X-ray Structure of Nucleoside Diphosphate Kinase Complexed with Thymidine Diphosphate and Mg^{2+} at 2-Å Resolution[†,‡]

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ABSTRACT: We report the crystal structure of nucleoside diphosphate kinase (NDP kinase) from *Dictyostelium discoideum* with thymidine diphosphate (dTDP) and Mg^{2+} bound at the active site. The structure has been refined to an *R*-factor of 18.3% at 2-Å resolution. The base stacks on the aromatic ring of Phe 64 near the protein surface and is wedged between the side chains of Phe 64 and Val 116. The sugar and the pyrophosphate are deeper inside the protein and make numerous H-bonds with protein side chains. There is no backbone interaction with the nucleotide. A Mg^{2+} ion bridges the α- and β-phosphates and interacts with the protein via water molecules. NDP kinase shows little specificity toward ribonucleotides and deoxyribonucleotides. This property, required by the enzyme biological function, can now be analyzed by comparing the crystal structures of free, ADP-ligated, and dTDP-ligated enzymes. The most significant differences are located in residues 60–64, which adapt their conformation to allow Phe 64 to stack on both types of bases. Nonspecific binding is achieved by the absence of polar interaction between the base and protein atoms. The ribose of ADP and the deoxyribose of dTDP occupy similar positions, their hydroxyl groups interacting with Lys 16 and Asn 119. The H-bond between Lys 16 and the $O_{2'}$ hydroxyl of ADP is replaced by a similar interaction with a water molecule in the dTDP complex. The β-phosphate position is the same for ADP and dTDP, suggesting that the mechanism of phosphate transfer is the same for all substrates of NDP kinase. The NDP kinase binding site is compared with other nucleotide binding proteins with low specificity toward the base and sugar. We discuss DNA binding to NDP kinase and the design of nucleotide analogues which might be better substrates of NDP kinase than those currently used as antiviral agents.

Nucleoside diphosphate kinase (NDP kinase) catalyzes the exchange of a γ-phosphate between nucleoside di- and triphosphates:

$$XDP + YTP \leftrightarrow XTP + YDP$$

The enzyme shows little specificity toward the X and Y bases and accepts both ribonucleotides and deoxyribonucleotides as substrates (Parks & Agarwal, 1973). The phosphate transfer operates via the phosphorylation of a histidine during catalysis. Biochemical and crystallographic data suggest a ping-pong mechanism, with a common binding site shared by the donor nucleoside triphosphate and the acceptor nucleoside diphosphate. Gene sequences from eukaryotic and bacterial sources available so far code for a chain of about 150 amino acids, with over 40% sequence identity (Hama *et al.*, 1991). The first crystal structure of NDP kinase was for a point mutant of the enzyme from slime mold *Dictyostelium discoideum* (Dumas *et al.*, 1992). The enzyme is a hexamer, with a central antiparallel β-sheet connected by α-helices, a topology similar to that of the allosteric domain of ATCase (Gouaux *et al.*, 1990). Further X-ray structures have revealed a closely similar fold for the enzyme from *Drosophila melanogaster* (Chiadmi *et al.*, 1993) and from the bacterium *Myxococcus xanthus* (Williams *et al.*, 1993), even though the latter is a tetramer.

The active site has been identified in crystal structures of NDP kinase–ADP complexes of the wild-type *Dictyostelium* (Moréra *et al.*, 1994) and *Myxococcus* (Williams *et al.*, 1993) enzymes. Each monomer binds one ADP molecule. The active site does not contain the classical mononucleotide binding fold found in other phosphoryl transfer proteins [reviewed in Schulz (1992)]. The nucleotide lies parallel to the edge of the β-sheet and interacts with protein side chains but not with backbone atoms. The base makes no polar interactions, while the ribose hydroxyls and phosphate moieties make numerous H-bonds with protein side chains.

We describe here the high-resolution crystal structure of wild-type *Dictyostelium* NDP kinase with thymidine diphosphate (dTDP) and Mg^{2+} bound at the active site. We show that this site is designed to bind nucleotides irrespective of the nature of the base and sugar and that the same catalytic mechanism may operate on ADP and dTDP. The crystal structure provides clues on the way mammalian NDP kinases bind DNA (Postel *et al.*, 1993) and for the structure-based design of biologically active antiviral nucleoside analogues, which should be efficiently phosphorylated by NDP kinase.

MATERIALS AND METHODS

Crystallization and Data Collection. Dictyostelium NDP kinase was overexpressed in *Escherichia coli* and purified as described in Lacombe *et al.* (1990). The protein sample was dialyzed against 20 mM $MgCl_2$ and 50 mM TRIS-HCl buffer, pH 8, and then incubated with 10 mM dTDP for 1 h. Hanging

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[‡] Atomic coordinates have been deposited in the Protein Data Bank (file name 1NDC).

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Table 1: Statistics on Crystallographic Data

diffraction data[a]	
resolution range	16–2 Å
measured intensities	71549
unique reflections	13562
completeness	99.6%
R_{merge}	6.8%
refinement[b]	
R_{cryst}	18.3%
resolution	16–2 Å
reflections	13527
protein atoms	1173
solvent atoms	105
average B	11.9 Å^2
geometry[c]	
bond distances	0.009 Å
bond angles	1.6°
peptide ω angle	1.8°

[a] Crystals are space group $R32$, $a = b = 79.8$ Å, $c = 160.6$ Å, $\alpha = \beta = 90°$, and $\gamma = 120°$. $R_{merge} = \sum|I(h)_i - \langle I(h)\rangle|/\sum\langle I(h)\rangle$. [b] $R_{cryst} = \sum||F_o| - |F_c||/\sum|F_o|$ calculated with XPLOR on all reflections with $F > 2\sigma$. [c] Average deviations from standard values.

drops were prepared by adding 8% PEG 6000 to this protein solution over pits containing 16% PEG 6000 in the same buffer. The protein concentration in the drop was 5 mg/mL. Crystals grew within 2 days at 18 °C and have a rhombic shape of maximal dimension 100 μm. X-ray diffraction data were collected on a single crystal kept at 4 °C on the W32 station of the LURE-DCI synchrotron radiation center (Orsay, France) at a wavelength of 0.91 Å, using an imaging plate system. Ninety-six degrees of rotation were collected in frames of 1.5° with 2-min exposure per frame. Crystals belong to rhombohedral space group $R32$ with rhombohedral cell parameters $a = b = c = 70.6$ Å and $\alpha = \beta = \gamma = 68.8°$ and hexagonal parameters $a = b = 79.8$ Å, $c = 160.6$ Å, $\alpha = \beta = 90°$, and $\gamma = 120°$ and have one subunit per asymmetric unit. Intensities were evaluated using MOSFLM (Leslie *et al.*, 1986) and processed with programs from the CCP4 suite (CCP4, Daresbury Laboratory, Daresbury, England). Statistics are reported in Table 1.

Structure Solution and Refinement. As dTDP·NDP kinase crystal cell parameters are close to those of the ADP·NDP kinase crystals which belong to space group $R3$ (Moréra *et al.*, 1994), intensities were initially integrated in this space group. The $R32$ symmetry was identified by the rotation function (Crowther, 1972) in the CCP4 suite. The NDP kinase subunit was then unambiguously positioned by overlapping NDP kinase hexamer symmetry axes with those of the crystal. Initial phases were calculated with NDP kinase coordinates from the ADP·NDP kinase complex refined at 2.2 Å (Moréra *et al.*, 1994) as a starting model, leading to an R-factor of 35%. The location of dTDP was clearly identifiable in the $2|F_o| - |F_c|$ density map at this stage and was built into the density. Rigid body refinement performed with XPLOR (Brünger *et al.*, 1987) produced only small shifts, indicating a similar quaternary structure. Further crystallographic refinement used the CCP4 program suite and the conjugate gradient facility in XPLOR using energy parameters from Engh *et al.* (1991). Electron density maps were examined with either FRODO (Jones, 1985), TURBO (Dr. A. Roussel and Dr. C. Cambillau, Marseille, France) or O (Jones *et al.*, 1991). Water sites were gradually added to the structure using $|F_o| - |F_c|$ maps and were accepted if they had strong density in the $2|F_o| - |F_c|$ map. The final R-factor value was 18.3% for the dTDP·Mg^{2+}·NDP kinase model including 105 water molecules. The final electron density is well-defined except for N-terminal residues 2–5 which are not visible, and residues 148–151 which only have backbone density. Only one non-glycine residue is outside permitted regions of the Ramachandran plot: Ile 120. Its unusual main-chain conformation is clearly defined by the electron density and has been observed in other NDP kinase X-ray structures.

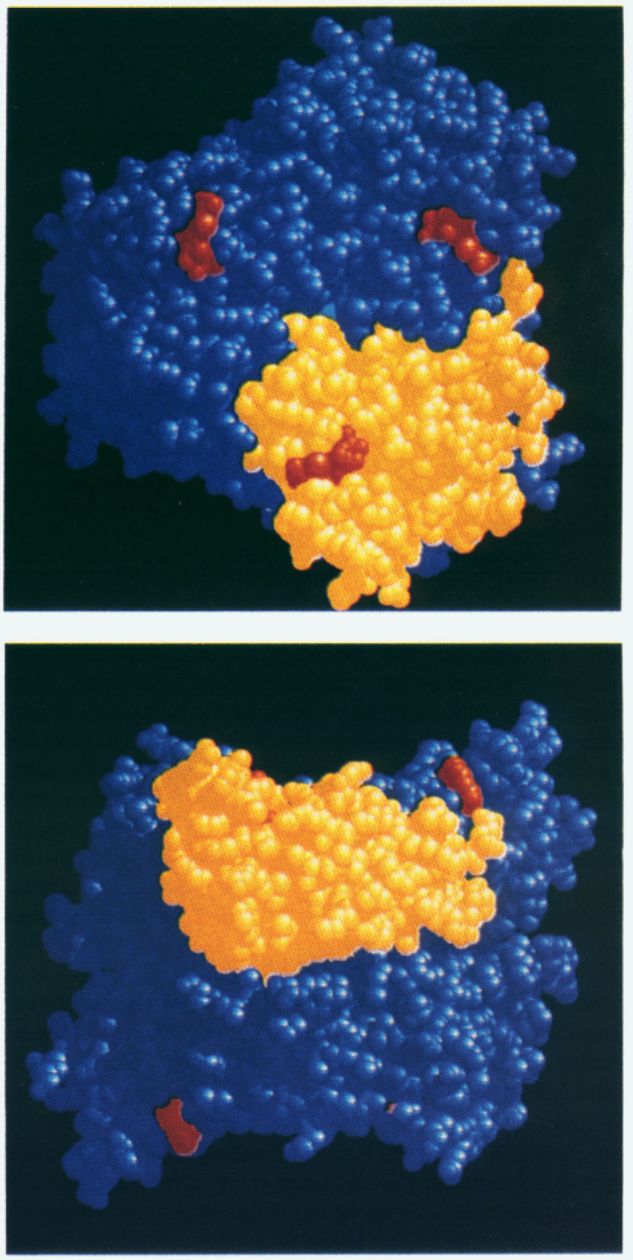

FIGURE 1: dTDP·NDP kinase complex. View along the 3-fold axis (top) and a 2-fold axis (bottom) of the hexamer. An individual subunit is shown in yellow. The nucleotide is in red. Drawn with RASMOL (written by R. Sayle).

RESULTS

Conformation and Binding of Thymidine Diphosphate. A general view of the hexamer with bound dTDP is shown in Figure 1, and details of the binding site are illustrated in Figure 2. The nucleoside moiety lies at the edge of the central β-sheet, next to strand β_4 that carries His 122, which is the phosphorylated residue. The base and sugar are in contact with helix α_2 on one side and with the loop connecting α_3 to β_4 on the other. We call this loop the Kpn loop in reference to the *Killer of prune* mutation in *Drosophila* NDP kinase (Biggs *et al.*, 1990). Phosphates interact with side chains

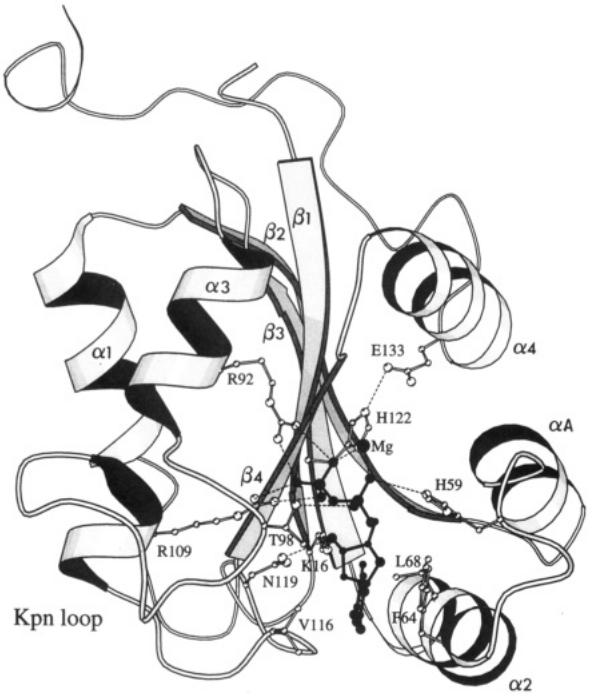

FIGURE 2: NDP kinase subunit with bound dTDP (full bonds) and Mg^{2+}. Secondary structure elements: β_1, 9–16; α_1, 25–35; β_2, 38–45; α_A, 49–55; α_2, 65–72; β_3, 77–83; α_3, 87–95; Kpn loop, 99–118; β_4, 120–124; α_4, 127–137. H-bonds are dashed. Drawn with MOLSCRIPT (Kraulis, 1991).

Table 2: Conformation and Accessibility to Solvent of Bound Nucleotide in the dTDP Complex and in the ADP Complex (Subunit B)

	free dTDP	free ADP	dTDP	ADP
dihedral angles				
glycosyl bond χ			−156	−137
sugar pucker P^a			31	31
C4′–C5′			−57	−61
accessible surface (Å²)[b]				
base	164	198	84	89
ribose	135	142	2	2
α-phosphate	68	73	30	32
β-phosphate	104	104	7	9
total	471	517	123	132

[a] The pseudorotation angle P is defined as in Saenger (1984). [b] Solvent-accessible surface areas were calculated with the program ASA (Prof. A. Lesk, Cambridge) and a probe size of 1.4 Å.

from helices α_2 and α_3 and with the Kpn loop. There is no direct nucleotide–protein backbone interaction. Conformational parameters of the nucleotide are listed in Table 2, and contacts with the protein in Table 3.

The thymine base is wedged between Phe 64 and Val 116 near the protein surface. It stacks parallel to the phenyl ring of Phe 64, with an interplane distance of 3.6 Å. The ring is buried in hydrophobic contacts with the side chains of Phe 64 and Val 116, while the methyl and keto groups point toward the solvent. There are no direct polar interactions from the pyrimidine ring to protein side chains. However, the exocyclic O_2 oxygen interacts via two water molecules with the carboxylate of the C-terminal Glu 155 from a 3-fold related subunit of the hexamer.

The deoxyribose ring pucker is $C_{3'}$-*endo*, with the glycosyl bond in *anti* conformation (Table 2). In contrast with the base, the sugar is totally buried in protein contacts. The $O_{4'}$ side of the ring makes nonpolar contacts with Leu 68. The $O_{3'}$ hydroxyl accepts H-bonds from Lys 16 and Asn 119. It also donates an internal H-bond to the O_7 β-phosphate oxygen.

Table 3: Nucleotide Contacts[a]

	protein	dTDP	ADP
base			
pyrimidine ring	Phe 64 ring	3.6	3.6
	Val 116 $C_{\gamma 1}$	3.6	3.5
ribose			
$O_{2'}$	Lys 16 N_ζ		2.6
	Asn 119 O_δ		3.3
$O_{3'}$	Lys 16 N_ζ	3.1	2.7
	Asn 119 N_δ	3.1	3.1
$O_{4'}$	Phe 64 ring	3.5	3.4
	Leu 68 $C_{\delta 2}$	3.5	3.2
α-phosphate			
O_{11}	His 59 N_δ	3.5	3.0
	Mg^{2+}	2.1	2.0
O_{12}	Thr 98 O_γ	3.4	3.9
β-phosphate			
O_{21}	Arg 92 $N_{\eta 2}$	3.0	2.9
	Mg^{2+}	2.1	2.3
O_{22}	Arg 92 $N_{\eta 1}$	2.9	4.4
	Thr 98 O_γ	3.3	3.1
	Arg 109 $N_{\eta 2}$	2.8	2.7
O_7	Arg 109 $N_{\eta 1}$	3.0	2.9
	Wat 246	2.6	2.9

[a] Polar bonds shorter than 3.4 Å and the shortest nonpolar contacts in the dTDP complex and in the ADP complex (subunit B) are reported.

The position of individual α- and β-phosphate oxygens is unambiguous in the electron density map (Figure 3). The α-phosphate remains accessible to solvent and makes rather long H-bonds with the side chains of His 59 and Thr 98. In contrast, the β-phosphate is buried and held by strong H-bonds to the side chains of Thr 98, Arg 92, and Arg 109. His 122 is distant by 4.9 Å from O_7, the nearest β-phosphate oxygen (Table 3). A well-defined water molecule, Wat 246, bridges this β-phosphate oxygen to His 122 N_δ and makes an additional H-bond with Tyr 56. The electron density displays very clearly the location of a magnesium ion that bridges the α- and β-phosphates (Figure 4). The metal ion has an octahedral coordination, contributed by the α- and β-phosphate O_{11} and O_{21} oxygens and four water molecules. Distances are in the expected range of 1.9–2.1 Å. The first coordination shell of the metal ion does not involve protein atoms. Asp 125 and Glu 58 carboxylates and Gly 123 carbonyl next to the active site histidine constitute the second coordination shell via water-mediated interactions.

Comparison of dTDP·NDP Kinase with Free NDP Kinase. A superposition of Cα positions of the free (Moréra *et al.*, 1994) and dTDP-ligated NDP kinase gives a root mean square deviation of 0.48 Å, indicating that the overall structure is the same (Figure 5). No quaternary structure change is observed when the hexamers are compared. Most differences are located in the 60–64 loop between helices α_A and α_2, which in free NDP kinase has *B*-factors higher than average. This segment is in "open" position in free NDP kinase, facilitating the entry of the nucleotide in the active site. In dTDP·NDP kinase, the loop has moved to enclose the nucleotide. Phe 64 moves over a distance of 2 Å in order to stack on the base. The rearrangement of residues 60–64 also brings Tyr 56 closer to the β-phosphate and may contribute to complete the active site.

In contrast, the side chains of Lys 16 and Asn 119 do not move upon binding the deoxyribose. As in free NDP kinase, Lys 16 and Asn 119 H-bond to each other, and the amide of Asn 119 is fixed by a H-bond to the backbone oxygen of Thr 98. Free and dTDP-bound NDP kinase share a common water molecule in the vicinity of Lys 16, held by additional H-bonds to the carbonyl of Gly 117 and the carboxylate of Asp 18. This water molecule makes a H-bond to Lys 16 in free NDP kinase.

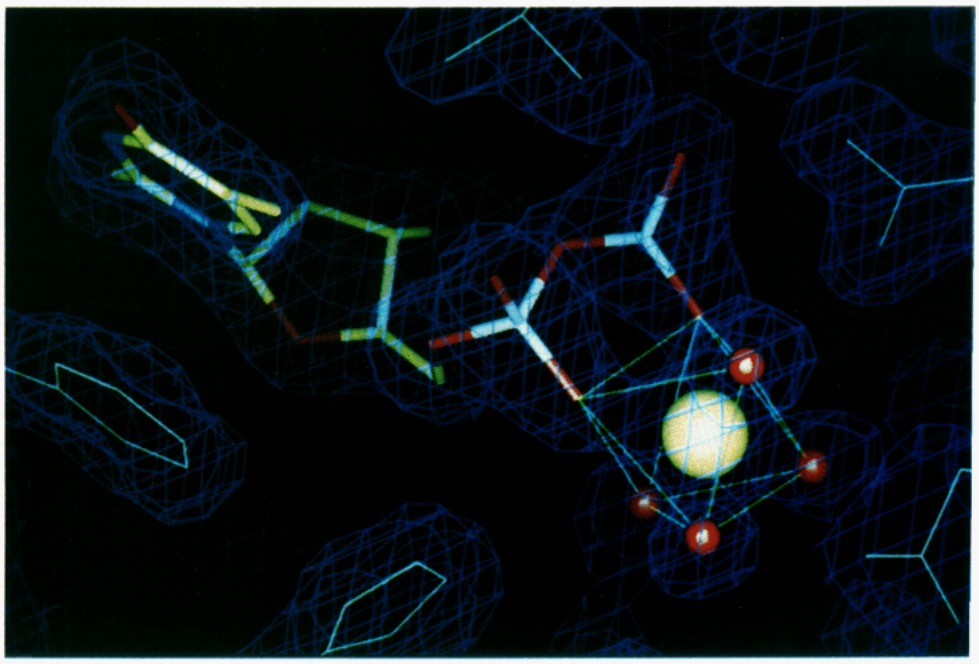

FIGURE 3: $2|F_o| - |F_c|$ electron density map contoured at 1σ showing dTDP and Mg^{2+} in the active site. The yellow sphere is Mg^{2+}; the red spheres are water molecules. The octahedral coordination of Mg^{2+} is emphasized by the green cage. Drawn with O (Jones *et al.*, 1991).

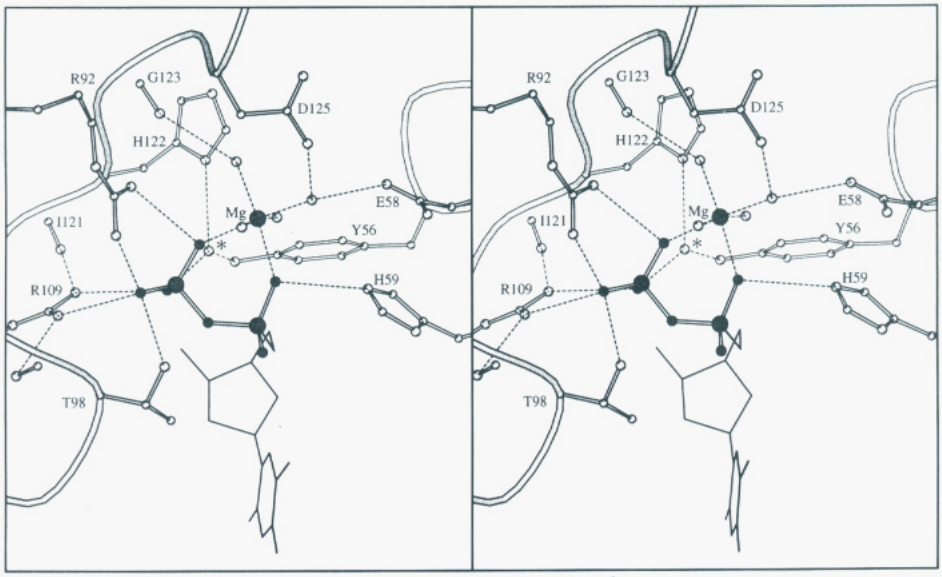

FIGURE 4: Phosphate–Mg^{2+} site with bound dTDP. Metal–oxygen bonds and H-bonds are dashed. White spheres are water molecules. Water 246 bridges the β-phosphate to His 122 and is marked with an asterisk. Stereo plot for direct viewing drawn with MOLSCRIPT (Kraulis, 1991).

Two basic residues are available to neutralize phosphate charges in the active site. In free and dTDP-bound NDP kinase, Arg 109 is fully extended and locked in place by H-bonds to the backbone oxygens of Val 97 and Ile 121. With two additional H-bonds to β-phosphate oxygens in dTDP·NDP kinase, its H-bond potential is saturated. In contrast, Arg 92 is not fixed in free NDP kinase and moves by about 2 Å to bind two β-phosphate oxygens. It may form at least one more H-bond, presumably with the γ-phosphate of the real substrate. The active site His 122 does not move. In both structures, its imidazole is held by a H-bond to Glu 133.

Comparison with the ADP·Mg^{2+} Complex. There are two molecules per asymmetric unit in the ADP·NDP kinase crystal structure, and their mode of binding is slightly different as the magnesium density is weak or absent in one subunit (Moréra *et al.*, 1994). Here we compare dTDP to ADP bound to the subunit with Mg^{2+}. The conformation of dTDP and its orientation in the active site are similar to those observed for ADP·NDP kinase (Table 2), and interacting groups are the same (Table 3). The root mean square deviation of Cα positions between dTDP·NDP kinase and ADP·NDP kinase is 0.35 Å, less than with free NDP kinase. The 60–64 polypeptide segment closes on the base in both structures, but the deviation between their Phe 64 is only 0.8 Å. Phe 64 adapts its position to stack above either the five-membered ring of adenine or the six-membered ring of thymine (Figure 6). dTDP exocyclic O_2 and ADP endocyclic N_3 atoms, which are H-bond acceptors in pyrimidines and purines, respectively, occupy a similar position relative to Phe 64. Both participate in water-mediated interactions with the carboxylate of Glu 155 from a neighbor subunit in the hexamer (Figure 7).

The sugar ring pucker and the pyrophosphate conformation are similar in dTDP and ADP (Figure 6). The absence of the $O_{2'}$ hydroxyl in dTDP has little effect, and the interactions of Lys 16 and Asn 119 with the $O_{3'}$ hydroxyl are conserved despite a small shift of 0.5 Å of the deoxyribose position. The

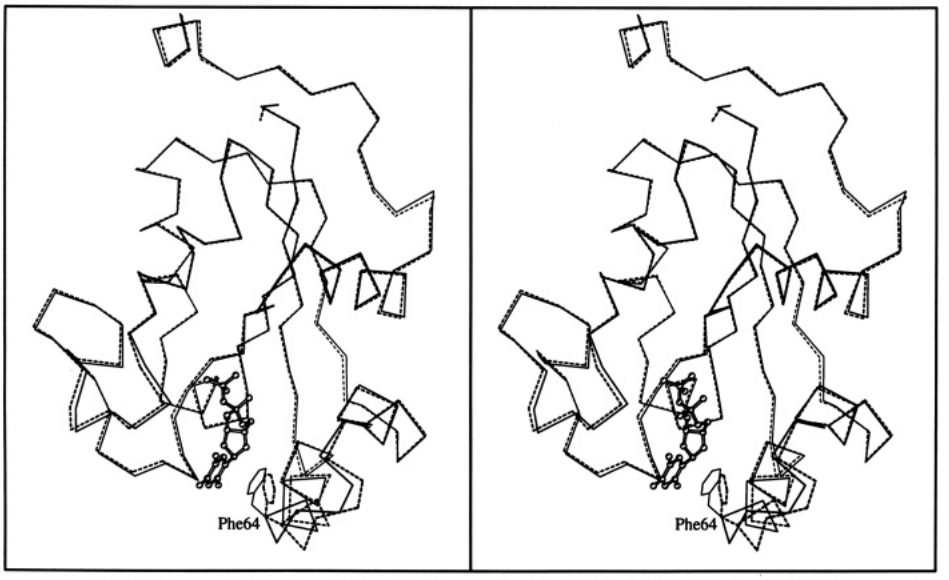

FIGURE 5: Comparison of free NDP kinase (dashes) and the dTDP complex (solid line). Phe 64, the 60–64 loop, and the first turn of helix α_2 are shown to move toward the bound nucleotide.

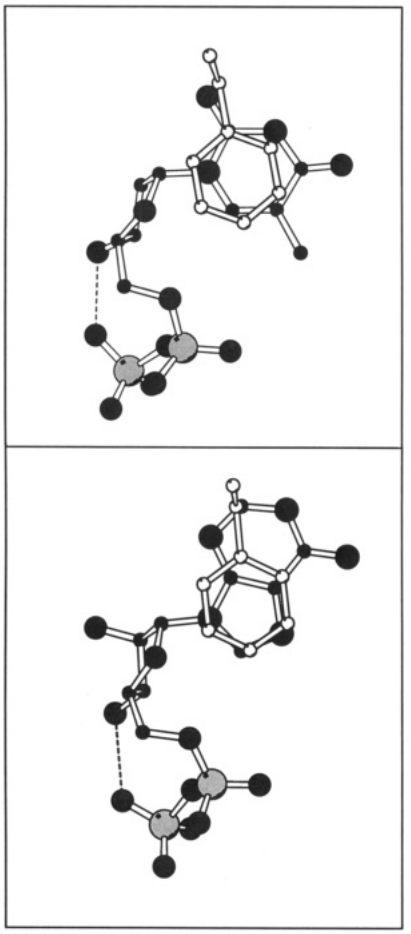

FIGURE 6: Stacking of Phe 64 on (a) dTDP and (b) ADP. Note the $C_{3'}$-*endo* ring pucker of the dTDP and ADP sugars and the H-bond between $O_{3'}$ and the β-phosphate (dashed).

position occupied by the $O_{2'}$ oxygen in ADP·NDP kinase is left empty in dTDP·NDP kinase (Figure 8). The H-bond between Lys 16 and the ribose $O_{2'}$ of ADP is compensated for by a H-bond to a water molecule in dTDP·NDP kinase. The $O_{3'}$ atoms from dTDP and ADP form an intramolecular H-bond with the β-phosphate, which constrains the pyrophosphate conformation.

Pyrophosphate interactions are very similar. The position of Mg^{2+} identified in the ADP complex is fully confirmed by its clear location in dTDP·NDP kinase. Both structures have a water molecule bridging the O_7 β-phosphate oxygen and the imidazole N_δ of the catalytic His 122.

Crystal Packing. The 2- and 3-fold symmetry axes of dTDP·NDP kinase hexamers superpose with the *R*32 crystal axes. Hexamers stack in staggered layers perpendicar to the 3-fold axis and make a single type of crystal contact. It involves the mobile 60–64 loop, with a salt link from Arg 62 to a symmetry-related Glu 61, and the Kpn loop with a H-bond between Arg 92 and the α-phosphate of a symmetry-related dTDP. The region near the nucleotide binding site, especially the 60–64 loop, is also involved in the packing of the $P6_322$ crystals of free *Dictyostelium* NDP kinase (Dumas *et al.*, 1992), the *R*3 crystals of ADP·NDP kinase (Moréra *et al.*, 1994), and the $P3_221$ crystals of the *Drosophila* enzyme (Chiadmi *et al.*, 1993). In all, contacts between hexamers block access to the binding site. Nevertheless, the nucleotide binds in a similar way in the *R*3 and *R*32 forms and also in *Myxococcus* NDP kinase, which has a completely different crystal packing (Williams *et al.*, 1993). Thus, the binding mode reported here is not guided by packing artifacts.

DISCUSSION

Pyrimidine vs Purine Binding. The broad specificity of NDP kinase toward ribonucleotides and deoxyribonucleotides is needed for equilibrating the ratio between diphosphate and triphosphate nucleotides in the cell (Parks & Agarwal, 1973). We can see how it is achieved by comparing the ways ADP and dTDP are bound. To that purpose, we may distinguish a base site, a sugar site, and a phosphate–Mg^{2+} site.

The flexible 60–64 polypeptide segment can adapt its position to the nature of the base. Its "open" position facilitates the entrance of the nucleotide in the active site, while the "closed" position stacks Phe 64 on purine and pyrimidines and wedges the base between the hydrophobic side chain of Val 116 and the aromatic ring. Distinctive exocyclic groups make no discriminating polar interaction with protein atoms. The *anti* conformation of the glycosyl bond exposes to solvent the far edge of the base opposite to ribose, thus accommodating bases of different size. This suggests that other aromatic compounds which bind to NDP kinase may use the same site, rose Bengal for instance (Lascu *et al.*, 1986).

The binding mode of adenine and thymine can readily be extended to other purine and pyrimidine bases. The methyl

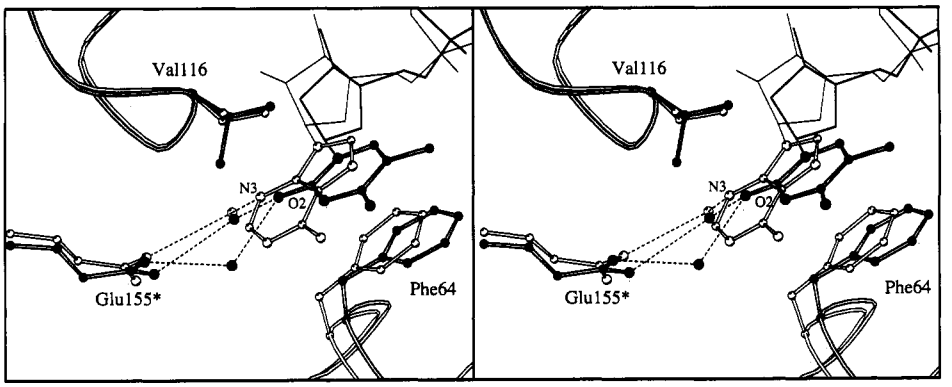

FIGURE 7: Base site. Superposition of thymine from the dTDP complex (full bonds) on adenine from the ADP complex (empty bonds). The base interacts through water molecules (spheres) with the C-terminal residue Glu 155 from a 3-fold related subunit. H-bonds are dashed.

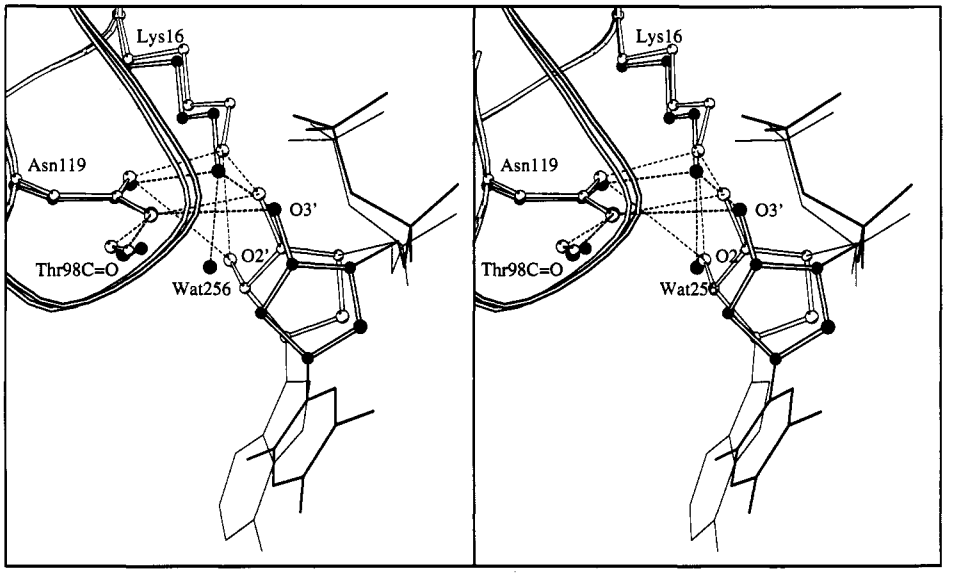

FIGURE 8: Sugar site. Superposition of deoxyribose from the dTDP complex (full bonds) on ribose from the ADP complex (empty bonds). Water 256 binds to Lys 16 in the dTDP complex.

group which differentiates thymine from uracil, the amino and imino groups of cytosine, and the imino and keto groups of guanine should be in the solvent. The keto group of cytosine should make a water-mediated interaction with Glu 155 as it does in thymine. The amino group on C_2 in guanine is a more interesting case, as it should replace a water molecule bridging the base to Glu 155 and could make a direct H-bond to the carboxylate. This interaction could possibly discriminate guanine from other bases, if it is stronger than water-mediated interactions observed in ADP and dTDP complexes. Glu 155 is conserved in eukaryotic NDP kinases but is absent in the bacterial *Myxococcus* enzyme which has a different quaternary structure and a shorter C-terminal sequence relative to eukaryotic sequences. Thus, if it takes place, this interaction is probably unessential to the phosphoryl transfer function of the enzyme.

Few proteins that bind both purine and pyrimidines have been analyzed in structural detail. The allosteric domain of *E. coli* aspartate transcarbamylase binds both ATP and CTP (Gouaux *et al.*, 1990) but in a different way from that of NDP kinase despite their similar topologies (Dumas *et al.*, 1992; Moréra *et al.*, 1994). Unlike NDP kinase, aspartate transcarbamylase discriminates between ATP, an allosteric activator, and the inhibitor CTP, although how it does so has not been fully elucidated yet. ATP is also an allosteric effector of bacterial phosphofructokinase. The allosteric site does not interact with adenine, in agreement with its low specificity (Shirakihara & Evans, 1988). Nucleases provide other examples for comparison. DNase I achieves its low sequence specificity by binding to the minor groove of the double helix without contacts with the bases (Weston *et al.*, 1992). Staphylococcal nuclease binds thymine in an hydrophobic pocket, making an edge-to-face interaction with a tyrosine side chain (Cotton *et al.*, 1979; Loll & Lattman, 1989). The NDP kinase nucleoside binding mode, where a phenylalanine and a branched hydrophobic side chain sandwich the base in an hydrophobic pocket, resembles the mononucleotide binding site of the P1 nuclease (Volbeda *et al.*, 1991) and the 3′,5′-exonuclease site of DNA polymerase I where the branched hydrophobic residue is a leucine (Beese & Steitz, 1991). Together with these, the structures of ADP and dTDP·NDP kinase complexes suggest that this is a general solution to the problem of nonspecific nucleotide binding.

Deoxyribose vs Ribose Binding. Recognition of the sugar is performed by two side chains, those of Lys 16 and Asn 119. Each contributes one H-bond to $O_{3'}$ of ADP and dTDP and a second H-bond to $O_{2'}$ of ADP. In dTDP·NDP kinase, the interaction of Lys 16 with the ribose $O_{2'}$ is replaced by a H-bond with a water molecule, while the location of the $O_{2'}$ atom is left empty. A comparison of the free and nucleotide-bound *Dictyostelium* NDP kinase with the *Drosophila* and *Myxococcus* enzymes, where these residues are conserved as in other known NDP kinases, suggests that Lys 16 and Asn 119 constitute a preformed, rigid template, held in place by a water molecule in the absence of nucleotide. This interaction is replaced by H-bonds with $O_{2'}$ and $O_{3'}$ ribose hydroxyls or

with the 3′ hydroxyl and a water molecule in deoxyribose. Thus NDP kinase appears to have double specificity for ribose and deoxyribose, rather than a lack of specificity.

At the nonspecific effector site of phosphofructokinase, the $O_{2'}$ of ATP makes no contacts with the protein (Shirakihara & Evans, 1988). The absence of $O_{2'}$ recognition is also suggested by the accessibility to solvent of the deoxyribose in P1 nuclease (Volbeda *et al.*, 1991), whereas purine nucleotide phosphorylase makes only van der Waals contacts with the $O_{2'}$ of iodoformycine (Ealick *et al.*, 1990). Specificity for deoxyribonucleotides is easily achieved by steric hindrance, as in thymidine phosphorylase·dTDP where a hydrophobic side chain forbids binding of a 2′-hydroxyl (Walter *et al.*, 1990). Since deoxyribonucleotides are present at only very low levels, specificity of ribonucleotide binding proteins for ribose may be of lesser relevance. As an example, the $p21^{ras}$ protein has been shown to contain mainly GDP but also a small amount of dGDP (Feuerstein *et al.*, 1987). Many protein–ribonucleotide crystal structures show the ribose hydroxyls to participate in H-bonds with carboxylates or carbonyls from the protein. In principle, the absence of the 2′-hydroxyl, by leaving a polar group impaired, should lower the affinity of these proteins for deoxyribonucleotides, unless a water molecule replaces the missing $O_{2'}$ as in NDP kinase.

Phosphate-Mg^{2+} Binding. The NDP kinase nucleotide binding site differs from the classical mononucleotide-binding fold [see Schultz (1992) for a review]. Its sequence does not possess the glycine-rich phosphate binding fingerprints. There are no main-chain amide nor helix dipole interactions with the phosphates, but two arginines are making the α- and β- and probably the γ-phosphate binding site. The conserved lysine of the G-protein fingerprint, which is assumed to assist the phosphoryl transfer by following the transferred group along the catalytic pathway (Schulz, 1992), bridges the β- and γ-phosphates in known structures. The strictly conserved lysine of the NDP kinase active site (Lys 16 here) is implicated in binding the ribose hydroxyls. We suggest that the H-bond to $O_{3'}$ breaks in order to bind to the phosphates. This would facilitate the release of the nucleoside diphosphate product from the phosphorylated enzyme.

As in most phosphoryl transfer proteins, the phosphate charges are shielded by a divalent cation bound to the nucleotide. A single magnesium ion bridges the β- and γ-phosphates of $p21^{ras}$ (Pai *et al.*, 1990; Milburn *et al.*, 1990) and completes its coordination with two water molecules and two hydroxyls from Thr or Ser side chains, which are in turn coordinated to Asp side chains. This coordination pattern of Mg^{2+} is remarkably similar in the G-proteins $p21^{ras}$, elongation factor Tu (Kjeldgaard *et al.*, 1993), and transducin α (Noel *et al.*, 1993), suggesting a conserved role for catalytic activity. In contrast, cAMP-dependent protein kinase binds two Mg^{2+} (Zheng *et al.*, 1993) or Mn^{2+} (Bossemeyer *et al.*, 1993) ions. One ion is coordinated to the β- and γ-phosphates of ATP and makes a charge interaction with an Asp side chain. The other one is liganded to the α- and γ-phosphates and to an Asn side chain. The crystal structure of phosphofructokinase with its reaction products gives another picture of Mg^{2+} binding (Shirakihara & Evans, 1988). At the active site of the closed subunit, the magnesium bridges the β-phosphate of ADP and the 1-phosphate of fructose 1,6-diphosphate. Its octahedral coordination is completed by an aspartate and three water molecules, two of which are in turn bound to an aspartate. In the open subunit, the magnesium ion is still coordinated to the Asp side chain, but it bridges the α- and β-phosphates of ADP. This suggested that the metal transits from the β/γ-phosphates of the substrate ATP to the α/β-phosphates of the product ADP (Shirakihara & Evans, 1988). In the ADP and dTDP·NDP kinase complexes, the protein provides no ligand atoms to the first coordination shell of Mg^{2+}. Asp 125, Glu 58, and Gly 123, in the second coordination shell, are conserved in all known NDP kinase structures. In our two structures, the β-phosphate and a water molecule are correctly positioned to perform *in line* phosphate transfer to His 122, the water molecule occupying the approximate position of the γ-phosphorus in the pentacoordinated transition state (Moréra *et al.*, 1994). As Mg^{2+} is only 4.4 Å from the putative γ-phosphorus location, it may chelate the α-, β-, and γ-phosphates in a tridentate coordination. This is compatible with the Mg^{2+} ion hoping from one pair of phosphate groups to another during phosphate transfer from ATP to His 122 and back, as proposed for phosphofructokinase.

DNA Binding to NDP Kinase. NDP kinases have been associated with functions that may be unrelated to their phosphotransferase activity. For instance, the gene encoding the *Drosophila* enzyme has been involved in larval differentiation (Biggs *et al.*, 1990), and a putative human metastasis suppressor gene was identified to *nm23*-H1 (Rosengard *et al.*, 1989), the gene encoding NDP kinase A (Gilles *et al.*, 1991). Recently, Postel *et al.* (1993) have identified one of the two human NDP kinases as a transcription factor for the *c-myc* protooncogene. The NDP kinase fold does not resemble other DNA-binding proteins of known structure, except perhaps the E2 domain of papilloma virus (Hedge *et al.*, 1991). Any model of the interaction is therefore speculative at this stage. However, we noted above that the base site between Phe 64 and Val 116 resembles that of the 3′,5′-exonuclease site of DNA polymerase I and of the P1 nuclease. Unlike NDP kinase, the substrate of these enzymes is not a mononucleotide, but DNA. The phenylalanine may help to unwind DNA by edging between consecutive base pairs (Beese & Steitz, 1991; Volbeda *et al.*, 1991). As a transcription factor, NDP kinase should also be expected to promote unwinding. This suggests that the NDP kinase mononucleotide binding site may be part of its DNA-binding site and that Phe 64 may contribute in locally denaturing duplex DNA into single-stranded DNA. In this context, the previouly noticed (Dumas *et al.*, 1992) resemblance of NDP kinase topology with that of the U1 small ribonucleoprotein A RNA-binding domain (Nagai *et al.*, 1990) is of particular interest.

Structure-Based Drug Design of Antiviral Nucleoside Analogues. Modified nucleosides lacking a 3′-hydroxyl are potential antiviral drugs targeted to reverse trancriptase, as they cause chain termination of viral DNA synthesis (De Clercq, 1992). Their biological activity depends upon their phosphorylation to the nucleoside triphosphates. The phosphorylation of several nucleoside diphosphate analogues by NDP kinase was shown to be slow *in vitro* and *in vivo* (Miller *et al.*, 1992; Karlsson *et al.*, 1990), suggesting that the enzyme may be rate-limiting for the synthesis of active compounds. Drug design of NDP kinase substrates or inhibitors could safely be based on the *Dictyostelium* structure, given that the active site and the residues involved in nucleotide binding are fully conserved in the human sequences. This approach is relevant to AIDS research. The triphosphate derivatives of azidothymidine (AZT) and dideoxyinosine (DDI), two major anti-HIV drugs, are substrates of reverse transcriptase which lack a 3′-hydroxyl. AZT triphosphate is a poor substrate for yeast NDP kinase (Kuby *et al.*, 1991) and for human NDP kinase (Lascu, preliminary results). This is easily understood

from the structure of the dTDP complex, which indicates that there is no room at the sugar site for the bulky azido group replacing $O_{3'}$. Binding to NDP kinase and phosphorylation of dideoxy compounds remain to be checked. We expect their affinity to be weak compared to deoxyribo- or ribonucleotides, due to the loss of $O_{3'}$ interactions with Lys 16 and Asn 119, and phosphate transfer to be inefficient due to the absence of the internal H-bond from $O_{3'}$ to the β-phosphate that constrains the position of the phosphate group being transferred. We suggest that nucleotide analogues where a similar bond can be made, and which can fit into the sugar site, should be better substrates of NDP kinase.

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