thymidylate kinase (PDB entry 1nn5), (*b*) tRNA-dependent kinase (3a4l), (*c*) myosin domain (3myk), (*d*) topoisomerase (1mx0), (*e*) adenylate kinase (1ank), (*f*) EphA kinase (3fxx). The H atoms are not present in the PDB files and are not shown here, but their locations can be inferred from the type of atoms engaged in hydrogen bonds. The figures were created with *PyMOL* (DeLano, 2002).

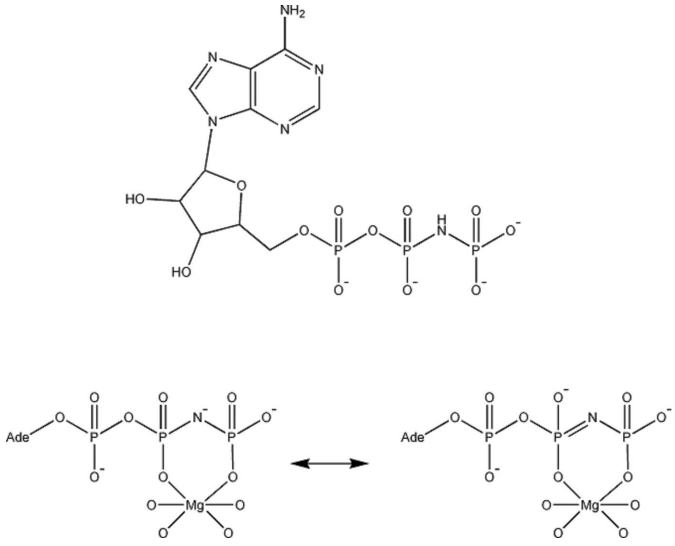

Figure 2
The anion of AMPPNP as it exists in aqueous solution and its complex formed with Mg^{2+} after deprotonation of the β,γ-bridging imide.

that in such cases the AMPPNP imide is deprotonated and acts as an acceptor in the observed hydrogen bonds.

The structures with AMPPNP deprotonated at the imide N atom are more typical of the situation in natural ATP-containing proteins since the β,γ-bridging O atom in ATP does not carry a proton and can only serve as a hydrogen-bond acceptor. In some structures, however, the AMPPNP imide is protonated since its hydrogen-bond counterpart can accept a proton but not donate one. For example, in the structure of ephrin receptor 3 (PDB entry 3fxx; Structural Genomics Consortium, unpublished work), illustrated in Fig. 1(*f*), the imide N atom of AMPPNP donates its proton to the peptide carbonyl O atom in the neighboring protein strand. This observation suggests that the AMPPNP molecule does not represent the same situation as in the natural complex with ATP.

Salts of both ATP and AMPPNP dissociate in aqueous solution forming ions with four negative charges, as illustrated in Fig. 2 (AMPPNP is commercially available as a tetralithium salt). However, coordination of Mg^{2+} neutralizes two of these charges and, in a suitable protein environment, the imide bridging the two terminal phosphates can lose its proton, leaving the AMPPNP–Mg complex with formally three negative charges. The negative charge at the bridging N atom is then stabilized by conjugation with the neighboring phosphates, as shown in Fig. 2. The effect of charge neutralization on the deprotonation of the imide seems to be supported by the fact that in obvious cases of deprotonation (Table 1) the oxygen metal ligands other than phosphate are neutral hydroxyl or water O atoms, but not negatively charged carboxyl groups. In the structure 1ank the negative charge of AMPPNP is partially compensated not by a metal ion but by a lysine.

In contrast to the methylene analog of ATP (AMPPCP), which does not show this effect, AMPPNP mimics ATP in relaxing muscle (Larsen *et al.*, 1969). It can be speculated that this property might be connected to the inability of AMPPCP to deprotonate and therefore the inability of the methylene group to accept hydrogen.

AMPPNP can therefore substitute for ATP in protein complexes, but the chemical properties of these two molecules are not identical. The imido group of AMPPNP seems to be able to deprotonate under certain conditions, but nevertheless prevents enzymatic hydrolysis of the terminal phosphate.

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