〈Original Article〉

Homology modeling and docking study of creatinine deiminase

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Summary Creatinine deiminase is commercially used in the diagnostic analysis of creatinine. The amino acid sequence of this enzyme was homologous to that of cytosine deaminase. For further understanding the catalytic function of the creatinine deiminase, a three-dimensional structure of the enzyme from *Tissierella creatinini* was constructed by homology modeling based on the X-ray structure of the cytosine deaminase from *Escherichia coli*. The enzyme-substrate interaction could also be discussed by constructing a molecular docking model. This would be of assistance in the improvement of the enzyme using protein engineering techniques.

Key words: Creatinine, Deiminase, Cytosine deaminase, Homology modeling, Molecular docking

1. Introduction

Creatinine deiminase (Cdi, EC 3.5.4.21) catalyses the hydrolyzation of creatinine to N-methylhydantoin and ammonia (Fig. 1). This enzyme is involved in the bacterial metabolism of creatinine with the related enzymes[1, 2]. Cdi is useful in the enzymatic assay of creatinine[3, 4], and is commercially used in the diagnostic analysis of creatinine. However, the enzymatic properties of Cdi were not yet clearly understood because the three-dimensional structure of the enzyme is still unknown.

This report shows a three-dimensional structure of Cdi modeled on the basis of its homology to the cytosine deaminase from *Escherichia coli* (CdaE)[5]. CdaE has been studied by X-ray crystallography, and then the substrate binding features of possible importance and the catalytic mechanism have been discussed[6]. The structure model of Cdi provides a reasonable starting point for analyzing the structure-function relationships.

2. Materials and methods

Homology search

Homology search analysis of the amino acid sequence of Cdi from *Tissierella creatinini* (CdiT)[7] was performed using the software GENETYX (Software Development Co., Ltd., Tokyo). The amino acid sequences of CdiT and CdaE (the DDBJ accession numbers are AX798055 and AY331712, respectively) were aligned to examine sequence identities and conserved sequences.

Homology modeling

Homology modeling was used to build the model

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of CdiT. The three-dimensional protein model was generated by the software MODELLER[8], based on the structure of CdaE (PDB ID: 3o7u). The program Pymol was used for molecular visualization. A model of CdiT binding site containing creatinine was obtained by superposing the coordinates of the active site of CdiT on that of CdaE containing the competitive inhibitor, phosphonocytosine. The coordinate for creatinine was then generated by superposing the positions of the carbonyl and imino groups on those of phosphonocytosine.

Molecular docking

A molecular docking study was performed using

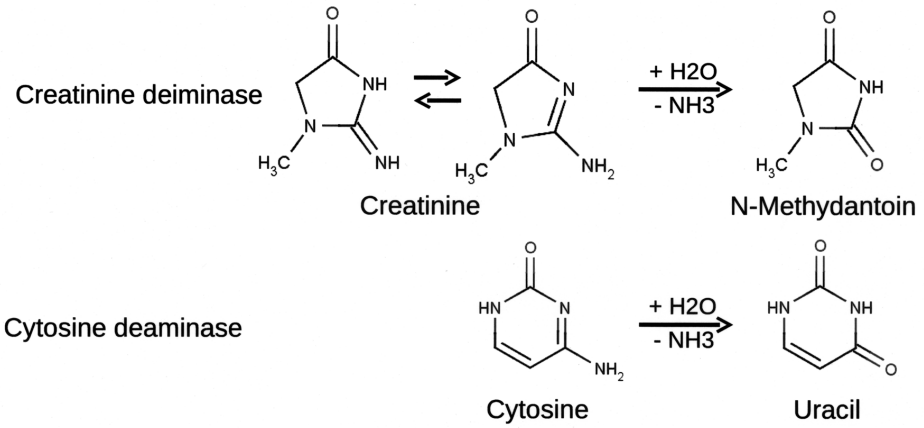

Fig. 1 Reactions of creatinine deiminase and cytosine deaminase.

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1'  INAKIYKNNE ATEILVEDGK IKEI---GNN LADCKEVIDL GGKMVTPPYV DPHLHLDYVY
    ***..  ...   .* ..*** *..*   ..  ..  .. .*  .  .*.**.* .**.*** .
1"  INARLPGEEG LWQIHLQDGK ISAIDAQSGV MPITENSLDA EQGLVIPPFV EPHIHLDTTQ

58'  TLAELGKTGA GSGTLFEAIE MWPVFKKTLT VESVKKLALK GVMDEVSQGV QHIRTHIDVT
     * .. . . . *  ****.** .*.  *  **  ..**. * . ... ....*. **.***.**.
61"  TAGQPNWNQS G--TLFEGIE RWAERKALLT HDDVKQRAWQ TLKWQIANGI QHVRTHVDVS

118'  DPKFTGLKAM LEMKEELKDI VDIQIVSFPQ QGMYTYKGGR ELVEEALKMG ADVVGGIPHY
      *...*.**** **.*.*.    .*.***.*** .*. .* .*  .*.****..* *****.***.
119"  DATLTALKAM LEVKQEVAPW IDLQIVAFPQ EGILSYPNGE ALLEEALRLG ADVVGAIPHF

178'  EPAREYGEMS VKATVELAMK YDKLIDVHCD ETDDPQARFI ELLNALVYLE GYGAKTSASH
      * .****  * .. * .** * **.******* *.** *.**. * ..**.. * * **...***
179"  EFTREYGVES LHKTFALAQK YDRLIDVHCD EIDDEQSRFV ETVAALAHHE GMGARVTASH

238'  TCSFGSADDS YAYRMIDLFK KSKINFISNP TENAYLQGRH DTYPKRRGLT RVKEFMEHGI
      * .. * ... *. *.. *.* .* ***..**   * .****  ********.* ****..* **
239"  TTAMHSYNGA YTSRLFRLLK MSGINFVANP LVNIHLQGRF DTYPKRRGIT RVKEMLESGI

298'  NVAFAQDSIN DPWYPMGNGN MMNILDNGIH LAQIMSPQDI EKDLDLITYN GARCLNIQDK
      ** *..*..  *****.*..* *...*. *.* . *.*.  .* ...*.***.. .** **.**
299"  NVCFGHDDVF DPWYPLGTAN MLQVLHMGLH VCQLMGYGQI NDGLNLITHH SARTLNLQD-

358'  YLLEVGKDSN FIVLNGDSPF DVIRNRANVL ACVRKG
      * ...*...* .*.* ... * *..*... *   .** *
358"  YGIAAGNSAN LIILPAENGF DALRRQVPVR YSVRGG
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Fig. 2 Amino acid sequence comparison of CdiT (upper: 406 amino acids) and CdaE (lower: 426 amino acids). Identical and similar residues are indicated by asterisks and dots, respectively. The central regions (286 amino acids) containing highly conservative sequences are surrounded by dotted lines. The four active site residues of CdaE are shown in grey color.

the software Autodock ver.4.2[9] on the basis of a grid-based docking procedure. The ligand structure was obtained from PubChem database. For the ligand, Gasteiger charge was calculated using the software Autodock Tools. The enzyme model obtained from the homology modeling was prepared with Autodock Tools, adding polar hydrogens and loading charges. The hydrogen atoms were added and optimized using the software Reduce[10]. The program AutoGrid settings were with 60 x 60 x 60 grid size and a grid spacing of 0.747 Å. Five billion conformations were evaluated by the Lamarckian genetic algorithm. The best docked conformer with the lowest free energy conformation was taken for discussion.

3. Results and discussion

As the first step in the building of a model, sequence analysis identified homologs for the CdiT protein sequence by searching the DDBJ website. Most of the highly homologous sequences were cytosine deaminases and their homologs. Cytosine deaminase (Cda) is a zinc-containing enzyme and catalyses the hydrolytic deamination of cytosine, forming uracil and ammonia. As shown in Fig. 1, its catalytic reaction appears to be similar to that of Cdi. In fact, Cda has been reported to be weakly reactable with creatinine[6]. Among the already known X-ray structures of Cda, CdaE was only from bacteria. Therefore, it was selected as a template for homology modeling. The sequence identity between CdiT (406 amino acid residues) and CdaE (426 amino acid residues) was approximately 46%, which was thought to be reasonable for model building. Especially, the central regions containing the important sequences for the substrate binding of CdaE exhibited a higher level of homology (53% identical residues / 286 amino acids) without any gap (Fig. 2). The active site of CdaE was composed of four residues, Q156, E217, H246, and D313. The residues correspond to Q152, E213, H242, and D309 in CdiT, respectively.

A three-dimensional model of CdiT was built by computer analysis based on the CdaE sequence and its X-ray structure. Overall structure of CdiT is shown in Fig. 3A. The central regions of CdiT and CdaE can be well superimposed with the root mean square deviation of atomic C α positions of 0.19 Å. To understand the enzyme-substrate interaction further, a structure of creatinine and a zinc ion atom were fitted in the putative active site of CdiT, based on the structural data of the CdaE-phosphonocytosine complex. Phosphonocytosine is a competitive inhibitor and binds to the active site of CdaE. As a result, the putative active site of CdiT composed of four residues is located around the substrate creatinine and zinc ion (Fig. 3B). Creatinine would be likely to form interactions with the side chains of the active site residues, as well as the zinc ion. In both CdiT and CdaE, their relative dispositions were conserved around the active site regions. The working model for the reaction mechanism for the deamination of cytosine has been proposed from the CdaE structure and the mutagenesis experiments[6]. Similarly, CdiT requires a single divalent cation in the active site for the coordination of the water molecule that will eventually attack creatinine. There are also three active site residues, E213, H242, and D309, which are required for the activation of this nucleophilic water and for subsequent proton transfer reactions. The metal-bound water molecule will be hydrogen bonded to D309 and H242. The side chain of H242 functions to shuttle the proton from the metal-bound water molecule to E213. When creatinine is bound in the active site, the metal-bound hydroxide will attack creatinine.

For the purpose of discussing the interaction between CdiT and creatinine, a molecular docking model was constructed using the software Autodock (Fig. 4). Molecular docking is a computational method that predicts how a ligand interacts with a protein, and plays an essential role in drug design. It is thought that the docking model also helps to enhance our understanding of the enzyme-substrate interaction. It was expected that molecular docking study would be useful for understanding enzyme reactions in the enzymatic assay field. The binding configuration of CdiT-creatinine predicted from molecular docking (Fig. 4B) was similar to that of the substrate-fitting model (Fig. 3B), although slightly different conditions were observed. The binding energy score of

creatinine was -5.6 kcal/mol. The Autodock prediction indicated that creatinine was able to effectively bind to CdiT, although no zinc ion was incorporated into the docking model. This result suggests that the apoenzyme of CdiT would play a role as a creatinine-binding protein, capable of using for creatinine determination.

Creatininase (Crn, creatinine amidohydrolase: EC3.5.2.10), which belongs to the degradation pathway of creatinine, an another creatinine-acting enzyme[11]. The enzyme reversibly hydrolyzes creatinine to creatine. As well as Cdi, Crn is useful in the enzymatic assay of creatinine. The Crn has been structurally characterized well and found to contain two zinc ions in its active site[12]. The K_m values of the three known Crn for creatinine were estimated to be 26 ~ 66 mM[13]. Those were much higher than the K_m of CdiT for creatinine, which was estimated to be 1.1

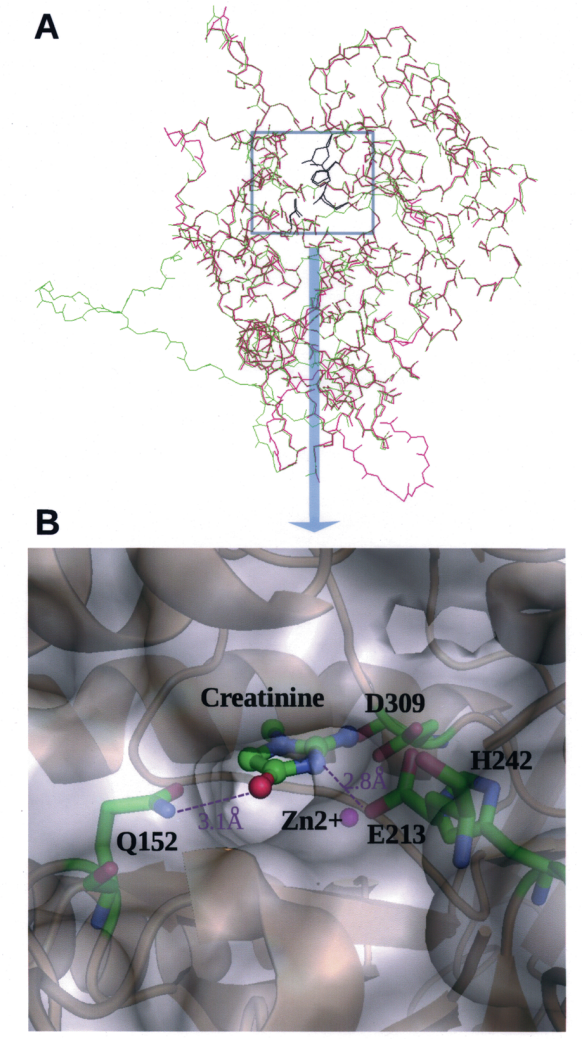

Fig. 3 Schematic diagrams of the three-dimensional structure of CdiT. (A) Superimposition of CdiT model (red) over CdaE (green). The active site residues are shown in black. (B) Close-up view of active site region. Creatinine and zinc are fitted, based on the structural data of the CdaE-inhibitor. The four active site residues are also represented by stick drawings.

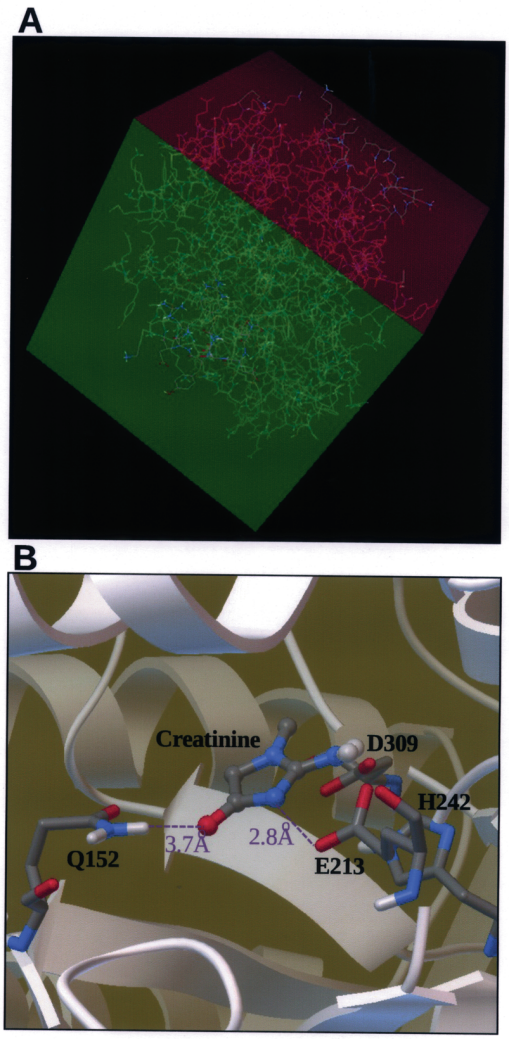

Fig. 4 Docking of creatinine with CdiT model. (A) Autodock grid map used in calculation. The program settings were performed as described in the Materials and methods section. (B) Close-up view of active site region. Creatinine and the four active site residues are represented by ball-stick and stick drawings, respectively.

mM[7]. Other commercially used Cdi also exhibited the same level of K_m values as that of CdiT. The higher substrate affinity of Cdi can be applied to the rapid determination of creatinine. The three-dimensional structure of Crn has been already determined[12], enabling comparison between 3D structures of Cdi and Crn. Structural interpretations of different substrate affinities and a study to analyze the substrate-binding using protein engineering techniques are now in progress.

The modeled 3D structure is very helpful to understand the structure-function relationships and mutational effects. The CdiT structure of the present study provides a reliable basis to predict mutations for desired effects on the activity and stability.

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