Analysis of the Local Sequences of Folding Sites in β Sandwich Proteins with Inter-Residue Average Distance Statistics

Yuko Ishizuka[1] and Takeshi Kikuchi*[,2]

[1]*Department of Chemistry and Bioscience, College of Industrial Technology, Kurashiki University of Science and the Arts, Kurashiki, Okayama, Japan*

[2]*Department of Bioinformatics, College of Life Sciences, Ritsumeikan University, Kusatsu, Shiga, Japan*

Abstract: The sequences of azurin and titin, β sandwich proteins, are analyzed based on inter-residue average distance statistics. A kind of predicted contact map based on inter-residue average distance statistics (Average Distance Map, ADM) is used to pinpoint regions of possible compact regions for two proteins. We compare predicted compact regions with the positions of the residues with experimental high ϕ values for these proteins reported in the literature. The results reveal that the regions predicted by ADMs correspond to the positions of residues with the high ϕ value. Furthermore, we perform random sampling of 3D conformations using these protein sequences with a potential derived from inter-residue average distance statistics. It is demonstrated that the residues with highest contact frequency during the simulations qualitatively correspond to the residues with the highest ϕ values for these proteins. Importantly, analysis with inter-residue average distance statistics predicts the properties of folding processes of the β sandwich proteins starting from only sequence information.

Keywords: β-sandwich protein, folding, inter-residue average distance statistics, ϕ values, azurin, titin.

INTRODUCTION

One of the ultimate goals of molecular biophysics or bioinformatics is to elucidate how the principle of protein folding is encoded in amino acid sequences. However, it is quite difficult to understand relationships between sequences and 3D structures of proteins, partly because structures of proteins are conserved better than their sequences as observed in so-called superfolds [1]. Among the various protein folds, the 3D structures of proteins with the β sandwich scaffold are rather complicated and the elucidation of the folding mechanisms is challenging [2]. Energetics of the β sheet structures has been extensively studied by several authors [3-10]. Recently, the regularity of β sandwich structures has been clarified [11-13] and the relationship of the regularity in β sandwich proteins and their folding mechanisms, has been recognized mainly through experimental ϕ value analyses. Portions with the regularity observed in β sandwich structures are related to the segments containing the residues with high ϕ values, i.e., the segments involved in the folding mechanism. Thus, β sandwich proteins are quite interesting for clarifying the relationship between 3D structures and sequences, and that is the reason why we treat β sandwich proteins in this work.

We focus on how we can extract information about folding mechanisms from the sequences of β sandwich proteins. In other words, the sequence specificity for the folding mechanisms of β sandwich proteins is considered. In particular, azurin and titin are taken as β sandwich proteins because detailed investigation of folding mechanisms, especially ϕ value analyses of azurin [14-19] and titin [20-24] have been performed.

There are also several theoretical and simulation studies on folding of azurin and titin. Zong *et al.* [25] predicted ϕ values of some residues in apo-azurin theoretically using the variational free energy functional; compared with the experimental data, the theoretical ϕ values coincide well with those from experiment (correlation coefficient of 0.90). However, their technique requires the native structure of a protein and does not clarify the characteristics of a local sequence related to the folding properties of a protein.

In the present work, we try to analyze the sequences of azurin and titin with the average distance map method and with simulations employing an inter-residue potential derived from inter-residue average distance statistics. It should be emphasized that the present work focuses on how folding information can be decoded in the sequences of azurin and titin. We demonstrate that average distance maps provide information on folding properties of proteins without any other information beside amino acid sequences. Actually, it was observed that differences in folding processes of homologous proteins within a family reflect on their average distance maps for the lipid binding protein family [26], the globin family [27] and the c-type lysozyme family [28].

On the other hand, Baker and coworkers [29, 30] and other authors [31, 32] found a strong correlation between values of contact order and folding rates of proteins exhibiting two-state folding. These works tried to predict protein folding rates from only sequence information, but all techniques predict just folding rates but not details of folding processes from amino acid sequences. Hence, the methods

*Address correspondence to this author at the Department of Bioinformatics, College of Life Sciences, Ritsumeikan University, 1-1-1 Nojihigashi, Kusatsu, Shiga 525-8577 Japan; Tel: +81-77-561-5909; Fax: +81-77-561-5203; E-mail: tkikuchi@sk.ritsumei.ac.jp

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azurin are 49-V and that of ϕ values is 50-L, i.e., very close, and that for titin the highest peak of the p(μ) values is at 58-L and that one of the highest peaks in the profile of ϕ values at 58-L, i.e., exactly the same position.

The locations of the key strands are compared with the region containing the residues with the high ϕ values and that with the highest p(μ) values for azurin and titin respectively. As mentioned above, the residues with high ϕ values are 31-V, 33-L in the 3th β strand (β3) and 50-L in the β4 in azurin as shown in Fig. (**8A**). 31-V and 33-L form hydrophobic packing with 48-W between β3 and β4 as shown in Fig. (**5A**). 50-L forms weak hydrophobic packing with 31-V. 97-F and 108-Y show a moderate magnitude of ϕ values (Fig. (**8A**)), and these residues are on the key strands β6 and β7 respectively forming weak hydrophobic packing (or these two residues interact *via* 102-L. see Fig. (**5B**). On the other hand, residues with high p(μ) values with >Average + 0.5s are in the region of 40-63. (In the present case, Average + 0.5s ≈ 0. see the figure legend of Fig. (**8A**)), and this region contains β4 and α helix (α1). As seen above, the residues with the highest p(μ) is 49-V that correspond very well to 50-L with the highest ϕ value. It should be noted that the p(μ) profile in Fig. (**8A**) shows the small peaks at the 29-F and 33-L, and these residues are almost same as the high ϕ value residues in β4 and β5, i.e., 31-V and 33-L. The p(μ) profile also shows a peak at 81-I, and there is a peak in the ϕ values at this residue.

For titin, the experimentally observed residues with the high ϕ values are 23-I, 49-I, 58-L, 60-L, and 73-F as shown in Fig. (**8B**). These residues are in the key strands β6 and β7, and 58-L and 60-L also form the hydrophobic packing with 71-V in β6 and β7 as shown in Fig. (**5D**). Furthermore, a peak at 23-I of the ϕ value profile (also on the key strand β3) and 23-I is involved in the hydrophobic packing between β3 and β4 as seen in Fig. (**5C**). On the other hand, residues with p(μ) values >Average+0.5s are 54-70 (Also in the present case, Average + 0.5s ≈ 0. see the figure legend of Fig. (**8A**) as in Fig. (**8B**) corresponding to the residues with high ϕ values and the interlocked pairs in key strands. This region contains β6 and a part of β7. The p(μ) profile also shows a peak at 47-C which is also very close to 49-I at which the ϕ profile denotes a peak.

p(μ) Values Fixing the Regions Predicted by ADMs to the Native 3D Structures

In the present study, the positions of the compact regions predicted by ADMs correspond to the folding transition structure forming region for the β sandwich proteins. Therefore, we are interested in how p(μ) values of each protein vary if the 3D structures of the predicted compact regions are fixed to the native structure. We also fix the 3D structures of the regular secondary structures to the native structure.

For azurin, we fixed the 3D structure of the region 19 – 61 and the conformations of the regions of all α helices and β strands (α1, β3 and β4). (Two regions are predicted by the ADM, i.e., 19 – 61 and 80 – 127. The η value of 80 – 127 is greater than that of 19 – 61; however, the highest peak is located in 19 – 61. This is a controversial result. In the present study, we take 19 – 61 to be fixed.) Then we performed the same calculations for p(μ) values. The result is shown in Fig. (**9A**).

The region with the broken line of the p(μ) profile in the figure denotes the region with fixed 3D native structure, and we mainly focus on change in the rest of the profile. A residue number underlined in the figure denotes a high ϕ value that we wish to focus on. The peaks of the p(μ) profile in β6 and β7 become higher compared with the profile in Fig. (**8A**), and the general tendency is getting closer to the ϕ profile. β6 and β7 form key strands, and this result suggests the participation of β6 and β7 with the folding consistent with the observation of the key strands. That is, this result suggests that β3 and β4 interacts with β6 and β7during the folding as long range interactions.

For titin, we fixed the 3D structure of the region 57-89 (predicted subdomain + C terminal two residues) and the conformations of all β strands (β6, β7 and β8) to the native structures. The simulation result is shown in Fig. (**9B**). The remarkable property is that the p(μ) values in the β3 region became higher compared with the profile in Fig. (**8B**) suggesting the participation of β3 in the folding; the correspond-

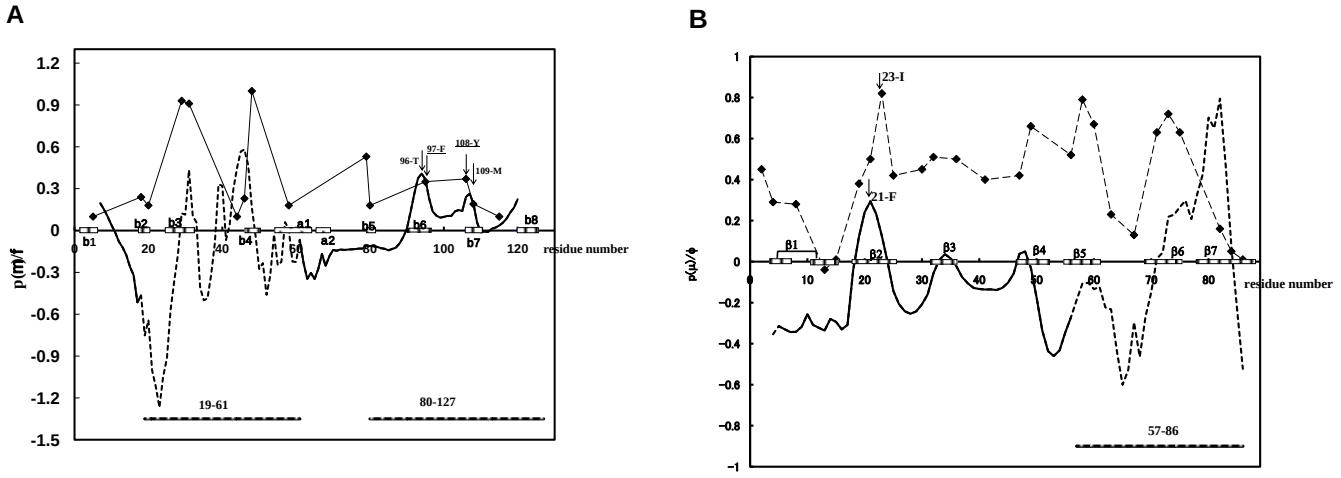

Fig. (9). Plots of p(μ) values for azurin (**A**) and titin (**B**) fixing the 3D structures of the compact regions predicted by ADMs (p(μ) profiles of these parts are drawn as broken lines in the figures) and the regular secondary structures to the native structures. An underlined residue name calls attention to the location of a peak in the ϕ profiles. ϕ profiles are indicated by filled diamonds and solid lines.

ing peak of the new p(μ) plot is 21-F which is close to 23-I which is a peak of the ϕ profile. β3 constitutes a key strand. Again, the long range interactions between β6-β8 and β3 are suggested in the folding of this protein.

For both azurin and titin, the positions of the new peaks appearing in Figs. (**9A** and **B**) are within three residues of the peaks observed in the experimental ϕ profiles.

DISCUSSION

For the β sandwich proteins treated in this study, azurin and titin, the ADMs predict the location of subdomains that include the residues with high ϕ values. These results mean that the regions in the sequences encode information on structure formation of these proteins are detected by ADMs.

Furthermore, the highest peak of the p(μ) plots corresponds well to that of the ϕ plots in the present cases. Therefore, a p(μ) plot specifies the location of the more significant areas in the region predicted by ADM. The whole profile of a p(μ) plot is not necessary to resemble to that of the corresponding ϕ plot. The most important point is the location of peaks. These results also mean that the average distance statistics contain information about initial 3D structure forming regions, i.e., folding sites. Thus, it is possible to specify which secondary structure elements are involved in the early folding event based on the knowledge of the location of secondary structures.

For example, let us suppose that we do not know the 3D structure and the secondary structures of azurin. Our ADM analysis predicts the compact regions 19-61 (η = 0.202) and 80-127 (η = 0.355) (Fig. (**7A**)). These two regions can be candidates for regions involved in structural formation. Although from the η value the region 80-127 is plausible to be compact during the early stage of folding, the p(μ) analysis predicts the region 44-59 to be a highly contacted region (Fig. (**8A**)). The knowledge of the location of the secondary structures allows us to predict that the β4 and a helix mainly pack as suggested from Fig. (**8A**). Furthermore, within the region 19-61, a peak of the p(μ) plot appears at β3. These observations lead us to predict that β3 and β4 pack together hydrophobically. These tentative predictions are consistent with the results of ϕ value and key strands analyses. A similar consideration can be applied to titin. From the sequence of titin, we predicted that the region 57 – 86 forms a compact region during a relatively early stage of folding (Fig. (**7B**)), and within this region, the segment 57 – 69 would be deeply involved in the folding (Fig. (**8B**)). If we know the location of β strands, it is also predictable that β6 and β7 pack together hydrophobically during folding (Fig. (**8B**)).

Furthermore, our method can specify the hydrophobic residues forming packing pairs in key strands. We observed that such a hydrophobic residue possessing a high ϕ value is also located within a few residues from those with a high p(μ) value as indicated in Fig. (**8**). Inversely, the hydrophobic residues in the segment with the highest p(μ) value can be considered as residues capable of forming packing pairs in a β sandwich protein.

Thus, the indices derived from average distance statistics correspond well to folding properties including folding transition states. It is remarkable that average distance statistics reflect some properties of the folding transition state of a protein, although average distance statistics probably include properties of a denatured conformation ensemble of a protein near the folding transition state. It should be emphasized again that the predictions based on ADMs and p(μ) values have been done without any 3D structure information.

As learned from Fig. (**9**), we can further predict a detailed folding mechanism if we fix the 3D structure of a predicted folding site and regular secondary regions to the native structure. That is, the main feature of the p(μ) profile becomes closer to the ϕ profile. In other words, a simulation with fixed partial structures reflects the folding process consistent with the folding transition state suggested from the ϕ value analyses. In particular, it is interesting that the peaks of the p(μ) profile within the segments corresponding to the key strands are getting higher, and thus key strands involved in folding are predictable from the present method. Thus, our method has potential to predict the location of the whole folding transition state area in a protein if we have knowledge of the partial 3D structure of a transition structure formation region.

The next interesting problem is whether the 3D structure of such a compact region in folding can be modeled. At least, for β sandwich proteins, 3D structures of transition

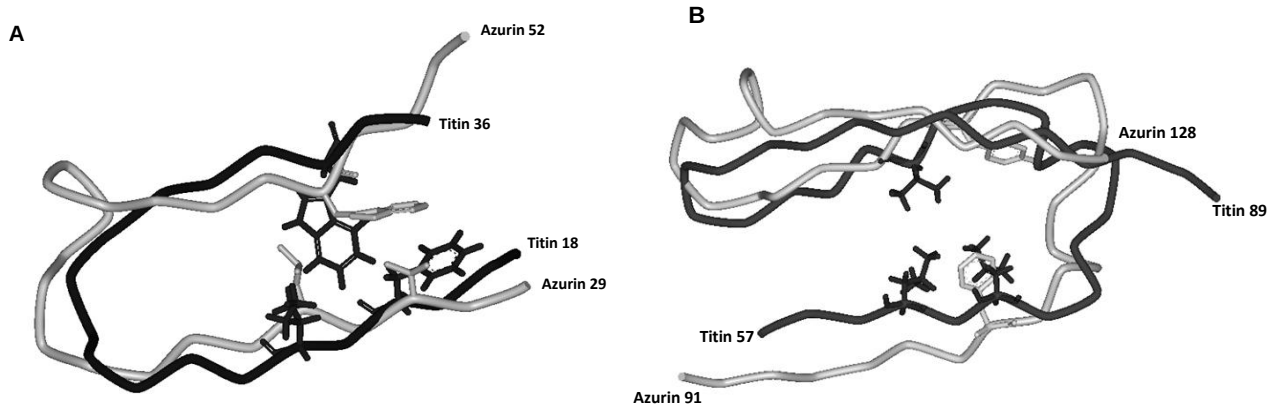

Fig. (10). A. Superposition of the 3D structures of azurin 29-52 (light gray) and titin 18-36 (dark gray). The rmsd value is 2.72 Å. **B**. Superposition of the 3D structures of azurin 91-128 (light gray) and titin 57-89 (dark gray). The rmsd value is 4.08 Å. The interlocked pairs of hydrophobic residues are also shown.

state formation areas might be similar. As an attempt, we compare the 3D structures between 29-52 corresponding to the packing pair of β3 and β4 in azurin and 18-36 corresponding to the packing pair of β3 and β4 in titin, and the region 91-128 of azurin containing the C-terminal 3 β strands within the predicted compact region 80-129 by the ADM and the region 57-89 predicted as a compact region by the ADM for titin. Illustrations of superposed structures are presented in Figs. (**10A** and **B**).

The alignments were performed so that the positions of the hydrophobic residues forming the interlocked pairs and secondary regions are superposed. The rmsd values of the Cα atoms are 2.72 Å and 4.08 Å, respectively, in the former superposition and in the latter. Thus, these rmsd values suggest that it is possible to model the 3D structure of the transition structure formation region of a β sandwich protein from an appropriate template structure.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers Web site along with the published article.

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