

STRUCTURE NOTE

Crystal Structure of Human Nucleoside Diphosphate Kinase A, a Metastasis Suppressor

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Introduction. Nucleoside diphosphate kinase (NDK) catalyzes the transfer of the γ -phosphoryl group from a nucleoside triphosphate (NTP) to a nucleoside diphosphate (NDP) by using ATP as a major phosphate donor. During the catalytic reaction, the enzyme is transiently phosphorylated on a conserved histidine residue. NDP kinases play a primary role in maintaining cellular pools of all NTPs but also in the regulation of important cellular processes.

In humans, the two isoforms NDK-A (NM23-H1) and NDK-B (NM23-H2) are closely related in amino acid sequence (88% identity) but display significant differences in cellular functions. NDK-A acts as a metastasis suppressor for some tumor types.¹ NDK-B, also known as PuF, binds to the promoter of the *c-myc* oncogene and activates its transcription.¹ These cellular functions of the two isoforms are independent of their NDP kinase activity, and two isoforms can form homo- and heterohexamers, resulting in different ratios of the respective subunits.¹

We present here the crystal structure of human NDK-A determined at 2.2 Å resolution. This enables a detailed structural comparison of NDK-A with NDK-B, contributing to understanding the difference in their cellular functions.

Materials and Methods. Crystallization and X-ray data collection have been reported elsewhere.² The crystals belong to the monoclinic space group $P2_1$ with cell parameters $a = 74.21$ Å, $b = 78.11$ Å, and $c = 82.29$ Å, and $\beta = 101.33^\circ$, and the asymmetric unit contains a whole hexamer. By using the NDK-B model² (PDB id code 1NUE) as the probe, the structure of NDK-A was solved by molecular replacement using the AMoRe program and refined using the X-PLOR. Noncrystallographic symmetry among the six subunits was restrained during refinement with an energy barrier of $300 \text{ kcal mol}^{-1} \text{ Å}^{-2}$. The refined model (Protein Data Bank ID code 1JXV) consists of 7295 non-hydrogen protein atoms from 894 amino acid residues of the hexamer and 191 water molecules in the asymmetric unit. The crystallographic R/R_{free} values are 20.7%/26.3% for reflections with $F_o > 2 \sigma$ in the resolution range of 6.0–2.2 Å. The average B-factor is 42.2 and 46.1 Å² for main-chain and side-chain atoms, respectively. More than 93.9 % of the residues are in the most favored regions of

the Ramachandran plot, and six residues (Ile116 in each subunit) are in disallowed regions. Residues 45–65 in subunit C and residues 57–64 in subunit F have poor electron density, and residues 1–3 are missing from all subunits.

Results and Discussion. Human NDK-A shares the same hexameric structure of D3 symmetry as NDK-B. The overall structure can be viewed as a dimer of two trimers or a trimer of three dimers (Fig. 1). And the six independent subunits in the asymmetric unit are virtually identical, with their C α positions being superimposed to within a root-mean-square deviation (RMSD) of 0.02 Å. NDK-A hexamer superimposes with two NDK-B structures^{3,4} determined with or without guanosine diphosphate (GDP) (PDB ID code 1NUE and 1NSK, respectively) with a RMS distance of 0.52 and 0.48 Å for all common C α atoms (residues 4–152), respectively. As shown in Figure 2, C α RMSD plots between uncomplexed structures of NDK-A and -B and GDP-complexed structure of NDK-B are similar and are different from that between uncomplexed structures of NDK-A and -B because of the nucleotide binding. The largest structural changes are observed in a segment (residues 45–69) encompassing a pair of surface helices (αA and $\alpha 2$). In comparison, the movements of $\alpha 3$ and the Kpn loop region (residues 96–116), the other constituent of nucleotide binding site, and a C-terminal extension (residues 135–152) are smaller. Comparisons of the RMS distance plot between two uncomplexed structures of NDK-A and -B (gray thick line in Fig. 2) and the RMS distance plots of the two uncomplexed structures of NDK-A and -B against the GDP-complexed structure of NDK-B (black and gray thin lines in Fig. 2, respectively) reveal that the nucleotide binding induces net conformational changes in residues 55–67 and 90–95. Only two

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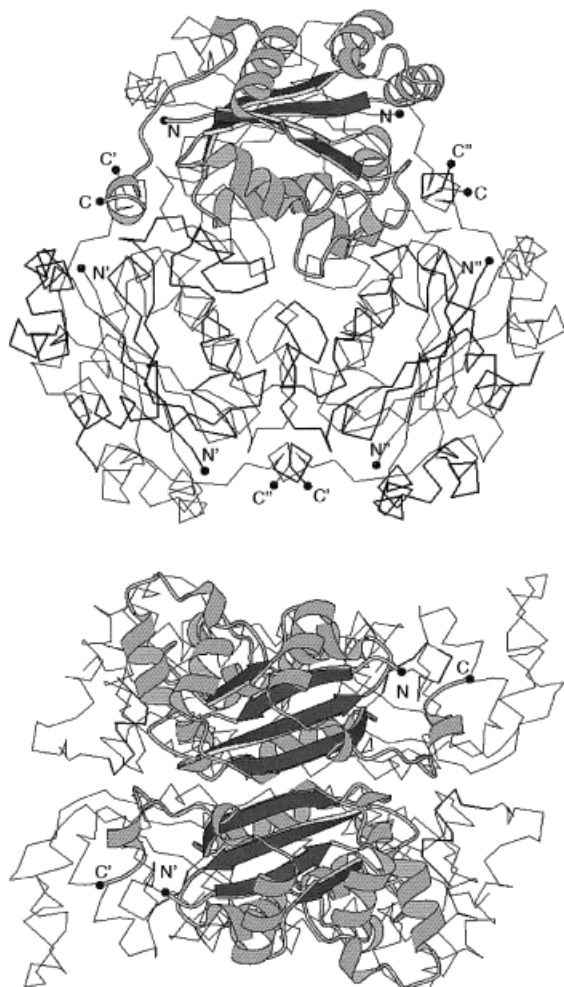


Fig. 1. NDK-A hexamer viewed along the threefold axis (**top**) and a twofold axis (**bottom**). Subunits are shown in α traces, except one (top) or two subunits (bottom) drawn in ribbon representation.

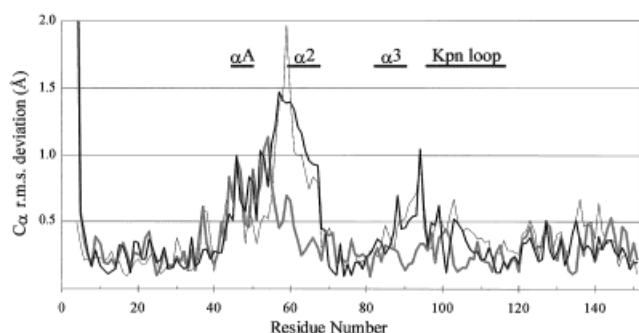


Fig. 2. RMSD plot (gray thick line, between uncomplexed NDK-A and -B; black line, between uncomplexed NDK-A and GDP-complexed NDK-B; gray thin line, between uncomplexed NDK-B and GDP-complexed NDK-B). A pair of hexamer structures are superimposed for 149 common α atoms and RMS distances for six subunits are averaged at each residue position.

residues (G37A and L38M), both located in the strand β_2 , of the 18 different residues (at positions 4, 37, 38, 41, 42, 46, 47, 50, 53, 62, 69, 124, 131, 135, 143, 147, 148, and 150) between isoforms A and B are involved in subunit contacts

in the dimer. The structural changes derived from them are small enough to accommodate heterohexamers in different ratios, suggesting that the subunit interactions in two homohexamers are essentially identical.

Despite high similarities in sequence and structure, NDK-A and NDK-B exhibit strikingly different cellular functions. Although NDK-B binds to a purine-rich sequence within the promoter of *c-myc* oncogene, NDK-A has no such property. Sixteen different residues between two isoforms, except the two involved in dimer interactions, are mainly located in the surface helices and the C-terminal extension. They are exposed on the surface of the hexamer, and six of them result in a change of net charge in the circumference of the hexamer. As a result, the surface nature of these regions of NDK-B is basic in contrast to the acidic character of NDK-A. This could explain the difference in DNA-binding property. It also suggests that DNA could bind to the side of the NDK-B hexamer.

The function of NDK-A as a metastasis suppressor is related with its histidine protein-kinase activity.¹ Biochemical studies of the S120G mutation found in aggressive neuroblastoma and the P96S mutation equivalent to the Kpn mutation of the *awd* gene, a *Drosophila* NDK homolog, showed that these residues are essential for the motility-suppression effect of NDK-A. Although the wild-type completely suppresses any cell movement and transfers the phosphoryl group to an aspartate residue on a 43-kDa protein, both mutants do not show such a wild-type effect.^{5,6} The differences are the absence of histidine-kinase activity of both mutants. Pro96 located near the threefold axis is involved in the trimer interaction, and Ser120 is in proximity to the catalytic His118; both residues are not solvent accessible in the hexamer. These mutants are known to alter the folding and stability of the protein in response to denaturation by heat and urea.^{7,8} Thus, these mutations are likely to affect the recognition of substrate molecules or the stability of protein complexes in a signaling pathway.

Conclusions. The structural differences that originated from two different residues in the subunit interface of human NDK-A and NDK-B are small enough to allow formation of heterohexamers. The differences in electrostatic surface potential and in DNA-binding properties between the two isoforms suggest the location of DNA binding site in NDK-B. The characteristic function of NDK-A as a metastasis suppressor may be related with its distinct surface features, which could possibly have an effect on recognition of substrates for its histidine protein-kinase activity and on formation of protein complexes.

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