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Title : Structure of Human Nucleoside DiPhosphate Kinase mutants
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The human NDPK-A : Nucleoside DiPhosphate Kinase isoform A (IBGC):
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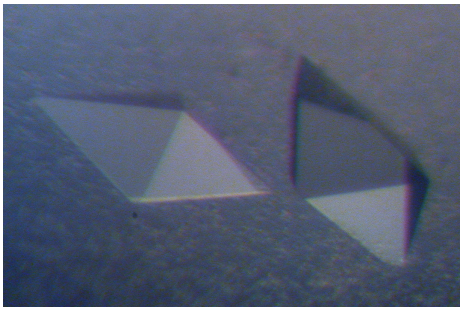
Nucleoside diphosphate kinase (NDPK) catalyses the transfer of the γ-phosphoryl group from a nucleoside triphosphate donor to a nucleoside diphosphate acceptor. The biological entity is a hexamer of 102 kDa. At least, two human NDP kinases have been identified :

- NDPK-A, product of the human *nm23-H1* gene, acts as a suppressor of metastasis for some tumour types.
- NDPK-B, product of the human *nm23-H2* gene, is both an enzyme and a transcription factor. It activates transcription of the *c-myc* oncogene independently of its catalytic function, by binding on the *c-myc* promoter.

The 3D structure of the NDPKs is well known (1 for a review). Recently, the structures of the wild type without bounded nucleotide (2) and of a double mutant H118G/F60W in complex with ADP, phosphate and calcium ions (3) of the human NDPK-A have been reported.
The Serine 120 is strictly conserved in all prokaryotic and eukaryotic NDP kinases. A serine to glycine mutation has been identified in several aggressive neuroblastomas. Biochemical studies of this S120G mutation showed that this residue is essential for the motility suppression effect of NDPK-A. Ser 120 is in proximity to the catalytic His118 and interacts with the Glu129 involved in the stabilisation of the His118.
The S120G mutant is almost as active as the wild-type enzyme, but its stability to denaturation is decreased. Lascu (4) showed that altered folding properties of the recombinant S120G mutant protein led to the accumulation of a molten globule intermediate.
To investigate the structural consequence of the S120G mutation, we have over-expressed the S120G mutant and the wild type versions of the human NDPK-A in *E. coli*, and purified the two proteins. Crystals were obtained for the two proteins in complex with ADP. Here, we present the two structures and their comparisons. Surprisingly, no significant changes were observed on the overall structure and in the neighbourhood of the mutation.

Crystallization



The wild-type and the S120G mutant were expressed using pET-21 vectors, and purified at 277 K. Crystals of wild type and S120G proteins were obtained by the sitting drop vapor diffusion method at 293 K. Bipyramidal shaped crystals appeared 1 day after mixing 2 µl of protein sample (protein 15 mg/ml, DTT 4 mM, ADP 10 mM, $MgCl_2$ 20 mM, Tris/HCl 20 mM pH 7.5) with 2 µl of reservoir solution (ammonium sulphate 2.4 M, MES 100 mM pH 6.0). Crystals were cryo-protected by adding 20% glycerol (v/v) to the mother liquor and flash-frozen in liquid nitrogen.

Data collection

A first X-ray diffraction dataset of the S120G variant collected at lab at 2.6 Å resolution was completed at 2.4 Å resolution at beamline BM30a of the ESRF (Grenoble, France). Data of

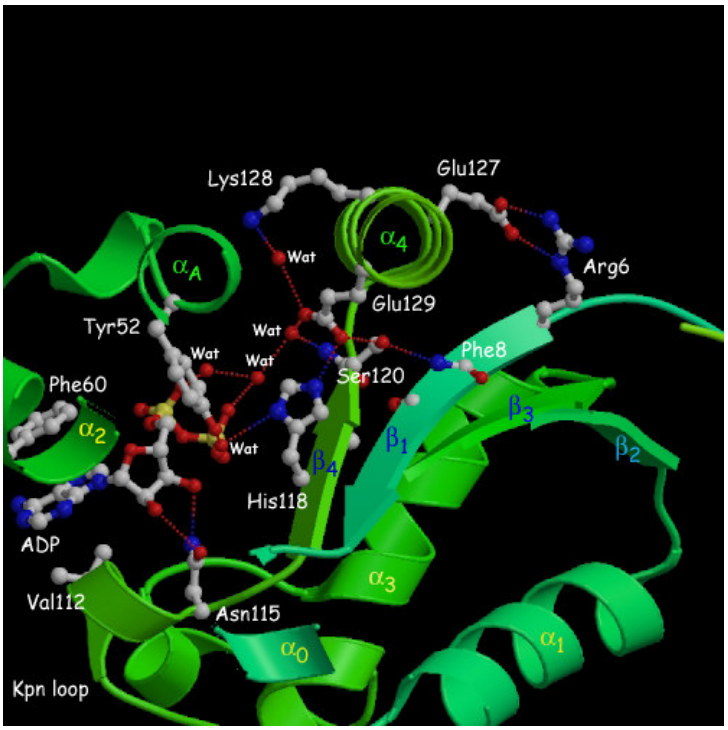
the wild type protein were collected from a single crystal at beamline ID29 of the ESRF. All the data were collected at 100 K and processed using MOSFLM and CCP4 program suite. Data collection and refinement statistics are gathered in table 1. The structure was solved using the trimer of the H118G/F60W variant (pdb 1UCN) of NDPK-A which crystallizes in the same space group and with similar unit cell parameters. Crystallographic refinement and model rebuilding were carried out using REFMAC and XtalView respectively.

Model

In the two structures (5), the asymmetric unit is made of a trimer, the three protomers are labelled A, B and C. The final model includes amino acids 2−152, with main and side chains unambiguously defined in the electron density except for residues around the catalytic site of protomer A. The N-terminal Met is missing in the *E. coli* recombinant protein. The hexamer can be generated by applying the 2 fold crystallographic operator (1-y, 1-x, ½-z) later noted #. The backbone dihedral angles of all of the non-Gly residues in each monomer, excepted Ile116, fall in the allowed regions of the Ramachandran plot. The refined models have been deposited at the Protein Data Bank.

The strong similarities between the two structures are in agreement with measured NDP kinase activities and indicate that the S120G specificities cannot be attributed to NDP kinase activity. These data do not allow to understand why this serine is a conserved residue and suggest that the S120G mutation may affect an other property of this multifunctional protein like interaction with other proteins or DNA. The S120G mutant characteristics could also be explained by the existence of a folding intermediate.

(1) Janin J, Dumas C, Morera S, Xu Y, Meyer P, Chiadmi M, Cherfils J. (2000) Three-dimensional structure of nucleoside diphosphate kinase. J Bioenerg Biomembr., 32(3) 215-25.
(2) Min K, Song HK, Chang C, Kim SY, Lee KJ, Suh SW. (2002) Crystal structure of human nucleoside diphosphate kinase A, a metastasis suppressor. Proteins., 46(3) 340-2.
(3) Chen Y, Gallois-Montbrun S, Schneider B, Veron M, Morera S, Deville-Bonne D, Janin J. (2003) Nucleotide binding to nucleoside diphosphate kinases: X-ray structure of human NDPK-A in complex with ADP and comparison to protein kinases. J Mol Biol., 332(4) 915-26.
(4) Lascu I, Schaertl S, Wang C, Sarger C, Giartosio A, Briand G, Lacombe ML, Konrad M. (1997) A point mutation of human nucleoside diphosphate kinase A found in aggressive neuroblastoma affects protein folding. J Biol Chem. 272(25) 15599-602.
(5) Giraud MF, Georgescauld F, Lascu I Dautant A (2006) Crystal structures of S120G mutant and wild type of human Nucleoside Diphosphate Kinase A in complex with ADP. Submitted to Journal of Bioenergetics and Biomembranes.



Slab view of the crystal structure of wild type of human NDPK-A in complex with ADP. Only some water molecules in the neighbourhood of the active site are added.

Table 1 Data collection and refinement statistics

	S120G	Wild Type
Data collection		
Space group	$P4_12_12$	$P4_12_12$
Cell dimensions		
a, *b*, *c* (Å)	114.82, 114.82, 89.72	114.26, 114.26, 89.79
α, β, γ (°)	90.00, 90.00, 90.00	90.00, 90.00, 90.00
Resolution (Å)	40.0-2.40 (2.53-2.40) *	18.0-2.15 (2.27-2.15) *
R_{sym}	0.10 (0.40)	0.08 (0.52)
$I / \sigma(I)$	21.4 (4.0)	10.2 (2.1)
Completeness (%)	97.1 (86.7)	97.2 (99.0)
Redundancy	10.9 (8.4)	3.2 (3.1)
Refinement		
Resolution (Å)	25.0 -2.40	18.0 – 2.15
No. reflections	22088	30827
R_{work} / R_{free}	0.21 / 0.28	0.24 / 0.29
No. atoms		
Protein	3588	3594
ADP	81	81
Water	151	190
B-factors		
Protein (Å^2)	51.43	46.18
ADP (Å^2)	76.62	50.01
Water (Å^2)	51.68	47.69
R.m.s deviations		
Bond lengths (Å)	0.010	0.010
Bond angles (°)	1.27	1.30

*Highest resolution shell is shown in parenthesis.