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RESEARCH PAPER

Molecular Docking of *Bacillus pumilus* Xylanase and Xylan Substrate Using Computer Modeling

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Abstract: *Bacillus pumilus* xylanase was cloned and sequenced. Based on the tertiary structure that originated from homology modeling, the potential active pocket was searched and ligand-protein docking was performed using relative softwares. The information extracted from the molecular docking was analyzed; several amino acid residues that may play a vital role in the xylanase catalytic reaction were obtained to instruct the further modification of xylanase directed-evolution.

Key Words: molecular docking; *Bacillus pumilus* xylanase; active pocket

Xylanase (E.C. 3.2.1.8) is applied to degrade xylan in the process of treating agricultural residues such as rice straw, corn cob etc. The research of microbial xylanase now focuses on the characteristics, enzyme production under different conditions, purification, gene expression and application in paper and pulp industries[1–3]. Thus, it is indispensable to investigate the catalytic mechanism between the action of xylanase and xylan substrate.

Molecular docking is defined as docking ligand to the active site of receptor, and searching a reasonable orientation and configuration to make an optimum match of the shape and interaction between the ligand and receptor. According to the lock and key principle, molecular docking can be used to screen compounds that match well with the active site of receptor in consideration of special and electrical characteristics. In drug design, molecular docking is mainly employed to search for molecules that bear relatively good affinity to biomacromolecules from compound database aiming to find new lead compounds. Meanwhile, molecular docking is thought as an effective technology to approach the interaction of proteins. Owing to a global consideration of the matching effect of ligand and receptor, molecular docking avoids the possibility of good local action but bad global combination as described in other methods[4,5].

In this research, based on the tertiary structure originating from the homology modeling of xylanase from *Bacillus pumilus*, molecular docking was exploited to model the action of xylanase with xylan; several potential active amino acids were obtained and the catalytic mechanism was discussed, and this established a foundation for further modification of xylanase directed-evolution.

1 Materials and methods

1.1 Materisals

Bacillus pumilus, DNA cloning kit, Pentium computer, Bioinformatic databases, Molsoft, visualizational softwares, such as Rasmol, spdbv, and chimera.

1.2 Methods and procedures

1.2.1 Xylanase gene cloning was operated according to molecule biology protocols, and sequencing was executed by Huanuo Company, Beijing. The sequenced nucleotides were translated into amino acids and aligned with different original xylanase sequences.

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1.2.2 Xylanase amino acid sequence was submitted to SWISS-MODEL servicer for homology modeling.1.2.3 IcmPocketFinder program was employed to search for potential substrate binding pockets based on the homology modeling structure.

1.2.3 Structures of xylan and xylanase were edited prior to docking. Docking was performed as per the instruction.

1.2.4 Data analysis.

2 Results and analysis

2.1 Xylanase sequencing and aligning

Gene cloning and sequencing results of *Bacillus pumilus* xylanase are shown in Fig. 1; NCBI ID is EF090270. The result of aligning with different orginal xylanase sequences by Clustal W is indicated in Fig. 2. The scores of Clustal W aligning were 30, 39, 44, 44, 49, 43, 44, and 66, respectively. Two reported conserved sequences, [PSA]-[LQ] -x-E-Y-Y-[LIVM](2)-[DE]-x-[FYWHN] and [LIVMF]-x(2)-E-[AG]-[YWG]-[QRFGS]-[SG]-[STAN]-G-xSAF] were closed in two large rectangles while the conservative Glu residues were closed in two small rectangles.

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ATGAATTTGAGAAAATTAAGACTGTTGTTTGTGATGTGTATTGGACTGACGCTTATACTG
ACGGCTGTACCAGCCCATGCGAGAACCATTACGAATAATGAAATGGGTAACCATAGCG
GGTACGATTATGAATTATGGAAGGATTATGGAAATACCTCGATGACACTCAATAACGGC
GGGGCATTTAGTGCAGGCTGGAACAATATCGGAAATGCTTTATTTAGAAAAGGGAAAA
AGTTTGATTCCACTAGAACTCACCATCAGCTTGGCAACATCTCCATCAATTACAACGCA
AGTTTTAACCCAGGCGGGAATTCCTATCTATGTGTCTATGGCTGGACACAATCTCCATTA
GCAGAATACTACATTGTTGATTCATGGGGCACGTATCGTCCAACAGGAGCGTATAAAGG
ATCATTTTATGCTGATGGAGGCACATATGACATTTATGAAACAACCCGTGTCAATCAGCC
TTCCATTATCGGGATCGCAACCTTCAAGCAATATTGGAGTGTACGTCAAACGAAACGTA
CAAGCGGAACGGTCTCCGTCAGTGCGCATTTTAGAAAATGGGAAAGCTTAGGGATGCC
AATGGGGAAAATGTATGAAACGGCATTTACTGTAGAAGGCTACCAAAGCAGCGGAAGT
GCAAATGTGATGACCAATCAGCTGTTTATTGGCAACATCCGAGCATCA
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Fig. 1 The sequencing result of *B. pumilus* xylanase

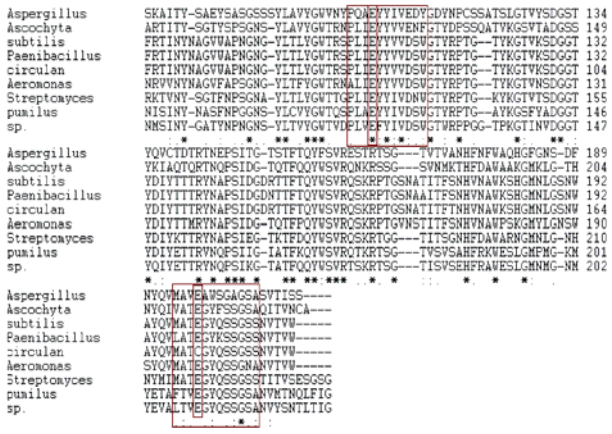

Fig. 2 The multi-alignment of xylanase from different origins

2.2 Homology modeling

Bacillus pumilus xylanase sequence was submitted to the Swiss-model server, and the rasmol software was used to visualize the resulting structure. As presented in Fig. 3, the tertiary structure contained two α-helix, twenty β-sheets, nineteen loops, and the final total energy was -8 600.9 kJ/mol.

2.3 Potential active pocket searching

One active pocket binding with substrate was found through the Molsoft programme based on the homology modeling structure of xylanase. As demostrated in Fig. 4, amino acids 23, 25, 50, 52, 54, 84-86, 90, 92, 99, 101, 109, 111,112, 131, 133, 136-139, 145, 147, 149, 188, and 190, denoted by different colors, were located around the active pocket, embodied as the solid.

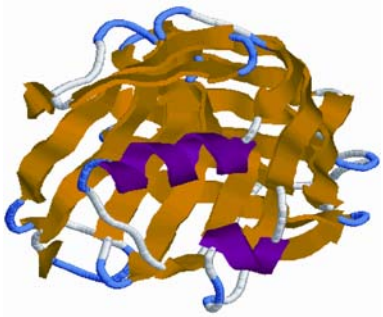

Fig. 3 The tertiary structure of *B. pumilus* xylanase from homology modeling lysate

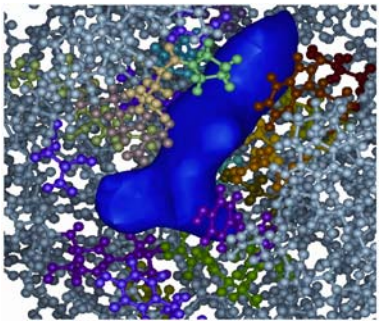

Fig. 4 The structure of active pocket of *B. pumilus* xylanase
The pocket space was represented by the blue solid, and different amino acids appearing around the pocket were denoted by different colors.

2.4 Molecular docking

Although there are still no reports about the crystal structure of *Bacillus pumilus* xylanase in PDB, the crystal structure of *Bacillus circulans* xylanase was reported with PDB id 1BCX. It was indicated that during the catalytic process, only two xylose residues could be fitted into the pocket while the rest of the xylan substrate extended beyond the active site cleft, waiting to enter the pocket two by two rings. In the ground of this cyatlytic mechanism, and for the sake of reducing computational complexity, only two xylose residues (Fig. 5) were taken as the docking ligand, and the structure was extracted from the 1BCX crystal structure, and edited with the spdbv software. *Bacillus pumilus* xylanase docking was executed as the protocols and the consequential structure was