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The Role of Glu367 in the Quaternary Structure of Goose δ-crystallin

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δ-Crystallin is a soluble structural protein that is recruited from argininosuccinate lyase through gene sharing to confer special refractive properties in avian eye lenses. The present study investigates the role of Glu367. This residue is located in the interface of the double dimer. Mutation in Glu367 caused no subtle changes in the conformation and stability of the secondary and tertiary structure as compared to wild-type protein. However, dissociated dimeric form was observed for E367A mutant protein. The dissociated dimers were unstable and prone to form protein aggregates. These results indicate that the interactions provided by E367 in the dimer-dimer interface of δ-crystallin are important for double dimer assembly.

Key words: Glu367, goose δ-crystallin

Abbreviations used: ASL, argininosuccinate lyase; GdmCl, guanidinium hydrochloride; CD, circular dichroism; T_m, mid-point temperature transition

INTRODUCTION

Argininosuccinate lyase (ASL) is a metabolic enzyme that catalyzes the reversible cleavage of argininosuccinate into arginine and fumarate.[1] δ-Crystallin is recruited from ASL to the eye lenses of reptiles and birds through a process called gene sharing, where it acts as the major soluble protein that confers special refractive properties.[2-6] δ-Crystallin ASL share about 70% sequence identity and even retain enzyme activity.[3,7-10]

δ-Crystallin is a homotetramer with a molar mass of about 200 kDa. The quaternary structure of δ-crystallin is assembled by two closely contacted dimers and each monomer has three domains (Fig. 1A).[9,11,12] The helix bundles in domain 2 are the major contact surface for subunit association. Hydrophobic interactions are the major forces for subunit assembly. In the presence of guanidinium chloride (GdmCl), unfolding of δ-crystallin occurs in multiple steps including subunit dissociation and then denaturation.[13,14] In this process, dissociated dimers are unstable and prone to form off-pathway aggregates.

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Disruption of the interaction of the subunit interface by mutagenesis results in disassembly of the double dimeric structure.[11] The present study investigates the role of E367. It is a residue located in the dimer-dimer interface that interacts with the neighboring subunit (Fig. 1). Site-directed mutagenesis and biophysical studies have indicated the important role of E367 in the stability of double dimer assembly.

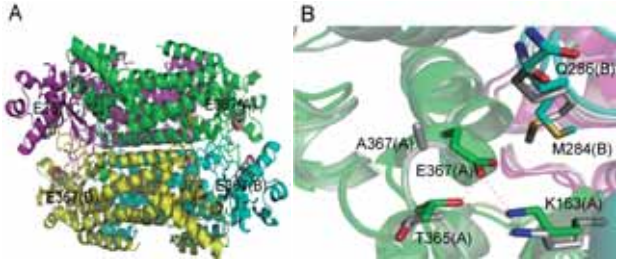

Fig. 1 Structure of δ-crystallin. (A) The quaternary structure of goose δ-crystallin (PDB accession no: 1XWO). Monomer A, B, C and D are shown as cartoons and are represented by green, cyan, magenta and yellow colors, respectively. E367 are highlighted as sphere models. (B) Superimposition of wild-type and E367A δ-crystallin. The colors for each monomer in E367A are shown as palegreen, palecyan, light pink and wheat. Residues are displayed as stick models in atom color. The carbon atoms for residues in E367A are highlighted in gray. The parentheses after each residue represent the defined subunit.

METHODS

Preparation of mutants

The mutant constructs E367A were generated by PCR amplification using the Stratagene QuikChange mutagenesis System with a template of pET-gδ[11] and complementary primer of 5'-gctctgacccctgcaatgctgtctact.

Protein expression and purification

Cultures containing the recombinant plasmid were fermented and crude extracts were prepared as previously reported.[11] The supernatants were loaded onto a Q-Sepharose anion exchange column (HiPrep 16/10 Q XL), pre-equilibrated in buffer A (50 mM Tris-HCl buffer, pH 7.5) and eluted with a linear gradient of 0 to 0.4 M NaCl. The pooled fractions were fractionated by 40-55% ammonium sulfate. The pellets were collected and dissolved in 5 ml buffer A and loaded onto a S-300 Sephacryl column (26 mm×85 cm). Fractions were analyzed by SDS-PAGE and Bradford analysis.

Circular dichroism studies

Circular dichroism (CD) spectra were obtained using a Jasco J-810 spectropolarimeter with a thermostatically controlled sample holder. Experiments were performed in 50 mM Tris-Cl buffer (pH 7.5) using a 1 mm path length cell for the far-UV region (200 to 250 nm). The thermostability of secondary structures was monitored by recording the ellipticity at 222 nm in a circulating bath with a programmable temperature controlled (Neslab) at a scan rate of 1.0 °C/min.

Fluorescence studies

The fluorescence spectra of δ-crystallin were measured using a Perkin-Elmer LS-50 luminescence spectrophotometer equipped with a thermostatically controlled sample holder. Intrinsic tryptophan fluorescence spectra of the protein were recorded with an excitation wavelength set at 295 nm. δ-Crystallin with various concentrations of GdmCl in 50 mM Tris-HCl buffer, with pH 7.5 was incubated at 25 °C overnight. The average emission wavelength was utilized for data analysis.[15]

Analytical ultracentrifugation studies

The sedimentation of the proteins were analyzed using a Beckman-Coulter (Palo Alto, CA) XL-A analytical ultracentrifuge (AUC) with an An50 Ti rotor. Sedimentation was performed at 20 °C using 42,000 r.p.m. in standard double sectors aluminum centerpieces. The radial scans were recorded at 5-min intervals for about 3 hrs. The SETFIT software was used for data analysis.[16]

Analyticl gel-filtration chromatography

Proteins were separated using Superdex 200 HR 10/30 column in a FPLC system. The equilibrium buffer was 50 mM Tris-HCl buffer (pH 7.5). Proteins were centrifuged before injection with or without incubation for 20 h at 37 °C.

Build mutant model

E367A mutant δ-crystallin was built using the protocol of the Built Mutants in Protein Modeling module (Accelrys Discovery Studio 2.1, Accelrys Inc.). The structure of the gooseδ-crystallin structure (PDB access no: 1XWO)[11] was used as a template. Energy minimization was then applied to the wild-type and E367A mutant model.

RESULTS AND DISCUSSION

Goose δ-crystallin has a double dimer quaternary structure. Disruptions of the interactions between subunit interfaces were assumed to affect the stability of subunit assembly. Hydrophobic interaction is the major force that holds the subunit association in δ-crystallin. However, the contribution of hydrogen bonding and ion pairs for proper assembly of double dimer is critical for functional protein. E367 is the residue that provided interaction between dimer-dimer interfaces. This residue is conservative in δ-crystallin between different species but is transformed to aspartate in ASL. In the structure, a water molecule that is located between double dimer interfaces stabilizes two associated subunits via hydrogen bonding with the OE2 atom in the carboxyl group of E367 in monomer A and with the carbonyl group of Q286 in monomer B (Fig. 1B). The side chain of E367 is also placed in a van-der Walls force distance to the side chain of M284. In addition, the OE1 atom of E367 is able to form hydrogen bonds with the OG1 atom of T365 and NZ atom of K163 to stabilize the interface between domain 2 and 3.

Substitution of E367 by alanine resulted in no changes in the secondary and tertiary structure (data not shown) but the quaternary structure was affected. Dissociation of dimeric δ-crystallin with molar mass of about 100 kDa was observed as judged by sedimentation velocity analysis (Fig. 2C). The result indicated the important role of E367 in stabilization of the quaternary structure of δ-crystallin as its location depicted in the structure of dimer-dimer interface.

Stability of protein conformation under environmental effects was determined. Measuring the variation of α-helix with the increasing temperature resulted in no subtle changes in the mid-point temperature transition (T_m) of E367A mutant as compared to wild-type protein (Fig. 2A). In the presence of variable concentration of GdmCl, the unfolding curve for E367A mutant nearly overlapped with that of wild-type protein (Fig. 2B). These results indicated that the variation of the secondary structure and the microenvironment of tryptophan for both proteins as similar under thermal and chemical denaturant treatment. The interaction that provided by E367 is assumed to be minor in conformational stability of δ-crystallin.

Mutation disrupted the interaction that was provided by the side-chain of E367 leading to dissociation of dimers from E367A (Fig. 1B). When E367A was incubated at 37 °C for 20 h, an elution peak at a retention volume of about 6 ml was shown (Fig. 2D). The result indicates that the dissociated dimers were not stable and tended to associate in different manners to form protein aggregate. The result was consistent with the N-terminal truncation study of δ–crystalline.[11] The interaction provided by the protruded N-terminus with neighboring monomers was interrupted. This resulted in dissociation of the double dimer and the subsequent formation of protein aggregate. Similarly, E367 has this function in subunit assembly. Interaction is required for proper alignment of the interfaces of the two dimers during the formation of native quaternary structure.

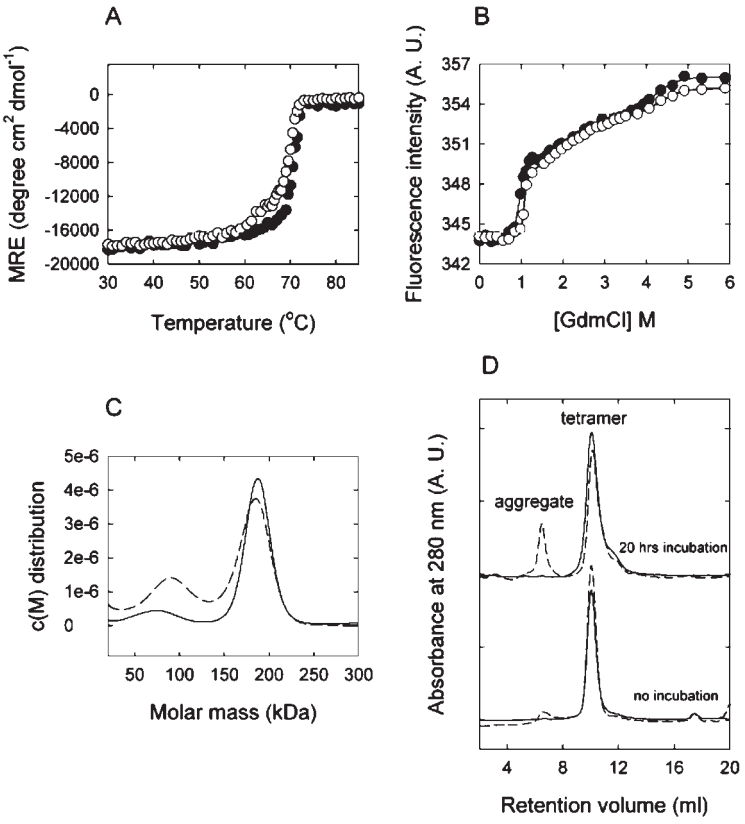

Fig. 2 Stability of the goose wild-type and E367A δ-crystallin. (A) Thermostability. Ellipticity at 222 nm was monitored at different temperatures. (B) Stability in the presence of GdmCl. Tryptophan fluorescence was measured at different GdmCl concentrations. (C) Sedimentation velocity experiments. The continuous molar mass distributions are present. (D) Gel-filtration chromatography. Elution profiles represent proteins with or without incubation at 37 °C for 20 hrs. Labels used: wild-type, open circle or solid line; E367A, solid circle or short-dash lines.

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