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Homology modeling of ρ-crystallin from bullfrog (*Rana catesbeiana*) lens☆

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This paper is dedicated to the memory of Professor Dr. Zafar H. Zaidi, Vice Chancellor, University of Karachi, who passed away on 7 January 2001

Abstract

ρ-Crystallins are major protein component found in the eye lenses of frogs of the genus *Rana*. Structural analysis has indicated that frog ρ-crystallins belong to aldo–keto reductase superfamily (AKRs) which include aldehyde and aldose reductases, prostaglandin F synthase and several detoxification enzymes. Members of AKRs catalyze the oxidation–reduction reaction over a range of substrates using NAD(P)(H) as a cofactor. In spite of higher structural similarity with AKRs and cofactor binding affinity, the ρ-crystallins were found to be catalytically inactive. This study presents comparative or homology modeling of ρ-crystallin from bullfrog (*Rana catesbeiana*) in presence and absence of cofactor NADP and a competitive inhibitor, testosterone. The predicted models are explored to examine the catalytic cleft, cofactor binding affinity characteristics and substrate binding pocket.

Keywords: Lens proteins; AKR superfamily; Homology modeling; ρ-Crystallin; α/β Barrel fold

1. Introduction

The refractive properties of the eye lens are maintained by abundant soluble lens proteins, crystallins. In some species, crystallins may account for 90% of total lens proteins [1]. Vertebrate lens crystallins are classified into α, β, and γ-crystallins. Apart from these, few additional crystallins have been identified in specie specific manner and are called 'Taxon specific' crystallins [2,3]. A remarkable feature of taxon specific crystallin is their similarity with metabolic enzymes [4]. Taxon specific crystallins appear to be a product of single gene with increased expression in lens while still serving the ancestral catalytic role with low expression in many other tissues [1]. Some taxon specific crystallins are easily identified as familiar enzymes while others appear to be novel enzymes discovered for the first time as crystallins. ρ-Crystallin, found in the lenses of frogs of genus *Rana*, is an example of a novel enzyme relevant crystallin [5]. A single polypeptide with a molecular weight of 38 kDa and a blocked N-terminal, ρ-crystallin has been identified from Bullfrog (*Rana catesbeiana*) and European common frog (*Rana temporaria*) [5–7]. Another member of same family was identified in Gecko (*Lepidodactylus lugubris*) lens, which was named as ρB-crystallin [8].

ρ-Crystallin is structurally similar to the aldo–keto reductase superfamily (AKRs). The AKR enzymes comprise of functionally divers gene family, which catalyze the NADPH dependant reduction of a variety of carbonyl compounds [9]. In vertebrate lens, aldose reductase is normally present at housekeeping levels and can convert sugars into corresponding alcohols through oxidation of NADPH. Aldose reductases have been implicated in initiating diabetic complications including sugar cataract [10,11] but their actual catalytic role in lens has been debatable [12]. In spite of higher structural similarity of ρ-crystallins with AKRs, ρ-crystallins have shown to be catalytically inactive, thus demonstrating gene duplication phenomena which allows one copy of the protein product to loose enzymatic activity and perform structural role [1]. Present communication deals with the prediction of three-dimensional structure of ρ-crystallin from bullfrog based on comparative modeling and the predicted structure is discussed with

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special reference to the active site and cofactor binding site.

2. Methodology

2.1. Sequence alignment

Amino acid sequence of ρ-crystallin from bullfrog (*R. catesbeiana*) was obtained from SWISS-PROT database [13]. Sequence homology searches were carried out using BLAST algorithm [14] against Protein Data Bank (PDB) [15]. Target-template alignment was created using ALIGN2D command of Modeller [16,17] which implements a global dynamic programming algorithm with a variable gap penalty.

2.2. Sequence comparison

Sequences homologous to ρ-crystallin were extracted from SWISS-PROT [13] and PDB [15] databases. Multiple sequence alignment of ρ-crystallin from bullfrog (*R. catesbeiana*), European common frog (*R. temporaria*), ρB-crystallin from Gecko (*L. lugubris*) and rat 3α-hydroxysteroid/dihyrodiol dehydrogenase (3α-HSD) was performed using CLUSTALW [18].

2.3. Model building, refinement and evaluation

Three-dimensional model of ρ-crystallin was constructed using crystal structure coordinates of rat 3α-HSD [19] (PDB code: 1AFS) as template using program Modeller [16,17]. This program is completely automated and is capable of generating energy minimized protein models by satisfying restraints on bond distances and dihedral angles. For ρ-crystallin model in presence of NADP and testosterone, hetero-atom command of Modeller was employed and model was constructed using coordinates of rat 3α-HSD [19]. Each model was subjected to various cycles of Modeller and the best possible model was selected. Model evaluation was conducted using ENERGY command of Modeller, program PROCHECK [20] and program WHATIF [21]. The secondary structure prediction of ρ-crystallin from bullfrog was carried out using the PHD method [22].

3. Results and discussion

3.1. Homology modeling of ρ-crystallin

ρ-Crystallin has 55% sequence identity with rat 3α-HSD. The structural model of ρ-crystallin was obtained using the known 2.5 Å resolution structure of homologous protein 3α-HSD (1AFS) [19]. The overall topology of the ρ-crystallin model is similar to X-ray structure of rat

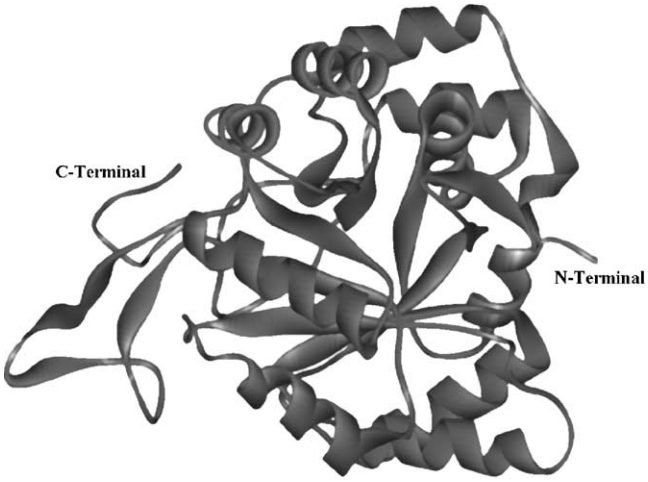

Fig. 1. Ribbon diagram of predicted three dimensional structure of ρ-crystallin from bullfrog showing the conserved $(\alpha/\beta)_8$ barrel motif common to all AKRs.

3α-HSD, which shows an $(\alpha/\beta)_8$ barrel fold shared by all members of AKR superfamily (Fig. 1). The fold consists of eight parallel β-strands each alternating with an α-helix running anti-parallel to the strands [23]. The chain forms the barrel repeating α/β units eight times with two additional helices which are not a part of the barrel. Comparison between AKRs and ρ-crystallins indicate that all functional residues are conserved in bullfrog ρ-crystallin with some minor differences. Multiple sequence alignment of ρ-crystallin from bullfrog, European frog, ρB-crystallin from Gecko and rat 3α-HSD is presented in Fig. 2. Eleven amino acid residues are highly conserved in primary structure of all AKRs [23]. These include Gly22, Gly45, Asp50, Lys84, Asp112, Pro119, Gly164, Asn167, Pro186, Gln190 and Ser271. All of these positions are conserved in ρ-crystallins. Among additionally conserved residues (i.e. Gly20, Tyr55, Gly62, Leu113, Trp148, Gly158, Glu192 and Arg276), only one exception (Tyr55Thr) was observed in ρ-crystallin.

3.2. Quality of the model

Quality of the model was assessed by using different validation tools. We performed proline puckering, packing quality, stereochemistry of main-chain and side-chain residues using program PROCHECK and program WHATIF. The results are presented in Table 1. The rmsd between template and the model using all main-chain atoms was found to be 0.119 and 0.148 in absence and presence of cofactor and substrate, respectively. Ramachandran plot statistics indicated that 93% of the main-chain dihedral angles are found in the most favorable region.

3.3. Catalytic triad

The AKRs catalyze the oxidation–reduction reaction over a range of substrates using NAD(P)(H) as a cofactor.

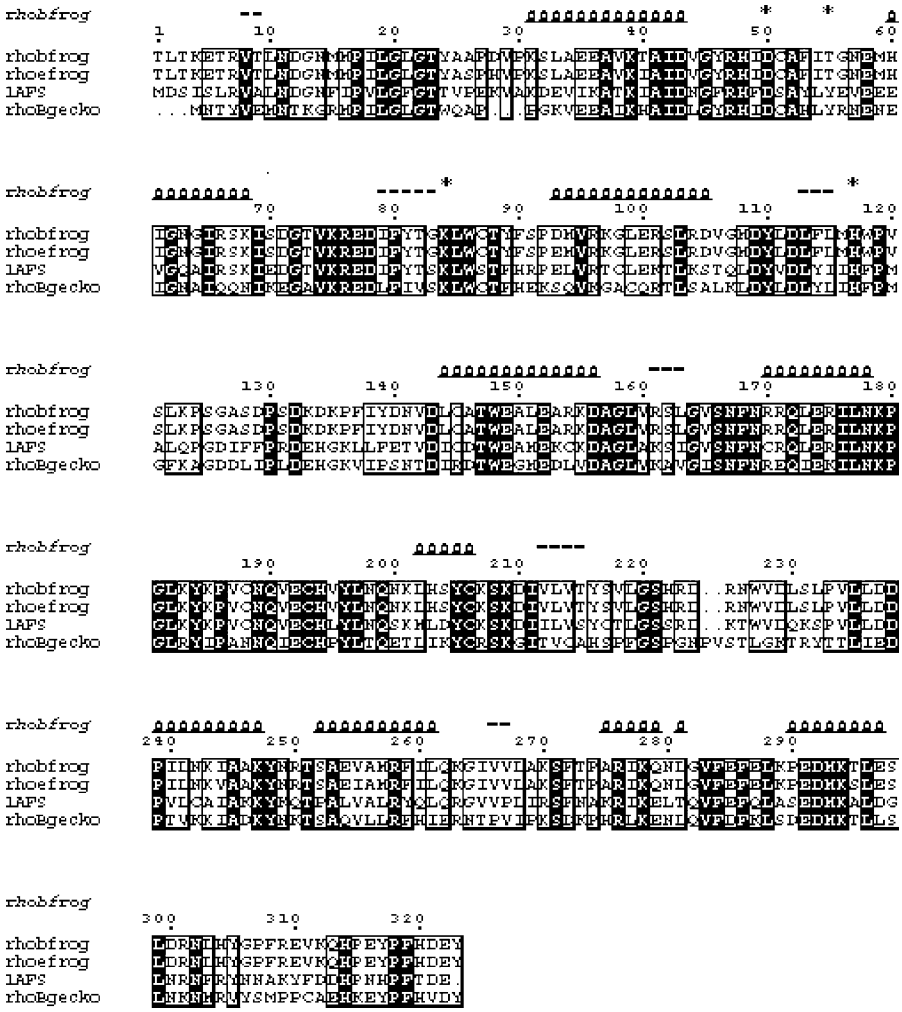

Fig. 2. Multiple sequence alignment of ρ-crystallins from bullfrog (rhobfrog, P17264), European frog (rhoefrog, P02532), Gecko (rhoBgecko, AJ245805) and rat 3α-hydroxysteroid/dihydrodiol dehydrogenase (1AFS). * represent residues important for catalytic activity; black and transparent boxes designate residues that are identical or highly conserved, respectively. Secondary structure motifs as determined for rhobfrog are indicated by circle (helix) or dash (b strands) above the alignment. The alignment figure was created by CLUSTALW.

Table 1
Quality of main-chain and side-chain parameters of modeled ρ-crystallin from bullfrog

Stereochemical parameter	No. of data points	Parameter value	Typical value	Band width	No. of bandwidths from mean
Stereochemistry of main-chain					
Percentage residues in A, B, L	281	93.2	76.6	10.0	1.7
Omega angle S.D.	318	2.7	6.0	3.0	−1.1
Bad contacts 100 residues	1	0.3	10.5	10.0	−1.0
Zeta angle S.D.	300	1.3	3.1	1.6	−1.1
Hydrogen bond energy S.D.	319	0	−0.6	0.3	2.1
Stereochemistry of side-chain					
Chi-1 gauche minus S.D.	45	5.6	22.7	6.5	−2.6
Chi-1 trans S.D.	97	8.8	22.7	5.3	−2.6
Chi-1 gauche plus S.D.	124	6.4	21.3	4.9	−3.0
Chi-1 pooled S.D.	266	7.4	22.0	4.8	−3.0
Chi-2 trans S.D.	63	7.7	23.1	5.0	−3.1

The model was verified at 2 Å resolution. A, B, L regions refer to percentage of residues in most favored regions of Ramachandaran plot which should be greater than 90% for good models. Omega angles are torsion angles of protein structure. The parameter value in table represents observed value for ρ-crystallin model compared with typical value obtained for well refined structures at same resolution. For further details, see Laskowski et al. [20].

Among the catalytic residues, Asp50, Tyr55, Lys84 and His117 are suggested to be important. Mutation analysis revealed that replacement of Tyr55 and Lys84 severely impairs the catalytic activity. The crystal structure analysis indicated that Lys84 is not in a proper position to act as general acid/base suggesting Tyr55 might be the acid in the catalytic mechanism. Site directed mutagenesis studies confirmed the functional importance of Tyr55 [24]. Catalytic activity in AKRs is facilitated by formation of a hydrogen bond/salt bridge between Asp50 and Lys84, which in turn promotes reduction of pK_a of tyrosine to aid proton donation. This hydrogen bond is retained in ρ-crystallin but Tyr55Thr replacement renders the molecule to be enzymatically inactive. Active site cleft for both proteins is shown in Fig. 3. The molecule of ρ-crystallin is slightly disoriented as another hydrogen bonds formed in 3α-HSD (Lys84-Tyr55) is replaced in ρ-crystallin (Lys84-Tyr24).

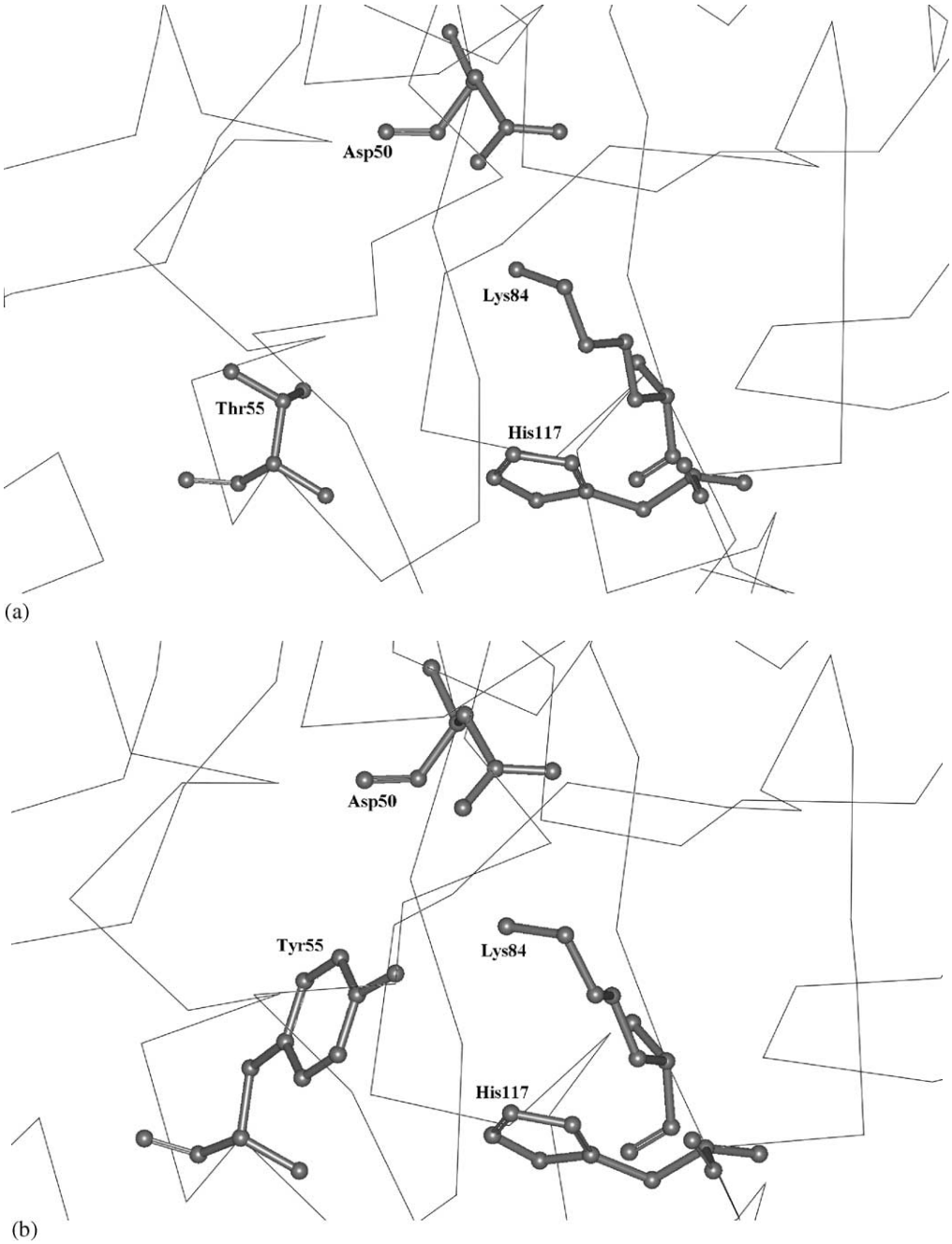

Fig. 3. Catalytic residues (drawn in ball and stick conformation) of predicted three-dimensional structure of ρ-crystallin (a) and crystal structure of rat 3α-HSD (b).

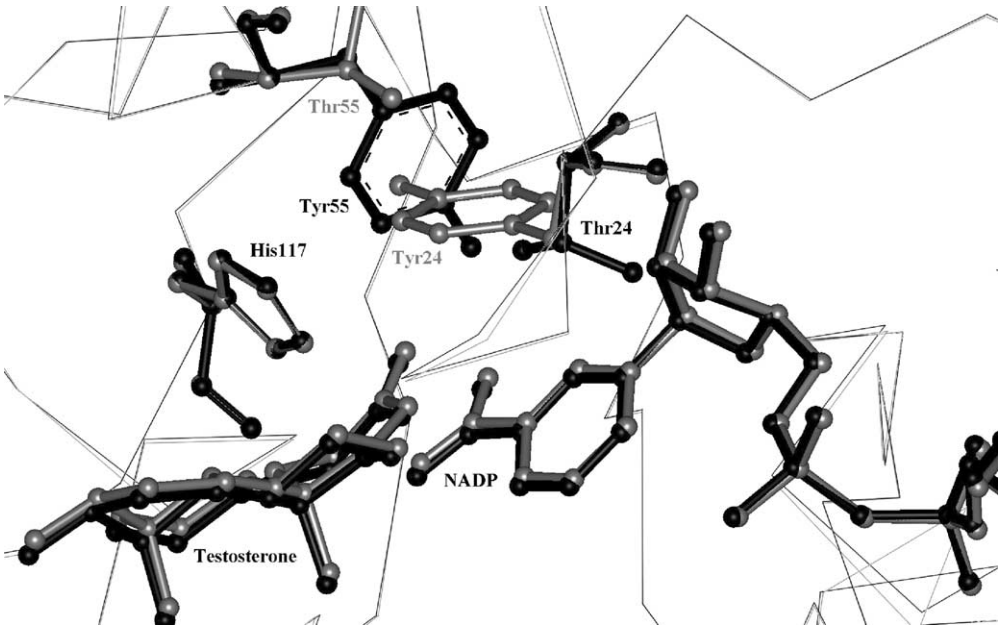

Fig. 4. Superposition of active site region of ρ-crystallin model with bound cofactor NADP and inhibitor testosterone (gray) with crystal structure of rat liver 3α-HSD (black). RMS deviation after superposition was 0.148 using all main-chain atoms.

3.4. Cofactor binding site and substrate cleft

The residues involved in cofactor binding pocket in 3α-HSD include Asp50, Asn167, Gln190, Ser271 and Arg276; all of which are conserved in ρ-crystallin suggesting a similar cofactor binding pocket. In order to examine the cofactor binding cleft and substrate binding pocket in ρ-crystallin, the coordinates of NADP and testosterone (a competitive inhibitor) bound to 3α-HSD were used to build a model of ρ-crystallin with bound cofactor and inhibitor. Constructed model showed same orientation as that of 3α-HSD (Fig. 4) with minor variation. In 3α-HSD, NADP is stabilized by forming thirteen hydrogen bonds with enzyme while in ρ-crystallin, six hydrogen bonds in total were observed; four of which are same as that of 3α-HSD. The catalytic mechanism of 3α-HSD suggests that the reactive ketone of steroid molecule must be located close to nicotinamide ring of NADP from which hydride transfer would take place and must also form two hydrogen bonds with conserved His117 and Tyr55. In ρ-crystallin structure, hydrogen bond with His117 is retained. The second hydrogen bond, however, is unlikely due to the replacement of Tyr55 with Thr55 in ρ-crystallin. Instead, C3 ketone forms the second hydrogen bond with Tyr24 which is present in the binding cavity. It is intriguing to note that residue at 24 position is claimed to be important to determine the efficacy of the catalytic activity. Among catalytically characterized 3α-HSDs, rat liver enzyme has the highest k_{cat} value [19]. In most of the hydroxysteroid dehydrogenases, this position is occupied by a Tyr residue as apposed to Thr24 in rat liver 3α-HSD. Crystal structure studies and kinetic data has indicated that Tyr24 decreases the rates of catalysis perhaps by altering the positioning of the steroid substrate while in rat liver 3α-HSD, Thr24 makes the enzyme catalytically efficient. Based upon these observations, we suggest that the presence of Tyr24 in ρ-crystallin may further contribute to the loss of enzymatic activity.

Although ρ-crystallin is catalytically inactive, it is interesting to note that it still retains NADP binding capability. Bullfrogs live on waterside which is ultraviolet radiation rich environment. There is a possibility that NADPH in lenses may absorb ultraviolet light and protect lens from toxicity caused by irradiation [25]. It has been suggested that cofactor binding capability might be important for contributing towards thermal stability of the lens [6]. It is likely that during evolution, loss of enzymatic activity may have been compromised over structural stability.

3.5. Structural–function relationship

Crystallins are group of proteins which are surprisingly diverse. They represent excellent example of gene duplication phenomenon. Among crystallins, ε-crystallin from duck lens, δ-crystallin from chicken lens, τ-crystallin from turtle lens, ξ-crystallin from tree frog lens and η-crystallin from elephant shrew lens retain intrinsic activity of lactate dehydrogenase, arginino succinate lyase, α-enolase, quinone reductase and retinaldehyde dehydrogenase (for review, see Wistow and Piatigorsky, 1988 [4]). Few other crystallins, however, are found to be catalytically inactive even though they retain most of the crucial residues. ρ-Crystallins studied so far are catalytically inactive. In case of bullfrog ρ-crystallin, this lack of catalytic activity may

be attributed to the Tyr55 replacement, however, possibility of involvement of other residues may not be ruled out. Analysis of ρB-crystallin from Gecko lens indicates that Tyr55 may not be exclusively responsible for the enzymatic activity. Gecko ρB-crystallin, which is more homologous to aldose reductase rather than to frog ρ-crystallin, has shown to retain all crucial residues required for catalytic activity but lacks catalytic activity [8]. It is therefore a possibility that either ρB-crystallin has different substrate specificity or some other amino acids are also involved. Another example is that of Ω-crystallin which is aldehyde dehydrogenase derived crystallin found in cephalopods [26,27]. Studies revealed that although scallop lens Ω-crystallin shows high degree of sequence similarity (> 64%) with human aldehyde dehydrogenases and preserves all crucial residues responsible for enzymatic activity, it lacks catalytic activity [28]. This enzymatic inactivity has been attributed to inability of Ω-crystallin to bind NADP presumably due to Ile282Val mutation in Ω-crystallins studied so far [28]. The examples cited above indeed call for further investigations to analyze the possibility of a different catalytic mechanism in these crystallins. However, it can be concluded that sequence similarity alone does not imply functional identity.

4. Conclusions

Aldo–keto reductase superfamily can be classified into three groups which include members of aldose reductases, aldehyde dehydrogenases and hydroxysteroid dehydrogenases. Various lens crystallin have been identified which are structurally similar to one of these groups. The members of AKR superfamily have been implicated in diabetic complications and are potential therapeutic targets [23]. 3α-HSDs are responsible for steroid hormone catabolism and bile acid synthesis and are also implicated in pathogenesis of benign hyperplasia and prostate cancer [19]. However, there has been little progress towards development of successful drug targets and specific inhibitors [23]. Before targeting individual AKRs for rational drug design, understanding of the relationship between structure and function in the superfamily is required [23]. Knowledge based approach such as comparative modeling can be used as an important tool to design site directed mutagenesis experiments, to build supramolecular structures, to perform molecular replacements and in rational drug designing/docking experiments [29]. The model presented in this study indicates that bullfrog ρ-crystallin acts as a natural mutant to aldo–keto reductase family. As a result of evolutionary pressure, a single gene has been recruited to perform dual role, resulting in two structurally similar but functionally different proteins. The functional diversity of AKRs members and yet their simultaneous relatedness together may prove useful in future to study the evolution of substrate specificity and to design potential drug targets.

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