

Prediction of Stability Factors at the domain interface of Human γ B Crystallin maintaining the Transparency of the Eye Lens

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Abstract

Objective: $\beta\gamma$ -crystallins are among the most long lived globular proteins known today. Interaction of the two domains through a hydrophobic interface is one of the major contributors to the stability of these crystallins. Changes in these interactions are either due to the amino acid substitutions or the changes in the orientations of the same amino acids leading to cataract formation. We have carried out a detailed analysis to observe the stabilizing effects of hydrophobic core residues at the domain interface of the predicted human γ B-crystallin structure.

Methods: Human γ B-crystallin model was built by Homology Modeling using MODELLER4 based on the crystal structure coordinates of bovine γ B-crystallin. In lens γ B-crystallin, there are six non polar residues, three each in the two domains which form a hydrophobic core at the domain interface. We performed mutational studies at position 56 and analyzed the changes in the protein structure. Three mutants (Phe $\rightarrow$ Trp, Phe $\rightarrow$ Ala, Phe $\rightarrow$ Asp) were constructed and analyzed for hydrogen bonding, ion pairs and accessibility by WHATIF web server.

Results: Being the largest amino acid among the six residues taking part in the hydrophobic interactions at the domain interface, Phe was predicted to be responsible for the greatest contribution to the stability at this region. Phe $\rightarrow$ Ala mutant showed the largest structural changes in the vicinity of the mutated residue.

Conclusion: The results obtained clearly emphasize the importance of hydrophobic interactions to the stability of crystallins. Mutations at the domain interface could decrease the interactions between these domains thus causing destability (JPMA 54:419;2004).

Introduction

One of the most critical questions regarding protein associations is the understanding of stabilization factors in protein complexes.¹ Protein stability results from the combined effects of certain non-covalent interactions in a polypeptide chain.² The relative strength of such interactions is not only important but the relationship between structural interactions and overall function is also of fundamental interest to the protein scientists.³ Some of these interactions include, hydrophobic effects⁴, hydrogen bonds⁵ and salt bridges.^{6,7}

It has been generally accepted that the principal driving force in protein folding is the hydrophobic effect⁴, which results in the burial of the hydrophobic residues in the core of the protein. More precisely this effect is defined as the process by which non polar groups are removed from contact with water.⁸

Crystallins are the major proteins in eye lens that contribute towards the maintenance of high refractive index and thus transparency. Changes in the structure of these crystallins therefore lead to opacity or cataract. These crystallins should therefore be highly stable. To have an insight about the stability features governing γ -crystallins, we carried out an elaborate analysis of one of these forces to predict its effect on the stability of the molecule.

Methods

Homology Modeling

Sequence Analysis: Primary sequence of human γ B-

crystallin (Accession No. CRGB-HUMAN) was retrieved from the SWISSPROT data bank.⁹ Sequence homology searches of the Protein databank, PDB¹⁰ using the basic BLAST algorithm¹¹ were carried out independently for each primary sequence. The highest sequence homology was found with bovine γ B-crystallin (PDB id: 4gcr). The crystal structure coordinates of bovine γ B-crystallin¹² were therefore used as template for constructing the homology models of human γ B-crystallins. The 3D coordinates of the template were extracted from the Protein Data Bank, PDB.¹⁰

Model building: The automated homology model building was performed using the protein structure-modeling program MODELLER 4.¹³ Reliability of the predicted homology models was assessed by the ENERGY command of the MODELLER. Evaluation was further carried out by PROCHECK¹⁴ and WHATCHECK.¹⁵ The details of model building are described elsewhere.¹⁶

Mutational Studies

In the present study, following mutations were carried out by the mutate command of the program, WHATIF.¹⁷

- Phe56 $\rightarrow$ Trp
- Phe56 $\rightarrow$ Ala
- Phe56 $\rightarrow$ Asp

Hydrogen bonding, surface accessibilities and ion pairs in the homology models were calculated by the HBONDS, ACCESS and ANACON menus of WHATIF.¹⁷

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Results

In the present study, we have carried out a mutation analysis to observe the role of a hydrophobic core residue, Phe 56 present at the domain interface of the predicted human $\gamma\beta$ -crystallin structure. Phe 56 was replaced by Ala, Trp and Asp. The local changes that occur in the surface accessibilities of amino acids in response to the incorporation of mutated residues are presented in Table.

Phe56→Trp Mutant

Only minor changes in the positions of amino acid residues present in the vicinity of the mutated Trp56 were observed in case of this mutation. There is a slight increase in the surface accessibilities of mutated Trp (Phe56 = 1.57Å² and Trp56 = 4.02Å²). Some of the amino acids moved to slightly more buried positions as compared to the original model. These include Arg58, Tyr134 and Arg168. No change in the accessibilities of Cys41, Met43, Ile81, Ile132, Leu145 and Val170 was observed.

Phe56→Ala Mutant

Ala56 was located in a slightly exposed position (6.47Å²) as compared to Phe56 (1.57Å²). The residues in the vicinity of Ala56 showed relatively greater changes in the environment of the mutated residue. Cys41, Met43, Arg58, Ile81, Ile132, Tyr134, Arg168 and Val170 show increases in their side chain accessibilities. Only Leu145 remained unchanged.

Phe56→Asp Mutant

Only slight alterations in the amino acid accessibilities were observed in case of this mutation. Very small increase in the accessibility of Asp56 was observed (Phe = 1.57Å²; Asp = 2.0Å²). The residues in the vicinity of Asp56 showed slight increases in the accessibilities. These include Cys41, Met43, Arg58, Ile81, Ile132, Tyr134, Arg168 and Val170. No change was seen in the accessibility of Leu145.

Discussion

$\beta\gamma$ -crystallins are among the most long lived globular proteins known today.¹⁸ Interaction of the two domains through a hydrophobic interface is one of the major contributors to the stability of these crystallins.¹⁹ In calf eye lens $\gamma\beta$ -crystallin, there are six non polar residues, three each in the two domains which form a hydrophobic core at the domain interface.² These residues are Met43, Phe56 and Ile81 in the N-terminal domain and Val132, Leu145 and Val170 in the C-terminal domain. We have carried out a detailed analysis to observe the stabilizing effects of similar hydrophobic core residues at the domain interface of the predicted human $\gamma\beta$ -crystallin structure. The changes that occur at the interface when these residues experience alterations in conformation or surface accessibility can be seen in the light of mutational studies performed at one or two of these sites. In the present study, we have applied this approach to predict the local structural changes resulting from point mutations at position 56. Being the largest amino acid among the six residues taking part in the hydrophobic interactions at the domain interface, Phe must be responsible for the greatest contribution to the stability at this region.

Phe present at position 56 was replaced by other non polar residues Ala and Trp and by a polar residue, Asp. Ala and Trp are non polar but they vary in size whereas Phe→Ala substitution might well give the picture of changes occurring when a non polar residue is replaced by an amino acid completely different in nature. The local changes that arise due to this mutation were also compared with the results of an experimental analysis performed with similar amino acid substitution by Palme et al.²

Table presents the local changes that occur in the surface accessibilities of amino acids in response to the incorporation of mutated residues. As expected the changes that arise from single mutation are small. Generally it has been observed that overall structure of proteins does not change upon the introduction of a point mutation. This is also true in case of $\gamma\beta$ -crystallin models of all the mutants. It has been observed that even if a mutated residue is located in the core of a closely packed protein, the mutated residues tries to adapt to its environment rather than the environment of the mutant itself.²⁰ Let us examine these structural perturbations in each of these mutants.

Phe56→Trp Mutant

The predicted model human γ -crystallin showed very minor changes in the positions of amino acid residues present

compared; Phe = $1.57\text{\AA}^2$, Trp = $4.02\text{\AA}^2$ (Figure 1a, b). The minor alterations in the neighboring amino acid are due to the bulkier side chain of Trp than that of Phe. Palme et al.² observed a very slight decrease in the stability of bovine $\gamma\beta$ -crystallin mutant which means that bulky Trp side chain is able to replace the comparatively smaller Phe side chain without causing a major disturbance to the domain interface. Thus domain pairing, a requisite for stability of two domains remains unaffected. The substitution of one hydrophobic residue with the other one of similar nature underlines the significance of hydrophobic interactions to protein stability.

Phe56→Ala Mutant

Introduction of small nonpolar alanine residue is expected to cause relatively larger structural changes than the Trp mutant. The residues in the vicinity of Ala in the mutated model therefore shows relatively greater changes in the environment of the mutated residue. The residues in the vicinity of Ala56 are much more exposed to the outer surface (Figure 2a, b). This change is likely to disturb the packed hydrophobic core thus decrease in stability of this mutated protein is expected. It is worth noting that Ala mutant of calf eye lens $\gamma\beta$ -crystallin was significantly destabilized when unfolding was monitored by fluorescence and spectral analysis.²

Phe56→Asp Mutant

A polar side chain when placed in hydrophobic interface should decrease the hydrophobic interactions by disturbing the neighboring residues to a large extent. Thus distinct structural difference is expected in the Asp mutant compared with Ala mutant. According to the results obtained in our study, the differences seen are very small even less than that of Ala mutant. When compared with the original model with Phe56, only slight alterations in the amino acid accessibilities were observed. Even the polar side chain of Asp showed very slight increase in its surface accessibility than that of non polar side chain of Phe; Phe = $1.57\text{\AA}^2$; Asp = $2.0\text{\AA}^2$ (Figure 3a, b). Experimental results of site directed mutations² also showed same degree of destabilization of crystallin structure in Asp and Ala mutants. The authors attributed it to the protonation of the carboxylate group of Asp as the experiment was carried out at low pH. It is expected that Asp side chain when uncharged might not cause a large perturbation in the hydrophobic environment.

The results obtained from the structural analyses of various mutants in this study are in accordance with the experimental results of site directed mutagenesis.² The results clearly emphasize the importance of hydrophobic interactions to the stability of crystallins. With smaller perturbations in the residues forming the hydrophobic environment, changes observed in the stability of the protein were less. The residues present at the interface connecting the two domains also show no change in the conformation. Stronger forces are therefore required to disturb the natural environment in the core of the native protein. It has been demonstrated that core mutations aimed at stabilization of protein by means of improved packing are normally unsuccessful.²¹

Proteins structures are highly optimized for functioning in their parent organism.²⁰ It seems extremely difficult to disturb the existing balance of forces in the core of a natural protein. In the case of γ -crystallins as well, domain pairing is highly dependent on the hydrophobic interactions at the domain interface. As the two domains fold independently, they seem to gain stability from domain pairing, forming highly stable structures.²² The models in this study support the observation of the effect of hydrophobic domain interactions being involved in the intrinsic stabilization of human γ -crystallins.

References

1. Xu D, Lin SL, Nussinov R. Protein binding versus protein folding: the role of hydrophilic bridges in protein associations. *J Mol Biol* 1997;265:68-84.
2. Palme S, Slingsby C, Jaenicke R. Mutational analysis of hydrophobic domain interactions in gamma B-crystallin from bovine eye lens. *Prot Sci* 1997;6:1529-36.
3. Sindelar CV, Hendsch ZS, Tidor B. Effects of salt bridges on protein structure and design. *Prot. Sci* 1998;7:1898-1914.
4. Lee B. The physical origin of the low solubility of nonpolar solutes in water. *Biopolymers* 1985;24:813-25.
5. Baker EN, Hubbard RE. Hydrogen bonding in globular proteins. *Prog Biophys Mol Biol* 1984;44:97-179.
6. Anderson DE, Becktel WJ, Dalquist FW. pH-induced denaturation of proteins: a single salt bridge contributes 3-5 kcal/mol to the free energy of folding of T4 lysozyme. *Biochemistry* 1990;27:8889-93.
7. Wimley WC, Gawrisch K, Creamer TP, et al. Direct measurement of salt-bridge solvation energies using a peptide model system: implications for protein stability. *Proc Natl Acad Sci USA* 1996 93 2985-90.
8. Doig AJ, Williams DH. Is the hydrophobic effect stabilizing or destabilizing in proteins? The contribution of disulphide bonds to protein stability. *J Mol Biol* 1991; 217:389-98.
9. Bairoch A, Apweiler R. The SWISS-PROT protein sequence data bank and its supplement TrEMBL. *Nucleic Acids Res* 1997;25:31-6.
10. Berman HM, Westbrook J, Feng Z, et al. The Protein Data Bank. *Nucleic Acids Res* 2000;28:235-42.
11. Altschul SF, Gish W, Miller W, et al. Basic local alignment search tool. *J Mol Biol* 1990;215:403-10.
12. Najmudin S, Nalin V, Driessen HPC, et al. Structure of the bovine eye lens protein gB (gII)-crystallin at 1.47Å. *Acta Cryst.* 1993;D49:223-33.
13. Sali A, Blundell TL. Comparative protein modelling by satisfaction of spatial restraints. *J Mol Biol* 1993;234:779-815.
14. Laskowski RA, McAurthur MW, Moss DS, et al. A program to check the stereochemical quality of protein structures. *J Appl Cryst* 1993;26:283-91.
15. Hooft RWW, Vriend G, Sander C, et al. Verification of protein structures: side-chain planarity. *Nature* 1993;381:272.
16. Salim A, Zaidi ZH. Homology models of human gamma crystallins: structural study of the extensive charge network in gamma crystallins. *Biochem Biophys Res Commun* 2003;300:624-30.
17. Vriend G. A molecular modelling and drug design program. *J Mol Graph* 1990;8 52-6.
18. Jaenicke R. Stability and folding of ultrastable proteins: eye lens crystallins and enzymes from thermophiles. *FASEB J.*, 1996; 10: 84-92.
19. Mayr EM, Jaenicke R, Glockshuber R. Domain interactions and connecting peptides in lens crystallins. *J. Mol. Biol* 1994;235:84-8.
20. de Fillipis V, Sander C, Vriend G. Predicting local structural changes that result from point mutations. *Prot Eng* 1994;7:1203-8.
21. Vriend G, Eijssink VGH. Prediction and analysis of structure, stability and unfolding of thermolysin-like proteases. *J Comp Aid Mol. Des*1993; 7:367-96.
22. Bennett MJ, Schlunegger MP, Eisenburg D. 3D domain swapping: a mechanism for oligomer assembly. *Prot Sci* 1995;4:2455-68.

Introduction