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6 Folding process in the presence of specific ligand

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Abstract

The analysis of hydrophobicity distribution assuming the presence of hydrophobic core present in central part of the protein body revealed its irregularity strongly related to the localization of ligand binding place. The ligand binding cavity shall be of high specificity versus the ligand to ensure the proper biological activity. This observation suggested that the ligand presence seems to be necessary during the protein folding process. The ligand and the folding protein influence themselves mutually

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ensuring the generation of the ligand binding cavity of high specificity. The influence of the ligand present in protein folding simulation is discussed in this chapter. The folding of ribonuclease simulated in the presence of the specific inhibitor revealed the magnitude of hydrophobicity irregularity as being strongly dependent on the distance versus the ligand locus in protein body. The structure of hemoglobin chains (α and β) folded in silico in the presence of hem molecule demonstrates the proper localization of histidines responsible for hem stabilization in the complex in the structure generated in silico.

Introduction

As was shown in former chapter, the generation of ligand binding cavity of high specificity may be ensured by the presence of ligand molecule during the folding process [1,2]. The two molecules: protein and ligand influencing mutually their structures produce the structure of protein with highly specific ligand binding cavity. Ligand molecule by itself may occupy the proper locus and orientation versus the local hydrophobicity in "*fuzzy oil drop*" environment. The highly hydrophobic ligand may occupy the central area of the "*fuzzy-oil-drop*" allowing the polypeptide folding around the ligand. The highly hydrophilic ligand is free to occupy the area close to the ellipsoid surface. The ligand of hydrophobic "dipole" character may orient itself with hydrophilic terminus exposed to the surface and hydrophobic terminus toward the center of the "*fuzzy-oil-drop*".

The hemoglobin chains: α and β were selected to check this hypothesis. The chain α and hem molecule and chain β also with hem molecule put together into the "*fuzzy-oil-drop*" environment is assumed to fold the way producing the molecule with cavity ready to bind the hem as the specific ligand. The conclusion of the observations and simulations presented in this chapter suggest that the presence of specific ligand is necessary during the folding process.

The ES (early stage) structural form was taken as the starting conformation for LS (late stage) simulation. Since two different simulations were performed, the one without ligand present is called LS and the one with the ligand molecule present in the folding environment is called LSL (late stage + ligand) in this paper.

Hem characteristics

The common scale of hydrophobic characteristics shall be applied to make parameterization of protein and ligand compatible. The hydrophobic characteristics of hem (as well as any other ligand) may be defined using the

localization of ligand in the protein-ligand complexes in respect to the local hydrophobicity. The protein-hem complexes available in PDB were localized (and oriented) in coordinate system according to the conditions for "*fuzzy-oil-drop*" model. The localization of hem in many hem-protein complexes was transformed to appropriate hydrophobicity density (the same procedure was applied for all amino acids) making the scale for protein and ligand compatible.

The hem molecule was divided in five sections (virtual amino acids) (Fig.1.). Each of them localized in all available hem-protein complexes in relation to the local hydrophobicity density produced the hydrophobic characteristics as it is shown in Tab.1. in relation to the amino acids.

This way of the hydrophobicity parameter definition makes the hydrophobicity scale compatible (common) for amino acids and ligands fragments.

The hem molecule was rigid in simulation. This molecule was free to translate in any direction and rotate around any axis in coordinate system. This flexibility made the mutual influence of folding protein and ligand possible.

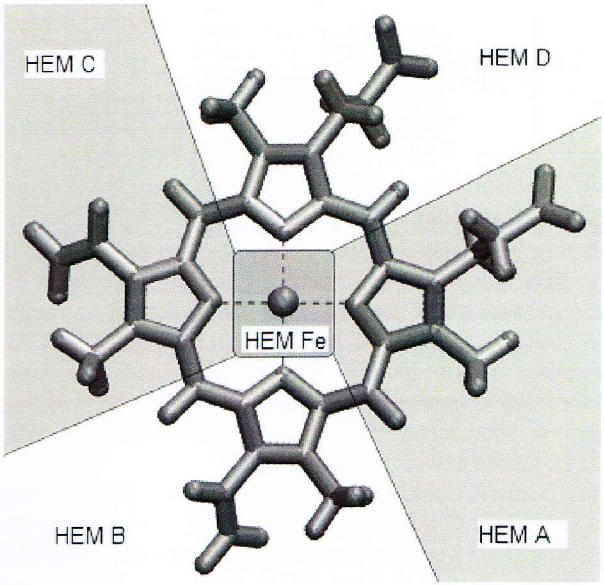

Figure 1. The "virtual amino acids "as distinguished in hem molecule: The hydrophobicity parameters were assigned for each part independently.

Table 1. Hydrophobicity scale for amino acids and pseudo-amino acids (fragments of ligand molecule – in our case hem molecule) calculated according to "*fuzzy-oil-drop*" (the hydrophobicity value according to the relative position of amino acid in the idealized "*fuzzy-oil-drop*").

Amino acid	Hydrophobicity
LYS	0.000
ASP	0.108
GLU	0.126
GLN	0.215
PRO	0.233
ASN	0.256
ARG	0.265
SER	0.314
THR	0.422
GLY	0.435
HEM_A	**0.532**
ALA	0.552
HEM_D	**0.574**
HIS	0.655
TYR	0.655
HEM_C	**0.716**
HEM_Fe	**0.726**
HEM_B	**0.763**
MET	0.825
LEU	0.834
TRP	0.874
VAL	0.892
ILE	0.942
PHE	0.982
CYS	1.000

Folding simulation

The starting structures of both chains were generated on the basis of contingency table (described in details in [3-6] and shortly in Chapter 4 of this book) summarizing the relation of tetrapeptide sequences and tetrapeptide structures expressed by letter codes (each letter identifies the local probability maximum in the limited conformational sub-space introduced for ES folding step) (see the Fig. 17 in Chapter 4 of this book).

The folding simulation was performed according to the procedure described in the Chapter 4 of this book.

The new element introduced in simulation of folding presented in this chapter is the presence of hem molecule during the folding process.

Two simulations were performed: one without ligand (LS) and the second – with the ligand present (LSL) in the folding environment. All monitored parameters are presented for both versions to make possible estimation of ligand influence on the folding process.

The monitoring of mutual orientation of helical fragments (Fig.2.) was of particular interest taking into account high percentage of helical structural character of this protein.

As it can be seen in Fig. 2, the presence of hem seems to help to reach the proper mutual orientation of B-C, E-F and G-H in α chain and B-C, C-D, D-E and F-G in β chain although the chains A-B in α chain and A-B, E-F and G-H in β chain appeared to be less correct in the presence of hem.

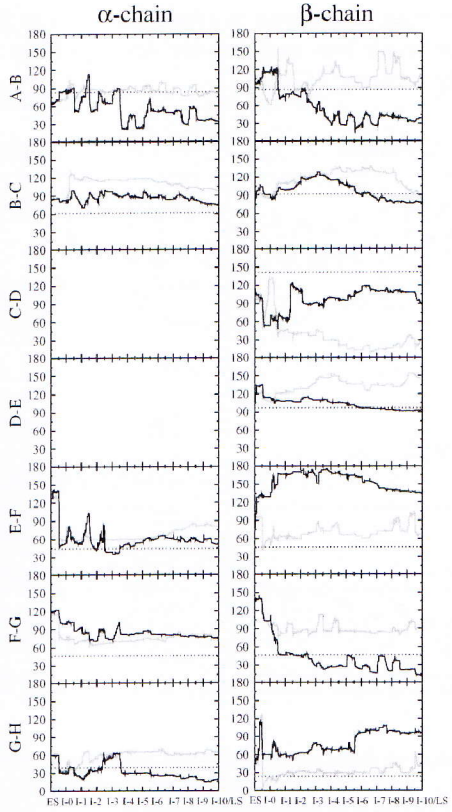

Figure 2. Change of the angle expressing the mutual orientation of particular helical fragments in hemoglobin during the simulation process. The gray line shows the value for simulation in the absence of hem and the black line for the simulation in the presence of hem. The horizontal line represents the value as observed in crystal form. (reproduced with permission – *Intern. J. Bioinformatics Research and Application* – Editor in Chief [1]).

The additional parameters: radius of gyration, total number of non-bonding contacts and solvent accessible area describing the molecule in the process of folding simulation are shown in Fig.3. Presentation of both versions (LS and LSL) makes possible estimation of ligand influence on the selected structural parameters.

The Rg radius of gyration seems to be reached in both chains of two forms of simulations. The NB – number of nonbonding contacts seems to be stable in α chain at the final part of simulation. Number of non-bonding contacts appeared even higher than in crystal structure in β chain in presence of hem. The best result was obtained for ASA (solvent accessible area) β chain folded in the presence of ligand.

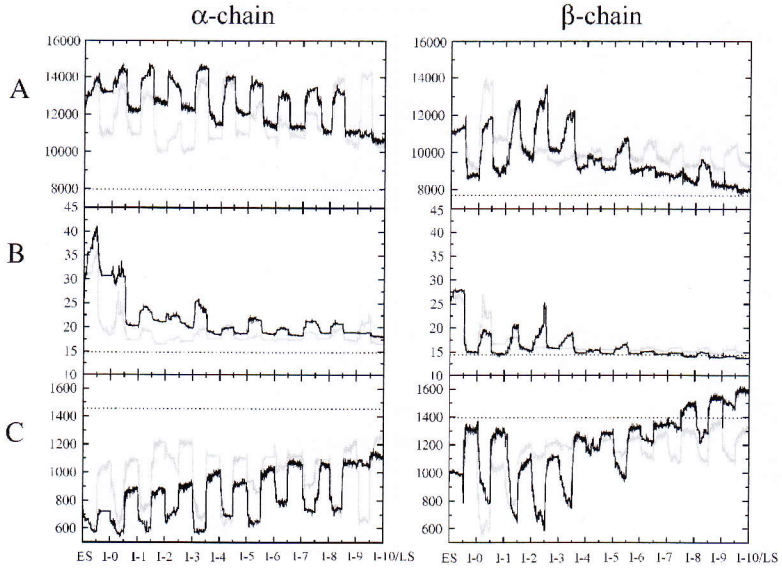

Figure 3. The parameters describing the structures of folding polypeptides. A – radius of gyration (R_g), B – number of non-bonding contacts (NB), C – solvent accessibility surface. The horizontal lines represent the value of the parameter under consideration as it appears in crystal form. The Black line – hem present, gray line – hem absent. The zig-zag form of the profiles comes form the optimization procedure which is the non-bonding energy minimization step followed by the hydrophobic interaction driven optimization procedure performed for decreased size of "*fuzzy-oil-drop*". ES – early stage intermediate, LS- late stage intermediate. (reproduced with permission – *Intern. J. Bioinformatics Research and Application* – Editor in Chief [1]).

The important characteristics, which is the monitoring the non-bonding interaction as they appeared in ES, LS and LSL in comparison to the contact maps for crystal forms of both chain are shown in Fig.4.

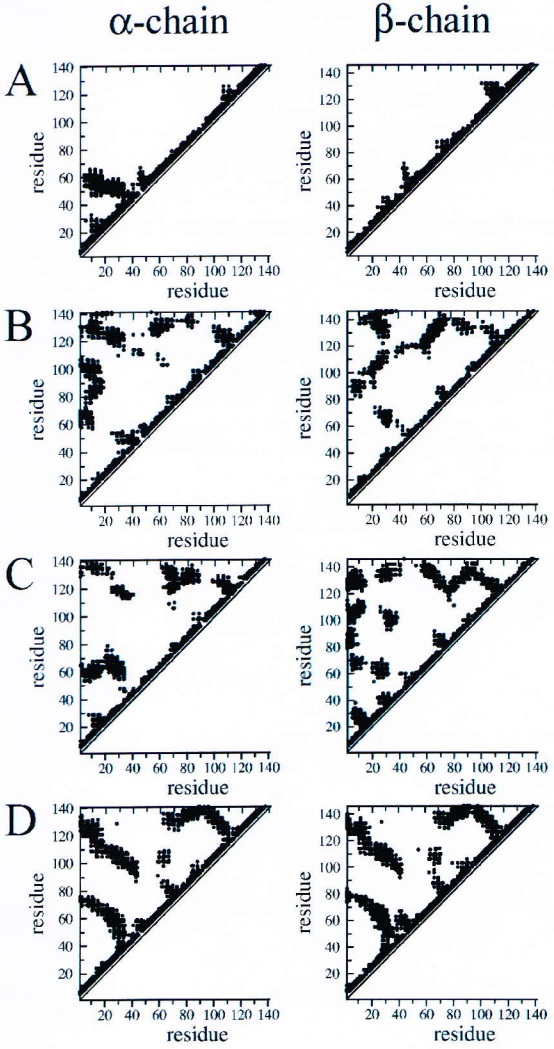

Figure 4. The contact maps for α and β chains as appeared in A - ES structural form, B - LS structural form, C – LSL structural form and D - in crystal form.

The distribution of points representing pair-wise interaction of residues in α and β chain seems to approach the distribution as it appears in crystal form. The part of correct contacts are observed even in ES structural form in the chain α. Particularly promising progress in non-bonding generation appeared in β chain in the presence of hem molecule.

The quantitative characteristics of non-bonding contacts together with RMS-D values are given in Tab.2.

The significant approach to the native structure in respect to number of non-bonding contacts was observed for β chain in the presence of hem molecule.

The spatial distribution of Cα atoms versus the geometrical center of the molecule is shown in Fig. 5.

Table 2. The non-bonding contacts (NB) characteristics. The NB calculated as percentage of the properly recognized non-bonding contacts versus the crystal structure. The RMS-D value is calculated for Cα atoms.

Chain	Structure	NB (%)	RMS-D – Cα
α	ES	40.37	22.49
	LS – hem absent	37.80	14.24
	LS – hem present	39.80	14.83
β	ES	37.69	25.02
	LS – hem absent	36.86	15.82
	LS – hem present	40.51	10.15

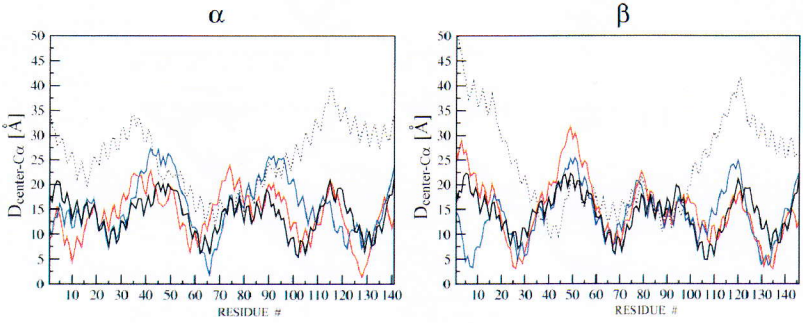

Figure 5. Profiles of $D_{center-C\alpha}$ for α and β chain as obtained after simulation of protein folding in ES, LS and LSL (reproduced with permission – *Intern. J. Bioinformatics Research and Application* – Editor in Chief [1]).

The comparison of Dcenter-Cα suggests significant approach of the results received after LSL simulation of β chain.

The important role played by His H58 and H87 in α chain and H63 and H92 in β chain as elements keeping the hem in a proper orientation versus the whole molecule can be analyzed in Fig.6.

The structures as they appeared in ES (early stage – the starting point for protein folding simulation) LS – late stage (post-simulation structural forms) with hem absent and present may be compared in Fig.7. The β chain folded in LSL simulation appeared to be significantly better approached to the crystal form.

The role of hem molecule in hydrophobicity distribution in both chains of hemoglobin is shown in Fig.8. The hydrophobicity distribution in molecule deprived of hem seems to be irregular with well defined cavity of hydrophobicity deficiency. The presence of hem molecule makes this distribution more similar to centric form (according to 3-D Gauss function).

The hydrophobicity density distribution in hemoglobin chains α and β are shown in Fig.9. The presence of hem ligand makes the distribution of hydrophobicity closer to the idealized one with the highest hydrophobicity density in the central area of the protein body.

The irregularities of observed hydrophobicity distribution (as seen in Fig.8.) versus the idealized one are still present. The hem binding cavity generation is under consideration in this paper. The α and β chains are engaged in other "activities" like for example generation of two α and two β

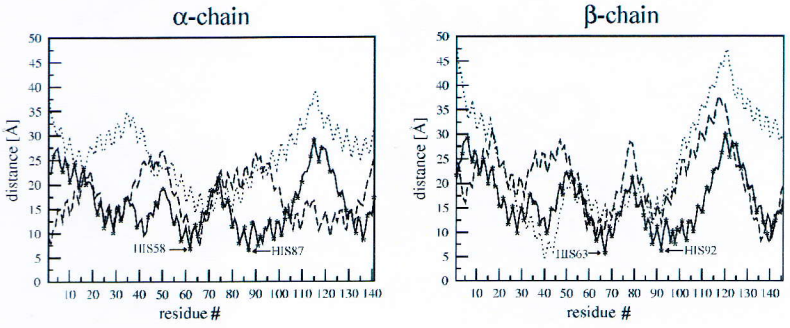

Figure 6. Profiles of the distances of sequential Cα atoms as calculated versus the geometric center of hem molecule. The solid line – crystal structure, dotted line – ES intermediate, dashed line – LSL folding simulation (reproduced with permission – *Intern. J. Bioinformatics Research and Application* – Editor in Chief [1]).

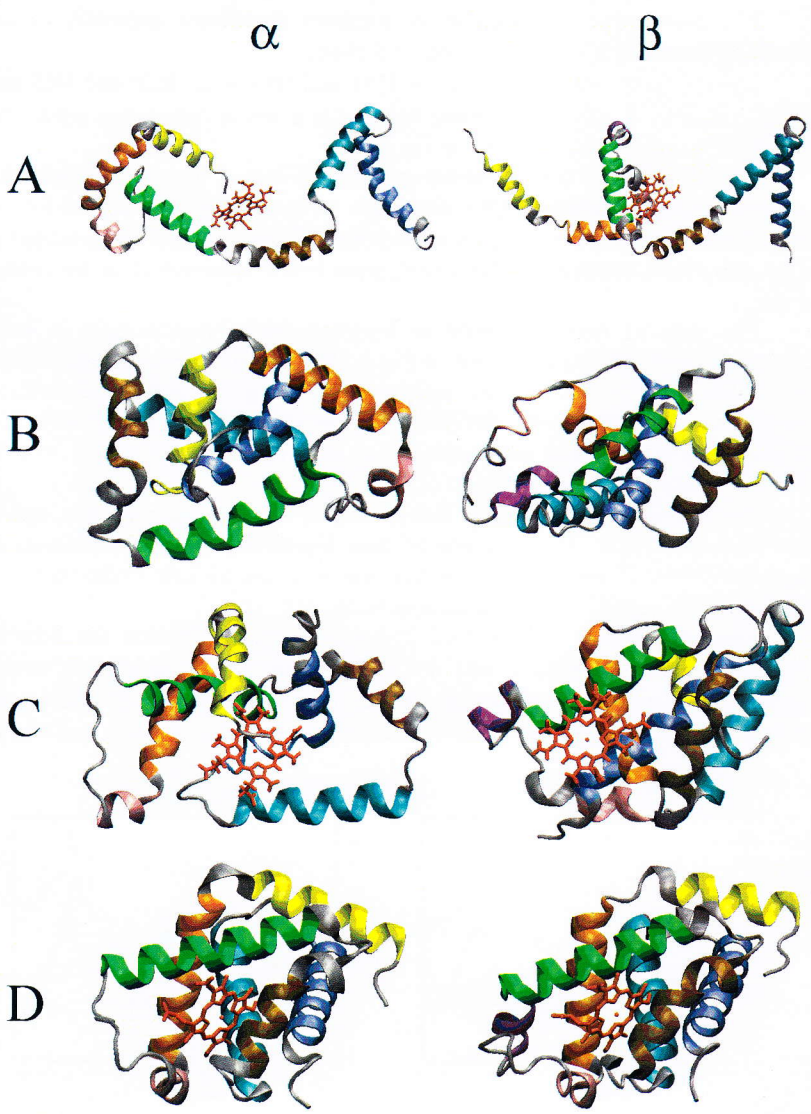

Figure 7. The structures as they appeared in: A - ES, B - LS, C – LSL and D - crystal structure. (reproduced with permission – *Intern. J. Bioinformatics Research and Application* – Editor in Chief [1]).

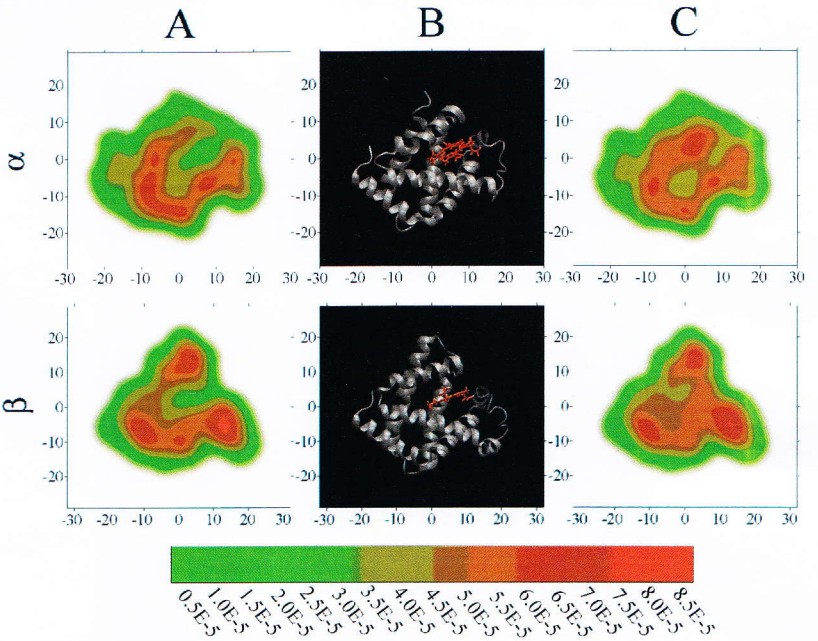

Figure 8. The participation of hem molecule in hydrophobic center generation shown in form of the central slice of the molecular body (XZ plane for Y=0.0). A - The chains deprived of hem molecules represent the hydrophobicity distribution of the cylindrical form. B – the structure of chains α and β with hem shown in red color. C - The hem presence makes the central part of the complex of "*fuzzy-oil-drop*" form with the central part of complex of high hydrophobicity. The orientation of molecules is identical for A and C presentation. The color scale expressing the hydrophobicity level given below the molecules presentation applied in pictures A and C.

chains complex. The mechanism of quaternary structure generation is also coded in the specific structure of each chain participating in hemoglobin construction. This is why other than hem binding site irregularities are expected to be present in these two chains.

The influence of hem molecule can be analyzed in a similar way as it was done for other protein-ligand complexes. The color scale visualizing the values distributed all over the molecule shows the ligand binding cavity of hydrophobicity deficiency character. The cavities of such characteristics did not appeared in the structures received according the simulation in the absence of hem-ligand.

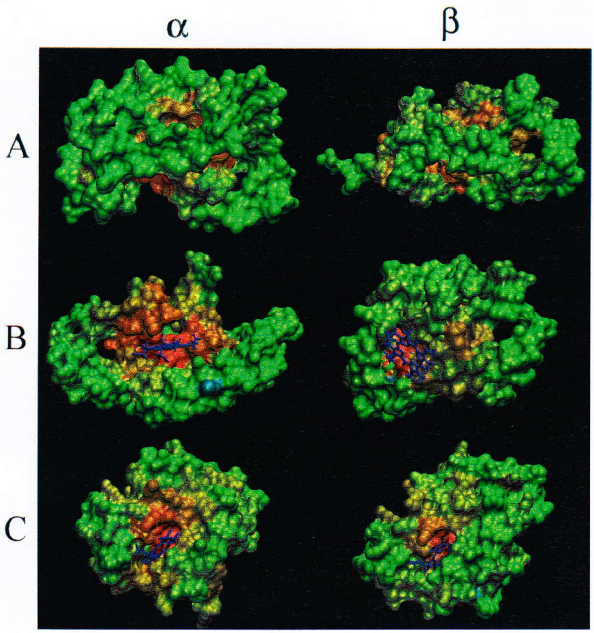

Figure 9. The distribution of hydrophobicity irregularity measured according to $\Delta\tilde{H}$. A – structures received according to LS folding intermediate folding, B – structures as appeared as result of LSL simulation, C – crystal structures. The value of $\Delta\tilde{H}$ was expressed using the color scale: the green color – the low difference between observed versus the idealized hydrophobicity distribution, red color – high value of $\Delta\tilde{H}$ expresses hydrophobicity deficiency, the blue color – the hydrophobicity excess. The hem molecule in dark blue color (reproduced with permission – *Intern. J. Bioinformatics Research and Application* – Editor in Chief [1]).

Simulation of folding process of ribonuclease A in the presence of CpA

Another example for folding protein in the presence of ligand is the ribonuclease A. Crystal structure of the ribonuclease A in complex with inhibitor CpA (PDB - 1RPG). The hydrophobicity of CpA molecule was calculated according to the procedure applied for hem molecule (the position of ligand in relation to the "*fuzzy-oil-drop*" model). The CpA molecule was treated as the set of five virtual amino acids (the parts distinguished according to the rigid aromatic rings and free rotation linker in between).

The hydrophobicity attributed to five fragments of CpA are presented in Tab.3.

The progress of non-bonding contacts map for ribonuclease A approach to crystal form in LS and LSL forms is shown in Fig.10.

The structures of ribonuclease A as received after simulation without CpA, in presence of CpA and crystal structure (1RPG is shown in Fig.11.).

Table 3. The hydrophobicity of virtual residues distinguished in CpA.

Virtual residue	Hydrophobicity
CpA – deoxycytydyl	0.579
CpA – desoxyribose	0.892
CpA – phosphodiester	0.797
CpA – desoxyribose	0.513
CpA – adenina	0.430

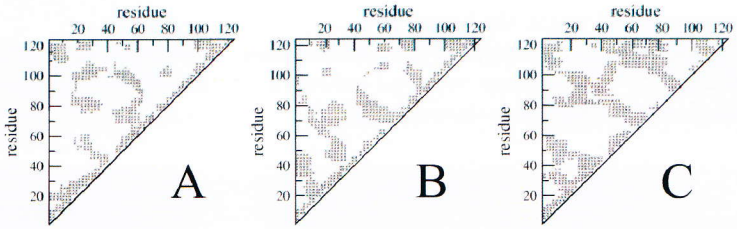

Figure 10. The non-bonding contact maps of ribonuclease A: A – LS simulation, B – LSL simulation, C – crystal structure.

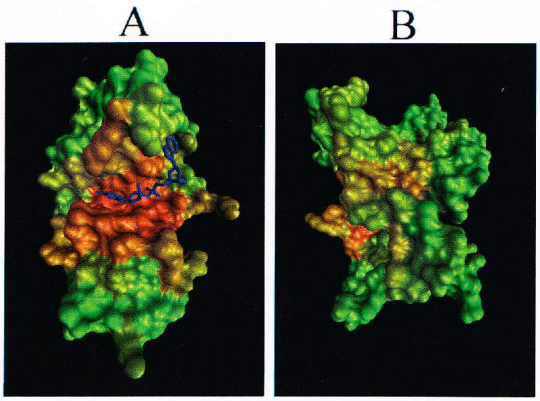

Figure 11. The structure of ribonuclease A A - as observed in crystal form and B - as received after the LS simulation of folding.

The structure of ribonuclease A as received after folding simulation according to "*fuzzy oil drop*" colored according to $\Delta\tilde{H}_j$ value.

The ligand binding cavity (hydrophobicity deficiency) was generated during the simulation of folding in the presence of ligand molecule (CpA). The structure received after folding simulation without the ligand present is shown in Chapter 4 of this book. The ligand binding cavity may be seen in LSL structural form. The general structure does not appeared to be satisfactory, although the influence of ligand on the protein structure seems to be of important character.

To measure the influence of ligand on binding cavity generation may be characterized also measuring the $\Delta\tilde{H}_j$ values as dependent on the distance versus the ligand position (the geometric center of ligand molecule). According to the "*fuzzy-oil-drop*" model the decrease of $\Delta\tilde{H}_j$ $\Delta\tilde{H}_j$ $\Delta\tilde{H}_j$ values with the distance increase versus the ligand.

It was measured for crystal form ribonuclease A (in complex with CpA) in relation to the structure received according to the LSL folding process simulation. This relation is shown in Fig.13.

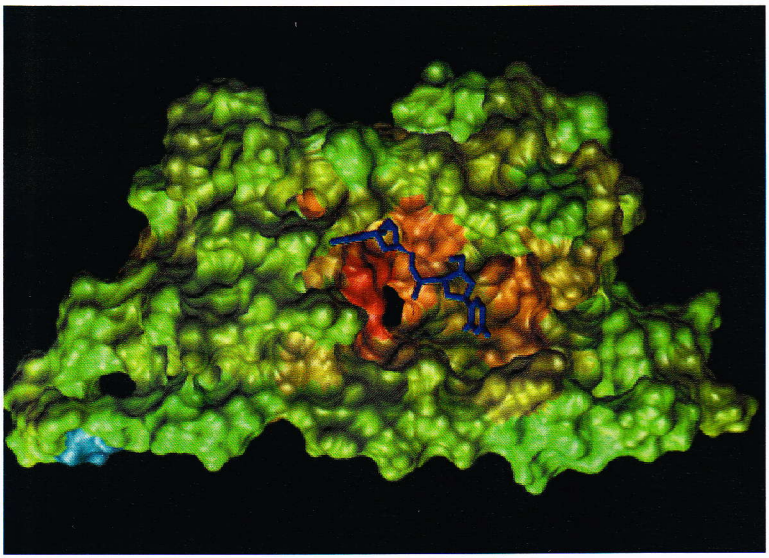

Figure 12. The ribonuclease A after LSL folding simulation in the presence of ligand (CpA) (ligand in dark blue color).

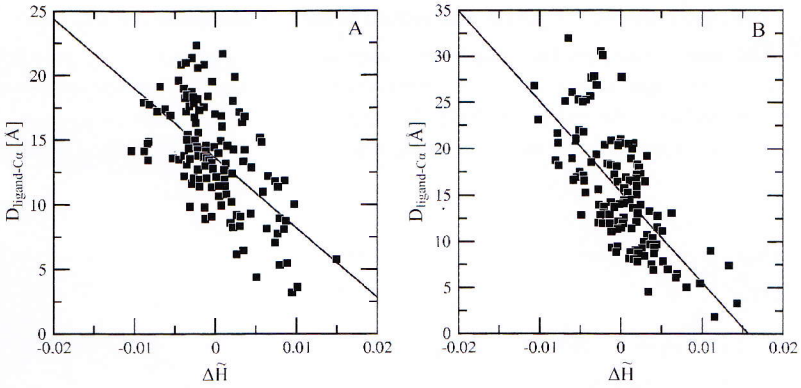

Figure 13. The relation between distance between particular residue (represented by its effective atom) and ligand position (geometric center) and $\Delta\tilde{H}_j$ (hydrophobicity irregularity versus the idealized "*fuzzy-oil-drop*"). A – the crystal structure - R^2 = 0.60. B – the structure as received as result of folding simulation in the "*fuzzy-oil-drop*" external force field of hydrophobic character with the ligand (CpA) present in the environment. R^2 = 0.69

The R^2 vales measuring the strength of the relation is equal 0.60 for crystal structure and 0.69 for "*fuzzy-oil-drop*" model. It suggests that the influence of ligand on the folding protein applied in model seems to be important.

Can be the biological function present in the structure generated in silico on the basis of "*fuzzy-oil-drop*".

The structure of TA0354_69_121 generated with the application of "*fuzzy-oil-drop*" was presented in the Chapter 4 of this book. This molecule does not interact with any specific ligand. This is the membrane protein. The general characteristics of this molecule (number of non-bonding contacts and RMS-D measuring the similarity between the target and model structures) and particularly analysis of its structure generated by LS simulation are given there.

The next step can be made in respect to biological function reconstruction. Assuming that the $\Delta\tilde{H}_j$ profile really visualizing the hydrophobicity density irregularity reveals the function related characteristics, the comparison of $\Delta\tilde{H}_j$ profiles of target and model structures of TA0354_69_121 may show to what extent the biological function can be estimated.

The $\Delta\tilde{H}_j$ profiles of target and model structures are shown in Fig.14.

The one-residue profile (Fig.14.A.) after smoothing procedure (Fig.14.b.) reveals very high similarity of hydrophobicity irregularity. On the basis of this comparison one may conclude that the biological function (whatever it represents) appeared to be very well represented in the *in silico* folded protein.

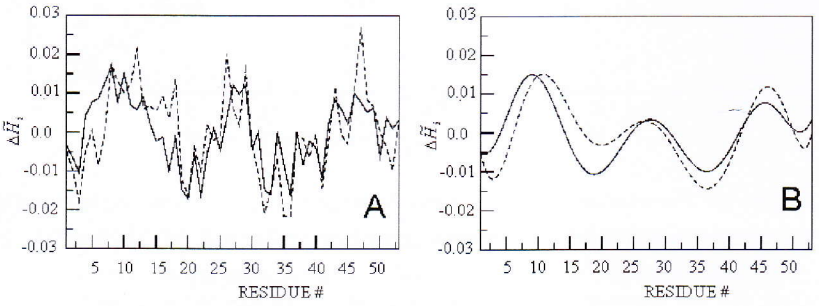

Figure 14. The $\Delta\tilde{H}_j$ profile for TA0354_69_121 as appeared for one-residue calculation and after smoothing procedure (B) taking the 5 residues window size.

Conclusions

The role of specific ligand as influencing the folding process was discussed in this Chapter. The correctly localized ligand binding cavity determines the protein's biological activity. The shape of the ligand binding cavity specificity makes possible also the *de novo* drug design [7-9].

The two examples of folding process simulation in the presence of specific ligand seem to support the assumption of active presence of ligand influencing the polypeptide chain folding process. The hydrophobic characteristics of ligands complexed with proteins and enabling their biological activity seems to be necessary.

The LSL simulation of α and β chains of hemoglobin revealed better approach received for β chain. These two chains represent very similar structures, although their folding process differ significantly [10,11]. The much lower stability is observed experimentally for α chain [12-14]. The structural instability of chains may influence the improper exposure of hem allowing the unexpected redox processes [12-14]. The AHSP mechanism protecting the hem molecule in α chain has been discussed recently [13]. The

observations described there suggest the necessity of additional support of AHSP polypeptide influencing particularly the position of His58 [12,14]. The differences between the α and β chains characteristics as presented in this chapter seem to be accordant with these experimental observations. The sequence of the evens in generating the quarternary structure suggests the necessity of the β chain to be folded earlier. It is able to stabilize the unstable α chain. This observation seems also to be highly accordant with the differentiation of these two chains in LSL simulation.

The experimental observation presented in [15] describing the presence of trigger factor which interacts with freshly synthesized polypeptide chain leaving the ribosomal channel suggest the folding process as directed one. The chaperonins seem to play similar role to the external force field introduced in "*fuzzy oil-drop*" model [16-18].

The experimental results of ribonuclease A folding [19] and analogous for cytochrome [20,21] indicated that the submiliseconds burst phase observed for these proteins reflects a solvent-dependent readjustment of the unfolded state ensemble, rather than the rapid formation of distinct folding intermediate. These experimental observations together with theoretical studies on protein folding [22,23] suggest that the initial step in globular protein folding is a long-range conformational search to find a topologically native-like transition state nucleus. The strong influence of hydrophobic interactions on rapid compaction of the polypeptide chain occurring at the early stage of the folding process was observed experimentally [21, 24-25].

Taking into account all mentioned discussions and experimental results the "*fuzzy-oil-drop*" model has been defined. The main assumption was to simulate *in silico* as much as possible the folding process rather than protein structure prediction. The general conclusion based on the presented results is that the protein folding process seems to be of aim oriented character. The influence of external environment as well as presence of other molecule including ligands in particular seems to direct the process toward the structure. The main goal for proteins is to be active. The specific structure ensuring the appropriate interactions of high specificity seems to be the result of directed process.

To perform the simulations according to the presented model large data base of ligands and particularly the parameters compatible with the force field applied for simulation is necessary. Such task has been undertaken. The database collecting the parameterization (including hydrophobicity parameterization) of molecules playing the role of ligands complexed to proteins as they appear in PDB has been currently organized and available on www.ligand.cm-uj.krakow.pl. These parameters may be applicable also for computer aided drug design.

The model oriented on folding process simulation the form of this process which was presented as follows:

$$U \Rightarrow ES \Rightarrow LS \Rightarrow N$$

needs to be corrected including the presence of ligand:

$$\begin{array}{c} \text{LIGAND} \\ \hookrightarrow \\ U \Rightarrow ES \Rightarrow LS \Rightarrow N \end{array}$$

and finally take the following form:

$$U \Rightarrow ES \Rightarrow LS \Rightarrow LSL \Rightarrow N$$

The moment of the ligand appearance in the folding environment is not defined so far for the "*fuzzy-oil-drop*" model. The large number of LSL simulations is planned to help finding the proper step to introduce the ligand into the folding environment. .

The observations presented in this work suggest that the presence of ligand during the LS step of multi-intermediate process seems to be necessary. The conformational changes in the hydrophobic environment ensure the hydrophobic center generation and localization of hydrophilic residues on the surface of the protein, although the ligand binding cavity of high specificity seems to be able to generate in the presence of the "matrix". This matrix can be represented by the natural ligand or by the similar molecule mimicking the natural ligand.

The presence of ligand was also recognized as critical for protein structure prediction in the CASP5 "blind" test [26].

The model to simulate the folding process rather than to predict the protein structure is presented in its complete form in Fig.15. The modifications of the general model are under consideration currently although the main ideas remain unchanged. The interpretation of contingency table is in focus of the attention. Simplification to the discrete form of the probability profile is necessary as well as the LS step and the hydrophobicity driven steps of optimization procedure is under modification. On the other hand the applicability of "*fuzzy-oil-drop*" and its limitations (in respect to the protein specificity) is under consideration. Further work seems to be necessary.

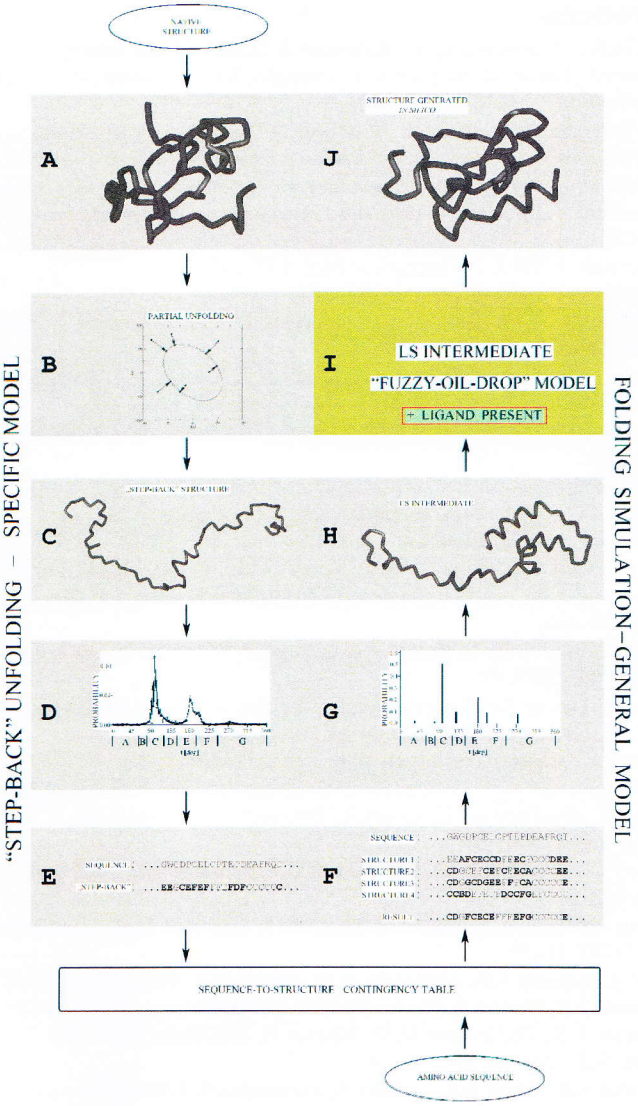

Figure 15. The complete model assumed to enable the simulation of protein folding process rather than protein structure. The step of particular interest is related to the ligand participation in final structure generation. The description of particular steps as in Fig.17 in Chapter 4 of this book.

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