Biological Science

6A/2007G137

Structural Analysis of Halophilic Nucleoside Diphosphate Kinase from *Halomonas* sp. 593

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Introduction

Nucleoside diphosphate kinase (NDK) is well known housekeeping enzyme that catalyzes the transfer of γ-phosphate from nucleoside triphosphates to nucleoside diphosphates. All known NDKs assemble into a different oligomeric structure depend on their origin; NDKs from eukaryotes, archaea, and gram-positive bacteria form homohexamer, while those from gram-negative bacteria form homotetramer. We have been studying the halophilic characteristics of NDKs from moderately halophilic bacteria *Halomonas* sp. 593 (HaNDK), and non-halophilic bacteria *Pseudomonas aeruginosa* (PaNDK). HaNDK and PaNDK show high sequence homology (78% identity) [1]. Despite their high sequence homology, we have shown that HaNDK forms dimer in solution in contrast to homotetramer of PaNDK like other NDK from gram-negative bacteria [2]. Our mutational analysis based on the sequence comparison of HaNDK with the tetrameric non-halophilic PaNDK elucidated that only one mutation at Glu134 to Ala in HaNDK (HaNDK/EA) resulted in conversion of the dimeric structure to the tetramer assembly. Here we report the structural analysis of wild type and mutant HaNDK. This structural study allows the elucidation the difference of oligomeric state at the atomic level.

X-ray diffraction studies of HaNDK

Out of the 300 crystallization conditions screened, two crystal forms (I, II) of wild HaNDK were obtained by using 30% (w/v) PEG4000 as precipitant. Form I and II belonged to the space group *R*3 and *C*2 with unit-cell parameters $a=b=112$, $c=126$ Å and $a=138$, $b=171$, $c=77.6$ Å, $\beta=93.5^{\circ}$, respectively. The calculated Matthews coefficient was approximately 4.8 and 2.9 Å^3 Da^{-1} assuming the presence of 1 and 2.5 dimer of HaNDK in the asymmetric unit of form I and II, respectively. Diffraction data of form I was collected at BL6A in PF, and was completed up to 2.3 Å resolution with completeness of 97.3 % and R_{merge} of 0.048. The structure was solved by molecular replacement using the monomer coordinates of NDK from *Myxococcus* xanthus (PDB ID: 1NHK). The present model of the HaNDK containing 62 waters was refined to a R_{cryst}/R_{free} of 0.263/0.289 at 2.3 Å resolution.

Two forms (III, IV) were also obtained in crystallization of the mutant HaNDK/EA. Form III and IV belonged to the space group *C*2 and *P*21 with unit-cell parameters $a=189$, $b=93.1$, $c=68.4$ Å, $\beta=104^{\circ}$, and $a=112$, $b=92.0$, $c=114$ Å, $\beta=94.7^{\circ}$, respectively. The calculated Matthews coefficient was approximately 2.3 Å^3 Da^{-1} assuming the presence of 2 and 4 tetramer of HaNDK/EA in the asymmetric unit of form III and IV, respectively. Diffraction data of form IV was collected at BL6A in PF, and was completed up to 2.5 Å resolution with completeness of 91.9 % and R_{merge} of 0.100. The structure was solved by molecular replacement using the coordinate of wild HaNDK. The present model of the HaNDK/EA was refined to a R_{cryst}/R_{free} of 0.231/0.298 at 2.5 Å resolution.

Crystal structure of HaNDK

The crystal structure of HaNDK and HaNDK/EA were determined by x-ray crystallography to 2.3Å and 2.5 Å resolution, respectively. Overall structure of HaNDK and HaNDK/EA are shown in Figure. The polypeptide chain of both HaNDK and HaNDK/EA were folded intoβαββα fold which showed quite similar folding to that of the other NDKs. However, the association of HaNDK showed simple dimer instead of tetramer or hexamer as seen in the other NDKs. On the other hand, x-ray structure of HaNDK/EA showed tetramer assembly (Figure). This comparison between wild and mutant structures showed the mutation of Glu134 to Ala in HaNDK leads to conversion of the protein from dimer to tetramer assembly, indicating that a single amino acid substitution at position 134 results in an alteration of the oligomeric structure of NDK.

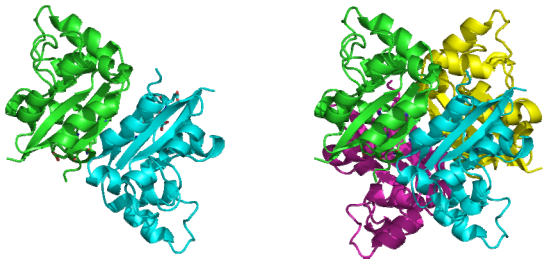

Figure. Oligomeric structure of HaNDK. Wild type HaNDK homodimer (left). Mutant HaNDK/EA homotetramer (right).

References

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