Adenosine Phosphonoacetic Acid is Slowly Metabolized by NDP Kinase

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Abstract: NDP kinase catalyzes the last step in the phosphorylation of nucleotides. It is also involved in the activation by cellular kinases of nucleoside analogs used in antiviral therapies. Adenosine phosphonoacetic acid, a close analog of ADP already proposed as an inhibitor of ribonucleotide reductase, was found to be a poor substrate for human NDP kinase, as well as a weak inhibitor with an equilibrium dissociation constant of 0.6 mM to be compared to 0.025 mM for ADP. The X-ray structure of a complex of adenosine phosphonoacetic acid and the NDP kinase from *Dictyostelium* was determined to 2.0 Å resolution showing that the analog adopts a binding mode similar to ADP, but that no magnesium ion is present at the active site. As ACP may also interfere with other cellular kinases, its potential as a drug targeting NDP kinase or ribonucleotide reductase is likely to be limited due to strong side effects. The design of new molecules with a narrower specificity and a stronger affinity will benefit from the detailed knowledge of the complex ACP-NDP kinase.

Key Words: Antiviral, nucleoside analog, adenosine phosphonoacetic acid, synthesis, NDP kinase inhibitor, nm23, phosphorylation.

INTRODUCTION

Nucleoside diphosphate (NDP) kinase (EC 2.7.4.6), found in all organisms and cells, catalyzes the phosphorylation of nucleoside diphosphates into triphosphates using ATP as the major phosphate donor. The enzyme participates in equilibrating the nucleotide pools for macromolecular synthesis. It participates both in the nucleoside salvage pathway and in nucleotide *de novo* synthesis, and it is involved in the cellular activation of antiviral nucleoside analogs [1], [2]. NDP kinase is transiently phosphorylated on an active site histidine as a part of the catalytic process [3].

The three dimensional structure of NDP kinases from several species has been determined at high resolution [4-8], showing the conservation of both the subunit fold and the active site. Eukaryotic NDP kinases are homohexamers made up of 17 kDa subunits with highly conserved sequences [9]. X-ray structures of complexes with ADP [8], [10], TDP [11] or GDP [12] demonstrated that the binding site is the same for all these nucleotides, and different from sites found in other nucleotide binding proteins. The di- or triphosphate moiety of NDP kinase-bound nucleotides adopts an unusual folded conformation, allowing an intramolecular hydrogen bond between the 3'OH of the ribose and the O_7 β-phosphate oxygen. This bond plays a major role in catalysis. As a consequence, diphosphorylated derivatives of nucleoside analogs used in antiviral therapy in which the 3'OH is absent or substituted, such as dideoxynucleotides diphosphate, are very poor substrates of NDP kinase [2], [13-15].

Few inhibitors have been described for NDP kinase. Cyclic AMP inhibits the enzyme [16], [17] but its affinity is rather poor ($K_I \approx 0.5$ mM). The best inhibitors so far are 3'-phosphorylated nucleotides such as 5'-phosphosulphate adenosine-3'-phosphate (PAPS), with a K_D of 10 μM [18]. In addition to mononucleotides, single-stranded oligonucleotides inhibit NDP kinase B [19], one of two major isoforms of the human enzyme. Human NDP kinases A and B have been implicated in a number of cellular functions apparently unrelated to nucleotide phosphorylation: an antimetastatic potential in tumoral cells, a role as a repair DNAse in apoptosis [20-22]. These functions make NDP kinase a potential therapeutic target, and the discovery of new inhibitors may represent a first step towards the design of drugs targeted to the enzyme. Here, adenosine phosphonoacetic acid (5'-*O*-(phosphono-acetyl) adenosine or ACP, Fig. (**1**), an ADP analog is presented, which was previously investigated by some of us as a potential inhibitor of ribonucleotide reductase [23]. ACP is shown to be both a weak inhibitor of NDP kinase, and a substrate that is converted to the triphosphate analog (ACP-P) by the enzyme. A 2 Å X-ray structure of ACP in complex with the NDP kinase from *Dictyostelium discoideum* is also presented which displays 58-60% sequence identity with human NDP kinases A and B [24]. As all the active site residues are conserved, *Dictyostelium* NDP kinase is a reliable model of

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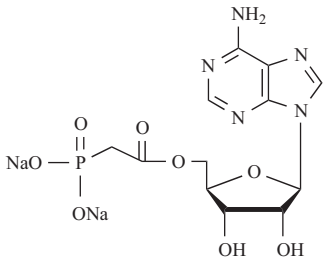

Fig. (1). Chemical formula of adenosine phosphonoacetic acid (ACP) as a sodium salt.

the human enzymes, and the knowledge of the interactions that ACP makes with that protein will be a great value for the design of new inhibitors and to define the pharmacophore.

EXPERIMENTAL

Preparation of 5'-O-(Phosphono-Acetyl)Adenosine (ACP)

ACP was prepared adapting the procedure previously described [23]. Briefly, adenosine was protected at the 2' and 3' positions as isopropylidene derivative. The protected adenosine was reacted with chloro acetyl chloride in pyridine at 0°C to give the corresponding ester at 5'-position in 23% yield after chromatographic purification. It was next converted into the corresponding iodine ester derivative by treatment with sodium iodide in acetonitrile (45% yield). The resulting 2',3'-*O*-isopropyliden-5'-*O*(-iodo-acetyl)-adenosine was then reacted with triethyl phosphite to give 2',3'-*O*-isopropyliden-5'-*O*-[(diethyl-phosphono)acetyl]-adenosine in 67% yield. This latter was finally deprotected by treatment with trimethylsilyl bromide (TMSBr), followed by Dowex Na^+ form (50x 2-100), to give ACP (27% yield). Analytical data were in agreement with those previously reported by us [23].

NDP Kinase Purification and Preparation of the Phosphorylated Enzyme

Wild type *Dictyostelium* NDP kinase and the H122G-F64W mutant were overexpressed in *E. coli* (XL1-Blue) as described [2]. The expression and purification of human NDP kinase were performed according to [13] for the A isoform and [19] for the B isoform. The phosphorylated enzyme was prepared as described [25]. The enzyme was first preincubated in buffer T (50 mM Tris-HCl pH 7.5 containing 5 mM $MgCl_2$ and 75 mM KCl) with a saturating amount of [γ^{32}P]-ATP (Amersham Biosciences). It was then freed of nucleotides by gel filtration on Sephadex G-25 in buffer T. The concentration of the phosphorylated enzyme and the absence of nucleotides in the preparation were checked from the absorbance spectrum of the protein. The phosphorylated enzyme was kept on ice and used within three hours.

Fluorescence Binding Studies

The affinity of ACP was measured by monitoring the variation of the intrinsic fluorescence of the H122G-F64W mutant of *Dictyostelium* NDP kinase upon nucleotide binding as described in [13]. All fluorescence measurements were performed at 20°C in buffer T on a PTI spectrofluorometer Quantamaster™. Titrations were achieved by successive additions of aliquots of a concentrated ligand solution to a 1 mL enzyme solution (1 µM) under continuous stirring, and the fluorescence was measured at 340 nm with excitation at 295 nm (2 nm excitation slit and 4 nm emission slit). After correction for dilution, data were fitted to a hyperbolic ligand-protein curve. The inner filter effect was negligible. Kaleidagraph (Abelbeck software) was used for all fittings.

NDP Kinase Activity Assays

NDP kinase activity was measured in two different ways. A spectroscopic assay using coupled enzymes contained 50 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 75 mM KCl, 1 mM phospho-enolpyruvate, 0.2 mM NADH, rabbit muscle pyruvate kinase (3U/mL, Roche), hog muscle lactate dehydrogenase (5 U/mL, Roche), bovine serum albumin 4 mg/mL, ATP 0.2 mM, dTDP 0.1 mM and NDP kinase, either human A or from *Dictyostelium* in amounts giving an initial rate less than 0.08 ΔA^{340} /min at 20°C. The spectroscopic assay monitored at 340 nm, the oxidation of NADP coupled ADP produced in the reaction catalyzed by NDP kinase. It was verified that ACP did not interfere with pyruvate kinase in the conditions of the assay. The reaction performed in 96-wells microplates in 250 µL (final volume) was monitored for 10 min at 340 nm with a microplate reader (BIO-TEK Elx808, Winooski, Vermont, USA). Data were fitted to the hyperbolic equation using Kaleidagraph software. In the radioactive assay, NDP kinase was labeled on the active site histidine by [γ^{32}P]-ATP 5 mM (400 Ci/mmol, Amersham Biosciences), followed by gel filtration to make the enzyme free of ATP, as described [25]. The phosphorylated enzyme (10 - 40 µM) was then incubated with ADP or ACP (0.5 – 3 mM) in buffer T for 1 - 20 min at 20°C. The reaction was stopped at –80°C during 1 min. After separation of the radioactive nucleotides by TLC on PEI cellulose (Macherey-Nagel, Germany), the radioactivity was quantified using a PhosphorImager (Molecular Dynamics).

Crystallization, X-Ray Diffraction Data Collection and Refinement

Dictyostelium NDP kinase was incubated with 10 mM ACP in 50 mM Tris-HCl buffer pH 7.5 containing 20 mM $MgCl_2$. Crystals were grown by the hanging drop vapor diffusion technique. Equal volumes of protein and well solution were equilibrated against 100 mM HEPES, pH 7.5 and 25% PEG 1000. The crystals appeared in one week. They belonged to the monoclinic space group $P2_1$ with unit cell dimensions a = 69.38 Å, b = 104.59 Å, c = 69.57 Å, α = 118.28°. The asymmetric unit contained a hexamer.

Crystals were cryoprotected with 30% ethylene glycol. X-ray diffraction data from a single crystal were collected at 1.0 Å on the BM14-UK CRG beamline of European Synchrotron Radiation Facility (Grenoble, France) with a MarResearch CCD detector. Data at 2.0 Å resolution were evaluated with DENZO and SCALEPACK programs [26] and further processed using the CCP4 program suite (CCP4,

1994). Overall statistics are given in Table **1**. The phases were calculated using the model of *Dictyostelium* NDP kinase, which yields isomorphous crystals in complex with 3'- fluoro- 2', 3'- dideoxy UDP [14]. The structure was refined with CNS (Brunger 1998) to an R-factor of 18.8% at 2.0 Å resolution (Table **1**). A 2Fo-Fc electron density map displayed with TURBO-FRODO [27] showed clear density for ACP in subunit A, but not in the other five subunits of the hexamer. The refinement library parameters for the phosphonate moiety of ACP were taken from those of the haptene ligand in [28]. Refinement confirmed that the binding site is fully occupied in subunit A. The occupancy was only 0.2 to 0.4 in the other five subunits of the crystal asymmetric unit, which were left empty in the final model. Water molecules were gradually inserted during conjugate gradient refinement. As in other structures of *Dictyostelium* NDP kinase, residues 2 to 5 are disordered and missing in the final model.

Table1. Statistics on Crystallographic Analysis

Parameter	Values
Diffraction data	
Space group	$P2_1$
Asymmetric unit	hexamer
Cell parameters a,b, c (Å), β(°)	69.4, 104.6, 69.6, 118.3
Resolution (Å)	2.0
Measured intensities	836,719
Unique reflections	58,517
I/σ	10.8
Completeness (%)	99.2
Rsym (%)[a]	8.0
Refinement	
Rcryst (%)[b]	18.7
Rfree (%)[c]	23.9
Reflections	58517
Protein atoms	6909
Solvent atoms	1147
Ligand atoms	30
Average B ($Å^2$)	29.2
Geometry	
Bond length (Å)	0.005
Bond angle (°)	1.26

[a] Rsym = $\sum| I_i - <I>| / \sum I_i$, where I_i is the intensity of a reflexion, and <I> is the average intensity of that reflexion.

[b] Rcryst = $\sum||Fobs| - |Fcalc|| / \sum |Fobs|$.

[c] 5% of the data were set aside for free R-factor calculation.

Atomic coordinates have been deposited at the Protein Data Bank under entry code 1S5Z.

RESULTS

Kinetic Studies of NDP Kinase in the Presence of ACP

The inhibition of NDP kinase by ACP was measured after a short incubation of the enzyme (2.4 nM) with various amounts of nucleotide analog. It was verified that ACP did not interfere with ADP in the pyruvate kinase reaction step of the coupled enzyme system. The data in Fig. (**2**) show that ACP is an inhibitor at millimolar concentrations, and that it is able to totally inhibit the enzyme with an apparent K_I of 1.3 mM in these conditions.

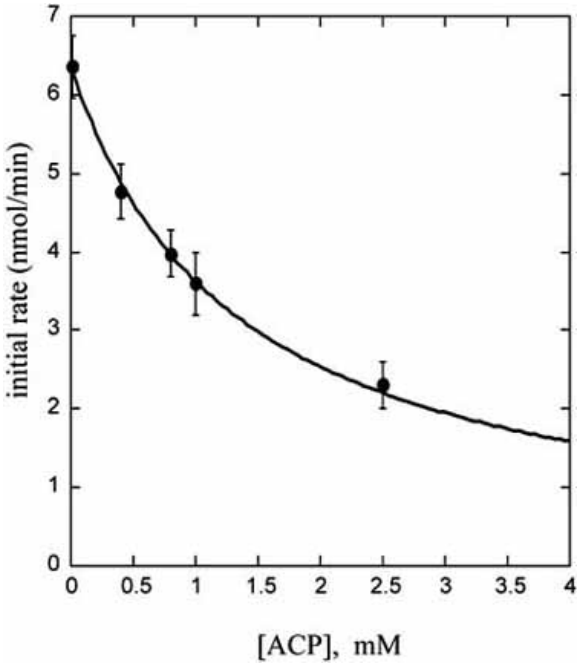

Fig. (2). Inhibition of NDP kinase activity by ACP.
The initial rate of human NDP kinase A (1.26 ng/test) activity was measured using the coupled enzyme assay at 0.1 mM dTDP and 0.2 mM ATP in the presence of the indicated ACP concentrations. The best fit of the data to an hyperbolic function is shown. It is compatible with total inhibition at infinite ACP concentration and yields an apparent K_I of 1.3 (+/- 02) mM.

The possibility that ACP is substrate for the enzyme, *i.e.* that it can be phosphorylated by NDP kinase was then explored. Fig. (**3**) shows the direct transfer to ACP of the [^{32}P] radioactivity carried by NDP kinase phosphorylated on its catalytic histidine. Phosphorylated NDP kinase did not migrate in the TLC experiment (lane 1). Whereas incubation with AMP instead of ACP had no effect (lane 2), ^{32}P was efficiently transferred upon incubation with ADP, yielding radioactive ATP (lane 3). Incubation with ACP was also found to lead to dephosphorylation of the enzyme and produce a new radioactive product, presumably 5'-O-(pyrophosphono-acetyl)adenosine (ACP-^{32}P), which migrates slightly above ATP (lanes 4 and 5). It is concluded that, like the β-phosphate of ADP, the phosphonate group of ACP can accept a phosphate from phosphorylated NDP kinase, and

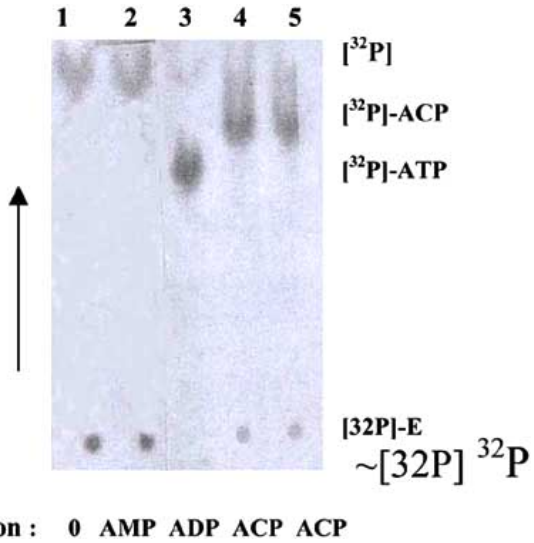

Fig. (3). Adenosine phosphonoacetic as a substrate for NDP kinase.

^{32}P-phosphorylated NDP kinase (14 μM subunit concentration) was incubated for 5 min, either alone (lane 1) or with the following ligand : 0.5 mM AMP (lane 2), 0.5 mM ADP (lane 3), 3 mM ACP (lanes 4 & 5). The mixtures were separated by TLC, and the plates were analyzed with a PhosphorImager. Labeled spots at the top of the picture show the inorganic phosphate produced by background hydrolysis of the phosphorylated enzyme under the conditions of the experiment. This experiment used NDP kinase from *Dictyostelium*, but the same results were obtained with human NDP kinase A.

therefore, the ACP is a substrate for NDP kinase. In the presence of an excess of ACP, the transfer reaction was completed in less than one minute. In the absence of Mg^{++}, the phospho-enzyme did not transfer the phosphate to ACP (result not shown).

Binding Studies of ACP to NDP Kinase

The binding of ACP to NDP kinase was monitored by fluorometric titration as previously reported for several nucleotides and analogs, using a mutant designed for that purpose [13]. The F64W-H122G NDP mutant of *Dictyostelium* NDP kinase lacks the active site His122, which prevents phosphate transfer, and it has a tryptophan in place of Phe64, yielding a fluorescence signal when it interacts with a nucleotide ligand. Titrations were performed by following the protein fluorescence intensity upon ligand addition. Like ADP binding [2], [13], the binding of ACP causes a quenching of the fluorescence intensity observed at 340 nm as shown on the fluorescence emission spectra (Fig 4, Inset). Fig. (**4**) is a titration curve for ACP binding to NDP kinase. Although saturation could not be reached, the fit of the curves indicates a 10% quenching at saturation and an equilibrium dissociation constant K_D of 0.6 mM. This value is similar to those reported for inhibitors like cyclic AMP and cyclic GMP [16], [17]. In comparison, the K_D values for ADP and ATP are 25 μM and 1 μM, respectively [13].

Structure of the ACP- NDP Kinase Complex

The X-ray structure of the ACP complex with *Dictyostelium* NDP kinase was determined to 2.0 Å resolution and refined to a R-factor of 18.7% (Rfree=23.9%, Table **1**) with good stereochemistry. The crystals are isomorphous with those of a previously analyzed complex with the UDP analog 3'- fluoro- 2', 3'- dideoxy UDP [14]. In both cases, the electron density shows that only one subunit (subunit A) of the hexamer binds the ligand at full occupancy. In the monoclinic crystal, the six subunits have slightly different environments due to contacts with neighboring protein molecules, and this may modulate their affinity for the ligand, leading to a low occupancy of some binding sites in spite of the high ligand concentration. Nucleotide binding to NDP kinase is known to induce changes in the polypeptide chain conformation [29]. In the ACP complex, chains B to F take the conformation seen in free NDP kinase (entry 1NPK, [30]; the RMS distance is 0.37 Å for all Cα atoms). In contrast, subunit A is closer to the complex with TDP (1NDP, [11]; RMS distance 0.6 Å). Although the active site geometry is essentially the same in all subunits, the description below of the bound ACP molecule is based on the high-occupancy subunit A.

Fig. (**5**) shows a general view of the active site of NDP kinase in the ACP complex and a comparison of the binding modes of ADP and ACP. The adenosine moiety of the analog takes the same conformation as in ADP bound to *Dictyostelium* NDP kinase [10] or to human NDPK-A [8]. The base is stacked between the Phe64 and Val116 side chains in *anti* orientation relative to the sugar. The ribose has a *C3'-endo* ring pucker and its 3'OH H-bonds to the side chains of Lys16 and Asn119. Lys16 also interacts with the 2'OH. The phosphonate group of ACP exactly replaces the β-phosphate of ADP, its phosphorus atom superimposing on Pβ within 0.4 Å. Like the β-phosphate, the phosphonate interacts with the Arg109 and Thr98 side chains. A water molecule bridges the phosphonate to the imidazole group of the active site His122, donating H-bonds to both groups. The water is at the expected position of a γ-phosphate being transferred between the histidine and a nucleotide substrate. Thus, the geometry of the active site and the mode of ACP binding observed in the crystal are compatible with phosphorylation of the phosphonate by the phospho-histidine intermediate.

The acetyl moiety of ACP replaces the α-phosphate of a natural nucleotide. Its carbonyl carbon is at 1.1 Å of the Pα position in bound ADP, and the carbonyl oxygen points toward the solvent making no interaction with protein groups. The phosphonate methylene is in almost eclipsed position relative to the ribose C5'. This conformation, which may involve some strain, allows the acetyl moiety to reproduce the folded geometry of the phosphate groups in ADP [8].

DISCUSSION

In view of the close similarities between ADP and adenosine phosphonoacetic acid (ACP), this latter compound was considered as a possible ligand for NDP kinase. The investigation was conducted by kinetic and X-ray studies.

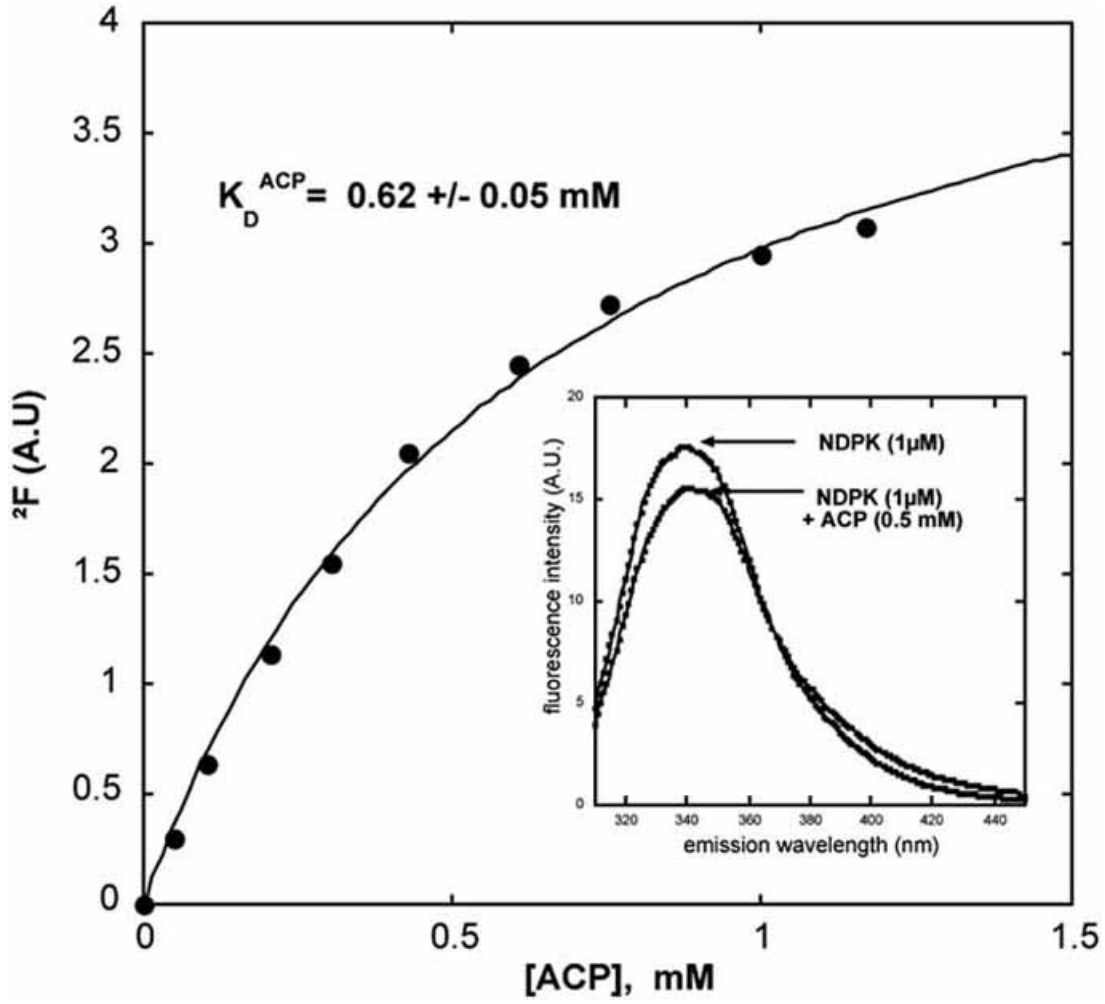

Fig. (4). Fluorescence titration of NDP kinase with ACP.

A quenching of the intrinsic fluorescence of the F64W-H122G mutant of *Dictyostelium* NDP kinase (1 μM subunit concentration) is observed upon binding of ACP at 20°C in buffer T, pH=7.5. The solid line represents the best fit of the data to an hyperbolic saturation curve with a K_D of 0.62 (+/- 0.05) mM (Table **2**). **Inset:** Fluorescence emission spectra of the mutant protein in buffer T and in the presence of 0.5 mM ACP, monitored upon excitation at 295 nm (excitation slit 2 mm, emission slit 4 nm).

The fluorescence quenching experiment showed that adenosine phosphonoacetic acid binds to the H122G-F64W mutant of *Dictyostelium* NDP kinase with a K_D of 0.6 mM, indicating a low affinity relative to ADP (K_D = 25 μM). When inhibition of the catalytic activity was tested on human NDP kinase A, the apparent inhibition constant (K_I) of ACP was 1.3 mM. This makes ACP a better inhibitor than AMP (K_I > 1 mM, result not shown), and comparable to cyclic AMP (K_I= 0.5 mM) [17], but significantly less efficient than PAPS, which has a K_I of 10 μM [18]. The relative affinities of ACP and ADP for NDP kinase point out a contribution of the α-phosphate to the interaction with the protein, the K_D ratio of 24 being equivalent to about 2 kcal/mol of free energy. The contribution of the γ-phosphate derived from the K_D ratio for ATP *vs*. ADP is similar.

The X-ray structure of the complex with *Dictyostelium* NDP kinase shows that ACP binds in the same way as ADP, makes the same interactions with active site groups, and altogether, it is a close analog of the natural substrates of the enzyme. There is however one major difference: no divalent ion is seen to interact with the analog. In the 2.0 Å resolution electron density map, the 10 electrons of Mg^{++} should be obvious. The absence of recognizable density indicates that even in subunit A, the metal ion has a low occupancy. All natural nucleotides bind to NDP kinase as a complex with a divalent ion (Mg^{++} in most experiments). When present, Mg^{++} ligates oxygens from the two phosphates of a bound nucleoside diphosphate, and of all three phosphates of a nucleoside triphosphate [8,29]. As the crystallization mixture contained 10 mM Mg^{++}, the affinity of ACP for the divalent ion in the crystalline complex must be very poor. The acetyl group is a weaker Mg^{++} ligand than the α-phosphate of a natural nucleotide, and although it may form a binding site for a divalent ion in combination with the phosphonate, this site is not appreciably occupied at 10 mM Mg^{++}. In return,

Table 2. Nucleotide Contacts [a]

Nucleotide atom	Protein atom	ACP	ADP[b]
Base			
Purine ring	Phe64 ring Val116 CG1	3.5 3.1	3.6 3.5
Ribose			
O2'	Lys16 NZ Asn119 OD1	3.2 3.4	2.6 3.3
O3'	Lys16 NZ Asn119 ND2	3.0 2.6	2.7 3.1
Phosphonate [c]			
O1B	Arg92 NH1 Thr98 NH2	- 3.1	2.9 -
O2B	Thr98 OG1 Arg109 NH1 Arg109 NH2	3.2 - 2.9	3.1 2.7 2.9
O3B	Ribose O3'	2.7	2.6

[a] polar bond and shortest non-polar contacts between NDP kinase and the ligand; distances are in Å units.

[b] ADP in complex with subunit B of *Dictyostelium* NDP kinase (Morera *et al*.).

[c] β-phosphate in ADP.

the poor Mg^{++} binding must affect the affinity of the enzyme for ACP. The K_D values cited above for natural nucleotides were measured in the presence of 5 mM Mg^{++}. As most other interactions are the same for ACP and ADP, the lack of Mg^{++} binding may be the prime cause for the high K_I and K_D of the analog.

It is shown here that ACP can be phosphorylated by NDP kinase. The transfer of a phosphate group from the phosphorylated enzyme to the phosphonate of ACP is demonstrated by the radioactive assay, but the turnover rate of the overall reaction with ATP as the phosphate donor is too low for the coupled enzyme assay. Thus, ACP is a substrate, but a very poor one. The X-ray structure of the complex indicates that the geometry of the active site and the conformation of the bound ACP are compatible with the transfer of a phosphate group from the active site histidine to the phosphonate. Remarkably, the phosphonate occupies the exact position of a β-phosphate, and it receives a H-bond from the 3'OH of the ribose. All other groups known to be involved in catalysis are present, except Mg^{++}. Nevertheless, the ACP phosphorylation reaction is Mg^{++} dependent, which suggests that a weak complex is formed between the divalent cation and ACP at the active site of the enzyme, with an occupancy too small to appear in the X-ray structure.

In conclusion, ACP was originally designed as a substrate analog and an inhibitor for ribonucleotide reductase, another major enzyme of macromolecular biosynthesis, and it may compete with ADP at the active site of other enzymes. The present study shows that, at least for NDP kinase, ACP is indeed a close analog of ADP, binding to NDP kinase active site and acting as a substrate, albeit a poor one. Moreover, although ACP showed a moderate inhibitory activity (IC_{50} = 0.5 mM) with ribonucleotide reductase, it was found completely inactive on cultured cells [23]. In the light of the present results this occurrence may be explained by processing of ACP by NDP kinase or other enzymes as well as by insufficient uptake by cells, as previously mentioned [23]. Its potential as a drug targeting NDP kinase in antiviral or anticancer therapy is therefore likely to be limited by side effects, which will require novel molecules with a narrower specificity. Their design will undoubtedly benefit from the detailed knowledge of the mode of ACP binding described in this study. The crystallographic and kinetic studies here described, confirmed the general hypothesis at the base of the design of ACP: the substitution of the bio-isosteric phosphonoacetyl moiety for the diphosphate group results in a compound that behaves as a ligand and a substrate of NDP kinase. The phosphonate, which occupies the exact position of a β-phosphate, is also more stable and thus more suitable for possible drug development. These findings can be usefully employed, together with X-ray structures of complexes with ADP, TDP or GDP, in the design by *in silico* techniques of new molecules making strong interactions with the active site, thereby resulting in potent ligands/inhibitors.

ATOMIC COORDINATES

Atomic coordinates have been deposited at the Protein data under entry code 1S5Z.

FINANCIAL SUPPORT

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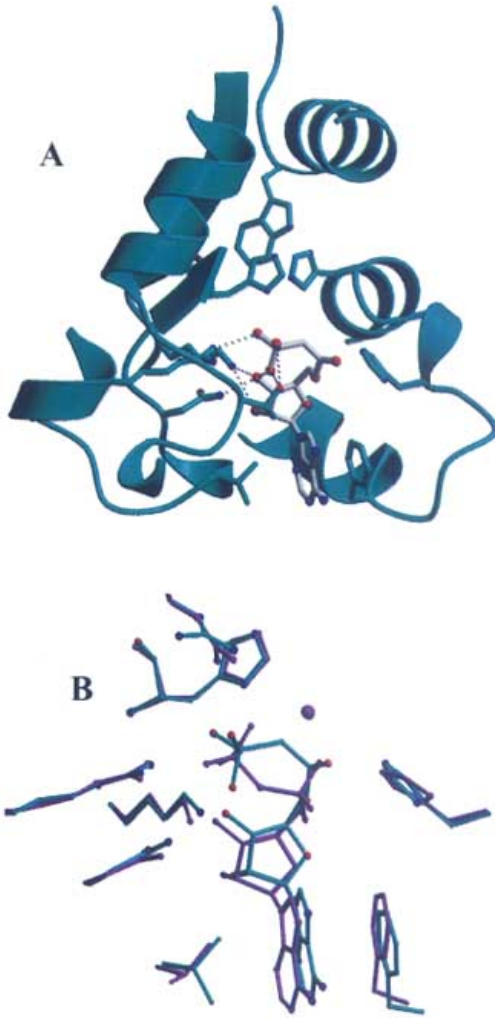

Fig. (5). Structure of the NDP kinase binding site and the mode of ACP binding.

(1) Part of subunit A in the 2.0 Å X-ray structure of the complex is represented as a ribbon diagram with the bound ACP molecule in grey bonds and interacting side chains in blue bonds. H-bonds are dashed. His122 is the active site histidine which is phosphorylated during the NDP kinase reaction. The adenine moiety of ACP is stacked on Phe64 and in Van der Waals contact with Val116. The ribose OH groups H-bond to Thr98, Asn119 and Lys16, the phosphonate group to Arg109 and the 3'OH.

(2) Superposition of ACP (thick blue bonds) in subunit A of the present structure, and ADP (thin purple bonds) in subunit B of *Dictyostelium* NDP kinase (PDB entry 1NDC; [15]). OA is the acetyl carbonyl oxygen in ACP. A water molecule (red dot) bridges the phosphonate (ACP) or β-phosphate (ADP) to the active site His122. Interacting side chains occupy the similar positions in the two structures, but Mg^{2+} (purple dot) is present only in the complex with ADP.

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ABBREVIATIONS

ACP = Adenosine phosphonoacetic acid

NDP = Nucleoside diphosphate

NTP = Nucleoside triphosphate

TLC = Thin layer chromatography

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