

Histidine 117 in the His-Gly-Ser-Asp motif is Required for the Biochemical Activities of Nucleoside Diphosphate Kinase of *Mycobacterium smegmatis*

Muthu Arumugam and Parthasarathi Ajitkumar*

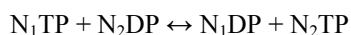
Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore-560012, India

Abstract: Nucleoside diphosphate kinase (NDK), which is widely conserved in both prokaryotes and eukaryotes, maintains a balanced pool of nucleotide triphosphates and their deoxy derivatives. NDKs from bacterial and other systems contain the conserved HGSD motif, where the His residue is required for the biochemical activities, namely the NTPase (ATPase and GTPase), NTP synthesising, and autophosphorylation activities of the enzyme. Amino acid sequence homology comparison of the NDK of *Mycobacterium smegmatis* (MsmNDK) with the NDKs of other bacterial genera showed the presence of H₁₁₇GSD motif. While the recombinant wild type MsmNDK showed the NTPase, NTP synthesising, and autophosphorylation activities, the H117Q mutation abolished the biochemical activities of the recombinant MsmNDK-H117Q mutant protein *in vitro*. These observations demonstrate that the H117 residue in the HGSD motif is required for the biochemical activities of MsmNDK.

Keywords: Nucleoside diphosphate kinase, *M. smegmatis*, Autophosphorylation, ATPase, GTPase, NTP synthesising activity.

INTRODUCTION

Maintenance of the level of nucleoside triphosphates (NTPs) as well as their corresponding deoxy derivatives (dNTPs) is crucial to all growth and developmental processes. The enzyme nucleoside diphosphate kinase (NDK), first discovered simultaneously by Krebs and Hems [1], and Berg and Joklik [2], utilises an autophosphorylated enzyme to mediate the transfer of 5' terminal phosphate from NTPs (mostly ATP) to nucleoside diphosphates (NDPs or their deoxy derivatives), to generate the respective NTPs, *via* a reversible mechanism, as given below [3-7].



Thus, the mechanism implies that NDK is a substrate non-specific enzyme [4-8], which can remove the gamma phosphate from ATP or GTP to show ATPase and GTPase activities, transfer the removed phosphate to NDPs to generate NTPs (NTP synthesising activity), through the phosphorylation of the His residue in the conserved HGSD motif (autophosphorylation activity) on the enzyme [8-10].

The primary function of NDK is to maintain NTP and dNTP concentrations, as NDK deficiency in *Escherichia coli* leads to nucleotide pool perturbation [4, 11], resulting in the incorporation of inappropriate nucleotides into DNA [11, 12]. *E. coli* NDK was also found to possess uracil-DNA glycosylase, AP-lyase, and 3'-phosphodiesterase activities *in vitro* [13]. Similarly, the human NDK homologues, NDK1, NDK5, NDK7 and NDK8 proteins, were found to possess 3'-5' exonuclease activity [14]. It has a role in the growth and differentiation in *Mycococcus xanthus* [15] and

Pseudomonas aeruginosa [9], and in tumor metastasis suppression in humans [16]. NDK in association with succinyl CoA synthetase in *P. aeruginosa* [17] and with pyruvate kinase, EF-Tu, and a cell wall protein in *Mycobacterium smegmatis* [18] synthesises specific NTP, required for the activity of the associated protein. In *Drosophila melanogaster*, NDK is involved in wing disc development [19]. The ATPase activity of the secreted form of *Mycobacterium tuberculosis* NDK (MtuNDK) causes cytotoxicity to macrophages [20]. MtuNDK was found to block phagosome maturation in murine Raw 264.7 macrophages [21]. In the present study, as the first step towards understanding the physiological roles of NDK in mycobacteria, we present evidence that the His residue in the His-Gly-Ser-Asp is required for all the biochemical activities of *Mycobacterium smegmatis* NDK *in vitro*.

MATERIALS AND METHODOLOGY

Bacterial Strains and Culture Media

E. coli JM109 and *E. coli* M15 cells were used for the propagation of recombinant clones and for the overexpression of protein, respectively. Luria broth with ampicillin (100 µg/ml) or kanamycin (25 µg/ml) was used for the selection and culturing of recombinant clones.

Cloning of *Msmndk* Gene

Full-length *Msmndk* gene (MSMEG_4627) was cloned in pBS(KS) vector between KpnI and EcoRI sites, after PCR amplification from *M. smegmatis* mc²155 genomic DNA using Msmndk-f2 and Msmndk-r2 primers (Table 1). The resultant pBS-Msmndk clone was sequence verified. The *Msmndk* ORF was then subcloned into pQE30 expression vector between KpnI and PstI sites, to obtain pQE30-Msmndk construct.

*Address correspondence to this author at the Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore-560012, India; Tel: 91-80-22932344; Fax: 91-80-23602697; E-mail: ajit@mcbli.iisc.ernet.in

Generation of MsmNDK-H117Q

The MsmNDK-H117Q mutant was generated by overlap extension PCR, using *Msndk* ORF-specific primers and *Msmndk* mutagenic primers (Table 1). Using ORF-specific forward primer (Msndk-f2) and mutagenic reverse primer (MsNDK-H117Qr) a 360 bp amplicon and using the forward primer (MsNDK-H117Qf) and ORF-specific reverse primer (Msndk-r2), another product of 60 bp were generated. These two mega primers were again used for the PCR reaction under the same condition for 5 cycles to generate megaprimers. Subsequently, the ORF-specific forward (Msndk-f2) and reverse primer (Msndk-r2) were introduced into the reaction mixture. The PCR reaction was then continued for 25 more cycles, and the PCR product was cloned in pBS-KS between KpnI and EcoRI sites, to get pBS(KS)-MsmNDK-H117Q. The mutation was confirmed using DNA sequencing. The mutant *Msmndk* ORF was then subcloned into pQE30, between KpnI and PstI sites, to generate pQE30-MsmNDK-H117Q mutant clone. Both the recombinant proteins 6xHis-MsmNDK and 6xHis-MsmNDK-H117Q were overexpressed and purified from soluble fraction to near homogeneity using Ni²⁺-NTA agarose affinity chromatography. The protein preparations were dialysed against lysis buffer containing 10% glycerol. Dialysis was carried out at 4°C with three changes of 500 ml buffer to remove imidazole and other salts, and the protein was stored at -75°C.

Circular Dichroism (CD) Spectroscopy

Circular Dichroism spectroscopy experiments were carried out using spectropolarimeter (JASCO J-715) measuring CD values over 200-260 nm wavelength range at every 0.5 nm interval, in 2 mm path-length cuvette. A concentration of 8.93 µM protein (4.2 mg/ml) was used in 1 ml of 1 mM Tris-HCl (pH 8.0) and 5 mM KCl buffer, per assay at room temperature. The CD values were processed and plotted as graph using Microsoft Excel 2003. CD spectrum was expressed in terms of mean residual ellipticity [22].

ATPase and GTPase Assays

ATPase and GTPase assays were carried out, as described [8]. In brief, 1 µg of NDK was incubated at 25°C in 20 µl of TMD buffer (50 mM Tris-HCl, 10 mM MgCl₂ and 1 mM DTT, pH 7.4) with 0.1 µCi of [γ -³²P]-ATP (6000 Ci/mmol) or [α -³²P]-GTP (3000 Ci/mmol) for 15 min or 30 min. The reaction was stopped with a final concentration of 2% SDS and 1 mM EDTA. The samples were heated at 75°C

for 5 min, one µl of the reaction mixture was spotted on a polyethyleneimine cellulose thin layer chromatography plate (PEI-TLC, Merck), and resolved using 0.75 M KH₂PO₄, as described [23]. The radioactive spots corresponding to [γ -³²P]-ATP and ³²Pi or of [α -³²P]-GTP and [α -³²P]-GDP were then visualised using Phosphorimager BioImage Analyser (FLA 2000, Fuji, Japan). The regions corresponding to ³²Pi or [α -³²P]-GDP on TLC were cut and counted in liquid scintillation counter. The counts per minute (CPM) values were plotted as bar graph, with standard deviation.

Autophosphorylation Assay

Autophosphorylation activity of the purified wild type MsmNDK and mutant MsmNDK-H117Q proteins was measured, as described [17]. In brief, 1 µg each of the purified proteins was incubated with 0.1 µCi [γ -³²P]-ATP (6000 Ci/mmol) in 20 µl of TMD buffer (50 mM Tris-HCl, pH 7.4, 10 mM MgCl₂, and 1 mM of dithiothreitol). The reaction was terminated after 10 min with 2 µl of 10% SDS. The samples were boiled for 10 min, fractionated on 12% SDS-PAGE, and analysed using autoradiography. The radioactivity in the protein bands in the dried gel was quantitated using liquid scintillation counter. The counts per minute (CPM) values were plotted as bar graph, with standard deviation.

NTP Synthesising Activity

NTP synthesising activity of MsmNDK was determined, as described [18]. Assay was carried out in 20 µl of TMD buffer containing, 1 µg of MsmNDK or MsmNDK-H117Q, 250 µM each of CDP and GDP. The reaction was initiated with the addition of 0.1 µCi [γ -³²P]-ATP (6000 Ci/mmol), along with of 125 µM non-radioactive ATP. After incubation at 30°C for 10 min, the reaction was stopped with final concentration of 2% SDS and 1 mM EDTA. The samples were heated at 75°C for 5 min, and one µl each of the reaction mixture was analysed on polyethyleneimine cellulose thin layer chromatography (PEI-TLC) plates, in 0.75 M KH₂PO₄, as described [23]. The positions of ATP, CTP, and GTP were identified using the R_f values for these NTPs in the solvent system. The radioactive GTP or CTP were then visualised using Phosphorimager Bioimage Analyser (FLA 2000, Fuji, Japan). The radioactive spot corresponding to the unhydrolysed γ -³²P-ATP was cut and counted in both cases. The counts per minute (CPM) values were plotted as bar graph with standard deviation.

Table 1. Oligonucleotide Primers Used

Name of the Oligo Primer	Sequence of the Oligo Primer	For Cloning
Msndk-f2	<u>cg</u> gggtaccgtgactgagcggaccctctgactatcaagcc	MsNDK ORF cloning
Msndk-r2	ccggaattcggcggggcctcgccggg	MsNDK ORF cloning
MsNDK-H117Qf	caccgaggacaatctctg tcagg gttccgattc	MsNDK-H117Q cloning
MsNDK-H117Qr	ctcgggcgaatcgaacc gtc cacgagattg	MsNDK-H117Q cloning

Note: Restriction enzyme sites are underlined. The mutation introduced is given in bold nucleotide letters.

RESULTS

Conservation of His-Gly-Ser-Asp Motif in MsmNDK

All the NDKs, including those of *Mycobacterium tuberculosis* and *Mycobacterium leprae*, contain the conserved motif, His-Gly-Ser-Asp (HGSD). The activity of hitherto biochemically characterised NDKs was known to be dependent on the His residue in the HGSD motif [5, 6, 20, 24-29]. Homology comparison of MsmNDK protein sequence with

the amino acid sequence of NDKs of a variety of bacterial genera and other organisms showed the presence of H₁₁₇GSD (Fig. 1). The possibility of the involvement of H117 residue in the biochemical activities of MsmNDK was examined using the wild type and the mutant, MsmNDK-H117Q, proteins (Fig. 2A, B). Far-UV CD spectroscopy did not show any gross alteration in the overall secondary structure of the mutant (Fig. 2C).



Fig. (1). Multiple sequence alignment of NDK proteins from diverse bacterial genera (Invariant amino acid residues HGSD is highlighted and active site H is indicated in bold letter). Multiple alignments were carried out using clustalW (<http://www.ebi.ac.uk/clustalw>). EcN:NDK from *Escherichia coli*, PaN: NDK from *Pseudomonas aeruginosa*, MxN: NDK from *Myxococcus xanthus*, MIN: NDK from *Mycobacterium leprae*, MtN: NDK from *Mycobacterium tuberculosis* (H37Rv), MsN: NDK from *Mycobacterium smegmatis* (mc²155), DdN: NDK from *Dictyostelium discoideum*.

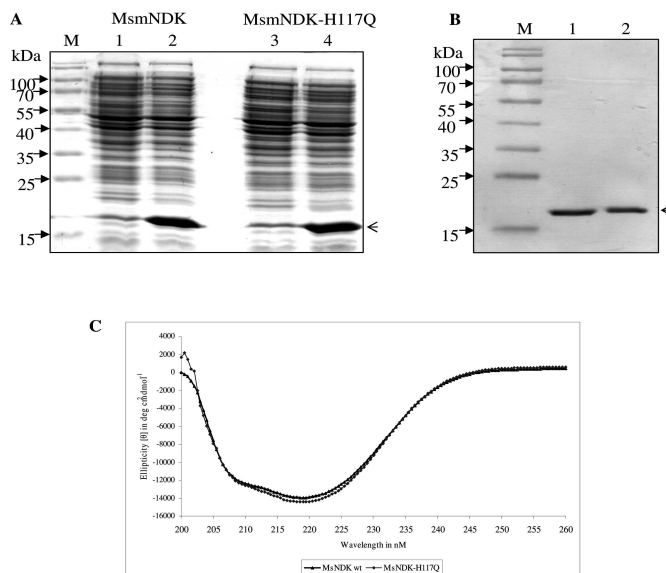


Fig. (2). Overexpression and purification profile of MsmNDK and MsmNDK-H117Q. **A.** Coomassie blue stained SDS-PAGE profile of overexpressed MsmNDK (2) and MsmNDK-H117Q (4). Lanes 1 and 3, respective uninduced samples. Arrow head indicates the overexpressed or purified protein. **B.** SDS-PAGE of recombinant purified MsmNDK wild type (1) and MsmNDK-H117Q (2) proteins; lane M represents molecular weight markers. **C.** Far-UV circular dichroism (CD) spectrum of MsmNDK and MsmNDK-H117Q.

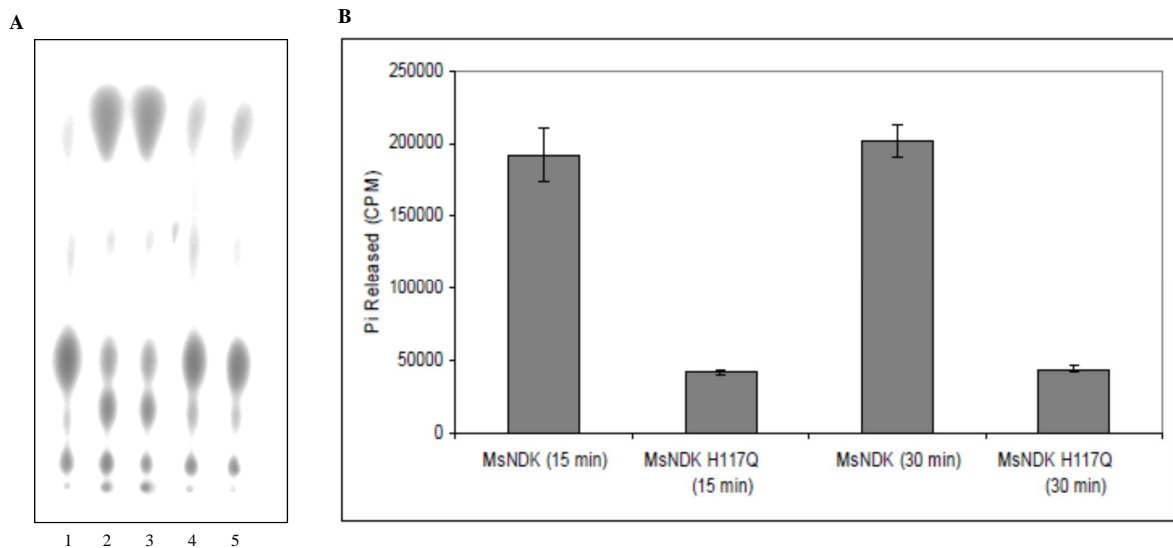


Fig. (3). ATPase activity of MsmNDK and MsmNDK-H117Q. **A.** Lane 1. ATP alone, Lane 2. MsmNDK at 15 min, Lane 3. MsmNDK at 30 min, Lane 4. MsmNDK-H117Q at 15 min, Lane 5. MsmNDK-H117Q at 30 min. **B.** Bar graph showing the ATPase activity of MsmNDK or MsmNDK-H117Q at 15 or 30 min. Assay was repeated independently three times for each sample. Counts per minute (CPM) were recorded and standard deviations were calculated.

ATPase, GTPase, and NTP Synthesising Activities

The ATPase and GTPase activities of the MsmNDK and MsmNDK-H117Q proteins were determined from the hydrolysis of γ - 32 P-ATP and α - 32 P-GTP. Quantitation of the 32 Pi released from γ - 32 P-ATP and the α - 32 P-GDP formed from α - 32 P-GTP by wild type MsmNDK and MsmNDK-H117Q, from the TLC profile, showed ATPase (Fig. 3A, B) and GTPase (Fig. 4A, B) activities by MsmNDK. In contrast, very low quantities of 32 Pi was released by MsNDK-H117Q, from γ - 32 P-ATP (Fig. 3A, B), and α - 32 P-GDP from α - 32 P-GTP (Fig. 4A, B), as compared to that by the wild type protein. The H117Q mutation abolished the ATPase and GTPase

activities to almost 4.75- and 2-fold, respectively. These observations confirmed that H117 is an essential residue required for the ATPase and GTPase activities of MsmNDK.

In the NTP synthesising activity, MsmNDK converted both GDP and CDP to GTP and CTP, respectively, using the 5' terminal phosphate from γ - 32 P-ATP (Fig. 5A). However, MsmNDK-H117Q showed negligible extent of conversion of GDP to GTP and CDP to CTP (Fig. 5A), showing that the H117Q mutation affected the activity (Fig. 5B). The almost complete abolition of ATP and GTP synthesis by the mutant indicated that H117 residue is essential for the NTP-synthesising activity.

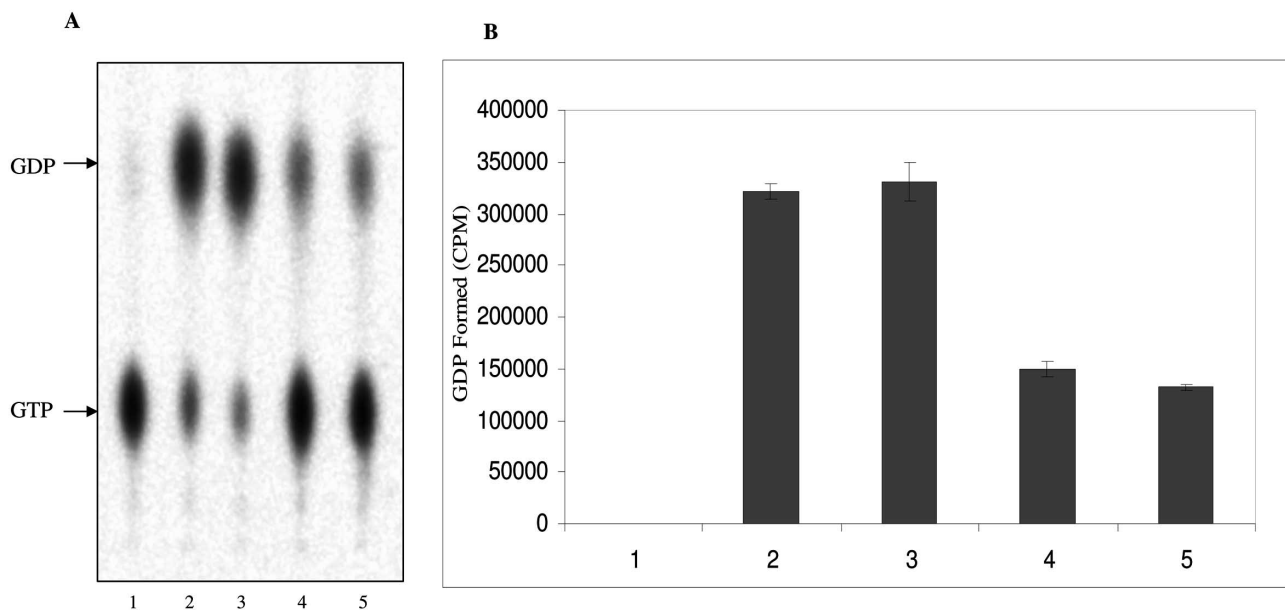


Fig. (4). GTPase activity of MsmNDK and MsmNDK-H117Q. **A.** Lane 1. GTP alone, Lane 2. MsmNDK at 15 min, Lane 3. MsmNDK at 30 min, Lane 4. MsmNDK-H117Q at 15 min, Lane 5. MsmNDK-H117Q at 30 min. **B.** Bar graph showing the GTPase activity in terms of α - 32 P-GDP formed by MsmNDK or MsmNDK-H117Q mutant at 15 or 30 min. Assay was repeated independently three times for each sample. Counts per minute (CPM) were recorded and standard deviations were calculated.

Autophosphorylation Activity

While transferring the γ -phosphate from ATP to an acceptor NDP, NDK, gets autophosphorylated at the His residue in the HGSD motif [25, 28, 30]. In order to verify whether the formation of autophosphorylated intermediate of MsmNDK is through H117, recombinant MsmNDK and MsmNDK-H117Q proteins, subsequent to incubation with $[\gamma\text{-}^{32}\text{P}]\text{-ATP}$ in the absence of NDP, were run on 12% SDS-PAGE and analysed using autoradiography. A 15 kDa phosphorylated band corresponding to MsmNDK was

observed, indicating that MsmNDK undergoes autophosphorylation (Fig. 6B). However, the mutation H117Q reduced the autophosphorylation activity, almost 5-fold, indicating that H117 is involved in the autophosphorylation (Fig. 6C; protein bands in Fig. 6A).

DISCUSSION

The present study demonstrates that MsmNDK, which is also conserved like other structurally and functionally characterised NDKs [9, 15, 18-20, 24-26, 31-41], possesses

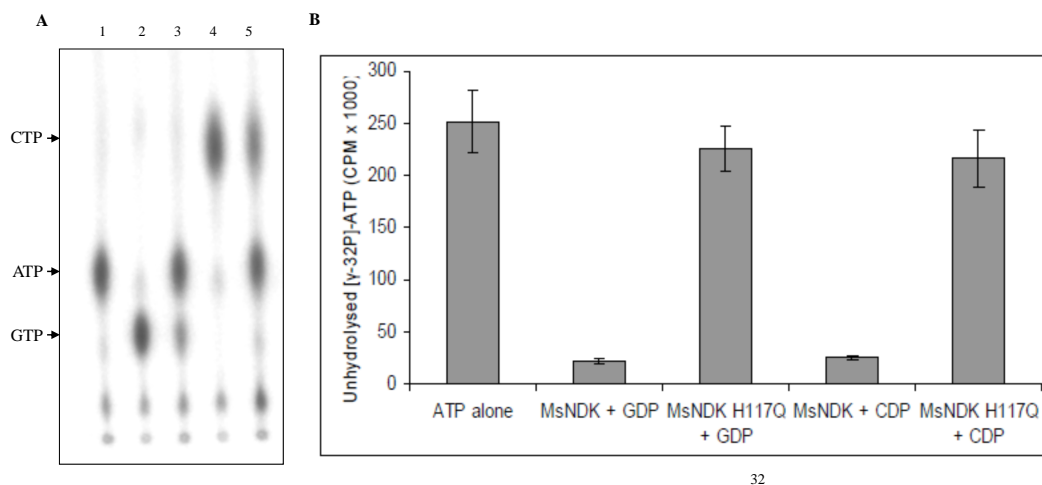


Fig. (5). NTP synthesising activity of MsmNDK and MsmNDK-H117Q. **A.** PEI-TLC profile of $[\gamma\text{-}^{32}\text{P}]\text{-ATP}$ alone (lane 1), and in the presence of $[\gamma\text{-}^{32}\text{P}]\text{-ATP}$: GDP + MsmNDK (lane 2), GDP + MsmNDK-H117Q (lane 3), CDP + MsmNDK (lane 4), and CDP + MsmNDK-H117Q (lane 5). **B.** Bar graph representing quantitation of unhydrolysed $[\gamma\text{-}^{32}\text{P}]\text{-ATP}$. The positions of ATP, CTP, and GTP were identified using the Rf values for these NTPs in the solvent system, 0.75M KH_2PO_4 . Assay was repeated independently three times for each sample. Counts per minute (CPM) were recorded and standard deviations were calculated.

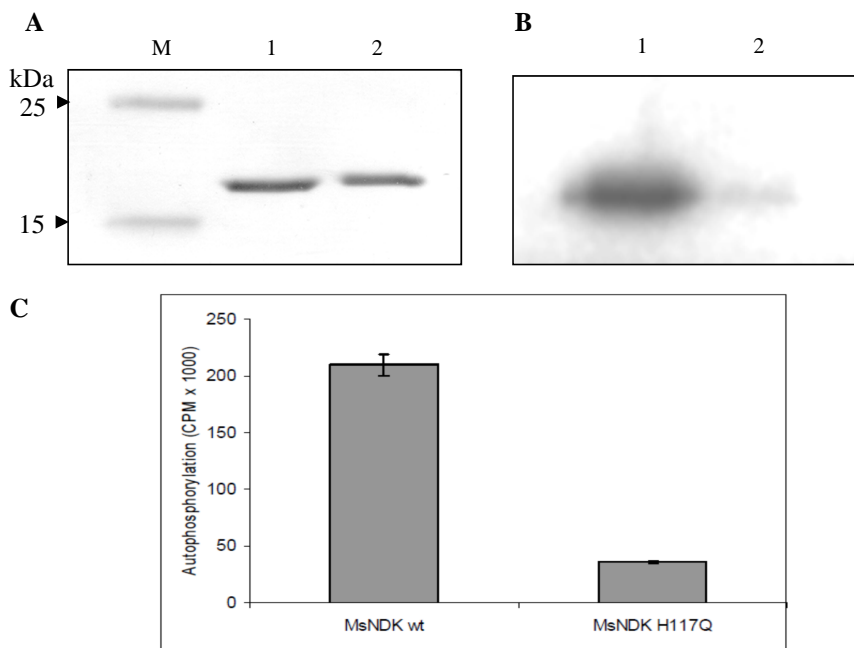


Fig. (6). Autophosphorylation assay of MsmNDK and MsmNDK-H117Q. **A.** Coomassie blue stained SDS-PAGE gel profile. Lane M, Mol Wt markers. Lane 1, MsmNDK (1 μg). Lane 2, MsmNDK-H117Q (1 μg). **B.** Autoradiograph of MsmNDK (lane 1) and MsmNDK-H117Q (lane 2) after autophosphorylation. **C.** Bar graph represents the quantitation of autophosphorylation of MsmNDK and MsmNDK-H117Q. Assay was repeated independently three times for each sample. Counts per minute (CPM) were recorded and standard deviations were calculated.

NTPase, NTP synthesising, and autophosphorylation activities. In the attempt to find out the requirement of H117 for the biochemical activities, the intention was to find out whether even the subtle change of His to Gln would be able to affect the activities. While Gln is neutral and polar, His is basic and polar. The dipole of the C-N bond in the amide group in Gln is stronger than the dipole in the C-N bond in His. The polarity scale of His is $(-) 0.49 \pm 0.15$, while that of Gln is $(+) 0.73 \pm 0.15$ [42, 43]. Further, the average volume of the side chains of Gln and His [44, 45], their relative hydrophilicity, and the net charge of their side chains, are comparable [46]. In fact, other research groups have also carried out the His-to-Gln conversion to show the role of His in the HGSD motif in the biochemical activities of the NDK of *Mycobacterium tuberculosis* (MtuNDK) [20] and of *Bacillus anthracis* (BanNDK) [47]. In view of these reasons, residues, such as Ala or Gly, were not considered as alternative to Gln for mutagenesis.

The NTPase, NTP synthesising, and autophosphorylation activities of MsmNDK are similar to those of NDKs from other bacterial genera and other organisms [20, 25, 30, 32, 48]. Although, the NTP synthesising activity of MsmNDK had earlier been shown [18], the requirement of H117 for these activities had not been demonstrated. The lack of complete abolition of GTPase activity in MsmNDK-H117Q might be indicative of other residues being involved in GTP hydrolysis. Studies using NDK mutants of *M. tuberculosis* (MtuNDK) indicated that besides His117, the residues, Lys10, His53, Tyr50, Arg86, and Arg104, are also involved in either phosphotransfer or autophosphorylation activity [28]. Incidentally, these five residues are conserved between MsmNDK and MtuNDK. This shows the strong possibility of the involvement of the additional residues in the biochemical activities, such as in the GTPase activity, of MsmNDK. Although MsmNDK binds GTP and hydrolyses it, the protein lacks the GXXGK and DXXG motifs of GTP binding proteins [49, 50], and NKKD, which is known to be involved in guanine base recognition [51], like in the case of MtuNDK [20].

Besides the histidine autophosphorylation, the *in vivo* serine phosphorylation of human NDK, NM23-H1, was found to correlate with the suppression of tumour metastatic potential in the TK melanoma cells, suggesting its biological relevance [52]. Similar serine residue phosphorylation was observed in the NDKs of rat mucosal mast cells [33], *Myxococcus xanthus* [30], *Dictyostelium discoideum* [53], and *Solanum chacoense* [54]. Except in *Myxococcus xanthus* [30], autophosphorylation of non-histidine residues has not been reported for NDKs from bacterial systems. Serine autophosphorylation of MsmNDK was not found *in vitro*. Taken together, the observations in the present study show that MsmNDK possesses all the biochemical activities that are characteristic of NDKs from other bacterial genera and that H117 in the HGSD motif is required for the biochemical activities.

CONFLICT OF INTEREST

None declared.

ACKNOWLEDGEMENTS

This work was supported by a Centre of Excellence in Tuberculosis Research part-grant from DBT. MA was a

CSIR SRF. The infrastructural facilities provided by the DBT Programme Support – Pathogen Biology - to Biological Sciences Division, IISc, UGC-CAS and DST-FIST support to the MCB Dep't, IISc, are acknowledged.

REFERENCES

- [1] Krebs, H.A.; Hems, R. Some reactions of adenosine and inosine phosphate in animals tissues. *Biochim. Biophys. Acta.*, **1953**, *12*, 172-180.
- [2] Berg, P.; Joklik, W.K. Transphosphorylation between nucleoside polyphosphates. *Nature*, **1953**, *172*, 1008-1009.
- [3] Parks, R.E., Jr.; Agarwal, R.P. Nucleoside Diphosphokinases. In: *The Enzyme*; Boyer, P.D., Ed., Academic Press: New York, **1973**, pp. 307-333.
- [4] Ray, N.B.; Mathews, C.K. Nucleoside diphosphokinase: a functional link between intermediary metabolism and nucleic acid synthesis. *Curr. Top. Cell. Regul.*, **1992**, *33*, 343-357.
- [5] Lecroisey, A.; Lascu, I.; Bominaar, A.; Veron, M.; Delepierre, M. Phosphorylation mechanism of nucleoside diphosphate kinase: ^{31}P -nuclear magnetic resonance studies. *Biochemistry*, **1995**, *34*, 12445-12450.
- [6] More'ra, S.; Chiadmi, M.; Lascu, I.; Janin, J. Mechanism of phosphate transfer by nucleoside diphosphate kinase: X-ray structures of the phosphohistidine intermediate of the enzymes from *Drosophila* and *Dictyostelium*. *Biochemistry*, **1995**, *34*, 11062-11070.
- [7] Lascu, I.; Gonin, P. The catalytic mechanism of nucleoside diphosphate kinases. *J. Bioenerg. Biomembr.*, **2000**, *32*, 237-246.
- [8] Chopade, B.A.; Shankar, S.; Sundin, G.W.; Mukhopadhyay, S.; Chakrabarty, A.M. Characterisation of membrane-associated *Pseudomonas aeruginosa* Ras-like protein Pra, a GTP-binding protein that forms complexes with truncated nucleoside diphosphate kinase and pyruvate kinase to modulate GTP synthesis. *J. Bacteriol.*, **1997**, *179*, 2181-2188.
- [9] Sundin, G.W.; Shankar, S.; Chugani, S.A.; Chopade, B.A.; Kavanaugh-Black, A.; Chakrabarty, A.M. Nucleoside diphosphate kinase from *Pseudomonas aeruginosa*: characterisation of the gene and its role in cellular growth and exopolysaccharide alginate synthesis. *Mol. Microbiol.*, **1996**, *20*, 965-979.
- [10] Shankar, S.; Kamath, S.; Chakrabarty, A.M. Two forms of the nucleoside diphosphate kinase of *Pseudomonas aeruginosa* 8830: altered specificity of the nucleoside triphosphate synthesis by the cell membrane-associated form of the truncated enzyme. *J. Bacteriol.*, **1996**, *178*, 1777-1781.
- [11] Bernard, M.A.; Ray, N.B.; Olcott, M.C.; Hendricks, S.P.; Mathews, C.K. Metabolic functions of microbial nucleoside diphosphate kinases. *J. Bioenerg. Biomembr.*, **2000**, *32*, 259-267.
- [12] Lu, Q.; Zhang, X.; Almula, N.; Mathews, C.K.; Inouye, M. The gene for nucleoside diphosphate kinase functions as a mutator gene in *Escherichia coli*. *J. Mol. Biol.*, **1995**, *254*, 337-341.
- [13] Postel, E.H.; Abramczyk, B.M. *Escherichia coli* nucleoside diphosphate kinase is a uracil-processing DNA repair nuclease. *Proc. Natl. Acad. Sci. USA.*, **2003**, *100*, 13247-13252.
- [14] Yoon, J.H.; Singh, P.; Lee, D.H.; Qiu, J.; Cai, S.; O'Connor, T.R.; Chen, Y.; Shen, B.; Pfeifer, G.P. Characterisation of the 3'-5' exonuclease activity found in human nucleoside diphosphate kinase 1 (NDK1) and several of its homologues. *Biochemistry*, **2005**, *44*, 15774-15786.
- [15] Munoz-Dorado, J.; Inouye, M.; Inouye, S. Nucleoside diphosphate kinase from *Myxococcus xanthus*. II. Biological characterization. *J. Biol. Chem.*, **1990**, *265*, 2707-2712.
- [16] Steeg, P.S.; Bevilacqua, G.; Kopper, L.; Thorgeirsson, U.P.; Talmadge, J.E.; Liotta, L.A.; Sobel, M.E. Evidence for a novel gene associated with low tumor metastatic potential. *J. Natl. Cancer Inst.*, **1988**, *80*, 200-204.
- [17] Kapatral, V.; Bina, X.; Chakrabarty, A.M. Succinyl coenzyme a synthetase of *Pseudomonas aeruginosa* with a broad specificity for nucleoside triphosphate (NTP) synthesis modulate specificity for NTP synthesis by the 12-kilodalton form of nucleoside diphosphate kinase. *J. Bacteriol.*, **2000**, *182*, 1333-1339.
- [18] Shankar, S.; Hershberger, C.D.; Chakrabarty, A.M. The nucleoside diphosphate kinase of *Mycobacterium smegmatis*: identification of proteins that modulate specificity of nucleoside triphosphate synthesis by the enzyme. *Mol. Microbiol.*, **1997**, *24*, 477-487.

- [19] Biggs, J.; Hersperger, E.; Steeg, P.S.; Liotta, L. A.; Serán, A. A *Drosophila* gene that is homologous to a mammalian gene associated with tumor metastasis codes for a nucleoside diphosphate kinase. *Cell*, **1990**, *63*, 933-940.
- [20] Chopra, P.; Singh, A.; Koul, A.; Ramachandran, S.; Drlica, K.; Tyagi, A.K. Singh, Y. Cytotoxic activity of nucleoside diphosphate kinase secreted from *Mycobacterium tuberculosis*. *Eur. J. Biochem.*, **2003**, *270*, 625-634.
- [21] Sun, J.; Wang, X.; Lau, A.; Liao, T.Y. A.; Buccì, C.; Hmama, Z. Mycobacterial nucleoside diphosphate kinase blocks phagosome maturation in murine Raw 264.7 macrophages. *PLoS ONE*, **2010**, *5*, e8769. doi:10.1371/journal.pone.0008769.
- [22] Greenfield, N.J. Using circular dichroism collected as a function of temperature to determine the thermodynamics of protein unfolding and binding interactions. *Nat. Protoc.*, **2006**, *6*, 2527-2533.
- [23] Cashel, M.; Lazzarini, R.A.; Kalbacher, B. An improved method for thin-layer chromatography of nucleotide mixtures containing 32P-labelled orthophosphate. *J. Chromatogr.*, **1969**, *40*, 103-109.
- [24] Gilles, A.M.; Presacan, E.; Vonica, A.; Lascu, I. Nucleoside diphosphate kinase from human erythrocytes. Structural characterisation of the two polypeptide chains responsible for heterogeneity of the hexameric enzyme. *J. Biol. Chem.*, **1991**, *266*, 8784-8789.
- [25] Almaula, N.; Lu, Q.; Delgado, J.; Belkin, S.; Inouye, M. Nucleoside diphosphate kinase from *Escherichia coli*. *J. Bacteriol.*, **1995**, *177*, 2524-2529.
- [26] Ogura, Y.; Yoshida, Y.; Ichimura, K.; Aoyagi, C.; Yabe, N.; Hasunuma, K. Isolation and characterization of *Neurospora crassa* nucleoside diphosphate kinase NDk-1. *Eur. J. Biochem.*, **1999**, *266*, 709-714.
- [27] Tepper, A.; Dammann, H.; Bominaar, A.A.; Veron, M. Investigation of the active site and the conformational stability of nucleoside diphosphate kinase by site-directed mutagenesis. *J. Biol. Chem.*, **1994**, *269*, 32175-32180.
- [28] Tiwari, S.; Kishan, K.V.R.; Chakrabarti, T.; Chakraborti, P.K. Amino acid residues involved in autophosphorylation and phosphotransfer activities are distinct in nucleoside diphosphate kinase from *Mycobacterium tuberculosis*. *J. Biol. Chem.*, **2004**, *279*, 43595-43603.
- [29] Shen, Y.; Kim, J.I.; Song, P.S. Autophosphorylation of Arabidopsis nucleoside diphosphate kinase 2 occurs only on its active histidine residue. *Biochemistry*, **2006**, *45*, 1946-1949.
- [30] Munoz-Dorado, J.; Alamula, N.; Inouye, S.; Inouye, M. Autophosphorylation of nucleoside diphosphate kinase from *Mycobacterium xanthus*. *J. Bacteriol.*, **1993**, *175*, 1176-1181.
- [31] Ginther, C.L.; Ingraham, J.L. Nucleoside diphosphokinase of *Salmonella typhimurium*. *J. Biol. Chem.*, **1974**, *249*, 3406-3411.
- [32] Lacombe, M.L.; Walley, V.; Troll, H.; Veron, M. Functional cloning of a nucleoside diphosphate kinase from *Dictyostelium discoideum*. *J. Biol. Chem.*, **1990**, *265*, 10012-10018.
- [33] Hemmerich, S.; Pecht, I. Oligomeric structure and autophosphorylation of nucleoside diphosphate kinase from rat mucosal mast cells. *Biochemistry*, **1992**, *31*, 4580-4587.
- [34] Fukuchi, T.; Nikawa, J.; Kimura, M.; Watanabe, W. Isolation, overexpression, and disruption of a *Saccharomyces cerevisiae* YNK gene encoding nucleoside diphosphate kinase. *Gene*, **1993**, *129*, 141-146.
- [35] Izumiya, H.; Yamamoto, M. Cloning and functional analysis of the ndk1 gene encoding nucleoside-diphosphate kinase in *Schizosaccharomyces pombe*. *J. Biol. Chem.*, **1995**, *270*, 27859-27864.
- [36] Brodbeck, M.; Rohling, A.; Wohlleben, W.; Thompson, C.J.; Susstrunk, U. Nucleoside-diphosphate kinase from *Streptomyces coelicolor*. *Eur. J. Biochem.*, **1996**, *239*, 208-213.
- [37] Lacombe, M.L.; Milon, L.; Munier, A.; Mehus, J.G.; Lambeth, D.O. The human Nm23/nucleoside diphosphate kinases. *J. Bioenerg. Biomembr.*, **2000**, *32*, 247-258.
- [38] Yonezawa, Y.; Tokunaga, H.; Ishibashi, M.; Tokunaga, M. Characterisation of nucleoside diphosphate kinase from moderately halophilic eubacteria. *Biosci. Biotechnol. Biochem.*, **2001**, *65*, 2343-2346.
- [39] Anderca, M.I.; Furuichi, T.; Pinontoan, R.; Muto, S. Identification of a Mitochondrial Nucleoside Diphosphate Kinase from the Green Alga *Dunaliella tertiolecta*. *Plant Cell Physiol.*, **2002**, *43*, 1276-1284.
- [40] Chen, Y.; Morera, S.; Mocan, J.; Lascu, I.; Janin, J. X-ray structure of *Mycobacterium tuberculosis* nucleoside diphosphate kinase. *Proteins*, **2002**, *47*, 556-557.
- [41] Hama, H.; Almaula, N.; Lerner, C.G.; Inouye, S.; Inouye, M. Nucleoside diphosphate kinase from *Escherichia coli*; its overproduction and sequence comparison with eukaryotic enzymes. *Gene*, **1991**, *105*, 31-36.
- [42] Guy, H. R. Amino acid side-chain partition energies and distribution of residues in soluble proteins. *Biophys. J.*, **1985**, *47*, 61-70.
- [43] Radzicka, A.; Wolfenden, R. Comparing the polarities of the amino acids: side-chain distribution coefficients between the vapor phase, cyclohexane, 1-octanol, and neutral aqueous solution. *Biochemistry*, **1988**, *27*, 1664-1670.
- [44] Chothia, C.; Janin, J. Principles of protein-protein recognition. *Nature*, **1975**, *256*, 705-708.
- [45] Hackel, M.; Hinz, H.J.; Hedwig, G.R. Partial molar volumes of proteins: amino acid side-chain contributions derived from the partial molar volumes of some tripeptides over the temperature range 10-90°C. *Biophys. Chem.*, **1999**, *82*, 35-50.
- [46] Creighton, T.E. *Proteins: Structures and Molecular Properties*, 2nd ed. Freeman and Company: New York, **1984**, pp. 1-20.
- [47] Misra, G.; Aggarwal, A.; Dube, D.; Zaman, M. S.; Singh, Y.; Ramachandran, R. Crystal structure of the *Bacillus anthracis* nucleoside diphosphate kinase and its characterisation reveals an enzyme adapted to perform under stress conditions. *Proteins*, **2009**, *76*, 496-506.
- [48] Zaborina, O.; Li, X.; Cheng, G.; Kapatral, V.; Chakraborty, A.M. Secretion of ATP-utilising enzymes, nucleoside diphosphate kinase and ATPase, by *Mycobacterium bovis* BCG: sequestration of ATP from macrophage P2Z receptors? *Mol. Microbiol.*, **1999**, *31*, 1333-1343.
- [49] McCormick, F.; Clark, B.F.C.; Cour, T.F.M.; Kjeldgaard, M.; Lauritsen, L.N.; Nyborg, J. A model for the tertiary structure of p21, the product of Ras oncogene. *Science*, **1985**, *230*, 78-82.
- [50] March, P.E. Membrane associated GTPases in bacteria. *Mol. Microbiol.*, **1992**, *6*, 1253-1257.
- [51] Dever, T.E.; Glynias, M.J.; Merrick, W.C. GTP binding domain: three consensus sequence elements with distinct spacing. *Proc. Natl Acad. Sci., USA*, **1987**, *84*, 1814-1818.
- [52] MacDonald, N.J.; De la Rosa, A.; Benedict, M.A.; Freije, J.M.; Krutsch, H.; Steeg, P.S. A serine phosphorylation of Nm23, and not its nucleoside diphosphate kinase activity, correlates with suppression of tumor metastatic potential. *J. Biol. Chem.*, **1993**, *268*, 25780-25789.
- [53] Bominaar, A.A.; Tepper, A.D.; Veron, M. Autophosphorylation of nucleoside diphosphate kinase on non-histidine residues. *FEBS Lett.*, **1994**, *353*, 5-8.
- [54] Dorion, S.; Dumas, F.; Rivoal, J. Autophosphorylation of *Solanum chacoense* cytosolic nucleoside diphosphate kinase on Ser117. *J. Exp. Bot.*, **2006**, *57*, 4079-4088.