Zoological Studies **33**(3): 177-185 (1994)

Zoological Studies

Review Article

Endogenous Enzymatic Activities of Taxon-Specific Lens Crystallins

Gu-Gang Chang[1,2] and Hwei-Jen Lee[1]

[1]*Department of Biochemistry, National Defense Medical Center, Taipei, Taiwan 100, R.O.C.*
[2]*Institute of Zoology, Academia Sinica, Taipei, Taiwan 115, R.O.C.*

CONTENTS

ABSTRACT

Gu-Gang Chang and Hwei-Jen Lee (1994) Endogenous enzymatic activities of taxon-specific lens crystallins. *Zoological Studies* **33**(3): 177-185. Eye lens crystallins were first thought to play only a structural role in lens transparency. The recent unexpected findings that some of the taxon-specific crystallins which have been isolated from eye lenses have similar amino acid sequences to some glycolytic enzymes and possess endogenous enzymatic activity raised some interesting questions: If there is no metabolism in a lens, what is the physiological meaning of these metabolic enzyme/crystallins in vivo? What is the evolutionary meaning of glycolytic enzyme employment as the lens structural proteins? In this review, we summarize recent progress on the characterization of enzyme/crystallins conducted in this laboratory. It includes duck and caiman ε-crystallin/lactate dehydrogenase, duck and chicken δ-crystallin/argininosuccinate lyase, octopus S-crystallin/glutathione S-transferase, and octopus Ω-crystallin/aldehyde dehydrogenase. We compare the kinetic and chemical mechanisms of these enzyme/crystallins with their cytosolic enzyme counterparts. Based on the chemical reactions catalyzed by these enzyme/cryatallins, we posit relationships between the possible physiological significance of these enzymatic reaction in vivo and stress. The possibilities of applying these enzyme/crystallins as natural mutants in molecular enzymology research are also discussed.

Key words: Lactate dehydrogenase, Argininosuccinate lyase, Glutathione transferase, Aldehyde dehydrogenase, Stress proteins.

INTRODUCTION

The eye lens is a specialized tissue. During the life span of an animal, the anterior epithelial cells continue to migrate laterally and differentiate into fiber cells, which overlay the older cells in concentric layers. During this process, the cell loses its nucleus and other organelles. Therefore, lens proteins have no metabolism; once synthesized they remain in the lens throughout the entire life span (DeJong et al. 1989, Wistow 1993). For this reason the crystallins were first thought to play only a structural role in lens transparency and optical clarity (DeJong et al. 1989).

Over the past decade the new DNA cloning techniques available to protein chemists have made it possible to delineate the primary amino acid sequence of a protein within a few months. When sequence analysis was performed with lens crystallins, among the remarkable findings was the information that some taxon-specific crystallins have amino acid sequences identical or related to some of the cytosolic enzymes (Table 1) (for some reviews, cf. Wistow and Piatigorsky 1987 1988 1990, Piatigorsky 1992, Wistow 1993). These surprising findings immediately raised intriguing questions about the physiological significance of the endogenous enzyme activity in vivo. Do these structural proteins undergo enzymatic activity? Does the enzymatic activity of the crystallins parallel its counterpart enzyme in the cytosol? What is the evolutionary meaning of employment of glycolytic enzymes as lens structural proteins? What factors influence the selection of catalytic molecules for structural purposes?

Vertebrate and invertebrate lens crystallins comprise a complex group of conserved structural proteins with distant evolutionary relationships (De Jong and Hendriks 1986, Wistow and Piatigorsky 1988). All vertebrates contain the alpha (α), beta (β) and gamma (γ) crystallins. Delta (δ) crystallin is found only among birds and reptiles. Other crystallins types occur in restricted taxonomic groups (Table 1). In this review, we limit our discussion to recent progress on the characterization of enzyme/crystallins conducted in this laboratory and the possible value in enzyme research of enzyme/crystallins' endogenous enzyme activity.

ENDOGENOUS ARGININOSUCCINATE LYASE ACTIVITY OF DUCK AND REPTILES δ-CRYSTALLIN

Kinetic mechanism of the endogenous argininosuccinate lyase activity of duck δ-crystallin

Our detection of endogenous argininosuccinate lyase activity of δ-crystallin in the duck lens δ-crys-

Table 1. Classification of Eye Lens Crystallins in Vertebrates and Invertebrates

Crystallin Components	Occurrence	Structural Relation
Ubiquitous		
α-crystallin	all vertebrates	molecular chaperones (heat-shock proteins)
β-crystallin	all vertebrates	protein S
γ-crystallin	all vertebrates	protein S (induced by osmotic shock)
Taxon-Specific		
δ-crystallin	birds and reptiles	argininosuccinate lyase
ε-crystallin	birds and reptiles	B4-lactate dehydrogenase
η-crystallin	elephant shrews	aldehyde dehydrogenase
λ-crystallin	rabbits and hares	hydroxyacyl-CoA dehydrogenase enoyl-CoA hydratase
μ-crystallin	some mammals	dehydrogenase?
ρ-crystallin	frogs	NADPH-reductase prostaglandin F-synthase
S-crystallin	cephalopods	glutathione S-transferase
τ-crystallin	turtles, lampreys, some fishes, birds, and reptiles	α-enolase
Ω-crystallin	octopi	aldehyde dehydrogenase
ζ-crystallin	guinea pigs	NADPH: quinone oxidoreductase

tallin was performed either by direct activity-staining in the gel (Lee et al. 1992a) or the enzyme activity in solution assay by monitoring the decomposition of argininosuccinate, which produces arginine and fumarate, the latter absorbs ultraviolet light at 240 nm due to its double bond (Lee et al. 1992b). Activity staining showed that duck δ-crystallin possessed enzymatic activity whereas that of chicken δ-crystallin was devoid of activity.

A detailed kinetic analysis of the argininosuccinate lyase activity of duck δ-crystallin was performed to elucidate the kinetic mechanism of this dual function crystallin. Variation of the enzymatic activity with argininosuccinate concentration in the forward reaction followed saturation kinetics with an apparent Michaelis constant for the substrate of 17 μM. In the reverse reaction, initial-velocity studies showed intercepting patterns. Inhibitions of the forward reaction by products (fumarate and arginine) were both noncompetitive with respect to argininosuccinate. Citrulline, an arginine analog, inhibited the enzyme activity in both directions and was competitive with respect to arginine but noncompetitive with respect to either fumarate or argininosuccinate. Succinate which inhibited the bovine argininosuccinate lyase, up to 300 mM did not affect δ-crystallin enzymatic activity. These results suggest a random Uni-Bi kinetic mechanism for the argininosuccinate lyase activity of duck δ-crystallin with various abortive δ-crystallin-argininosuccinate-arginine, δ-crystallin-argininosuccinate-fumarate, and δ-crystallin-argininosuccinate-citrulline ternary complex formations (Lee et al. 1992b) (Fig. 1).

The proposed random Uni-Bi kinetic mechanism was confirmed by chemical modification (Lee et al. 1993). The argininosuccinate lyase activity of duck δ-crystallin was inactivated by diethyl pyrocarbonate at 0°C and pH 7.5. The inactivation followed pseudo-first order kinetics after appropriate correction for the decomposition of the reagent during the modification period. L-Arginine, L-norvaline, or L-citrulline protected the argininosuccinate lyase activity of δ-crystallins from diethyl pyrocarbonate inactivation. The dissociation constants for the δ-crystallin-L-arginine and δ-crystallin-L-citrulline binary complexes, determined by the protection experiments, were 4.2 and 0.12 mM, respectively. Fumarate alone had no protective effect. However, fumarate with L-arginine offered synergistic protection with a ligand binding interaction factor (α) of 0.12. The double-protection data supported a random Uni-Bi kinetic mechanism. Our results indicated that only one histidyl residue per subunit was modified by the reagent. This super-active histidine has a pK_a value of ~6.8 and acts as a general acid-base catalyst in the enzyme reaction mechanism.

Recently, we have prepared a deuterium labeled argininosuccinate by enzymatically reacting fumarate and arginine in D_2O. We did not observe an isotope exchange effect when comparing

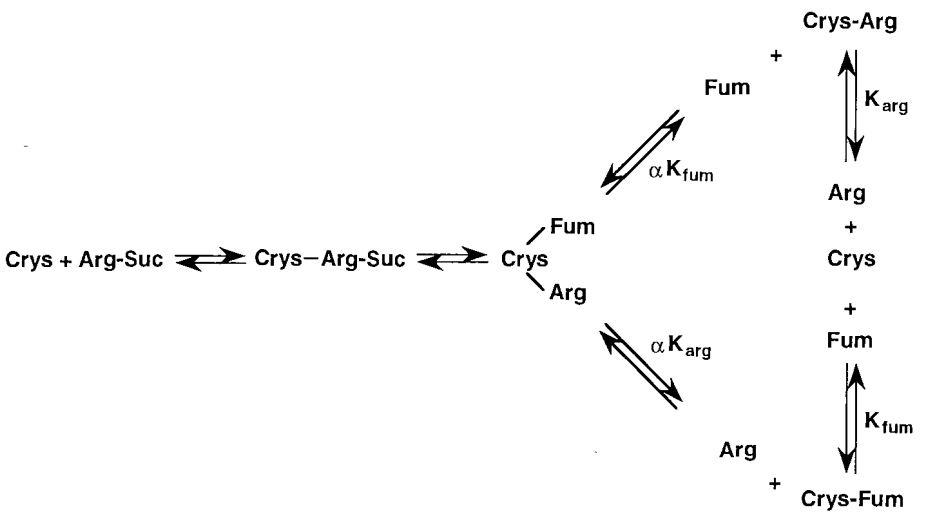

Fig. 1. Proposed random Uni-Bi kinetic mechanism for the argininosuccinate lyase activity of duck lens δ-crystallin. Crys: duck δ-crystallin, Arg-Suc: argininosuccinate, Arg: arginine, Fum: fumarate, Cit: citrulline. δ-crystallin was proposed to bind with L-arginine and fumarate in a random manner forming δ-crystallin-L-arginine and δ-crystallin-fumarate binary complexes with dissociation constants of K_{arg} and K_{fum}, respectively. Binding of either ligand favored the binding of the other with interacting factor of α, and formed the L-arginine-δ-crystallin-fumarate ternary complex.

the pH-rate of protonium- or deuterium-substrate (Lee and Chang, unpublished results). Our experimental data mesh with an E1cB mechanism for the argininosuccinate lyase with the essential histidyl residue close to the arginine binding domain of δ-crystallin (Fig. 2). L-Citrulline, after binding to this domain, might possibly form an extra hydrogen bond with the essential histidyl residue.

Characterization of the multiple forms of duck δ-crystallin

When the SDS-PAGE-judged homogeneous duck δ-crystallins were subjected to electrophoresis under native conditions or isoelectric focusing, multiple forms were detected and further, divided into δa- and δb- subgroups (Lee et al. 1922b). All of the multiple forms possessed endogenous argininosuccinate lyase activity with activation energy ~12.5 kcal/mol. δb-crystallins showed a higher value of enzyme activity than did the δa-crystallins. Slightly different kinetic parameters were observed for the δb-crystallins multiple forms. Further, δa-crystallins could be divided into two subgroups according to their kinetic parameters. There is a 12-fold difference in the k_{cat} value between these two subgroups. The δa-crystallin also has a lower K_m value than does the δb-crystallin.

When examined by polyacrylamide gel electrophoresis under reducing conditions in the presence of sodium dodecyl sulfate, the multiple forms were shown to be composed of subunits with similar M_r values of 55,000. All of these forms showed the same antigenicity toward the rabbit anti-duck δ-crystallin antiserum; however, different carbonyl contents were observed for these forms indicating that the origin of these multiple forms was due to post-translational oxidative modification or glycation of the protein molecules. Protein modifications resulted in a change of intrinsic tryptophan and tyrosine fluorescence. Those forms with higher pI values were shown to be much more thermostable than those with lower pI values.

Recently we have found that some of the multiple forms resulting from N-terminal amino acid truncation (Lee et al., unpublished results). Such findings confirmed oxidative modification and peptide bond cleavage as the reason for the observed multiple forms.

ENDOGENOUS LACTATE DEHYDROGENASE ACTIVITY OF DUCK OR CAIMAN ε-CRYSTALLIN

We have characterized the kinetic mechanism of duck ε-crystallin as possessing endogenous lactate dehydrogenase activity (Chiou et al. 1990). We provided conclusive evidence demonstrating that duck or caiman ε-crystallin is the heart-type

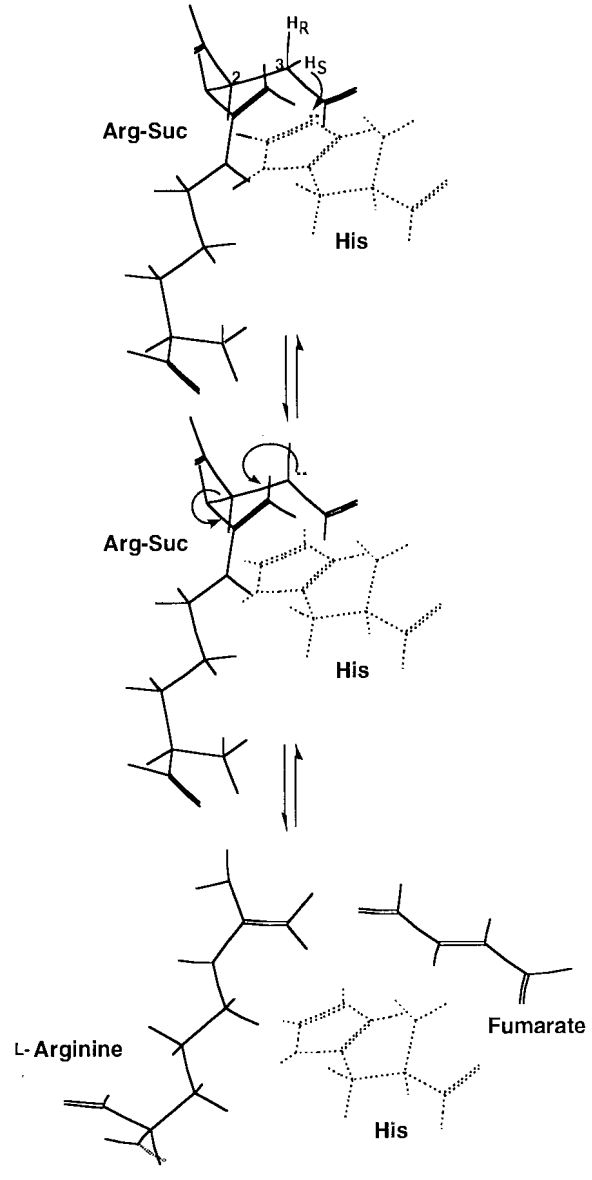

Fig. 2. Proposed E1cB chemical mechanism for the endogenous argininosuccinate lyase activity of duck lens δ-crystallin. The E1cB mechanism proceeds in two steps. First, a histidine residue in its conjugated base form abstracts the Hs proton in C3 of argininosuccinate, forming a carbanion intermediate. Second, the carbanion intermediate provides the driving force for the expulsion of the fumarate group. The carbanion intermediate is stabilized by the positive charge in the guanidino group, which provides an electron-withdrawing center (Abeles et al. 1992). The substrates are denoted by solid lines and the essential histidine residue depicted with dashed lines.

lactate dehydrogenase (Chiou et al. 1990 1991a); additionally, strong substrate inhibition by pyruvate and L-lactate was observed. The K_m^{Lact}/K_m^{Pyr} ratio for the ε-crystallin was similar to that for the heart-type lactate dehydrogenase, but was 2-3 times smaller than that for the muscle-type lactate dehydrogenase. Enzymatic reactions follow a compulsory ordered Bi-Bi sequential kinetic mechanism with NADH as the leading substrate followed by pyruvate (Fig. 3). The products L-lactate and NAD^+ are sequentially released. The catalyzed reaction is shown to have a higher rate in the formation of L-lactate and NAD^+. The substrate inhibition mechanism was demonstrated to be a formation of ε-crystallin-NAD^+-pyruvate and ε-crystallin-NADH-L-lactate abortive ternary complexes in forward and reverse reactions, respectively (Chang et al. 1991).

The structural requirements for the substrate and coenzyme of ε-crystallin are similar to those of other dehydrogenases, additionally the carboxamide carbonyl group of the nicotinamide moiety is important for coenzyme activity (Chang et al. 1991). The pyruvate structure can be varied considerably without losing its activity as a substrate. The carboxyl group could be esterified and the methyl group could be halogenated. However, if the methyl group was replaced with an amino group, the oxamate could no longer serve as a substrate.

Recently we have embedded the ε-crystallin into a reverse micellar system prepared by dissolving the surfactant AOT [sodium bis(2-ethylhexyl) sulfosuccinate] in isooctane (2,2,4-trimethylpentane). The tetrameric protein was found to dissociate to various associated forms (monomer, dimer, trimer, tetramer, and octamer) and each form was enzymatically active (Lee and Chang, unpublished results), which provides a foundation for the further pursuit of the physiological function of the LDH quaternary structure.

ENDOGENOUS GLUTATHIONE TRANSFERASE ACTIVITY OF CEPHALOPODS S-CRYSTALLIN

S-Crystallin isolated from octopus or squid lens extract was found to have similar amino acid sequences with glutathione S-transferase (GST) (Tomarev and Zinovieva 1988, Tomarev et al. 1991). We found that the octopus S-crystallin possessed endogenous glutathione S-transferase activity albeit severly decreased. We have purified both S-crystallin and digestive gland GST from the octopus to an apparent homogeneity (Tang et al., unpublished results). The specific GST activity of purified S-crystallin and digestive gland GST were found to be 0.096 and 236 μmol/min/μg protein, respectively.

Steady-state kinetics was used to investigate the kinetic mechanism of the endogenous glutathione S-transferase activity of octopus S-crystallin. Biphasic double reciprocal plots were obtained for both the glutathione and the electrophilic substrate 1-chloro-2,4-dinitrobenzene. Substrate inhibition was only observed for 1-chloro-2,4-dinitrobenzene with a K_{si} of 19.6 mM. The initial-velocity studies indicated that the enzyme reaction conformed to a steady-state random Bi-Bi kinetic mechanism. This is consistent with the glutathione S-transferase from other sources (Fig. 4).

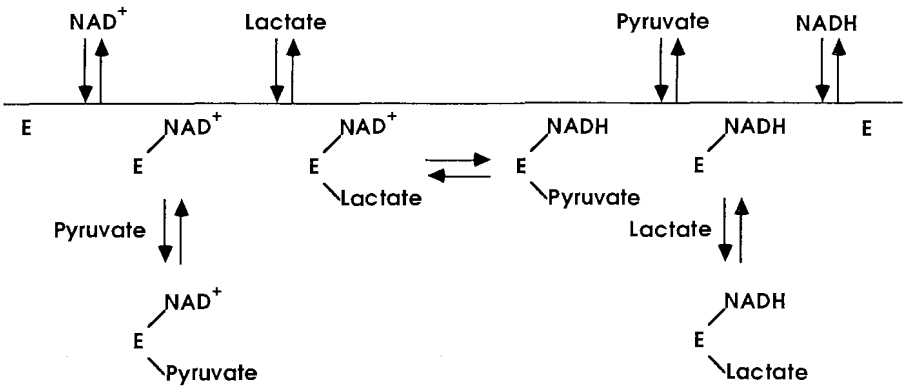

Fig. 3. Proposed sequential order Bi-Bi kinetic mechanism for the lactate dehydrogenase activity of duck lens ε-crystallin (From Chiou et al., 1990). The compulsory-order mechanism requires that both substrates be added before the first product is released, and that the addition of substrates and release of products follow an obligatory order as depicted in the scheme. Substrate inhibitions are shown at the bottom of the scheme.

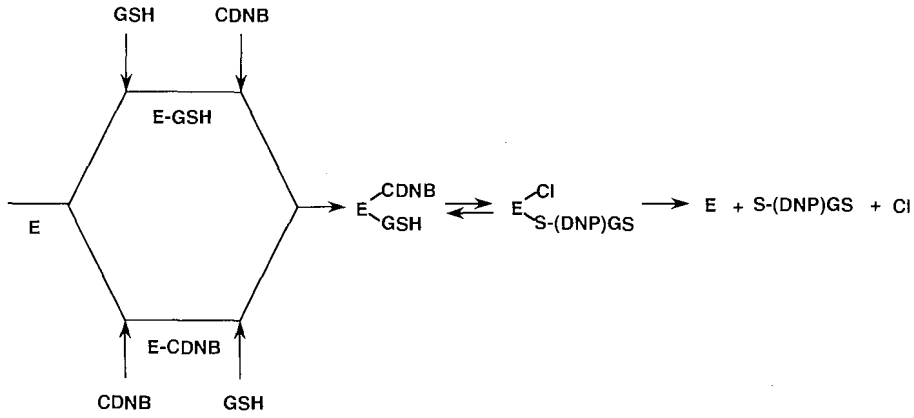

Fig. 4. Proposed sequential random Bi-Bi kinetic mechanism for the glutathione transferase activity of octopus lens S-crystallin. The endogenous GST activity of the S-crystallin is very low; no attempt was made to determine the product inhibition patterns, whether the products are released in order or randomly is as yet undetermined.

The sequence of twenty N-terminal amino acid residues of S-crystallin was determined by an automatic protein sequenator. Digestive gland GST and S-crystallin both had 55% identity and 80% homology. In comparison with other types of GST, after proper alignment, both digestive gland GST and S-crystallin of octopus were similar to alpha (α) or pi (π) type GST. Among the five compounds that we employed (GST type specificity in parenthesis), 1-chloro-2,4-dinitrobenzene (α/π), ethacrynic acid (π), bromosulfophthalein (μ), trans-4-phenyl-3-buten-2-one (μ), and cumene hydroperoxide (α), only 1-chloro-2,4-dinitrobenzene and ethacrynic acid exhibited activity for both proteins. The results of N-terminal amino acid sequence and substrate specificity analyses suggest that the octopus S-crystallin belongs to a π-type isoenzyme of glutathione S-transferase.

OTHER ENDOGENOUS ENZYME ACTIVITY OF CRYSTALLINS

A recent article by Zinovieva et al. (1993) claimed the octopus Ω-crystallin had no enzyme activity. However, our results definitely showed the octopus Ω-crystallin possesses an endogenous aldehyde dehydrogenase activity, which has an even higher specific activity than does the digestive gland aldehyde dehydrogenase of the same animal (Lee and Chang, unpublished results). Aldehyde dehydrogenase is an enzyme with a wide substrate specificity. The failure of Zinovieva et al. (1993) to detect the aldehyde dehydrogenase activity of Ω-crystallin was due to employment of the wrong substrate concentration range.

There are many other enzyme/crystallins yet to be characterized (see Table 1). It may be premature at this stage to posit the true physiological meaning other than structural role of the enzyme/crystallins. However, with the available experimental data in hand, it may be relevant to propose the possible physiological functions of the enzyme activity of crystallins in vivo.

POSSIBLE PHYSIOLOGICAL SIGNIFICANCE OF ENDOGENOUS ENZYME ACTIVITY OF CRYSTALLINS

The impact of enzymatically active ε-crystallin/LDH and τ-crystallin/enolase on duck lens metabolism was investigated by comparing metabolite profile of the duck lens with that of the calf, which does not contain enzyme/crystallins. The duck lens contains higher concentrations of ATP, α-glycerophosphate, and NAD(H) than does the calf lens (Reddy et al. 1993). The ATP alterations and α-glycerophosphate concentrations appear to be related to high [NAD] and high [NADH]/[NAD] ratios. Reddy et al. (1993) postulated that the selection of LDH to serve a structural role in the lens may be related to its capacity to bind the coenzyme that increases the protein stability, alters the net charge, and the electrostatic potential, thereby modifying their structural role in the maintenance of lens transparency.

More enzyme/crystallins, although structurally related to some cytosolic enzymes, possess little or are void of enzymatic activity. For example,

chicken δ-crystallin does not possess argininosuccinate lyase activity and the catalytic constants of S-crystallin were three orders of magnitude lower than those of the digestive gland GST. These results cast doubt on the physiological significance of enzymatic activity in vivo. Squid GST was proposed to be used as a lens structural protein for its solubility, stability, and optical transparency and not enzymatic activity per se (Doolittle 1988). This condition may also hold true for octopus S-crystallin or other enzyme/crystallins. The physiological significance of the endogenous enzyme activity per se in crystallins is still open to debate.

Possible physiological significance of multiple forms of crystallins

Multiplicity seems to be a general phenomenon for crystallins. We have demonstrated that the multiplicity of duck δ-crystallin is due to post-translational oxidative degradation (Lee et al., unpublished results). We have also found that both GST and S-crystallin isolated from the octopus showed multiple forms. N-terminal amino acid sequence analysis indicated that these multiple forms also resulted from post-translational modification (Tang et al., unpublished results). These multiplicities may have physiological significance for crystallins. During its life span, animals are exposed to many kinds of stress. Various factors may cause denaturation of lens proteins, which may in turn cause a decrease in protein solubility leading to the formation of cataracts. If the lens consisted of a single protein, the possibility of protein aggregation due to environmental alterations would increase. Proteins with varied optical properties, after appropriate packing, allow light of various wavelengths to pass through the lens. In this regard, multiple forms, each with slightly varied physical properties, may benefit the animal during adaption to environmental changes, this in turn offers an evolutional advantage.

Relationship of crystallins with stress proteins

All crystallins are stress connected proteins. α-Crystallin has a high amino acid sequence homologous with small heat-shock proteins, it is a molecular chaperone that protects cells from stress-induced damage (DeJong et al. 1988, Rao et al. 1993). β- or γ-Crystallins are proteins S of *Myxococcus xanthus*, a stress protein induced by osmotic shock (Wistow et al. 1985). Many taxon-specific enzyme/crystallins are detoxification enzymes, e.g., ε-crystallin/lactate dehydrogenase, ζ-NADPH: quinone oxidoreductase (Rao et al. 1992), Ω-crystallin/aldehyde dehydrogenase involved in alcohol detoxification; S-crystallin/glutathione transferase in protection against electrophilic substances or peroxide damage. In view of the fact that the lens lacks the homeostatic responses through hormones or neural responses, crystallins may function as a constitutive stress protein, conferring protection against the various endogenous or exogenous threats to which the lens is exposed.

UTILIZATION OF CRYSTALLINS IN THE STRUCTURE-FUNCTION RELATIONSHIP STUDIES OF ENZYMES

Giving some enzyme/crystallins have little enzyme activity when compared to their counterpart enzymes in cells, then these proteins provide ideal natural mutants for the study of the structural and functional relationship of enzyme molecules.

Comparison of duck and chicken δ-crystallin

The avian δ-crystallin gene locus consisted of two tandemly arranged highly homologous genes, δ1 and δ2 (Nickerson et al. 1986). While only the δ1 gene was expressed in the chicken (Nickerson et al. 1986), both the δ1 and the δ2 genes were expressed in the duck (Wistow and Piatigorsky 1990, Barbosa et al. 1991). Among the two gene products, only those from the δ2 gene possessed the endogenous argininosuccinate lyase activity (Barbosa et al. 1991). Chicken lens δ-crystallin showed only 0.4 ~ 0.8% enzymatic activity as compared to duck δ-crystallin under identical assay conditions. Biochemical comparison of δ-crystallin from these two species revealed distinct differences in their structural and kinetic properties. His^{89} of argininosuccinate lyase was proposed to act as a general acid-base catalyst in the active site (Barbosa et al. 1991). However, the presence of a histidine residue at position 89 of the pigeon δ-crystallin, which has little enzyme activity, suggests that the above conjecture has to be revised (Lin and Chiou 1992). Further studies on the expression of pigeon δ-crystallin and site-directed mutagenesis are necessary to further define this problem.

Comparison of octopus digestive gland GST and S-crystallin

S-Crystallin provides an excellent natural mutant

of GST. Mammalian GST-π, GST-μ and GST-α crystal structures were recently defined (Reinemer et al. 1991 1992, Ji et al. 1992, Sinning et al. 1993). Although the amino acid sequence identity between different gene type subunits is only ~30%, the structural topology including subunit interactions is similar. This similarity in structure allows these proteins to utilize the same catalytic mechanism as implicated by both the putative active-site geometry as well as kinetic and spectral analyses (Reinemer et al. 1992, Ji et al. 1992, Chen et al. 1988, Graminski et al. 1989, Atkins et al. 1993). Glutathione thiolate anion was demonstrated to be the active GSH species and Tyr^{7} was involved in the ionization of enzyme-bound GSH. Arg^{13}, Gln^{62}, and Asp^{96} were involved in polar interactions with GSH (Reinemer et al. 1991 1992). Both Tyr^{7} and Arg^{13} are reserved in S-crystallin. In this sense other critical amino acids must be intimately involved in the catalytic reaction or in the maintenance of active site correct conformation. Detailed structural comparison between the octopus digestive gland GST and lens S-crystallin will provide important information about the structure-function relationship of GST.

Acknowledgements: This work was supported by the National Science Council, Republic of China (NSC 83-0418-B016-019 to G.-G.C. and NSC 82-0412-B016-013 to H.-J.L.).

REFERENCES

Abeles RH, PA Frey, WP Jencks. 1992. Biochemistry. Boston: Jones and Bartlett, pp. 503-525.

Atkins WM, RW Wang, AW Bird, DJ Newton, AYH Lu. 1993. The catalytic mechanism of glutathione S-transferase (GST). Spectroscopic determination of the pK_a of Tyr-9 in rat a 1-1 GST. J. Biol. Chem. **268:** 19188-19191.

Barbosa P, GJ Wistow, M Cialkowski, J Piatigorsky, WE O'Brien. 1991. Analysis of naturally occurring and site-directed mutations in the argininosuccinate lyase gene. J. Biol. Chem. **266:** 22319-22322.

Chang GG, SM Huang, SH Chiou. 1991. Kinetic mechanism of the endogenous lactate dehydrogenase of duck ε-crystallin. Arch. Biochem. Biophys. **284:** 285-291.

Chen WJ, GF Graminski, RN Armstrong. 1988. Dissection of the catalytic mechanism of isozyme 4-4 of glutathione S-transferase with alternative substrates. Biochemistry **27:** 647-654.

Chiou SH, HJ Lee, GG Chang. 1990. Kinetic analysis of duck ε-crystallin, a lens structural protein with lactate dehydrogenase activity. Biochem. J. **267:** 51-58.

Chiou SH, HJ Lee, SM Huang, GG Chang. 1991a. Kinetic comparison of caiman ε-crystallins with authentic lactate dehydrogenases of vertebrates. J. Protein Chem. **10:** 161-166.

Chiou SH, HJ Lee, H Chu, TA Lai, GG Chang. 1991b. Screening and kinetic analysis of δ-crystallin with endogenous argininosuccinate lyase activity in the lenses of vertebrates. Biochem. Int. **25:** 705-713.

DeJong WW, W Hendrik. 1986. The eye lens crystallins: Ambiguity as evolutionary strategy. J. Mol. Evol. **24:** 121-129.

DeJong WW, JAM Leunissen, PJM Leenen, A Zweers, M Versteeg. 1988. Dogfish α-crystallin sequence. Comparison with small heat shock protein and schistosoma egg antigen. J. Biol. Chem. **263:** 5141-5149.

DeJong WW, W Hendriks, JWM Mulders, H Bloemendal. 1989. Evolution of eye lens crystallins: the stress connection. Trends Biochem. Sci. **14:** 365-368.

Doolittle RF. 1988. More molecular opportunism. Nature **336:** 18.

Graminski GF, Y Kubo, RN Armstrong. 1989. Spectroscopic and kinetic evidence for the thiolate anion of glutathione at the active site of glutathione S-transferase. Biochemistry **28:** 3562-3568.

Ji X, P Zhang, RN Armstrong, GL Gilliland. 1992. The three-dimensional structure of a glutathione S-transferase from Mu gene class. Structure analysis of the binary complex of isoenzyme 3-3 and glutathione at 2.2-Å resolution. Biochemistry **31:** 10169-10184.

Lee HJ, SH Chiou, GG Chang. 1992a. Staining of argininosuccinate lyase activity in polyacrylamide gel. J. Biochem. Biophys. Meth. **24:** 205-214.

Lee HJ, SH Chiou, GG Chang. 1992b. Biochemical characterization and kinetic analysis of duck δ-crystallin with endogenous argininosuccinate lyase activity. Biochem. J. **283:** 597-603.

Lee HJ, SH Chiou, GG Chang. 1993. Inactivation of the endogenous argininosuccinate lyase activity of duck δ-crystallin by modification of an essential histidyl residue with diethyl pyrocarbonate. Biochem. J. **293:** 537-544.

Lin CW, SH Chiou. 1992. Sequence analysis of pigeon δ-crystallin gene and its deduced primary structure. Comparison of avian δ-crystallins with and without endogenous argininosuccinate lyase activity. FEBS Lett. **311:** 276-280.

Nickerson JM, EF Wawrousek, T Borras, JW Hawkins, BL Norman, DR Filpula, JW Nagle, AH Ally, J Piatigorsky. 1986. Sequence of the chicken $\delta 2$ crystallin gene and its intergenic spacer. Extreme homology with the $\delta 1$ crystallin gene. J. Biol. Chem. **261:** 552-557.

Piatigorsky J. 1992. Lens crystallins. Innovation associated with changes in gene regulation. J. Biol. Chem. **267:** 4277-4280.

Piatigorsky J, GJ Wistow. 1989. Enzyme/crystallins: Gene sharing as an evolutionary strategy. Cell **57:** 197-199.

Piatigorsky J, GJ Wistow. 1991. The recruitment of crystallins: New functions precede gene duplication. Science **252:** 1078-1079.

Rao PV, CM Krishna, JS Zigler Jr. 1992. Identification and characterization of the enzymatic activity of ζ-crystallin from guinea pig lens. J. Biol. Chem. 267: 96-102.

Rao PV, J Horwitz, JS Zigler Jr. 1993. Alpha-crystallin, a molecular chaperone, forms a stable complex with carbonic anhydrase upon heat denaturation. Biochem. Biophys. Res. Commun. **190:** 786-793.

Reddy MC, B Groth-Vasselli, A Sharon, PN Farnsworth. 1993. Effect of catalytically active recruited crystallins on lens metabolism. FEBS Lett. **335:** 285-288.

Reinemer P, HW Dirr, R Ladenstein, J Schäffer, O Gallay, R Huber. 1991. The three-dimensional structure of class π

glutathione S-transferase in complex with glutathione sulfonate at 2.3 Å resolution. EMBO J. **10:** 1997-2005.
Reinemer P, HW Dirr, R Ladenstein, R Huber, ML Bello, G Federici, MW Parker. 1992. Three-dimensional structure of class π glutathione S-transferase from Human placenta in complex with S-hexylglutathione at 2.8 Å resolution. J. Mol. Biol. **227:** 214-226.
Sinning I, GJ Kleywegt, B Mannervik, PG Board, TA Jones. 1993. The active site in class alpha glutathione transferases. *In* Structure and Function of Glutathione Transferases, eds. KD Tew, CB Pickett, TJ Mantle, B Mannervik, JD Hayes. Boca Raton: CRC Press, pp. 75-85.
Tomarev SI, RD Zinovieva. 1988. Squid major lens polypeptides are homologous to glutathione S-transferases subunits. Nature **336:** 86-88.
Tomarev SI, RD Zinovieva, J Piatigorsky. 1991. Crystallins of the octopus lens. Recruitment from detoxification enzyme. J. Biol. Chem. **266:** 24226-24231.
Wistow G. 1993. Lens crystallins: Gene recruitment and evolutionary dynamism. Trends Biochem. Sci. **18:** 301-306.
Wistow G, J Piatigorsky. 1987. Recruitment of enzymes as lens structural proteins. Science **236:** 1554-1556.
Wistow G, J Piatigorsky. 1988. Lens crystallins: The evolution and expression of proteins for a highly specialized tissue. Annu. Rev. Biochem. **57:** 479-504.
Wistow G, J Piatigorsky. 1990. Gene conversion and splice-site slippage in the argininosuccinate lyase/δ-crystallins of the duck lens: Members of an enzyme superfamily. Gene **96:** 263-270.
Wistow G, L Summers, T Blundell. 1985. *Myxococcus xanthus* spore coat proteins may have a similar structure to vertebrate lens $\beta\ \gamma$-crystallins. Nature **315:** 771-773.
Zinovieva RD, SI Tamarev, J Piatigorsky. 1993. Aldehyde dehydrogenase-derived Ω-crystallins of squid and octopus. Specialization for lens expression. J. Biol. Chem. **268:** 11449-11455.

種屬特異性晶體蛋白之酶活性

張 固 剛[1,2]　李 惠 珍[1]

動物眼球水晶體中所含之各種晶體蛋白，過去一直被認爲只具結構性之功能，負責水晶體之透明度確保光線通透，達到視網膜上產生清晰之影像。隨著生化技術之進展，蛋白質之胺基酸定序工作較前簡單而有效率，隨著各種蛋白質之胺基酸序列陸續訂定而發現某些種屬特異性晶體蛋白其胺基酸序與胞漿內糖解酶相同或甚爲相似，因此衍生之疑問包括：這些結構與酶分子相同或相似的晶體蛋白是否亦具酶活性？水晶體內並無代謝反應，這些晶體蛋白如具酶活性，其生理意義何在？另外，在生物進化過程中如何將職司代謝反應的酶分子用於眼球中專司結構性功能？本文專就具有類似酶分子結構之晶體蛋白加以討論。主要重點在鴨及鱷魚ε－晶體蛋白／乳酸去氫酶，鴨，雞δ－晶體蛋白／精胺醯丁二酸分解酶，章魚S－晶體蛋白／麩胱甘肽轉移酶等酶活性之分析鑑定，同時與細胞內職司代謝反應之酶分子比較，經由其負責之化學反應推測其酶活性可能之生理意義。另外，我們亦討論這些晶體蛋白作爲變異酶分子在酶學研究上之應用價值。

關鍵詞：乳酸去氫酶，精胺醯丁二酸分解酶，麩胱甘肽轉移酶，醛去氫酶，壓力蛋白。

[1] 國防醫學院生物化學研究所
[2] 中央研究院動物研究所