PROTEIN STRUCTURE AND FOLDING:

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Detection of Protein-Protein Interactions among Lens Crystallins in a Mammalian Two-hybrid System Assay*

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α-Crystallin consists of two subunits, αA and αB, and each can form an oligomer by itself or with the other. The aggregation arises from interdomain interactions. However, it is not known whether such interactions also exist among α-, β-, and γ-crystallins. This heterogeneous crystallin interaction is far weaker than the homogeneous crystallin interaction and is difficult to detect by conventional spectroscopic measurements. We used a mammalian two-hybrid system in this study. The major crystallin components, αA-, αB-, βB2-, and γC-crystallin genes, were subcloned into the DNA binding domain and transcription activation domain vectors of the two-hybrid system, and they were cotransfected along with a chloramphenicol acetyltransferase (CAT) reporter vector into HeLa cells. Chloramphenicol acetyltransferase activity indicated that there were interactions between αA- (or αB-) and βB2- or γC-crystallins but with an intensity of one-third that of αA-αB interactions. Hsp27, a member of the family of the small heat-shock proteins, showed a similar interaction property with αB-crystallin. Using the N- and C-terminal domain-truncated mutants, we demonstrated that both domains were important in the αA-crystallin self-interaction, but that only the C-terminal domain was important in the αB-crystallin self-interaction. These results show that the two-hybrid system can detect interactions among various crystallins and may be used in mapping interaction domains.

The lens proteins consist of three major crystallins designated α-, β-, and γ-crystallins (1). They have distinct structures and/or functions. However, little is known about whether they interact with one another. From the functional viewpoint that cellular functions arise basically from the formation of protein complexes, it is reasonable to assume that protein-protein interactions exist among the proteins in lens cells, and that the interactions are the basic mechanisms for lens transparency. Traditionally, a short range order among crystallins was thought to be sufficient to explain lens transparency (2–4). Some attempts to detect the protein-protein interactions among crystallins at high concentrations using biochemical and spectroscopic studies (5–9) have been complicated by excluded-volume effects (10). Detection of protein-protein interactions by spectroscopy, such as circular dichroism and fluorescence, usually is based on conformational changes, which at high concentrations may be appreciable even in the absence of interactions. Other techniques such as light scattering, which is not conformation-specific, provide some convincing evidence for protein-protein interactions among crystallins (11, 12). The report by Mach *et al.* (11) indicates that the ability of α-crystallin to associate is inhibited by the presence of γ- or β-crystallin, possibly through the interaction between α-crystallin and γ- or β-crystallin. A more recent report also supports protein-protein interactions among crystallins by showing an enhanced thermodynamic stability of α-crystallin in the presence of γ-crystallin or a mixture of γ- and β-crystallins (12). However, evidence for protein-protein interactions from these two reports is indirect. To obtain direct evidence, we turn to a two-hybrid system assay, which is an *in vivo* assay and is physiologically more relevant than *in vitro* study.

The earlier two-hybrid system designed in a yeast host (13, 14) exploits the modular nature of a transcriptional activator that contains two domains, a DNA-binding domain (BD)[1] and a transcription-activation domain (AD). Reconstitution of these domains in *trans* restores their activity. This objective is achieved by making two fusion proteins. The first fusion is between the BD and a bait protein, and the second fusion is between AD and a prey or target protein. Both fusions are expressed in the presence of a reporter gene. The interaction of these two fused proteins restores activation of the transcriptional activator, and turning on the reporter gene allows the cells to be identified. So far there are only two reports of crystallin interactions in the yeast two-hybrid system (15, 16). The first of which is a study of protein-protein interactions between αB-crystallin and its C- and N-terminal truncated mutants as well as some nonspecific mutants. The major finding is that the conserved C-terminal domain is essential for the interaction (15). The second study deals with the interaction between αB-crystallin and full-length and fragmented Hsp27, and again the C-terminal region of Hsp27 is responsible for interactions (16). Hsp27 and αB-crystallin are two members of the highly homologous small heat-shock proteins (17). Although these two reports present some interesting results, they are far from comprehensive and do not include β- and γ-crystallins. In addition, the yeast two-hybrid system has significant limitations. The host yeast, although a eukaryote, is far removed from human, mammalian, and higher eukaryotic organisms. Therefore, the use of a mammalian system for studying lens protein-protein interactions may be more appropriate than the use of the yeast system (18). Mammalian proteins are likely to retain their native conformation in a mammalian host, and the results would probably represent biologically significant interactions. In the present studies, we used a mammalian two-hybrid

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[1] The abbreviations used are: BD, binding domain; AD, transcription-activation domain; CAT, chloramphenicol acetyltransferase; Hsp, heat-shock protein.

TABLE I
The 5′ and 3′ primers for subcloning experiments
The underlined sequences are *Eco*RI and *Xba*I restriction sites for 5′ and 3′ primers, respectively.

	5′ Primer	3′ Primer
αA	CGGAATTCATGGACGTGACCATC	GCTCTAGATTAGGACGAGGGAGC
αB	CACCTAGAATTCATGGACATCGCC	GCAATCTAGACTATTTCTTGGGGGCT
βB2	CAGGAATTCATGGCCTCAGATCAC	CATGGTCTAGAGGGCACTAGTTGG
γC	GGAATTCATGGGGAAGATCACCTTC	CGGTAGTGTTAATCTAGATTAAT
αAn	CGGAATTCATGGACGTGACCATC	GCTCTAGATGTCCCGGTCGGATCG
αAc	GAGGTTGAATTCGACCGGGACAAG	CGCTCTAGATTAGGACGAGGGAGC
αBn	CACCTAGAATTCATGGACATCGCC	TCCAGTGTTCTAGAAGCTGGGTGC
αBc	GGAATTCCTGGAGAAGGACAGGTTC	GCAATCTAGACTATTTCTTGGGGGCT
Hsp27	CGGAATTCATGACCGAGCGCCGCGT	GCTCTAGATTTACTTGGCGGCAGT

system to assay the protein-protein interactions among the three major crystallins and with a small heat-shock protein Hsp27. We have cloned αA-, αB-, βB2-, and γC-crystallins (19, 20) and used them in this study. αA-, βB2-, and γC-Crystallins are the major components of α-, β-, and γ-crystallins, respectively (21). Our results indicate that there are specific interactions among these crystallins in both homogenous and heterogeneous systems.

EXPERIMENTAL PROCEDURES

Materials—The mammalian Matchmaker two-hybrid assay kit, obtained from CLONTECH (Palo Alto, CA), contains three vectors, pM-cloning vector for the DNA binding domain of the GAL4, pVP16-cloning vector for the transcriptional activation domain of VP16, and pG5CAT reporter vector. Cell culture reagents were purchased from Invitrogen. The monoclonal antibodies specific to GAL4 DNA-BD and to GAL4-AD were purchased from CLONTECH.

Plasmid Constructs—Two sets of constructs were generated from the two vectors, pM and pVP16. αA-, αB-, βB2-, and γC-Crystallin genes were subcloned from previously prepared plasmids pAED4-αA, pAED4-αB, pET-βB2, and pET-γC (19, 20) into the pM and pVP16 vectors. PCR was performed for each gene using the appropriate 5′ and 3′ primers (see Table I). All of the primers were incorporated with *Eco*RI and *Xba*I restriction sites, and the PCR products were digested by the restriction enzyme *Eco*RI and *Xba*I and subcloned into BD and AD vectors, respectively, to yield plasmids pM-αA, pM-αB, pM-βB2, and pM-γC and pVP-αA, pVP-αB, pVP-βB2, and pVP-γC. All constructs were verified by Sanger sequencing in an ABI Automatic Sequencing System (PerkinElmer Applied Biosystems Inc, Foster City, CA) at Brigham and Women's Hospital automatic sequencing and genotyping facilities. The primers were custom-synthesized by Invitrogen.

For the N-terminal gene fragments (αAn with amino acid residues 1–68 and αBn with amino acid residues 1–70) and the C-terminal gene fragments (αAc with amino acid residues 68–173 and αBc with amino acid residues 70–175), a similar PCR was performed using the appropriate primers (Table I), and each PCR product was subcloned into pVP16 vector.

For subcloning of the Hsp27 full-length gene, PCR was performed using the plasmid pT7T3D-Pac (ATCC 834387, American Type Culture Collection, Manassas, VA) as a template and the two primers (Table I). The PCR product was subcloned into pM and pVP16 vectors.

Tissue Culture and Transfections—HeLa cells were grown in Dulbecco's modified Eagle's medium (low glucose, GlutaMAX) supplemented with 10% fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 μg/ml) at 37 °C with 5% CO_2. Cells were seeded at ~6.6×10^4/well in six well plates 1 day before transfection. Transfections were performed with the LipofectAMINE. The detailed protocol was provided in the kit. All three plasmids were cotransfected into HeLa cells. 2.0 μg of both the pM-based plasmids and VP16-based plasmids, 1.0 μg of reporter plasmid pG5CAT, and 6.0 μl of LipofectAMINEreagent were used per well. After cells were cultured for 72 h at 37 °C in 5% CO_2, the cells were harvested and lysed for CAT activity assay using the CAT enzyme-linked immunosorbant assay kit (Roche Molecular Biochemicals). Each experiment was done in duplicate, and three independent transfections were performed.

Basal control (pM and pVP16), X-control (pM and pVP16-X), and Y-control (pM and pVIP-Y) were included. The inclusion of X- and Y-controls was to ensure that X or Y protein did not function autonomously as a transcriptional activator. The data for basal control were used for the conversion of CAT activity to -fold activation.

CAT Assay—The transfected cells were lysed, and the cell extracts (containing CAT enzyme) were added to the wells of a microtiter plate, which was precoated with a polyclonal antibody to CAT. All CAT in the cell extracts bound to the polyclonal antibody to CAT on the plate surface. A digoxigenin-labeled antibody to CAT was added to bind to CAT, and an antibody to digoxigenin conjugated to peroxidase was added to bind to the digoxigenin. Finally, the peroxidase substrate ABTS was added. The peroxidase enzyme catalyzed the cleavage of the substrate, yielding a colored reaction product. The absorbance of the sample was determined with an enzyme-linked immunosorbent assay reader and was directly correlated to the level of CAT present in the cell extracts. The results were normalized with respect to total protein concentration determined by the Pierce BCA assay (22).

Western Blot Analysis—Expressions of αA-, αB-, βB2-, γC-crystallins, and Hsp27-BD fusion and AD fusion proteins were analyzed by Western blot analysis. Proteins from cell lysates were separated by SDS-PAGE and transferred to a nitrocellulose membrane. The membrane was blocked with 5% nonfat dry milk in Tris-buffered saline buffer for 30 min followed by incubation with monoclonal antibody specific to GAL4 DNA-BD or VP16-AD (1:1000 dilution) at 4 °C for 16 h. The membrane was then washed and incubated for 1 h with horseradish peroxidase-conjugated anti-mouse antibody (Bio-Rad) in Tris-buffered saline buffer (1:1000 dilution) at room temperature. This incubation was followed by three 10-min washes in Tween 20-Tris-buffered saline. Protein bands were detected by enhanced ECL.

Immunoprecipitation and Immunoblot Analysis—2 μg of both the pM-based plasmids and VP16-based plasmids were cotransfected into HeLa cells. 48 h after transfection, cells were washed twice with ice-cold phosphate-buffered saline and incubated in lysis buffer containing 50 mM Tris-HCl, pH 8.0, 120 mM NaCl, 0.5% Nonidet P-40, 1% Triton X-100, 1 μg/ml leupeptin, 1 μg/ml aprotinin, 100 μg/ml phenylmethylsulfonyl fluoride, 1 mM sodium vanadate, 1 mM sodium fluoride, and 1 mM EDTA for 30 min on ice and then sonicated for 10 s. Particulate matter was removed by centrifugation at 12,000 *g* for 10 min at 4 °C. Immunoprecipitation was carried out with mixing at 4 °C for 16 h with antibodies (anti-αA-, αB-, βB2- and γC-crystallin polyclonal antibodies). A slurry of protein A/protein G agarose beads (Santa Cruz Biotechnology, Santa Cruz, CA) was added, and the system was rotated for 2 h. The beads were washed five times with lysis buffer. The resulting immunoprecipitated immunocomplexes were solubilized and boiled in 1× SDS buffer for 5 min. Proteins were separated by SDS-PAGE and transferred to membranes. Blots were probed with monoclonal antibody specific to DNA-BD horseradish peroxidase-conjugated anti-mouse secondary antibody and detected by ECL.

Immunofluorescence Cell Staining—Transfected cells were examined for the distribution of BD and AD fusion proteins. HeLa cells were grown and transfected on chamber slides. Cells were fixed in 4% paraformaldehyde, permeabilized in 0.2% Triton X-100, and blocked with 1% bovine serum albumin in phosphate-buffered saline followed by incubation with polyclonal antibodies to αA-, αB-, βB2- and γC-crystallins and monoclonal antibody to Hsp27, respectively. The slides were then washed and incubated for 1 h in the dark with green fluorescein-conjugated goat anti-rabbit IgG and goat anti-mouse IgG secondary antibody (Santa Cruz Biotechnology) at 1:200 dilutions. The cells were viewed with a fluorescence microscope (Eclipse TE300, Nikon Corp., Tokyo, Japan) equipped with a high performance C-imaging system (Compix, Tualatin, OR).

RESULTS

Heterogeneous Interactions among αA-, αB-, βB2-, and γC-Crystallins—The CAT activities for various heterogeneous systems, such as αA-αB and αA-βB2, indicated a very efficient interaction between αA- and αB-crystallins (Fig. 1*A*). The in-

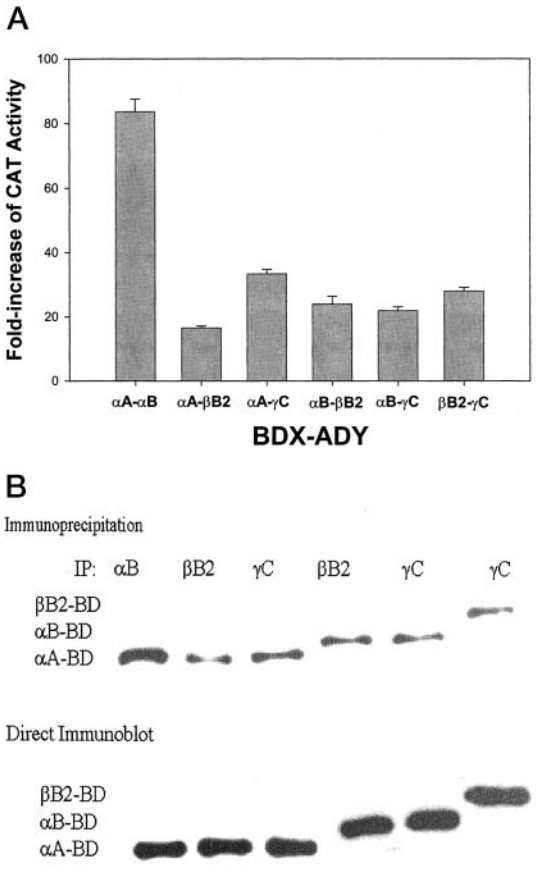

FIG. 1. **Heterogeneous interactions among lens crystallins.** *A*, CAT assays. The CAT activity values are normalized for total protein concentrations and are expressed as -fold increase relative to basal control (pM or pVP16 vector without insert). The values are averages from three separate experiments (± S.D.) on the binding domain of X control (*BDX*) and transcription-activation of Y control (*ADY*) systems. Similar results were obtained for the BD-Y and AD-X systems. *B*, coimmunoprecipitation and Western blot. Cell lysates were first immunoprecipitated with polyclonal antibody specific to αB-, or βB2-, or γC-crystallins, respectively. The complexes were separated by SDS-PAGE and immunoblotted with monoclonal antibody specific to GAL4 DNA-BD (*top*). Immunoblotting was performed directly on lysates using monoclonal antibody specific to GAL4 DNA-BD (*bottom*).

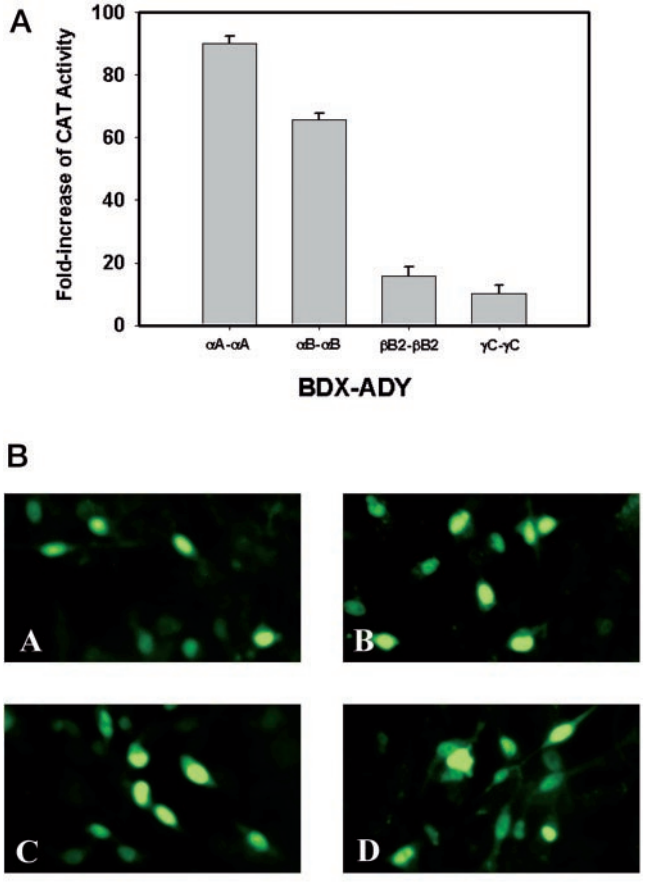

FIG. 2. **Homogeneous interactions of αA-, αB-, βB2-, and γC-crystallins.** *A*, the CAT assays. The CAT values are expressed as -fold activation relative to the basal controls (vectors without inserts) and normalized for the total protein concentrations. The values are averages from three independent experiments (± S.D.). *B*, immunofluorescence cell staining. Nuclear localization of BD-αA (*panel A*) or BD-αB (*panel C*) or AD-αA (*panel B*) or AD-αB (*panel D*) fusion proteins is demonstrated. HeLa cells transfected with αA-AD or αA-BD were stained with polyclonal antibody specific to αA- or αB-crystallin and green-fluorescence-conjugated secondary antibody and were visualized by immunofluorescence.

teractions of other heterogeneous systems were rather weak but significantly greater than those of the controls.

The same intense interaction was observed when the two proteins were cloned in reverse order (*e.g.* αB-αA and βB2-αA) (data not shown), indicating that the interactions were not vector-specific. The results of coimmunoprecipitation confirmed the observed interactions (Fig. 1*B*, *top panel*). Western blots indicated complex formation between two crystallins, and the αA-αB system showed the strongest staining intensity, which was consistent with the results of the two-hybrid system assays. The pattern of direct immunoblotting with monoclonal antibody specific to DNA-BD indicated that fusion proteins were expressed fairly equally (Fig. 1*B*, *bottom panel*).

Self-interactions of αA-, αB-, βB2-, and γC-Crystallins—As assessed by two-hybrid system (Fig. 2*A*), both αA- and αB-crystallins showed considerable self-interaction, whereas βB2- and γC-crystallins showed a rather weak self-interaction. These results were expected, because both αA- and αB-crystallins are oligomer proteins. There are intersubunit interactions responsible for the oligomerization (23, 24). Immunofluorescence cell staining indicated that the majority of αA-AD or αB-AD and αA-BD or αB-BD fusion proteins were located in the nucleus (Fig. 2*B*), suggesting that either oligomer αA- and αB-crystallin were able to enter the nucleus or the freshly expressed αA- and αB-crystallins were in a monomer state. Other fusion proteins showed a similar nuclear localization (data not shown).

Interactions between Hsp27 and Crystallins—There was a strong interaction between Hsp27 and αA- or αB-crystallin but a weak interaction between Hsp27 and βB2-crystallin and a very weak or no interaction between Hsp27 and γC-crystallin (Fig. 3*A*), results confirmed by immunoprecipitation assays (Fig. 3*B*, *top*). The direct immunoblotting with monoclonal antibody specific to GAL4 DNA-BD showed an expression of almost equal levels for various BD fusion proteins (Fig. 3*B*, *bottom*).

Interactions Involving the N-terminal and C-terminal Truncated αA- and αB-Crystallins—The interaction between the wild-type αA-crystallin and the αAn mutant was slightly greater than that between αA-crystallin and the αAc mutant, indicating that the N-terminal domain was more important than the C-terminal domain in the αA-crystallin self-interaction (Fig. 4). However, for αB-crystallin the reverse was true; the interaction between the wild-type αB-crystallin and the αBc mutant was much greater than that between αB-crystallin and αBn mutant. For cross-interactions, αA-crystallin interacted with αBc far more than it did with αBn, and αB-crystallin interacted with αAn far more than it did with αAc. These results suggest that both the N- and C-terminal domains of

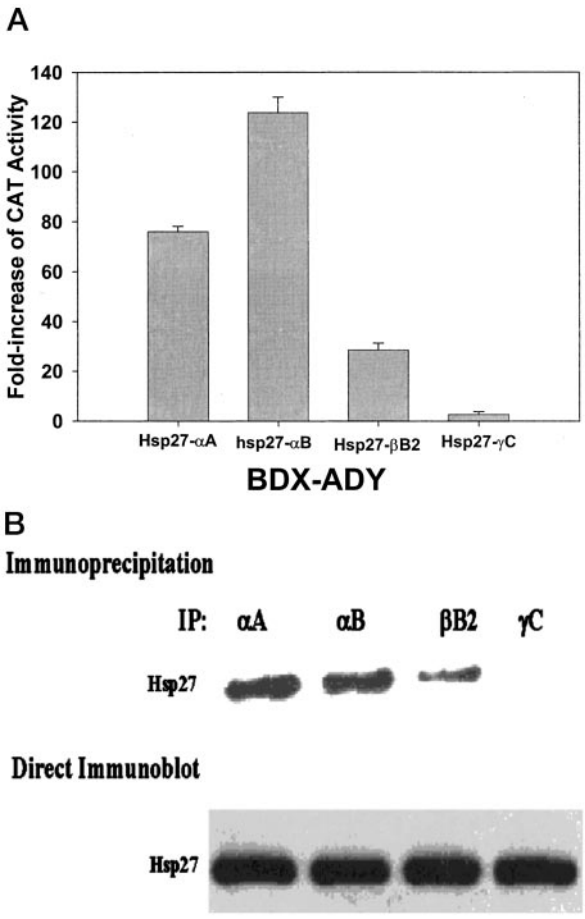

FIG. 3. **Interactions between Hsp27 and various crystallins.** *A*, CAT assays. The CAT activity values are expressed as -fold activation relative to the basal controls (vectors without inserts) and normalized for the total protein concentrations. The values are averages from three independent experiments (± S.D.). *B*, coimmunoprecipitation and Western blot. Cell lysates were immunoprecipitated first with polyclonal antibody specific to αB-, or βB2-, or γC-crystallin. The complexes were separated by SDS-PAGE and immunoblotted with monoclonal antibody specific to GAL4 DNA-BD (*top*). An aliquot of cell lysates was immunoblotted with a GAL4 BD monoclonal antibody to verify the fusion protein expression (*bottom*).

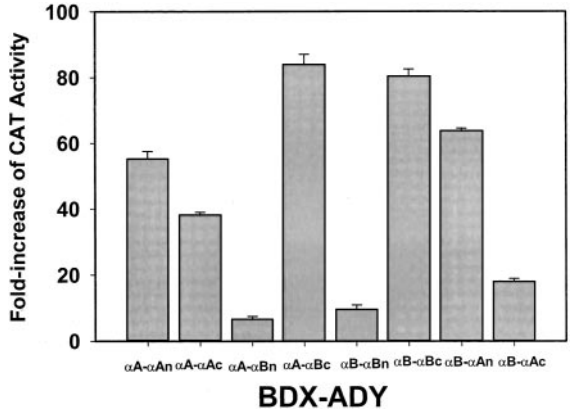

FIG. 4. **CAT activities for interactions between αA- or αB-crystallin and N- or C-terminal truncated mutants (αAn, αAc, αBn, and αBc).** The activity values for the BD-αA (or αB) and AD-mutant systems. The values are expressed as fold-activation relative to the basal controls (vectors without inserts) and normalized for the total protein concentrations. The values were averages from three independent experiments.

αA-crystallin but only the C-terminal domain of αB-crystallin are involved in the interactions that lead to oligomerization. It is to be noted that αAc and αBc mutants include the "α-crystallin" domain, which contains most of the conserved residues (25) and has been reported to contain regions important in chaperone function (24).

DISCUSSION

Earlier reports of the presence of heterogeneous interactions among α-, β-, and γ-crystallins are either controversial or the evidence is indirect (5, 6, 11, 12). Our data from two-hybrid system assays provide direct evidence for such interactions and are further supported by the results of coimmunoprecipitation. The discrepancy may arise from many factors including *in vivo versus in vitro* environments, protein concentrations, and sensitivity of measurements. Among those factors, the most important difference is environment. In biochemical and spectroscopic measurements, the proteins used had undergone many steps of purification in which their conformation may have been altered from those *in vivo* and thus may have influenced their interactions. The factor of protein concentration may not be important, because concentrations of fusion proteins in the two-hybrid system are not higher than those of *in vitro* studies (5, 6, 11, 12). The estimated concentrations of fusion proteins are less than 0.5 mg/ml (total protein concentrations of lysates are approximately 0.5 mg/ml). Furthermore, the interactions detected in the two-hybrid system appear not to be concentration-dependent. The difference of sensitivity apparently is an important factor; the two-hybrid system assay is more sensitive than *in vitro* measurements and can detect even weak and transient interactions.

The present finding of detected heterogeneous interactions among crystallins of different classes (α- and β-crystallins, α- and γ-crystallins, or β- and γ-crystallins) is the first such report of *in vivo* assay. Because the two-hybrid system assays are highly specific, the observed heterogeneous interactions cannot be nonspecific. Compared with homogeneous αA-αA and αB-αB interactions, which are known to be attributed to intersubunit domain interactions, the heterogeneous interactions are rather weak. From earlier studies (15, 23, 24), subunit domain interaction and interactions among negatively charged amino acid residues are suggested to be responsible for oligomerization of αB-crystallin. For detailed consideration of various interactions in α-crystallin, we use the reported X-crystallographic structure of a small heat-shock protein Mj Hsp16.5 (23), in which 24 subunits form a hollow spherical complex of octahedral symmetry with eight trigonal and six square windows. Each subunit has nine β-strands arranged into two anti-parallel β-sheets. In the formation of a dimer, the building block for an oligomer, one of the strands from one subunit interacts with a β-sheet of the neighboring subunit to form an intersubunit composite β-sheet. In this 2-fold structure, hydrogen bonds are the main force. In oligomerization, 3- and 4-fold symmetrical structures are formed through interactions among negatively charged amino acid residues. A structure similar to that of hsp16.5 has been constructed for αB-crystallin based on homology to Mj Hsp16.5 and site-specific mutagenesis (24). The strong CAT activities for homogeneous systems of αA- and αB-crystallins must arise from these domain and charge interactions. In contrast, the heterogeneous interactions among αB-, β-, and γ-crystallins were weak and may be explained in terms of the facts that β- and γ-crystallins have a unique Greek motif structure (26, 27) and are unable to form domain interactions with other crystallins. Whether the heterogeneous interactions among α-, β-, and γ-crystallins are due to charge interactions may be resolved by site-specific mutagenesis. Because highly sensitive two-hybrid system assay can detect even transient interactions, it is possible that the detected heterogeneous interactions are transient. The two-hybrid system assay alone

cannot differentiate between a transient and a weak interaction, a further study with mutation in the domain region, which affects a weak but not a transient interaction, is required.

The other two homogeneous systems, βB2-βB2 and γC-γC, did not show strong CAT activity. βB2-Crystallin is a dimeric protein, but its CAT activity was almost the same as that of the monomeric γC-crystallin (Fig. 2*A*). The most likely explanation is that βB2-crystallin, like βA3-crystallin, is in a reversible monomer-dimer equilibrium and favors the monomer state at low concentration (28).

The interaction between αA- and αB-crystallin is very important physiologically. The quaternary structure of oligomer αA- or αB-crystallin is in a dynamic state; the subunits constantly undergo subunit exchange (29–31). A study of αA-crystallin knockout mice indicates that the lens develops opacity early and that most of the αB-crystallins are in the inclusion body (32); αB-crystallin expressed alone is relatively unstable. Heating experiments also indicate that αB-crystallin is thermally less stable than αA-crystallin (19, 30, 33). The stability of αB-crystallin is increased by the addition of αA-crystallin. A 3:1 ratio of αA-crystallin:αB-crystallin in the mixture, which coincides with the ratio in the mammalian lens, is found to be the most stable combination (30, 33). Based on the above observations, a question arises on the state of αB-crystallin in the nonlenticular tissues; is it also in an insoluble state? For example, we do not know the state of αB-crystallin in the neurotoxic plaques of patients with Alzheimer's disease (34, 35), in which αB-crystallin but not αA-crystallin colocalizes with amyloid peptides.

The results of studies with N- and C-terminal truncated mutants indicate the importance of the C-terminal domain in oligomerization of αB-crystallin and of the N-terminal domain in the oligomerization of αA-crystallin, consistent with previous reports (15, 36, 37). From our present data and those of Bova *et al.* (36), oligomerization potency appears to correlate with CAT activity. It is not clear what makes αAn more potent than αBn in oligomerization. Depending on the evaluation methods, there is either no β-strand or only one β-strand in the N-terminal domain in αA- and αB-crystallins (23, 24, 38, 39), and there would not be any interacting surfaces of β-sheets. Regardless of the nature of interaction of the N-terminal domain, it is likely that oligomerization needs both N- and C-terminal domains to interact cooperatively, so that almost the same sizes of oligomers are produced for the homogeneous αA- and αB-crystallins or for the heterogeneous α-crystallin.

The two-hybrid system assays have many applications including mapping the interaction surfaces using site-directed mutagenesis (15, 40). Such mapping will be particularly useful in the structural study of αA- and αB-crystallins, of which three-dimensional structures have not been determined. Previous use of spin-labeling, site-directed mutagenesis, and chaperone activity in the detection of β-strands provides enormous information regarding αA-crystallin structure (38). Instead of determining chaperone function, the use of the two-hybrid system to detect protein-protein interactions may provide a more precise evaluation. From the data of β-strands in the protein polypeptides (39), an amino acid in a particular β-strand may be replaced with proline, a strong β-strand breaker (41). By repeating the process for each β-strand, the contribution of individual β-strands to protein-protein interaction may be assessed.

The two-hybrid system assay can also be used to determine whether cataract mutant genes alter the protein-protein interactions. The information may demonstrate an important role for protein-protein interactions in lens transparency. Many cataract genes have been detected, including CRYAA (R116C) in autosomal dominant congenital zonular central nuclear cataract (42), CRYAB (R120G) in desmin-related myopathy (43), CRYBB2 (truncation of 51 amino acids from C-terminal) in cerulean cataract (44, 45), CRYGC (T5P) in Coppock-like cataract (46), CRYGD (R58H) in aculeiform cataract (46), and CRYGD (R14C) in juvenile-onset punctate cataract (47, 48). Many reports have indicated structural changes because of gene mutation (49, 50). Recently, we have also observed conformational change, destabilization, and insolubilization of the γC-crystallin T5P mutant.[2] It will be interesting to determine whether these gene mutations also alter protein-protein interactions.

In conclusion, we have demonstrated that the lens crystallins interact both homogenously and heterogeneously. The interactions among αA-, βB2- and γC-crystallins, however, are far less intense than the interaction between αA- and αB-crystallins. The significance of protein-protein interactions is that structural changes such as those caused by posttranslational modification and gene mutation may alter the interactions and thus affect protein solubility and lens transparency.

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