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# NMR Signal Assignments of Human Adenylate Kinase 1 (hAK1) and its R138A Mutant (hAK1R138A)

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**Abstract** Adenylate kinase (AK) enzyme which acts as the catalyst of reversible high energy phosphorylation reaction between ATP and AMP which associate with energetic metabolism and nucleic acid synthesis and signal transmission. This enzyme has three distinct domains: Core, AMP binding domain (AMPbd) and Lid domain (LID). The primary role of AMPbd and LID is associated with conformational changes due to flexibility of two domains. Three dimensional structure of human AK1 has not been confirmed and various mutation experiments have been done to determine the active sites. In this study, AK1R138A which is changed arginine[138] of LID domain with alanine[138] was made and conducted with NMR experiments, backbone dynamics analysis and mo-lecular docking dynamic simulation to find the cause of structural change and substrate binding site. Synthetic human muscle type adenylate kinase 1 (hAK1) and its mutant (AK1R138A) were re-combinded with E. coli and expressed in M9 cell. Expressed proteins were purified and finally gained at 0.520 mM hAK1 and 0.252 mM AK1R138A. Multinuclear multidimensional NMR experiments including HNCA, HN(CO)CA, were conducted for amino acid sequence analysis and signal assignments of $^{1}H$-$^{15}N$ HSQC spectrum. Our chemical shift perturbation data is shown LID domain residues and around alanine[138] and per-turbation value(0.22ppm) of valine[179] is consid-ered as inter-communication effect with LID domain and the structural change between hAK1 and AK1R138A.

**Keywords** HNCA, HN(CO)CA, HSQC, NMR, AK1

## Introduction

Adenylate kinase(AK) are ubiquitous small enzymes, catalyzes a reversible phosphorylation reaction between ATP and AMP which play a key role in the energetic metabolism and nucleic acid synthesis and signal transmission.[1,2]

$$ATP * Mg^{2+} + AMP \Leftrightarrow ADP * Mg^{2+} + ADP$$

AK has two kinds of iso-forms: short form (1~190 residues) and long form (214~238 residues).[3] In general, the short form has high enzymatic activity and exists in eukaryotic cytosol whereas the long form has low activity and exists in mitochondria and bacteria.[4,5] This enzyme has three distinct domains: Core, AMP binding domain (AMPbd) and Lid domain (LID). The primary role of AMPbd and LID

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is associated with conformational changes due to flexibility of two domains. In despite of extensive effort by X-ray crystallographic and $^{1}$H-NMR studies, the substrate binding site has been a controversial issue. Various methods have been reported for understanding of activity relationships with mutant.[6-13] AK1 LID domain is consist of only 11 residues(K131 ~ D141), forming a highly flexible loop. Loop or domain motions are widely recognized as key elements in the catalytic mechanism of the enzyme. Under such situations, AK mutation research has contributed to understand their reaction mechanism and activity relationship.[14-16] In addition, various studies for enzyme activities have been reported. One of well-known studies is about human muscle-type cytosolic AK (hAK1: M.W=21,700, 194 amino acid) carrying 6 conserved arginine residues as shown in Table 1. [17]

**Table 1.** Primary sequence of the human muscle type AK1 and conserved 6 arginine residues.

| | | | |
|---|---|---|---|
| 10 | 20 | 30 | 40 |
| MEEKLKKTKIIFVVGGPGSGKGTQCEKIVQKYGYTHLSTG | | | |
| 50 | 60 | 70 | 80 |
| DLLRSEVSSGSARGKKLSEIMEKGQLVPLETVLDMLRDAM | | | |
| 90 | 100 | 110 | 120 |
| VAKVNTSKGFLIDGYPREVQQGEEFERRIGQPTLLLYVDA | | | |
| 130 | 140 | 150 | 160 |
| GPETMTRRLLKRGETSGRVDDNEETIKKRLETYYKATEPV | | | |
| 170 | 180 | 190 | |
| IAFYEKRGIVRKVNAEGSVDEVFSQVCTHLDALK | | | |

Human AK1 (EC 2.7.4.3) has 13 Arg residues and mutation was made for Arg-138 sequence in this study. These Arg residues were known to have some important role in substrate binding through the electrostatic interaction. In this work, we prepared Arg mutants, in which each Arg[138] residues in the AMPbd was substituted by ala residue which non-polar amino acid (R138A). We made signal assignments for native hAK1 and its single mutant AK1R138A by using the multinuclear multidimensional NMR experiments including 3D-NMR HNCA, HN(CO)CA and 2D- NMR $^{1}$H-$^{15}$N HSQC experiment for amino acid sequence analysis and signal assignments of spectrum.[11] In this studies, we attempted to elucidate the structural features of substrate-binding sites by using $^{1}$H-$^{15}$N NMR signal assignments and chemical shift perturbation.

## Experimental Methods

*Materials*- The human adenylate kinase1 (hAK1) and mutant (AK138A) cells in this study obtained from institute of molecular immunology lab (Hanyang Uni versity). The hAK1 and its single mutant AKR138A were overproduced in E. coli strains M9 and JM109 cell respectively, Cells were cultured in LB medium and M9 medium with 100μg/ml ampicillin (Amp) and kanamycin (Kan). For $^{15}$N-labeling protein, we put 0.5g $^{15}NH_4Cl$ in 1 liter of M9 medium and kept the flask then in a shaking incubator at 37˚C. After 15hr, 1ml cultured cell was added to 50ml of fresh LB medium and Amp, Kan for identification of expression rate. The growth rate of LB medium and M9 medium were checked with UV absorbance at value (0.4, 500λ). IPTG was injected in M9 medium and then incubated for overnight. Affinity chromatography having column contain divalent Ni ion-resin were used for purification. Buffers system was used during affinity chromatography containing washing buffer (Imidazole 20 mM, HEPES 20 Mm, NaCl 0.5 M, pH 7.9) and elution buffer (Imidazole 250 mM, HEPES 20 mM, NaCl 0.5M, pH 7.9). Total 43 fractions were collected. The dialysis was done in 1 liter 0.5×PBS buffer at pH 7.1 (adjusted with 1N HCl acid solution) by using dialysis membrane (MWCO 6-8,000, Flat width 40 mm). Lastly the wild and mutant AK concentration were checked with absorbance at 562 nm by using standard bovine serum albumin (BSA) and pierce reagent (BCA, A & B in the ratio of 50:1). The hAK1 and R138Aconcentration were found to be 0.520 mM, 0.252 mM, respectively, which is quite good enough for the NMR experiments

*NMR experiments*- The NMR experiments were carried out on 800MHz Bruker spectrometer at 298 K. All reagents used in protein sampling were obtained from the Cambridge isotope Laboratories and

Sigma-Aldrich. The 300 µl NMR samples for hAK1 and R138A (pH 7.1) were prepared by taking 280 µl protein and 20 µl $D_2O$ in shigemi 5mm symmetrical NMR micro-tube in 2M Tris-buffer (pH 7.9) solution.$^{15}N$ spin-lattice ($R_1$= $1/T_1$) and spin-spin ($R_2$= $1/T_2$) relaxation rate constants were measured by using sensitivity enhanced pulse sequence, 2048 x 128complex points in the $F_2$ and $F_1$ dimension respectively. The heteronuclear $^1H$-$^{15}N$ HSQC values were obtained from the spectrum peaks intensities ratio, acquired the NOE spectrums in the absence and presence of proton saturation. The scan number (nt) and number of increment (ni) used during HSQC experiments were 16 and 128.The NMR data were processed using NMRpipe /NMRdraw and analyzed with NMRViewJ version (8.0.b49).

## Results

*$^1H$-$^{15}N$ HSQC Spectrum assignment*- The $^1H$-$^{15}N$ HSQC was analyzed for both hAK1 and mutant AK1R138A. The HSQC signal data were determined using 3D-NMR HNCA and HN(CO)CA spectrums. Selected $^{15}N$ chemical shift slices extracted from

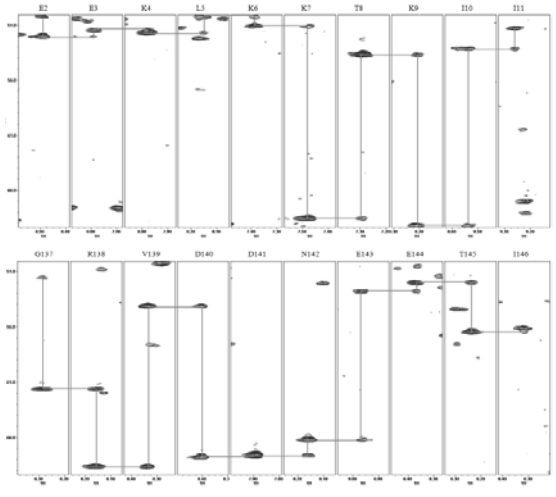

**Figure 1.** Selected $^{15}N$ chemical shift slices extracted from 3D HNCA and HN(CO)CA spectra of hAK1. The Sequential backbone resonance assignment for E[2]-I[11] (up) and G[137]-I[146] (down, mutation site) peptide stretch is illustrated.

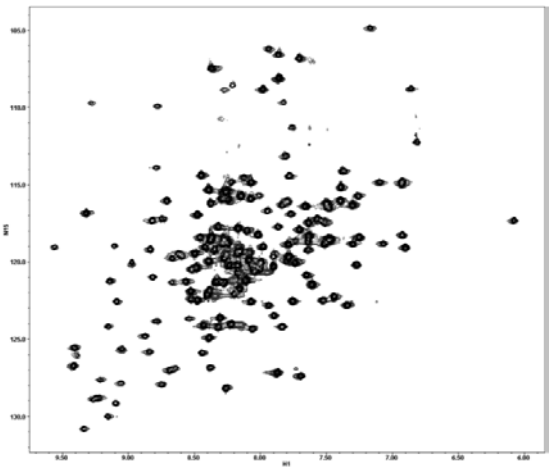

**Figure 2.** $^1H$-$^{15}N$ HSQC superimposition of $^1H$-$^{15}N$ HSQC spectra of hAK1 (black) and AK1R138A (grey).

3D-NMR HNCA and HN(CO)CA spectra of hAK1 and its mutant AK1R138A. The sequential backbone resonance assignments for initial site (G[2]-I[11]) and mutation site (G[137]- I[146]) peptide are shown in Figure 1.

HSQC data were assigned for backbone amide groups of 188 non-proline residues (P17, P68, P96, P112, P122, P159) for wild type and mutant AK1R138A respectively. The ATP-binding P-loop (G20, K21, G22) amide groups were not observed in the HSQC spectrum that the P-loop residues are more expose towards the solvent or due to its high flexibility undergoes various conformation changes in the absence of any bound ligand.[18]HSQC spectrum of hAK1 and mutant AKR138A were superimposed on each other as shown in Figure 2.

Zoomed $^1H$-$^{15}N$ HSQC spectra of arginine mutation

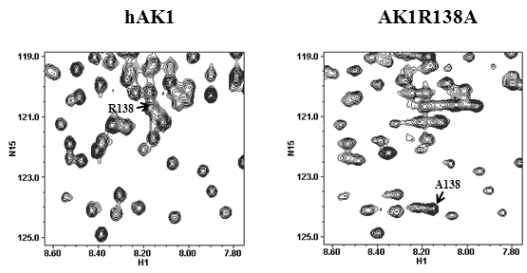


**Figure 3.** Representative 2D-NMR $^1H$-$^{15}N$ HSQC spectra of Mutated residues Arg[138] of hAK1 (left) and Ala[138] of AK1R138A (right).

residue of hAK1 and AK1R138A are shown in Figure 3.

*Chemical-shift perturbation of AK1 & AK1R138A*- Chemical shift perturbation value was also calculated for hAK1 to observe the conformational effect upon mutation. Chemical shift perturbation analysis may determine the effect of mutation residues from enzyme structure. It may suggest that the trend of fundamental structural changes. The chemical shift perturbation values were calculated by using following equation.

$$\Delta\text{shift} = [(\Delta^{1}\text{H shift}) + (0.2 \times \Delta^{15}\text{N shift})]$$

The chemical shift perturbations value due to mutation and upon binding with various ligands were plot as a function of residue numbers for hAK1 and mutant as depicted in Figure 4.

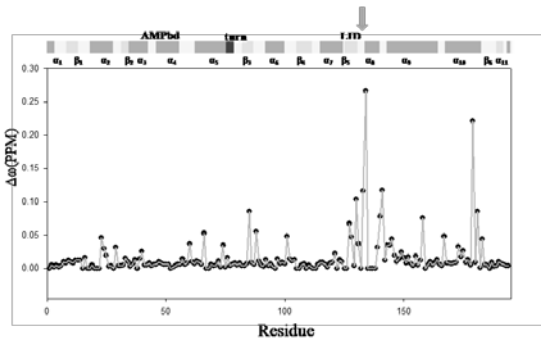


**Figure 4.** Chemical-shift perturbations are relative to hAK1 and are calculated as the absolute value of a normalized sum of nitrogen and proton perturbations according to $\delta_{\omega} = |0.2\,\Delta^{15}\text{N} + \Delta^{1}\text{H}|$(ppm), An arrow indicates the mutation point.

From the value, chemical shift perturbation of most residues was not observed. Tyr[23] residue area of turn site and ATP binding site and Leu[66] residue area of AMP binding site showing large chemical shift perturbation was detected. The α-helix site from Val[179] residue in LID domain and the terminal of enzyme to Lys[194] residue in c-terminal also has large chemical shift perturbation in a wide range. The largest perturbation of hAK1 and AK1R138A was shown in Arg[132] residue involved in binding with ATP except the mutation site Arg[138] residue. This perturbation may indicate that these Arg[132] and Arg[138] residues participate in substrate binding with ATP and are involved in enzyme activity.

## Discussion

By using 3D-NMR peak assignments and $^{1}$H-$^{15}$N HSQC NMR spectral changes for $^{15}$N-labeled AK1 and its mutant R138A which a non-polar mutant alanine[138] was replaced for arginine[138] site, we were able to examine the structural perturbation difference of human AK1 and its mutant AK1R138A. In this study, the chemical perturbation value for mutant Ala[138] was changed up to 0.1ppm compare to that of native Arg[138] located in LID domain. In addition, big changes around 0.2 ppm in chemical shift perturbation were observed in α11 and β6 portion of mutant AK1R138A. This conformational fluctuation of substrate binding site may affect to catalytic activity and mobility of the enzyme.
Detail structural studies and mechanism of enzyme activities about AK1 and its mutant with substrate binding model will be published elsewhere.

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