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Molecular analysis of c-terminus structure for elucidating the stabilization effect of site-specific immobilization

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Abstracts

C-terminus specific immobilization often results in a increased structural stability resistant to various denaturation factors. In order to elucidate the immobilization effect on the c-terminus in molecular level, we made over 200 protein data set from Protein Data Bank(PDB), analyzed c-terminus structure of each protein, and investigated the structural relationship with the stabilizing factors such as hydrogen bond, ion pairs, cation pi, disulfide bond, solvation free energy, surface area, flexibility and so on.

Introduction

Immobilization very often results in a greatly increased stability resistant to various denaturation factors[1]: extreme pH and temperature value, high ionic strength, denaturating regents, protease, and so on. Recently, site-specific immobilization has received much attention since it has several merits such as good mass transfer, intact active-site conformation, low denaturation probability, no random orientation, and structural stabilization compared with random immobilization[2]. Such site-specific immobilization method of proteins on a surface, a matrix, or a bead also can play crucial role in developing the better systems for biomolecule interactions such as biosensor, biochip, high throughput screening etc.

The candidate targets for immobilization are surface residues with functional groups such as lysine, arginine, aspartic acid, glutamic acid, histidine, cysteine, tyrosine, N-terminus and C-terminus[3]. However, the multiple point-attachment sites made it difficult to get the merits of site-specific immobilization. Therefore, site-specific immobilization using the power of molecular biology and genetic technology has emerged and dominated[2].

Nowadays, c-terminus specific immobilization methods such as 6x tag system, biotin-avidin system and surface display system has been very famous and applicable[3),4)]. Many researches have showed that such c-terminus immobilizations enhance the stability of many proteins. However, there has been no attempt to elucidate the stabilization effect of site-specific immobilization in the view of molecular level.

In this study, many c-terminus structures were investigated by computational calculation. Also, the structural relationships with other stabilizing factors were analysed statistically.

Material and Methods

Data set

A data set of over 200 non-homologous x-ray crystal protein structures of single chains with a resolution of >=1.5 and <=2.0 and an R factor of <=0.20 and containing all non-hydrogen atoms was used for the analysis of packing. The original list was obtained as described by Hobohm and Sander[2)] from PDB and culled to meet the all the non-hydrogen atom criterion and to remove ASX or GLX amino acid residues or other non-standard amino acid residues and to remove membrane proteins.

Structural analysis

Solvent accessible area, the ratio of surface area to normal surface area, and determination of inner residue or outter residue are calculated by Getarea 1.1. The number of hydrogen bond, αcarbon- flexibility and solvation free energy are calculated by Protable on the Biopolymer. The number of salt bridge interaction, the number of cation pi and the number of disulfide bond are calculated by Protein Explorer package 2.1.

Results and Discussion

All the residual surface area and solvent accessibility were calculated by Getarea 1.1. As shown in the Fig 1, the surface area and solvent accessibility c-terminus have higher value than other residues.

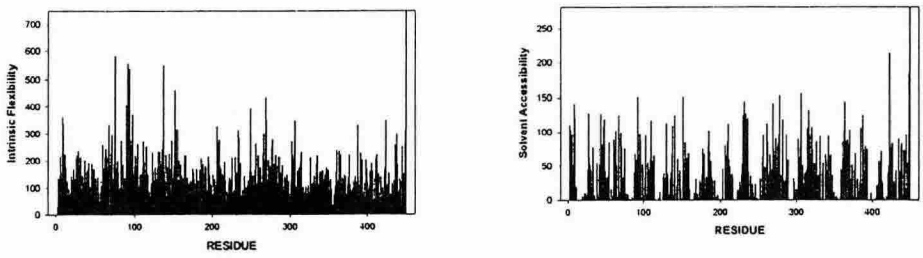

Fig 1. The surface area and solvent accessibility of all the residues of a protein

All the residual intrinsic flexibility and surface energy were calculated by Protable on the Biopolymer module. As shown in the Fig 2, the flexibility and absolute solvation free energy of c-terminus have higher value than other residues.

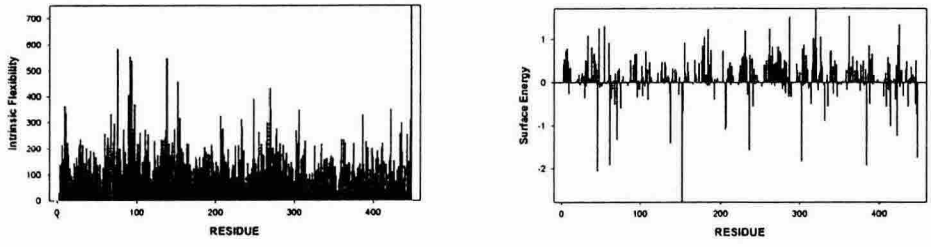

Fig 2. The flexibility and surface energy of all the residues of a protein

The number of salt-bridge, cation pi and disulfide bond were calculated by Protein Explorer package 2.1. The probability of such bonds in c-terminus have fewer value. Moreover, the probability of H-bond in the c-terminus is lower than other residues

For the statistical analysis of c-terminus structure, a standard index, the requisites of which are the representative of structure, the good relation with other stabilizing factors and the overcome of individual residual length, was

defined and used. Residual compactness, the ratio of residual surface area to residual volume, was used as the standard index for structural analysis. As shown in Fig 3. the c-terminus compactness has higher value than others.

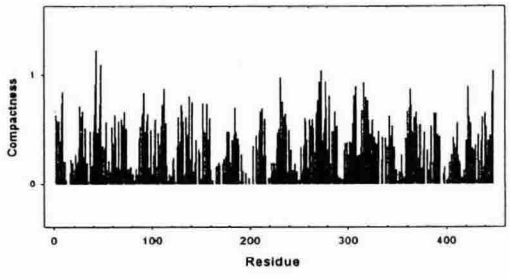

Fig 3. The compactness value of all the residues of a protein

Through the statistical analysis, it was resulted that c-terminus has very large surface area, large solvent accessibility, high flexibility, big absolute solvation energy and big compactness. In addition, c-terminus has few probability of the stabilizing forces such as salt bridge, cation pi, disulfide bond and hydrogen bond.

In conclusion, c-terminus has higher probability to become unstable than other residue of a protein. Therefore, site-specific immobilization would be more effective on the c-terminus structure.

Reference

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