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ORIGINAL PAPER

Crystal Structures of S120G Mutant and Wild Type of Human Nucleoside Diphosphate Kinase A in Complex with ADP

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Abstract *Nm23* was the first metastasis suppressor gene identified. This gene encodes a NDP kinase that also exhibits other properties like histidine protein kinase and interactions with proteins and DNA. The S120G mutant of NDPK-A has been identified in aggressive neuroblastomas and has been found to reduce the metastasis suppressor effect of *Nm23*. In order to understand the differences between the wild type and the S120G mutant, we have determined the structure of both mutant and wild type NDPK-A in complex with ADP. Our results reveal that there are no significant changes between the two enzyme versions even in the surroundings of the catalytic histidine that is required for NDP kinase activity. This suggests that the S120G mutation may affect an other protein property than NDP kinase activity.

Keywords Nm23-H1 · NDPK-A · S120G · Crystal structure

Abbreviations

NDPK:	nucleoside diphosphate kinase
NDPK-A:	nucleoside diphosphate kinase isoform A
NDP:	nucleoside diphosphate
ADP:	adenosine diphosphate
rmsd:	root mean square deviation. Human NDPK-A numbering was used

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Introduction

Nm23 was the first metastasis suppressor gene identified. At least four catalysis and regulatory functions have been attributed to proteins of this gene family (Ouatas et al., 2003) (i) NDP kinase activity via its catalytic histidine, (ii) serine or histidine protein kinase activities, (iii) interactions with several proteins and (iv) interaction with DNA (Ma et al., 2002). Despite numerous trials, the biochemical mechanism whereby Nm23 protein family suppresses tumor metastasis remains unknown.

A serine 120 to glycine (S120G) mutation of Nm23-H1 (NDPK-A) has been identified in several aggressive neuroblastomas (Chang et al., 1994). Over-expression of wild type Nm23-H1 suppressed motility in vitro, but S120G site-directed mutant recombinant protein failed to inhibit motility (Mc Donald et al., 1996). The Ser120 is strictly conserved in all prokaryotic and eukaryotic NDP kinases because Ser120 seems directly involved in the catalytic mechanism stabilizing the Glu129 that interacts with the catalytic His118 (Tepper et al., 1994). Biochemical studies showed that this mutant S120G retained about 50% NDP kinase activity of the wild-type enzyme and its stability towards denaturation is decreased (Chang et al., 1996). The altered folding properties of the recombinant S120G mutant protein leading to the accumulation of a molten globule intermediate (Lascu et al., 1997) could suggest that the 3D structure of the mutant may be also altered. The 3D structure of the NDPK family is well known (Janin et al., 2000). Recently, the structures of the wild type without bounded nucleotide (Min et al., 2002) and of a double mutant H118G/F60W in complex with ADP, phosphate and calcium ions (Chen et al., 2003) of the human NDPK-A were reported. 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.

Experimental

Crystallization

The wild-type and the S120G mutant were expressed using pET-21 vectors, and purified at 277 K as described elsewhere (Lascu et al., 1997; Mocan et al., 2006). 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

First X-ray diffraction data of the S120G variant were collected at 2.6 Å resolution using a Rigaku RU300 rotating-anode source operating at 50 kV and 90 mA and a Mar-Research 345dtb image-plate detector. This data set was completed by a second one 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 (Leslie, 1992) and CCP4 program suite (CCP4, 1994). 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 (Chen et al., 2003). Crystallographic refinement and model rebuilding were carried out using REFMAC (Murshudov et al., 1997) and XtalView (MacRee, 1999) respectively. Figure was drawn using MOLSCRIPT (Kraulis, 1991).

Atomic coordinates

In the two structures, 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 $(1-y, 1-x, 1/2-z)$ 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.

Results and discussion

The rms deviations of the Cα atoms between chains are less than 0.4 Å for both proteins with the maximum deviations occurring within the α_A–α_2 helices. When the wild type and the S120G Cα were fitted, the rmsd was 0.35 Å. For the wild type, the rmsd between the ADP complex and the nucleotide free (pdb 1JXV) structures was 0.56 Å with the most important 2 Å movement located again in the loop between α_A and α_2.

The mean temperature factors are lower for protomer B (36.1 $Å^2$) than protomer C (45.0 $Å^2$) and than protomer A (57.5 $Å^2$). They are obviously correlated with more or less well defined electron densities. Thus protomer B was taken as the reference. Such differences have already been observed in human Nm23-H3 (pdb 1ZS6) and NDPK-A H118G/F60W variant (Chen et al., 2003) both in complex with ADP. They cannot be explained by crystal contacts or a too high symmetry space group ($P4_12_12$ instead of $P4_1$) but could be the consequence of slightly lower occupancy of ADP into the binding site of protomer A. These temperature factor variations were not observed for the apo protein due to high NCS restrains (Min et al., 2002). Although Mg^{2+} was present in the crystallization solution, only weak density peaks were observed in the vicinity of α and β phosphate groups of ADP where such cations are found in available NDP complex structures. These peaks were attributed to water molecules.

The hydrogen bond between the Oγ atom of Ser120 and the backbone nitrogen atom of Phe8 (3.13 Å) strengthens the antiparallel β sheet at the beginning of strand β_1 and the end of strand β_4. One of the Oε atom of the Glu129 is coordinated (i) to the hydroxyl group of Ser120 (2.55 Å) and (ii) to the Nε of the catalytic His118 (2.77 Å). These two interactions clearly occur above and below out of the carboxylate plane (2.3 and 1.9 Å respectively). Although this oxygen atom lies close to the His128 imidazole plane (0.3 Å), the carboxylate and imidazole planes make an angle of about 60°. In all available X-ray structures of NDPKs, the conserved Glu129 belonging to helix α4 adopts always the same very favoured gauche$^-$/trans/trans rotamer conformation. This interaction could stabilize the His118 sidechain which adopts in the two structures a slightly unfavourable conformation (χ_1 of about 155°).

On the other hand, the other Oε atom is hydrogen bonded with two water molecules (2.5 and 2.8 Å) lying close to the carboxylate plane (0.4 and 0.37 Å respectively). These two

Table 1 Data collection and refinement statistics

Data collection	S120G	Wild type
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)[a]	18.0–2.15 (2.27–2.15)[a]
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

[a]Highest resolution shell is shown in parenthesis.

water molecules belong to a hydrogen bond network. The first one interacts directly with backbone NH of Asp121 and via water molecules with Asp121 carboxylate group and ADP phosphate group, the second one with Nζ of Lys128. This binding scheme indicates the preferential localization of the negative charge on the oxygen atom interacting with the Nε nitrogen of His118.

In the two structures, the ADP binding interactions in the cleft and the conformation of the active site are similar. This is in agreement with the close measured activities. The most significant difference we observed between wild type and S120G structures is a shift at Ala126 (0.4/0.6 Å for $C_{\alpha/\beta}$ respectively) towards Gly120 in order to partially fill the void left by the substituted Ser120 sidechain (Fig. 1). Subsequently this displacement induces the move of about 0.3 Å on C_α positions of two solvent accessible glutamic acid side chains (Glu124 and Glu127). At the beginning of the helix α4, the hydroxyl group of Ser122 and Ser125 are hydrogen bonded with the exposed backbone NH of Ser125 and with the carboxyl group of Asp121 respectively.

Each NDPK-A subunit contains 3 cysteine residues. Within a hexamer, there are three putative disulfide bridges between two symmetry related Cys145 from chain A to B$^\#$; chain B to A$^\#$ and chain C to C$^\#$ ($d_{C\beta-C\beta} = 5.31$ and 5.10 Å respectively). Cys145 belongs to the loop before the C_{term} one turn helix. At the N-terminal extremity, the Cys4 are solvent accessible and could have formed, in the crystal, a disulfide bridge between two symmetry related ($y-1$, $1+x$, $-z$) Cys4 of chain A of a neighbouring hexamer. Surprisingly, it was the buried Cys109 from Kpn loop that has been identified by mass spectrometry after trypsic digestion as forming disulfide bridge under oxidative stress (Song et al., 2000).

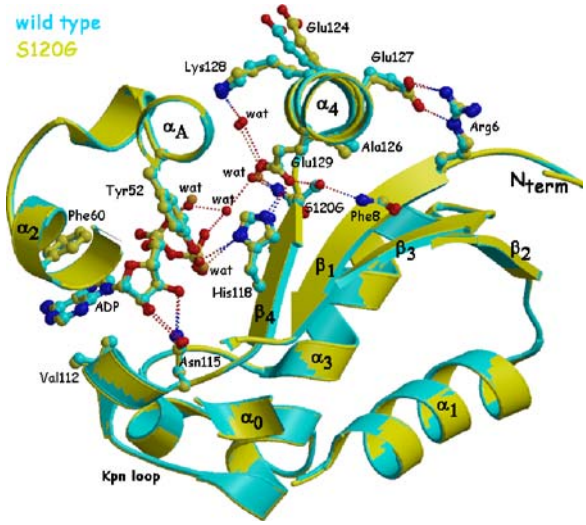

Fig. 1 Slab view of the crystal structures of wild type (cyan) and S120G mutant (yellow) of human NDPK-A in complex with ADP. Only the water molecules in the neighbourhood of the mutation are added and coloured in red and orange for the wild type and the S120G respectively.

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 as suggested in a companion paper (Lascu I., this issue).

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