ISSN 0233–7657. Biopolymers and Cell. 2011. Vol. 27. N 6. P. 432–435

Current scenario on computer-aided metalloenzymes designing

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The metalloenzymes are proteins with enzymatic activity which contain metals tightly bound in their active sites to display a chemical action. This review describes the recent developments and success of using computational methods and algorithms for designing industrial enzymes. A recent approach based on functional amino acids or peptides as characteristic molecular moieties and their conservations, has led to a significant expansion of the field of enzyme designing or enzyme mimics. Evolutionary conservation is accounted to consider designing enzymes while the metalloenzymes are a major concern due to their extensive role in catalytic activity and stability. The enzymes from methanogens may provide useful biocatalysts and may be even more valuable for biotransformation reactions, but their biotechnological applications are restricted. Therefore, a method based on the evolutionary hypothesis of conserved domain of sequences obtained from methanogens would make a significant interest in synthetic enzyme biotechnology.

Keywords: metalloenzymes designing, methanogens, evolutionary conservation.

The transitions metals are essential cofactors in the physiology of all organisms. Unfortunately, naturally available enzymes are usually not optimally suited for the industrial applications due to a lower stability of the enzymes under process conditions [1]. The protein engineering is an alternative strategy for changing different enzyme properties simultaneously [2]. The development of enzyme technology has been recently shown by a progress in the theory concerning a mode of enzymes function and how it is related to their primary structure through the formation and configuration of their 3D structures [2, 3]. Generally, an enzyme design is based on the knowledge about the structure, architecture and functional properties of native enzymes. It is well known that enzymes contain a binding site and a catalytic site consisting of two or more catalytic amino acid groups [4, 5]. Exploitation of the diverse reactivity of metal center cofactors has presented a profitable strategy to introduce catalytic activity into proteins. Computer-aided enzyme modeling has taken an important effort to design metalloenzymes so as to perform chemical reactivity with good catalytic efficiency in biotransformation processes.

Several different potential reactions toward a single substrate are often exhibited on a metal centre for designing and engineering enzymes [6, 7]. The successful design of small monomeric proteins [8], protein oligomers [9], and redesign of natural proteins to confer novel functionality have been achieved earlier. The generation of active biocatalysts from dramatically reduced amino acid alphabets provides a strong support for the idea that the primordial enzymes were made from only a handful of building blocks [9]. The success of current protein design methods is based largely on optimizing the packing of atoms. The proposed natural design properties were not necessary conditions for producing

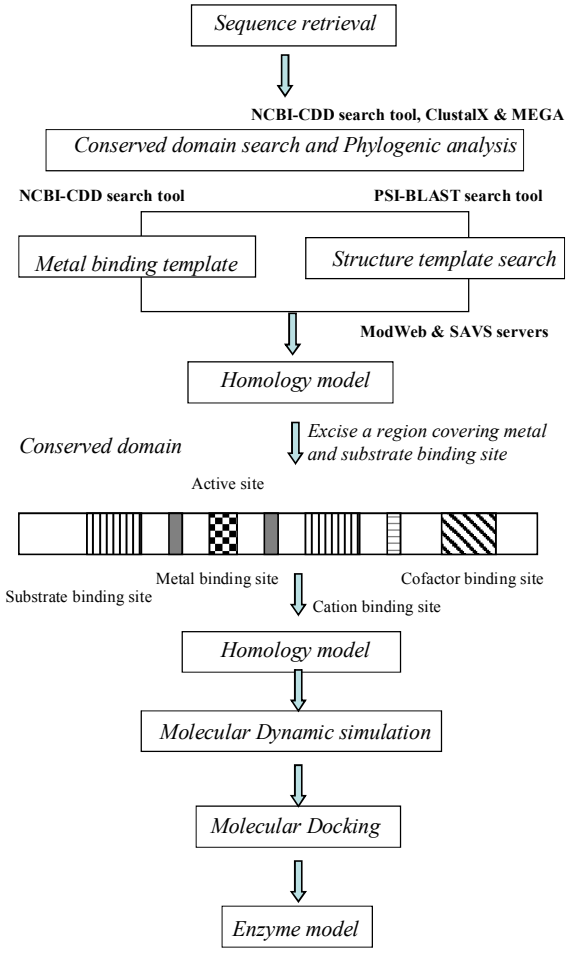
List of enzymes computationally designed

Enzyme	Approach	Reference
Mn-superoxide dismutase	CAChe system	[25]
Mn-superoxide dismutase	Molecular mechanics method	[26]
Mn-superoxide dismutase	DEZYMER algorithm	[7]
Nuclease	Chemical modification of protein scaffold	[27]
Protease	Chemical modification of protein scaffold	[27]
Deoxyribose-phosphate aldolase	Recapitulation of active sites of native enzymes	[28]
Isochorismate pyruvate lyase	QM/MM methods	[29]
Chorismate mutase	Computing empirical valence bonds	[30]
Proteinase K	Machine learning algorithms	[32]
β-Glycosidases	Amino acid replacements	[33]
L-Aminoacylase	Alternation of metal ions	[34]
Co-dependent β-methylaspartate mutase	Molecular-evolution directed approach	[11]
Cs-dependent formyl-tetrahydrofolate ligase	Molecular-evolution directed approach	[12]

well-folded and perhaps even functional artificial proteins [10]. Chellapandi and Balachandramohan have introduced an *in silico* approach to design similar biocatalysts from small molecule mimics of enzyme active sites by combining in a small molecule with emphasis to evolutionary conservation of sequences [11, 12].

Structural homology-based approach is a powerful approach, which has produced a number of new designed metalloproteins [6, 13–15]. Dezymer [16, 17] and ORBIT [18] were the first computer based approaches developed for designing metalloenzymes. Metal Search [19] program has been developed to aid for designing metal-binding sites into proteins [20]. Structure-based computational design techniques have also been used to construct catalytically active sites in proteins of known structure [10]. An increasing effort has been made to combine rational design features into Darwinian evolutionary protocols [21, 23]. TransCent program has been developed for supporting the transfer of active sites from one enzyme to an alternative scaffold [24]. Therefore, to effectively develop a rational modeling paradigm for enzymes, detailed understanding of the mechanism of a rate determining step in the catalytic process and a comprehensive database of chemical structures with their rate data are required so far. The list of enzymes computationally designed is presented in Table. Manganese superoxide dismutase by molecular mechanics calculations (CAChe system) [25, 26] and rational design using DEZYMER algorithm [7], nuclease and protease by modification of protein scaffold [27], deoxyribose-phosphate aldolase by recapitulation of active sites of native enzymes [28], isochorismate pyruvate lyase by quantum mechanics/molecular mechanics [29], and chorismate mutase by computing empirical valence bonds [30] have already been successfully obtained by computational approaches. However, these approaches are more complex and used altered protein scaffold or amino acids. Unfortunately the resulting models were significantly less effective than the corresponding natural enzymes [31] and the reasons for rather limited success are not completely clear [30]. A few successful experimental enzyme designs have been made for proteinase K invariant by machine learning algorithms [32], β-glycosidases by amino acid replacements [34], L-aminoacylase by alternation of metal ions [34] in the recent years. Using evolutionary conservation of catalytic domain, cobalt-dependent β-methylaspartate mutase [11] and cesium-dependent formyltetrahydrofolate ligase [12] constructs from the sequences of archaea [35] have been already designed, and the designing strategy is schematically represented in Figure. Chellapandi has comprehensively reviewed the enzyme engineering and designing algorithms and associated computational programs in conceptual trends, which will ensure a competitive edge in developing improved enzymes [36].

The application of wide range of archaeon enzymes and the usage of organisms themselves in biotechnology are fairly restricted due to the complicated purification strategies and lack of expression systems. How-



A schematic representation of enzyme designing strategy used in this study

ever, the increasing interest in applying enzymes in industrial processes has spurred the search for biocatalysts with new or improved properties. The use of biotransformation in industry will raise as it has been claimed that a doubling of the number of industrially established biocatalytic processes every decade is probable.

Acknowledgement. The author is thankful to the University Grants Commission, New Delhi, India, for financial assistance (32-559/2006) to carry out the work.

П. Челлапанді

Сучасний сценарій комп'ютерного конструювання металоферментів

Резюме

Металоферменти – це білки, що функціонують як ферменти. Вони містять метали, які взаємодіють в активних сайтах, що забезпечує їхню хімічну активність. У представленому огляді описано останні розробки та успішне застосування комп'ютерних методів і алгоритмів для конструювання промислових ферментів. Використання сучасного підходу на основі функціональних амінокислот або пептидів як характерних молекулярних компонентів та їхньої консервативності дозволило значно розширити сферу конструювання або імітування ферментів. Еволюційна консервативність важлива при конструюванні ферментів, серед яких основну зацікавленість представляють металоферменти через їхню суттєву роль у каталітичній активності та стабільності. Ферменти метаногенів можуть слугувати біокаталізаторами і навіть бути цінніщими для реакцій біотрансформації, однак їхнє застосування у біотехнології обмежене. Зважаючи на це, метод, оснований на еволюційній гіпотезі про консервативний домен послідовностей, отриманих із метаногенів, може стати корисним у сфері біотехнології синтетичних ферментів.

Ключові слова: комп'ютерне конструювання металоферменти, метаногени, еволюційна консервативність.

П. Челлапанди

Современный сценарий компьютерного конструирования металлоферментов

Резюме

Металлоферменты – это белки, функционирующие как ферменты. Они содержат металлы, взаимодействующие в активных сайтах, что обеспечивает их химическую активность. В данном обзоре описаны последние разработки и успешное применение компьютерных методов и алгоритмов для конструирования промышленных ферментов. Использование современного подхода на основе функциональных аминокислот или пептидов как характерных молекулярных компонентов и их консервативности позволило значительно расширить сферу конструирования или имитирования ферментов. Эволюционная консервативность важна при конструировании ферментов, среди которых основной интерес представляют металлоферменты благодаря их значительной роли в каталитической активности и стабильности. Ферменты метаногенов могут служить биокатализаторами и даже быть более ценными для реакций биотрансформации, но их применение в биотехнологии ограничено. Вследствие этого метод, основанный на эволюционной гипотезе о консервативном домене последовательностей, полученных из метаногенов, может стать полезным в сфере биотехнологии синтетических ферментов.

Ключевые слова: компьютерное конструирование металлоферментов, метаногены, эволюционная консервативность.

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UDC 577.325
Received 10.05.11