

Crystal Structure of Nucleoside Diphosphate Kinase from Rice

Nucleoside diphosphate kinase (NDK) is a ubiquitous enzyme found in all organisms and cell types, including human. We found that NDK is involved in and required for coleoptile elongation during seed germination and the early stages of seedling growth. The NDK structure of rice, also the first structure from plant systems, provides the structural information needed to understand the functional significance of this enzyme during growth and development in rice as well as other plants.

Nucleoside diphosphate kinase (NDK) can catalyze the γ -phosphate of nucleoside triphosphate to transfer onto β -phosphate of other nucleoside diphosphate. The reaction step can be described as below (Parks *et al.*, 1973):



In this reaction, NDK~P is the intermediate species and the phosphorylation of histidine residue in activity site of the NDK embarked for the most part of phosphate transfer process. In most other ATP-dependent kinase phosphorylation mechanisms, the accepters snatch the γ -phosphate of donors directly, which is very different from above two-step ping-pong mechanism of NDK (a) (b). NDK functions efficiently and has the K_{cat} value of the order of 10^3 s^{-1} which implies that each step takes place at a time scale of less than 1ms. Another characteristic of NDK is its none-specificity: the phosphate donor N_1TP and acceptor N_2TP can be any of nucleotide or deoxynucleosides. Generally, ATP plays the role of donor in reaction (a), and the reaction (b) can produce all other (deoxy)nucleoside triphosphates ((deoxy)NTPs) needed for RNA and DNA synthesis, such as GTP and dATP. NDKs are ubiquitous in all organisms and all cell types, where they have a major part in maintaining the pool of intracellular NTPs.

Previous research results have shown that NDK participates in photoreceptor operation in fungi and plant systems, the coleoptile elongation in rice, the microorganism growth and signal transduction. A plant periodical indicated an up-regulation of NDK gene by wounding in tomatoes, and sugarcane NDK may be modulated by heat shock. Recently, an experiment showed that transferring antisense NDK gene into rice decreased

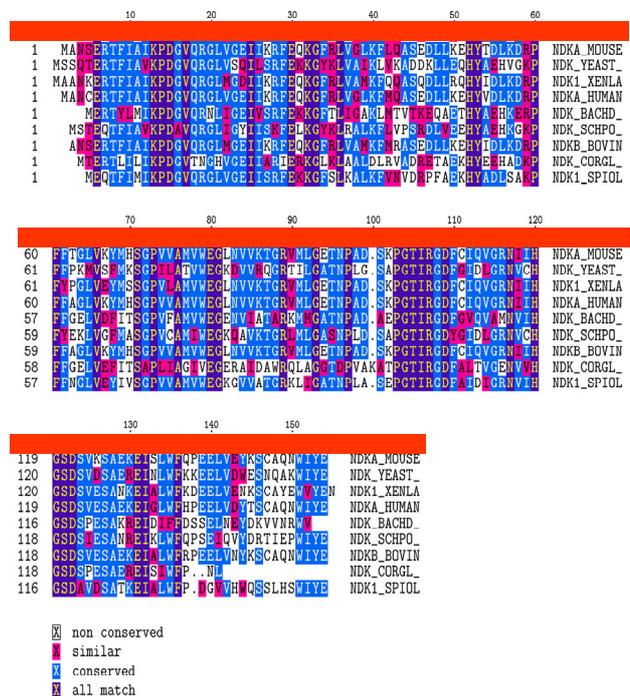


Fig. 1: Sequence alignment and comparison of NDKs from different species. From the top to bottom: rice, mouse, yeast, bovine, human, *Bacillus halodurans*, Fission yeast, bovine, *Brevibacterium flavum*, and spinach.

expressing amount of NDK by suppressing mRNA of NDK. The result exhibited the NDK-suppressed rice are shorter than normal one, which implied NDK is related to rice development. Analysis of coleoptile elongation, NDK enzyme activities, and the cell size in non-transformants and anti-NDK plants showed that the cell elongation process was predominantly inhibited in epidermal cells of coleoptile in antisense plants. This is the first evidence to show that the gene engaged in nucleotide homeostasis is responsible for cell elongation process in higher plants. Cell elongation mediated by phytohormones and/or phytochrome

has also been extensively studied. It was assumed that receptor stimulated NDK contributed to the mediation of hormone action produce GTP for activation of GTP-binding protein. This process is considered as an important step of mediating of cell physiology. It is also possible that NDK interacts with G-protein according to our previous study on microorganism system despite of only 48% sequence homology between microorganism and rice.

For the process of rice sprouting, a special phenomenon is observed that the rice can still sprout restrictedly for growth of leaf sheath under anaerobic conditions in which the rice plant is only capable of carrying out molecular respiration; therefore, the production of ATP molecules will be very limited. Thus, NDK must be very active in generating NTP to supply other necessary physiological activities. Although Several NDK genes have been cloned in plants, such as spinach, tomato, pea, oat, and rice, the structural-functional relationship of this protein during plant growth and development remains puzzling, especially for coleoptile elongation during seed germination and the early stages of seedlings growth. In this paper, we present the X-ray crystal structure of NDK with a molecular weight of 17 kDa from rice at 2.5 Å resolution to further understand the biological significance of NDK in rice development on the structural basis. To be our knowledge, it would be the first crystal structure of nucleoside diphosphate kinase from the plant system.

Overall fold of the NDK monomer consists of a four-stranded β -sheet whose surfaces are partially covered by six α -helices, which is based on $\alpha\beta$ sandwich fold. The α/β area contains four anti-parallel β sheets and two connected α helices. In the respect of topology, the order of this four anti-parallel sheet is $\beta_2\beta_3\beta_1\beta_4$, and α_1 , α_3 connect with β_1 , β_2 and β_3 , β_4 , respectively. This basis $\beta\alpha\beta\beta\alpha\beta$ -like structure is called $\alpha\beta$ sandwich. The $\beta\alpha\beta\beta\alpha\beta$ notion emphasizes that the folding consists of two $\beta\alpha\beta$ which are internal symmetric. In rNDK two β -sheet are parallel in $\beta\alpha\beta$ unit and antiparallel in each $\beta\alpha\beta$. Beside the $\alpha\beta$ sandwich, rNDK contains other reprehensive structures: αA - α_2 hairpin, α_4 kpn loop and external C-terminal, which dominate about 60 amino acids. αA - α_2 hairpin and α_4 kpn loop are involved in enzyme activity and will be discussed later. In general, the polypeptide chain starts with a disordered N-terminal residue in the order of $\beta_1\alpha_0\alpha_1\beta_2<\alpha A-\alpha_2>\beta_3\alpha_3<\alpha_4>\beta_4\alpha_5$, where $<>$ is for differentiation from $\alpha\beta$ sandwich (Fig. 2). Furthermore, superimposed twelve molecules in the asymmetric unit show that, except for the N- and C-terminal

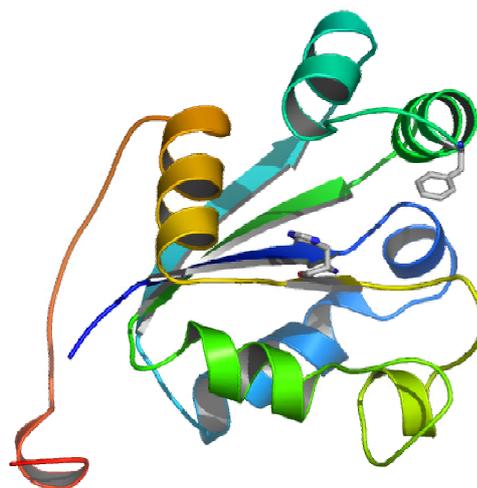


Fig 2: Ribbon drawing of NDK monomer structure. The active sites His116 and Phe58 (in ball-and-stick) are located on β_4 and αA , respectively.

deviations, all αA - α_2 area has more structural variations and higher temperature factors indicating the high flexibility.

The sequence alignment and comparison show the rice NDK has highly conservation and similarity among those from other species (Fig. 2). The NDK active site residues are highly conserved based on all the NDK sequence alignment and the complex structure of substrate and NDK from *Myxococcus xanthus* and *Dictyostelium discoideum*. The amino acids which involve the NDK and substrate interaction are conserved in all known NDK complex and in rice NDK sequence. Analysis of known NDK complex and rice NDK structures shows that their αA - α_2 regions all have higher temperature factors, which implies that this area is flexible for substrate entrance. Superimposed structures of rNDK and known NDK-substrate complex illustrate the pseudo-modeling active site with similar orientations of critical conserved amino acids of the enzyme (Figure 3). It is very likely that the active site of rNDK would be also at the similar location between αA - α_2 region and kpn loop among these homologous structures, i.e., the conserved active site residues His116 and Phe58 located on β_4 and αA , respectively (Figure 2). Moreover, the nucleotide-binding site in a cleft between the head and Kpn loop form a highly positive potential area (Figure 3).

There are twelve NDK molecules in one asymmetric unit of crystals, in which two hexamers interact with each other (Fig. 4). Each hexamer has a non-crystallographic 2-fold symmetry locating between the two plate-alike trimers and non-

crystallographic 3-fold symmetry lying in the central of three dumbbell-like dimers. Intermolecular interactions that form a trimer involve mainly the residues of the C-terminal region and the Kpn loop. These 2- and 3-fold symmetry axes are perpendicular with each other. Although NDK sequences in different species are highly conservative, there are various in their tertiary structures. Five crystal structures of NDKs from eukaryotes were previously determined, including those of *D. discoideum*, *Drosophila*, and human, as well as those from the prokaryotes *M. xanthus* and *Escherichia coli*. Recently, the first structure of an NDK from *Bacillus halodenitrificans*, a moderate

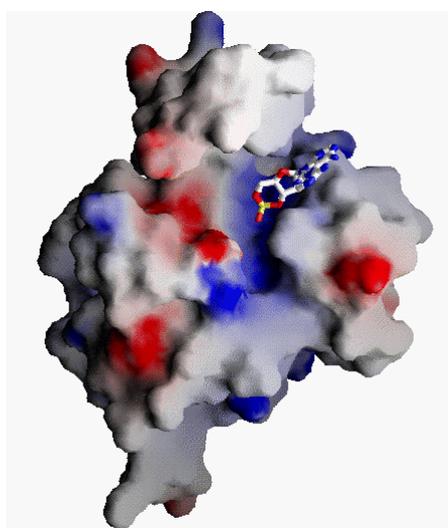


Fig. 3: The molecular surface of the enzyme is colored according to the electrostatic potential. The darkest blue denotes a potential of $15 \text{ Kcal mol}^{-1} \text{ atomic charge unit}^{-1}$ and the darkest red denotes a potential of $-15 \text{ Kcal mol}^{-1} \text{ atomic charge unit}^{-1}$. The modeling ADP-binding site is in a highly positive potential cleft between the head (αA - $\alpha 2$) and Kpn loop.

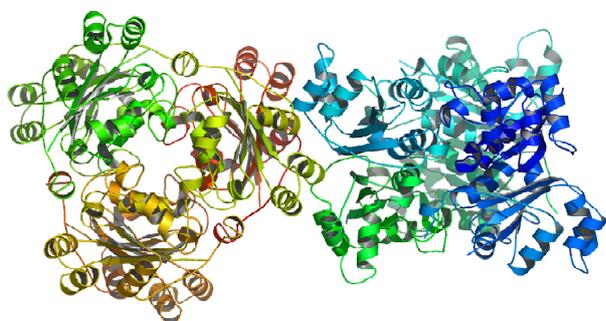


Fig. 4: Twelve NDK molecules packing in asymmetric unit: two hexamers interact with each other. The hexamer consists of three dimers with crystallographic 3-fold axes perpendicular to 2-fold axes.

halophile and Gram-positive bacterium, was determined as a hexamer. Interestingly, among these structures the prokaryotes NDKs all form tetramers, while the enzymes from the eukaryotes, which are 5-7 residues longer at the C-terminus extending out to the other monomer of dimer, have been shown to be hexamers instead of tetramers. However, both hexameric and tetrameric molecule packing have the similar dimeric interaction even though the overall packing is very different.

Molecular packing shows in the asymmetric unit that two hexamers are not perpendicular each other. The rice NDK is the first NDK structure to show a large number (more than 6) of enzyme molecules packing in the crystal. Inspection of the contact distance within 4 \AA shows that the amino acids from 134 to 140 involve the major close contacts between these two different-orientated hexamers. Coincidentally, those residues in rice also present the major structural difference from other NDKs in various species, indicating the strong relevance to 12-mer formation.

BEAMLINES

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EXPERIMENTAL STATION

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