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ζ-Crystallin versus other members of the alcohol dehydrogenase super-family

Variability as a functional characteristic

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Species variability of the lens protein ζ-crystallin was correlated with those of alcohol dehydrogenases of classes I and III and sorbitol dehydrogenase in the same protein family. The extent of overall variability, nature of residues conserved, and patterns of segment variability, all fall within the limits typical of the 'variable' group of medium-chain alcohol dehydrogenases. This shows that ζ-crystallin is subject to restrictions similar to those of classical liver alcohol dehydrogenase and therefore derived from a metabolically active enzyme like other enzyme crystallins. Special residues at the active site, however, differ substantially, including an apparent lack of a zinc-binding site. This is compatible with altered functional properties and makes the spread within this medium-chain dehydrogenase family resemble the wide spread within the short-chain dehydrogenases. Schematic plotting is useful for illustrating the differences between 'variable' and 'constant' enzymes.

Crystallin; Alcohol dehydrogenase; Structure–function relationship; Segment variability; Glycine conservation

1. INTRODUCTION

ζ-Crystallin is an intriguing protein, initially detected in guinea pig lenses [1] and structurally characterized as a cDNA [2]. Like other taxon-specific crystallins, it was interpreted as a recruitment [3,4] of an enzyme for a lens structural protein and would then represent a product of a gene with dual functions ('gene sharing') and possibly multiple mRNAs, or of a duplication and special regulation [5]. Significantly, ζ-crystallin was found to be a distant homologue of the enzyme alcohol dehydrogenase, but considerably altered, lacking a large internal segment and residues typical of the dehydrogenase [6]. Consequently, the enzymatic properties of ζ-crystallin and its relationships with alcohol dehydrogenase were not easily interpreted, except to conclude that the coenzyme-binding segment was the one most conserved [6]. Deletion of an exon in this segment is associated with congenital cataract formation in guinea pigs [7–9]. ζ-Crystallin has since been found not to be taxon-specific, but also to occur as a major protein in the lens of the camel [10]. The appearance of this protein as a crystallin in two evolutionarily distant species is atypical of enzyme crystallins. Occurrence in mammalian liver and kidney has also been proven [8–11], and present evidence indicates that ζ-crystallin is expressed at low enzymatic levels in various tissues, including lens, in a variety of mammals. An enzyme activity has been defined, for ζ-crystallin to constitute a quinone reductase using NADPH [12,13]. In the case of the bovine protein, originally isolated as a DNA binding protein and named RF-36, other activities have been reported [14–16]. Only recently, when sequence data were obtained (Du Bois, Lavers and Chen, in preparation), it was found to be a homologue of ζ-crystallin. Like the human and mouse proteins (and in contrast to the guinea pig protein), the bovine protein is present in the lens at very low levels. The importance of any of the activities reported for ζ-crystallin remains an enigma, and the nature of the active site is unknown.

Further evaluation of ζ-crystallin is now possible, since species variants (human, mouse, bovine) have been characterized [2,17] (Du Bois, Lavers and Chen, in preparation), and the nature of the species variability has proved to be a characteristic property of several dehydrogenases, dividing the alcohol dehydrogenase family members into two types, constituting 'variable' and 'constant' enzymes [18], where different segment similarities allow distinction of active site segments, subunit interactions, and other special units [19]. In the

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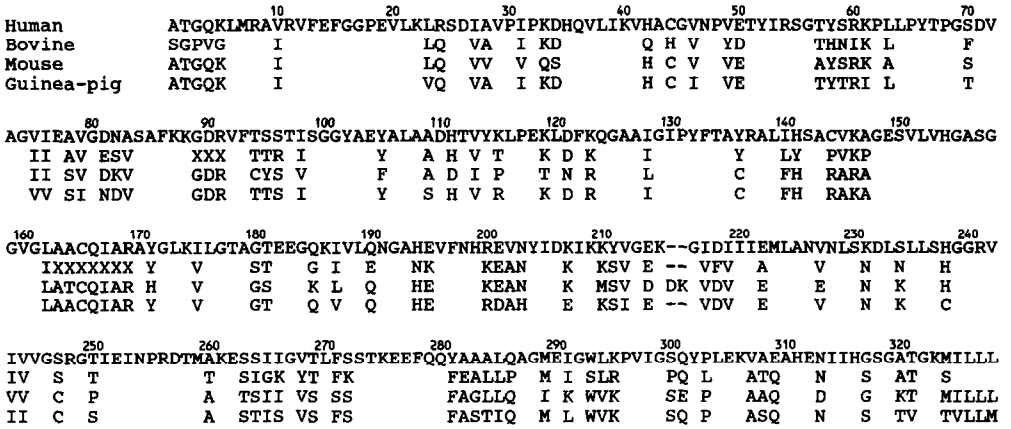

Fig. 1. Aligned species variants of ζ-crystallin. The top line, representing the human structure, is continuous. Remaining lines have designations only where any of them is different from the top line. –, gap; X, any amino acid.

present work, we therefore analyze the structure–function relationships of ζ-crystallin variability towards the alcohol dehydrogenase family at large. Results are conclusive and define specific properties of the ζ-crystallin molecule.

2. MATERIALS AND METHODS

ζ-Crystallin structures from four different species, guinea pig [2], human, mouse (Gonzalez, Rao and Zigler Jr., in preparation), and bovine (Du Bois, Lavers and Chen, in preparation), were evaluated in relation to similar species variants within the two best characterized classes of mammalian alcohol dehydrogenase and the single class of sorbitol dehydrogenase. The two alcohol dehydrogenase classes are the class I enzyme (human, horse, mouse, rabbit [19–21], which is the classical, ethanol-active alcohol dehydrogenase of liver, with known structural and functional properties down to the fish line [22], and the class III enzyme, which is equivalent to the glutathione-dependent formaldehyde dehydrogenase [23], recently also traced in structure and enzymatic properties down to the fish line [24].

Programs were developed for on-screen inspection and alignment of multiple amino acid sequences and for calculation and construction of plots representing structural variabilities.

3. RESULTS AND DISCUSSION

3.1. *ζ-Crystallin is a variable protein, but in a manner characteristic of the enzyme family*

Overall, the ζ-crystallin residue differences between a primate, an ungulate, and two rodents (human, bovine, mouse, guinea pig) constitute 17–21% (Fig. 1). This is the same level (Table I) as that for similar species variants of class I alcohol dehydrogenase (human, horse, mouse, rabbit) and sorbitol dehydrogenase (human, sheep, rat), but clearly different from that for class III alcohol dehydrogenase (human, horse, rat). These two levels of human/rodent species variability are typical of several dehydrogenases in general [18]. Consequently, it may be concluded that ζ-crystallin belongs to the variable group within the alcohol dehydrogenase super-family. It does so in a typical manner, is not hyper-variable and does not exhibit deviating overall values.

3.2. *Residues conserved*

The most strictly conserved residue within the ζ-crystallin variants is glycine (Table II). This pattern is typical of the alcohol dehydrogenase super-family, where glycine is most often conserved because of space restrictions [19]. In many cases, including when ζ-crystallin is compared with alcohol dehydrogenases [9] rather than with its own species variants, and when the different classes of alcohol dehydrogenase are compared, this is even more pronounced. Notably, however, ζ-crystallin shows little conservation in Cys and Trp residues, in fact bovine ζ-crystallin appears to have neither. With the exception of Cys, the most conserved and the least conserved residues are the same in ζ-crystallin as in the three dehydrogenases (Table II). The Cys deviation is explained by the fact that Cys is a functional zinc ligand in the non-crystallin proteins but not in ζ-crystallin (section 3.4, below). It is concluded that residue-wise, ζ-crystallin overall conservation behaves like that of a

Table I

Extent of human/rodent variability for ζ-crystallin versus that for other members of the medium-chain alcohol dehydrogenase family

For ζ-crystallin, the rodent line used in the calculations is guinea pig, for alcohol dehydrogenase class I (ADH I), class III (ADH III) and sorbitol dehydrogenase (SDH) it is rat. Values given are those excluding all gap positions in the calculations. However, use of different rodent species gives only marginally different values (for class I alcohol dehydrogenase, the human/mouse residue difference is 15%). Similarly, different alignments or gap inclusions give small alterations (cf. 18 instead of 17 in [18]).

Protein	Species differences human/rodent (%)
ζ-crystallin	17
ADH I	17
ADH III	6
SDH	17

Table II

Numbers of strictly conserved residues within the species variants of the ζ-crystallins compared with those in other members of the medium-chain alcohol dehydrogenase family

(A) The four residues most extensively conserved in ζ-crystallin and values for the same residues in the other enzymes (B) The four least conserved in ζ-crystallin, and values for the same residues in the other enzymes. As shown, Gly is top-conserved in all cases, and, except for Cys, both groups of residues are largely similar for all four proteins. Abbreviations as in Table I.

	Residues conserved			
Residue	ζ-crystallin	ADH I	ADH II	SDH
(A)				
Gly	31	34	40	32
Ala	19	21	31	24
Leu	19	21	19	26
Glu	15	14	23	18
(B)				
Met	3	3	7	6
Gln	3	5	5	3
Cys	0	13	13	10
Trp	0	2	4	2

typical enzyme member of the alcohol dehydrogenase super-family, suggesting that conformational restrictions are roughly the same in ζ-crystallin as in the dehydrogenase enzymes.

3.3. *Patterns of segment conservations*

ζ-Crystallin variability exhibits a fairly even distribution along the entire protein chain (Fig. 2). As expected from the finding that ζ-crystallin is a 'variable' protein (cf. 3.1), like sorbitol dehydrogenase and class I alcohol dehydrogenase, the pattern is clearly different from that of class III alcohol dehydrogenase (Fig. 2). However, it is similar to that for the other enzymes and has a somewhat patchy distribution, although not as pronounced as for the class I alcohol dehydrogenase (Fig. 2). This overall distribution of positional variability suggests that similar restrictions on structural variations apply to ζ-crystallin as to the dehydrogenases. In addition, the schematic representation of the species variations exceptionally well illustrates the different extent of variability between the class I/III forms (Fig. 2), and the three regions with extensive variability in alcohol dehydrogenase (Fig. 3).

3.4. *Residues/positions of special functional importance*

ζ-Crystallin does not have the active site residues typical of alcohol dehydrogenase [6]. However, it does have the classical GXXGXXG pattern (positions 155–161; Fig. 1) characteristic for the coenzyme-binding fold [24,25], and this region is in the area of maximal conservation (indicated in Fig. 2). Consequently, the fact that ζ-crystallin lacks alcohol dehydrogenase activity, but binds coenzyme (NADPH) highlights the importance of the conserved segment. A deletion mutant lacking 34

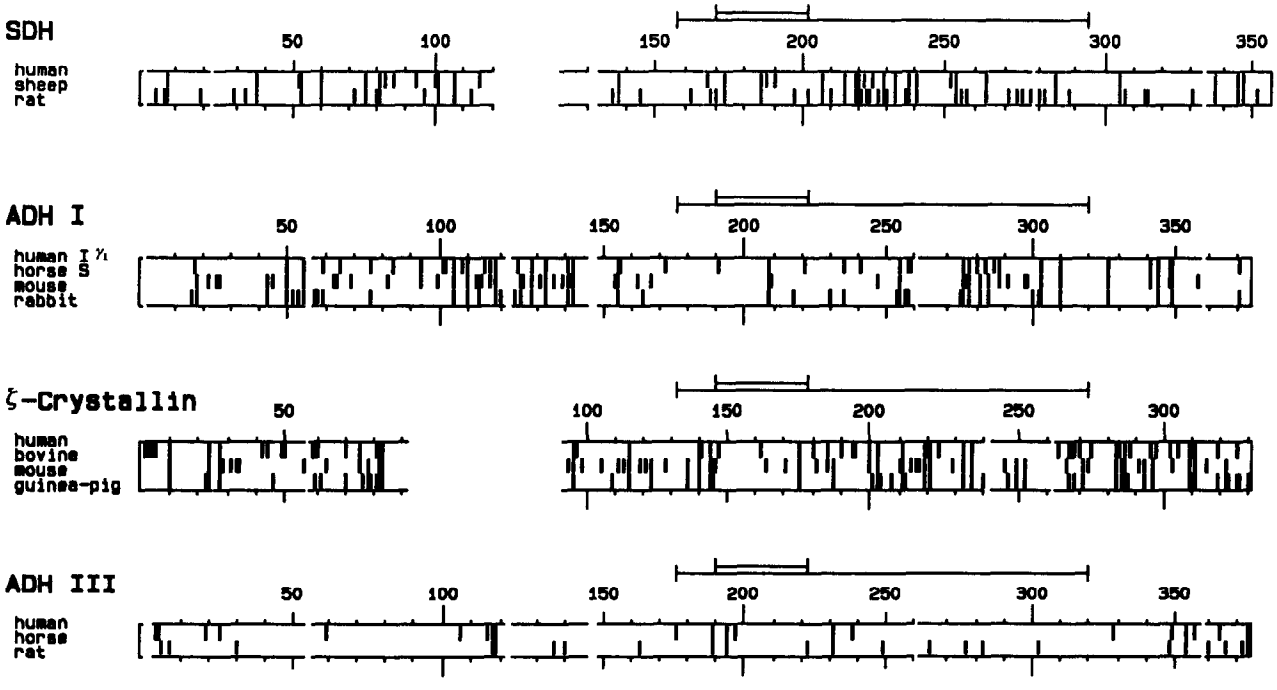

Fig. 2. Schematic representation of species variants of ζ-crystallin compared to those of alcohol dehydrogenases of classes I (ADH I) and III (ADH III) and sorbitol dehydrogenase (SDH). For each protein, the residue variability of the characterized forms towards the human variant are represented by black bars. The considerably larger conservation of class III than class I is clearly visible. The line above each protein box indicates the coenzyme-binding domain, and the double-line its central β-strands of great importance in coenzyme-binding (borders as given in [6,27]).

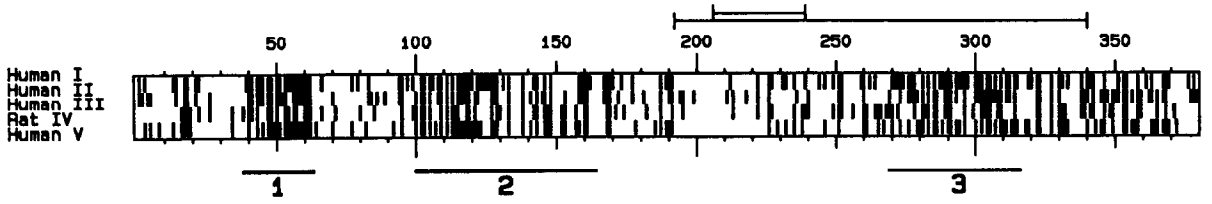

Fig. 3. Positions with non-conserved residues between the classes of alcohol dehydrogenase. In contrast to Fig. 1, positions marked are those representing class differences, rather than species differences. The three most variable segments are indicated by numbers, and are also faintly visible in Fig. 2 for the alcohol dehydrogenase class I enzymes. 1 denotes a segment contributing zinc ligands to the active site, 2 the segment around the second zinc atom, and 3 the area of subunit interactions.

amino acid residues of that part is associated with early cataract formation [7–9].

Because ζ-crystallin has extensive differences toward alcohol dehydrogenase, and an unknown three-dimensional structure, computer modelling as done for other enzymes within the alcohol dehydrogenase super-family [27], is difficult and not easily interpretable. However, one further observation of functional relevance is obvious from the comparisons. This concerns sites for possible metal-binding, which is of special interest because both the alcohol and polyol dehydrogenases are zinc metalloenzymes [28,29]. The lack in ζ-crystallin of an exact equivalence of the alcohol dehydrogenase zinc-binding sites had already been established [6], but other alternatives for zinc-binding have not been evaluated. This is now possible to do with respect to current rules regarding protein zinc-binding sites; in general, they have Cys, His, Glu, and Asp residues [30,31]. The ζ-crystallins do not have any conserved potential zinc-binding site. When only the guinea pig ζ-crystallin structure was known [6], His^{203}-X-X-X-Glu^{207} could be considered because that segment exhibited the spacing required [30,31], but with new structures available from other species we now find that neither His^{203} nor Glu^{207} is conserved. Although the segment Glu^{312}-X-X-X-His^{316} has the spacing required of a potential zinc ligand and is conserved, it is close to the C-terminus and therefore lacks a more distant third ligand [cf. [30,31]). Consequently, the ζ-crystallin species variants now known, and the predictive rules, when combined, do not give support for any zinc-binding site in ζ-crystallin. Therefore, it appears likely that ζ-crystallin lacks the metal-binding characteristics of the other enzymes within the alcohol dehydrogenase family, i.e. alcohol dehydrogenases of all classes [19], sorbitol dehydrogenase [28], and threonine dehydrogenase [29].

3.5. *Functional conclusions*

The fact that all ζ-crystallin properties now studied, i.e. extent of variability (section 3.1), type of residues conserved (section 3.2), and patterns of segments conserved (section 3.3), agree with corresponding properties of classical alcohol dehydrogenase shows that ζ-crystallin is a typical member of the variable group of alcohol dehydrogenases. Restrictions on the variability in all these respects are identical to those of the classical liver enzyme. Hence, a similarly defined function for ζ-crystallin would appear likely and supports the view that ζ-crystallin constitutes a true enzyme recruited for functions in the lens, in a manner generally similar to those of enzyme crystallins in general. Nevertheless, additional functions and different roles for the ζ-crystallins in different species should not be excluded. It may be significant that bovine ζ-crystallin has other activities and a low abundance in the lens [14–16].

The pronounced differences in specific residues at the active site, including the apparent absence of zinc sites (section 3.4), establish that ζ-crystallin has a basically different active site than that found in alcohol dehydrogenases, in spite of the similar overall properties. This is compatible with the lack of hitherto discernable dehydrogenase activity in ζ-crystallin and the presence of a reductase activity [11–13]. This presence of different activity types in one protein family, as evidenced by ζ-crystallin and dehydrogenases in the medium-chain alcohol dehydrogenase family, makes the functional properties of this whole family resemble those of other protein families, including the short-chain dehydrogenases, which also include different activity types, and functional residues different form those typical of the zinc enzyme [32].

Combined, the present results support the view of ζ-crystallin as an enzyme crystallin, and gives the medium-chain dehydrogenase family functional and overall properties exhibiting a wide spread.

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