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Improved Prediction for the Structure of the Dimeric Transmembrane Domain of Glycophorin A Obtained Through Global Searching

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ABSTRACT **A more global search method, using fewer assumptions, has been used to predict the structure of the dimeric transmembrane region of the protein glycophorin A. The resulting model significantly differs from that previously determined. In particular, the arrangement between the two transmembrane helices is now more symmetric resulting in improved interaction energies and an increased buried surface area. An increase in the van der Waals interaction energy due to tighter packing compensates for the loss of the interhelical hydrogen bond observed between Thr-87 of each helix in the previous model.**

Key words: membrane proteins, α-helices, packing, two-stage model, computational searching, molecular dynamics

INTRODUCTION

There are many integral membrane proteins whose functions depend upon the successful formation of oligomers through interactions between transmembrane α-helices.[1] However, high-resolution structural information about such integral membrane proteins has proved difficult to obtain using conventional structure determination techniques (x-ray crystallography, electron microscopy, or solution nuclear magnetic resonance). The two-stage model for the folding of polytopic α-helical membrane proteins proposes that individually stable transmembrane helices interact with one another to form the folded conformation without changes in secondary structure.[2] If this is so, conformational searches can be simplified by maximizing the interaction energy (or complementarity) between the independently folded secondary structure elements. While the energy of the system alone may not be a reliable indicator of model correctness, the careful consideration of mutagenesis data[3,4] can allow a critical, independent test of any proposed model.[5,6]

The application of global computer searching in combination with mutagenesis data has been previously used to determine a model for the transmembrane domain of the dimeric protein glycophorin A (GpA),[5] which has been shown to possess a highly specific dimerization motif.[7] This study predicted a right-handed interaction between the two transmembrane α-helices. The model had a crossing angle of −30° and an average shift between the two helices along the long helical axes of 2 Å producing an out-of-register packing arrangement. It was concluded that this shift is essential to allow optimal packing between the helices with this right-handed crossing angle. This asymmetry also allowed the formation of an inter-helical hydrogen bond between $O_{\gamma 1}$ of Thr-87 and $H_{\gamma 1}$ of Thr-87 of the opposite chain, stabilizing the interaction between the two helices.

We have revisited the modeling of the GpA in the light of the development of a new global searching method, which we applied to modeling of the transmembrane pentamer phospholamban.[6] The searching method differs from the previous one by improving the sampling of all possible packing interactions between helices. In addition, a more intensive simulated annealing procedure has been employed to facilitate finding the global minimum. The number of assumptions used in the modeling was reduced by eliminating the initial supercoiling imposed on the helices. Application of the revised global search method to the GpA transmembrane sequence has produced a model which, although still possessing a right-handed crossing angle between the helices, shows significant differences from the original model.

METHODS

Differences between the new global conformational search method and the previous ones are described in the following. A detailed description of the new method can be found in reference.[6] A nonbonded cutoff of 13 Å was used for all calculations, compared to 15 Å in the original method. A switching function was applied to the van der Waals terms between 10 Å and 12 Å, and a shifting function was

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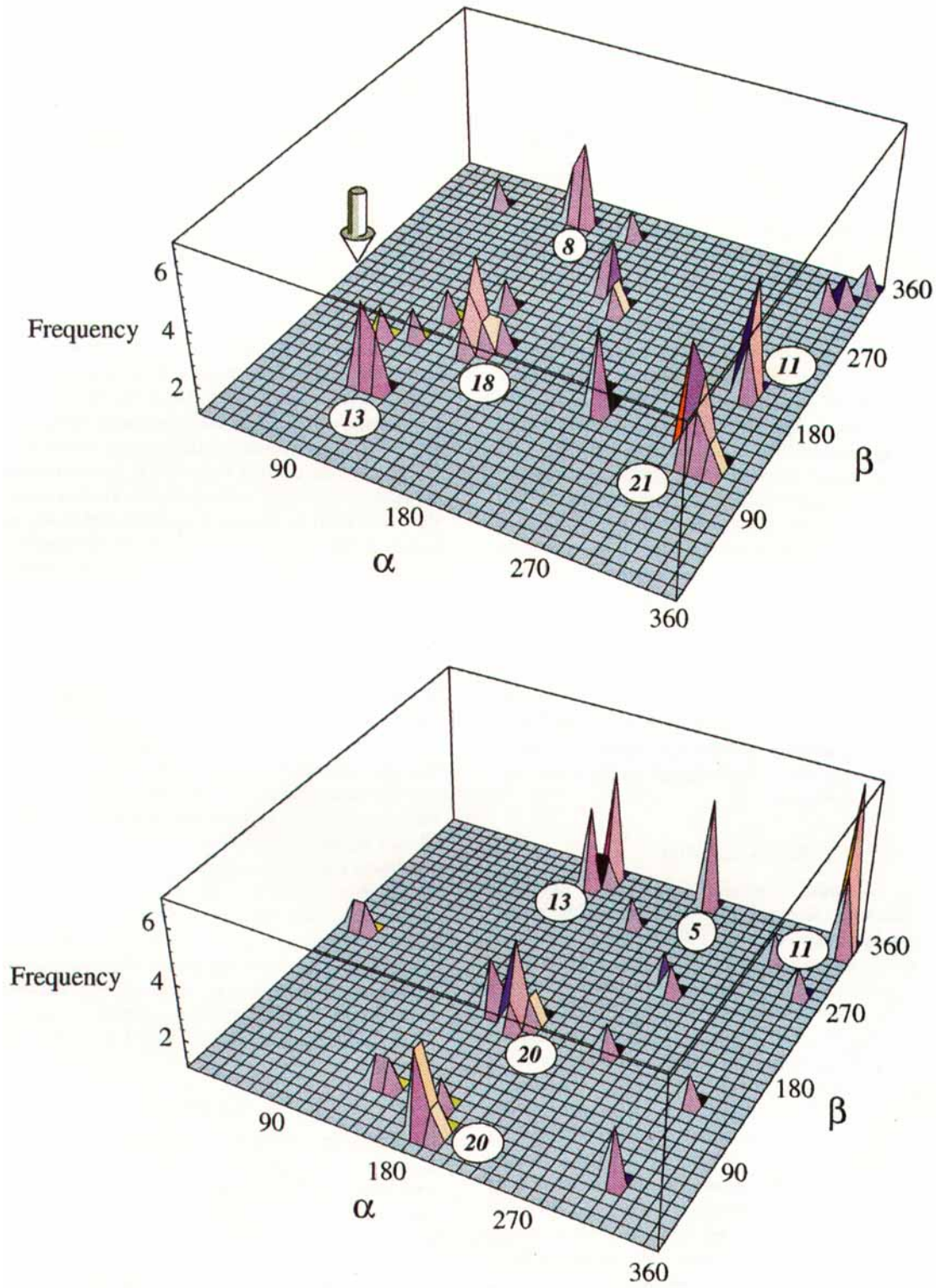

Fig. 1. Frequency of structures obtained by simulated annealing found in α/β rotation space, averaged over a 10° by 10° grid. The number of structures present per peak is shown for the 10 most populated peaks. **A:** Results for right-handed helical pairs. **B:** Results for left-handed helical pairs. The arrow marks the only cluster of structures which is consistent with the mutagenesis data.[3] See references[5,6] for details of the mutational analysis.

applied to the electrostatic term between 10 Å and 12 Å, compared to truncation at the cutoff distance in the original method. Distance restraints of 3.2 Å, instead of the 2.9 Å used previously, were applied between O_i and N_{i+4} in order to maintain an α-helical conformation during the systematic search pro-

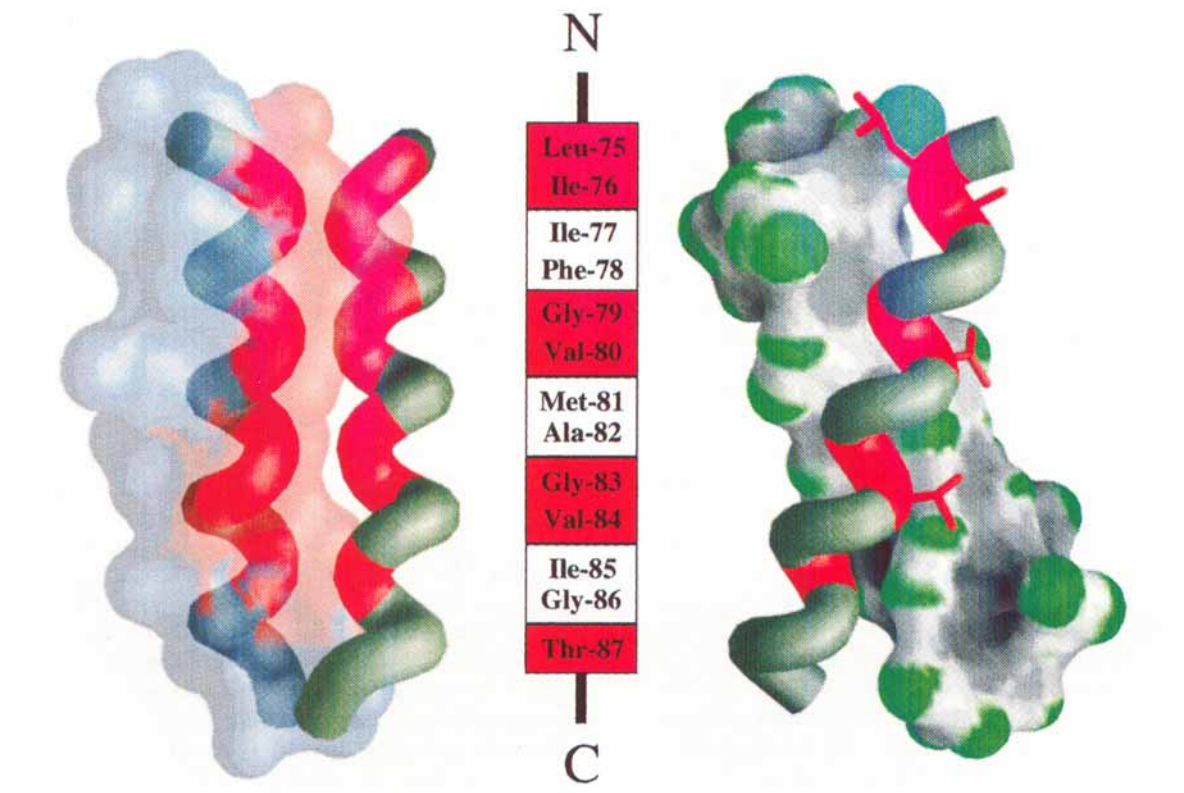

Fig. 2. The interaction between the two transmembrane α-helices in the glycophorin A dimer. In the center is the sequence encompassing the dimerization motif, the residues which are sensitive to mutation are highlighted in red throughout the figure. The leftmost image shows the highly symmetric nature of the interaction. The rightmost image is rotated 90°, and shows the nature of the interface between the two helices. A groove formed by the Gly-Val residues at the center of the sequence allows a very close contact between the helices at Gly-79 and Gly-83. These images were generated with the program Grasp.[11]

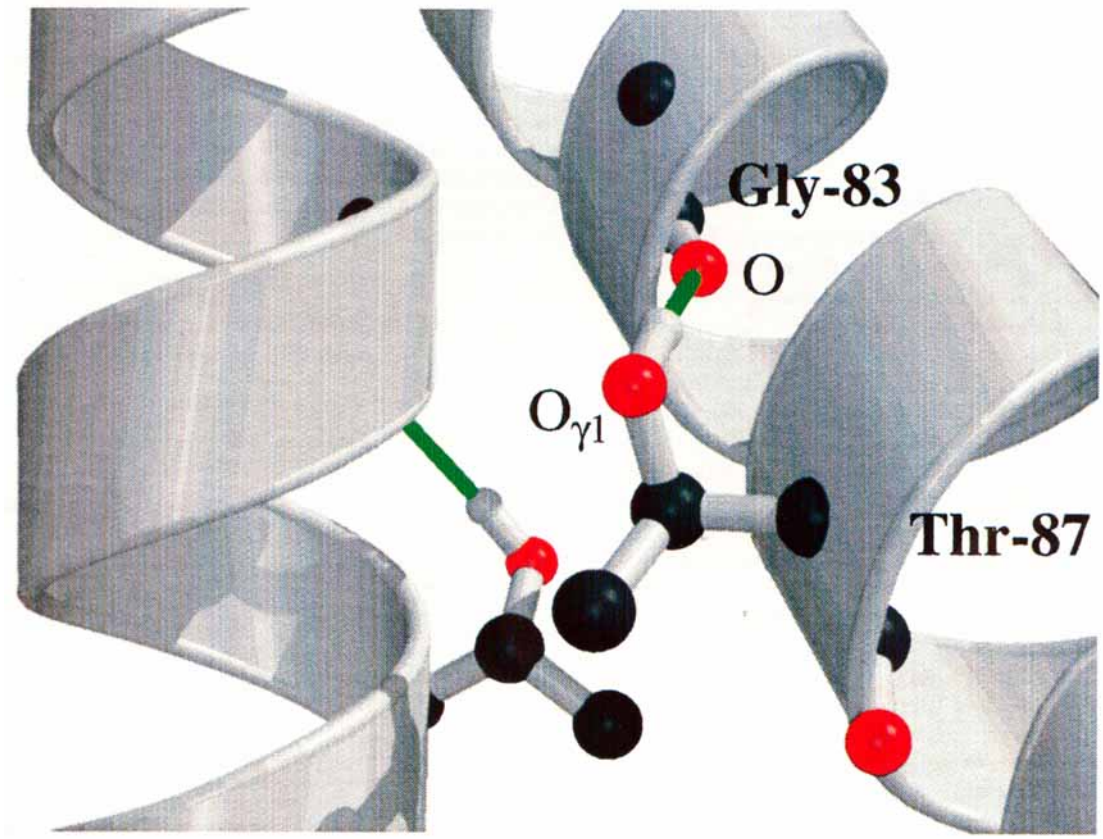

Fig. 3. The side chain to main chain hydrogen bonding observed for Thr-87 of each helix. Hydrogen bonds (shown in green) are formed between $H_{\gamma 1}$ of Thr-87 and the main chain O of Gly-83. These intra-helical hydrogen bonds allow a close inter-helical packing between Gly-83 of each helix. This image was generated using the programs Molscript and Raster3D.[12,13]

cedure. The initial coordinates were those of a canonical α-helix (3.6 residues per turn). In contrast to reference,[5] no prior supercoiling was applied to α-helices, helices were allowed to reach a stable conformation during the annealing procedure (a coiled-coil interaction would occur if it were energetically favorable). The system was simulated more extensively using molecular dynamics for 5,000 steps at 600K with a timestep of 1 fs. This was followed by simulation, again 5,000 steps, at the lower temperature of 300K with a timestep of 2 fs. An exhaustive search of the possible interactions between the parallel helices was carried out. The search was carried out over the whole possible two-body rotational interaction space ($\alpha = 0°$ to 360°, $\beta = 0°$ to 360°, where α and β are the rotation angles about the long axis of the two helices relative to some arbitary starting position), whereas in the previous method, the search was restricted to the region around the two-fold dyad axis. The frequency of sampling was 45° for both α and β. Tests showed that initial crossing angles (Ω) between +20° and −20° often became trapped in local minima around 0°, therefore, the starting structures were canonical α-helices with either a left-handed (+50°) or right-handed (−50°) crossing angle. These initial crossing angles allow convergence to stable left-handed and right-handed coiled-coil structures, including configurations close to $\Omega = 0°$. In order to further increase sampling, four trials were carried out from the same starting position, using different initial random velocities in each case. The search generated a total of 512 independent configurations for the GpA dimer which were analyzed as outlined previously.[5,6]

RESULTS

Several groups of stable structures were found by the search (Fig. 1) but only one of these has packing interactions compatible with the mutagenesis data (indicated by the arrow). The average structure has a crossing angle of −45° and an average shift along the long helical axes of 0.15 Å. This renders the model more symmetric and more closely packed than the previous model (Fig. 2). The closest approach between the helices is at Gly-79, where the axes of the helices are separated by only 6.2 Å. This should be compared to the previous model which had a larger helical separation of 6.9 Å. Crucial to the increased symmetry of the dimer is the absence of the inter-helical hydrogen bond at Thr-87. Instead, the $H_{\gamma 1}$ of each Thr-87 side chain hydrogen bonds back the main chain O of Gly-83 on the same helix (Fig. 3). The length of this hydrogen bond, from the $O_{\gamma 1}$ of Thr-87 to the main chain O of Gly-83, is 2.85 Å. This side chain to main chain hydrogen bonding is commonly observed for both Thr and Ser residues in high resolution crystal structures.[8] The disappearance of this interhelical hydrogen bond allows the helices to make a closer approach, with the helices rotated some 10° from the previous model. This rotation allows a very close interhelical interaction between Gly-79 and Val-80, and Gly-83 and Val-84, through a "ridges-into-grooves" type packing.[9]

DISCUSSION

Several characteristics of the new model lead us to believe that it is an improvement over the previous one. The model has a significantly increased packing interaction with 340 $Å^2$ of buried surface area at the interface compared to 280 $Å^2$ in the previous model. There is a concommitant gain in Lennard-Jones interaction energy between the helices; −48 kcal/mol compared to −42 kcal/mol, which compensates for the loss of the interhelical hydrogen bond (−4 kcal/mol). The model is also consistent with solid-state NMR measurements which place an upper bound of 5 Å ± 1 Å on two interhelical distances.[10] The distance between Val-80 and Gly-79 is 5.3 Å in the original model while the distance between Val-84 and Gly-83 is 7.2 Å, clearly violating the experimentally measured distance. In contrast, in the new model the distances are 4.4 Å and 4.7 Å, respectively, both of which are within the experimental error of the NMR distance measurements.

This new modeling study of GpA again illustrates both the power of global computer search methods and the need for additional information, such as mutagenesis data. It also highlights the need for a complete global search of packing interactions in order to find the optimum structure. On the basis of this latest model, it is predicted that the transmembrane dimer of GpA will be very closely packed due to specific interactions between residues Gly-79 and Val-80, and Gly-83 and Val-84, and will not contain an interhelical hydrogen bond between Thr-87. The coordinates for the predicted structure will be deposited with the Brookhaven Protein Database.

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