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Interactions between Important Regulatory Proteins and Human αB Crystallin†

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ABSTRACT: Protein pin arrays assessed interactions between αB crystallin and 12 regulatory proteins, including EGF, FGF-2, IGF-1, NGF-*β*, TGF-*β*, VEGF, insulin, *β*-catenin, caspase-3, caspase-8, Bcl-2, and Bcl-x_L, which are important in cellular differentiation, proliferation, signaling, cytoskeletal assembly, and apoptosis. FGF-2, NGF-*β*, VEGF, insulin, and *β*-catenin had strong interactions with human αB crystallin peptides, and the αB crystallin interactive sequences for these proteins were identified. The seven remaining proteins (EGF, IGF-1, TGF-*β*, caspase-3, caspase-8, BCl-2, and Bcl-x_L) did not interact with αB crystallin. The αB crystallin sequences that interacted with FGF-2, NGF-*β*, VEGF, insulin, and *β*-catenin overlapped with sequences that selectively interact with partially unfolded proteins, suggesting a common function for αB crystallin in chaperone activity and the regulation of cell growth and differentiation. Chaperone assays conducted with full-length αB crystallin and synthetic αB crystallin peptides confirmed the ability of αB crystallin to protect against the aggregation of FGF-2 and VEGF, suggesting that αB crystallin protects these proteins against unfolding and aggregation under conditions of stress. This is the first report in which sequences involved in interactions with regulatory proteins, including FGF-2, NGF-*β*, VEGF, insulin, and *β*-catenin, were identified in a small heat shock protein.

Small heat shock proteins (sHSPs)[1] make up a class of molecular chaperones with molecular masses of <43 kDa that display a low degree of sequence similarity, contain a highly conserved immunoglobulin-like structural fold called the α crystallin core domain, and are upregulated in response to cellular stress (*1*−*5*). Human αB crystallin is the archetype for sHSPs and "unfolding response proteins" (URP) that respond to protein unfolding and misfolding under conditions of cellular stress (*6*, *7*). The molecular chaperone activity of sHSPs, which involves the recognition, binding, solubility, and refolding of a wide range of structurally unrelated proteins in various states of unfolding, is considered their most prominent functional role in vivo (*8*−*11*). In addition to recognizing, binding, and stabilizing structurally compromised proteins during or after various forms of stress, sHSPs interact with growth factors and filaments under physiological conditions, suggesting a functional role for sHSPs in normal development (*6*, *9*, *12*−*16*). sHSPs play an important role in organizing and stabilizing cytoskeleton filament networks (*8*, *10*, *14*, *17*−*26*). Mammalian sHSPs, including αB crystallin, sHSP20, sHSP22, sHSP25/27, sHSPB2, sHSPB3, cvHSP, sHSPB9, and αA crystallin, are constitutively expressed during development and are believed to be important in cellular processes that include cell morphogenesis, inflammatory response, cell proliferation, mitogenesis, platelet function, and cell differentiation (*9*, *11*, *27*−*37*). The interactions between sHSPs and proteins involved in regulatory pathways have not been studied systematically, and surface domains that mediate these interactions in sHSPs have not been identified.

Under physiological conditions, αB crystallin is a soluble cytosolic protein. Under conditions of stress or in pathologies like Alzheimer's disease, Parkinson's disease, and age-related macular degeneration (AMD) (*38*, *39*), αB crystallin migrates into the nucleus and colocalizes in extracellular plaques, in Lewy bodies, in Drusen, or with the plasma membrane (*40*−*49*). αB crystallin localizes with *β*-catenin and other regulatory proteins involved in actin dynamics and cell adhesion at the leading edges of migrating lens epithelial cells (*50*). Cell migration is a dynamic process that involves rapid conformational changes in proteins that require protective chaperone-like activities to prevent aggregation. It is likely that αB crystallin is a chaperone for *β*-catenin and other regulatory proteins involved in cell migration under conditions of mechanical stress and strain. FGF-2 lacks the classical signaling sequences required for ER−Golgi mediated secretion, and an alternate hypothesis for its extracellular secretion and trafficking may involve chaperone proteins (*51*). αB crystallin associates with the Golgi apparatus (*52*, *53*) and has a high affinity for phospholipid vesicles (*47*). An association with αB crystallin may sequester FGF-2 molecules into phospholipid vesicles, enabling their transport and secretion, especially under conditions of stress. The presence of αB crystallin in blood plasma and cerebrospinal

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[1] Abbreviations: sHSP, small heat shock protein; EGF, epidermal growth factor; FGF-2, fibroblast growth factor; IGF-1, insulin-like growth factor; NGF-*β*, nerve growth factor; TGF-*β*, transforming growth factor; VEGF, vascular endothelial growth factor; MOE, Molecular Operating Environment.

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Table 1: Proteins and Antibodies Used in the Pin Array and Chaperone Assays

protein or peptide	catalog no.	supplier	molecular mass (kDa)	concentration (μM)
rhEGF	RDI-115	Research Diagnostics	6.2	0.2419
rhFGF-2	234-FSE	R&D Systems	17.4	0.0052
rhFGF-2	00081101	National Cancer Institute	16	3.12
rhIGF-1	RDI-111	Research Diagnostics	7.6	0.1974
rhNGF-β	N 1408	Sigma	27	0.0185
rhTGF-1β	89091902	National Cancer Institute	25	0.0003
rhVEGF	32203	National Cancer Institute	34	0.2206
rh insulin	I-0259	Sigma	5.8	1.5497
rhBcl-2	827-BC	R&D Systems	27	0.0926
rhBcl-xL	894-BX	R&D Systems	28	0.0893
rhcaspase-3	707-C3/CF	R&D Systems	28	0.0179
rhcaspase-8	218769	Calbiochem	55	0.0005
rhβ-catenin	n/a[a]	Purified	86	0.0249
rh αB crystallin	n/a[a]	Purified	20.2	n/a[a]
DRFSVNLDVKHFS	custom synthesis	Advanced ChemTech	1.6	n/a[a]
LTITSSLSDGV	custom synthesis	GenScript Corp.	1.1	n/a[a]
FISREFHR	custom synthesis	GenScript Corp.	1.1	n/a[a]
ERTIPITRE	custom synthesis	GenScript Corp.	1.1	n/a[a]

antibody	catalog no.	supplier	primary Ab dilution	secondary Ab dilution
rabbit polyclonal anti-EGF	RDI-EGFabrP	Research Diagnostics	400	10000
mouse monoclonal anti-FGF-β	F6162	Sigma	40000	40000
rabbit polyclonal anti-IGF-1	RDI-ILGF1abrP	Research Diagnostics	400	20000
rabbit polyclonal anti-NGF-β	RDI-NGFabrP	Research Diagnostics	2000	10000
rabbit polyclonal anti-TGF-β	3711	Cell Signaling Technology	5000	40000
rabbit polyclonal anti-VEGF	RDI-VEGFabrP	Research Diagnostics	5000	20000
mouse monoclonal anti-insulin	I2018	Sigma	500	20000
mouse monoclonal anti-Bcl-2	MAB827	R&D Systems	2000	20000
mouse monoclonal anti-Bcl-xL	2300-MC	R&D Systems	5000	40000
mouse monoclonal anti-caspase-3	MAB707	R&D Systems	5000	40000
rabbit polyclonal anti-caspase-8	51-1100	Zymed	5000	40000
mouse monoclonal anti-β-catenin	RDI-BCATENINabm	Research Diagnostics	250	20000
rabbit anti-mouse IgG-HRP	A 9044	Sigma	n/a[a]	n/a[a]
goat anti-rabbit IgG-HRP	SAB-300	Stressgen	n/a[a]	n/a[a]

[a] Not applicable.

fluid, and the increased level of expression of αB crystallin after stroke and under neurological conditions, suggest that similar pathways may mediate the stabilization and secretion of VEGF, NGF-β, and insulin (*54*, *55*). The intracellular and extracellular physiological actions of αB crystallin and the specific function of individual interactive sequences in the folding, stability, and function of signaling molecules like FGF, VEGF, and β-catenin are an important area for future studies of sHSPs in normal cell activities and in disease.

This study used protein pin arrays to identify interactive sequences in human αB crystallin for selected proteins reported to be involved in the cellular pathways that are important for cell differentiation (*12*, *31*, *37*, *50*, *56*−*61*). Protein pin array analysis determined that human αB crystallin contained interactive sequences for FGF-2, NGF-β, VEGF, insulin, and β-catenin, while EGF, IGF-1, TGF-β, Bcl-2, Bcl-x_L, caspase-3, and caspase-8 had no interactions with αB crystallin. The αB crystallin sequences that had the strongest interactions with FGF-2, NGF-β, VEGF, insulin, and β-catenin overlapped with previously identified αB crystallin chaperone and filament interactive sequences, suggesting that αB crystallin may be important for the folding and processing of these proteins in vivo. Chaperone assays conducted with full-length wt human αB crystallin and synthesized αB crystallin interactive sequences $_{73}$DRFSVNLDVKHFS$_{85}$, $_{113}$FISREFHR$_{120}$, $_{131}$LTITSSLSDGV$_{141}$, and $_{156}$ERTIPITRE$_{164}$ confirmed that αB crystallin protected FGF-2 and VEGF from unfolding and aggregation through its surface interactive sequences.

EXPERIMENTAL PROCEDURES

ELISA-Based Protein Pin Array Assay. Proteins and antibodies used in the protein pin array assay are listed in Table 1. The protein pin array assays were performed as described previously with each protein being assayed two or three times (*61*). The loss of efficiency of the protein pin array was measured as described previously and was found to be insignificant for up to 30 assays (*60*, *61*). For each target protein, the pin array quantifies differences in interactions between peptides on the pin array and the target protein (*61*). When a new target protein is used, the antibody reactions and affinities change so quantitative comparisons are difficult to make for different target proteins. Quantitative and systematic validation of the pin array results using SPR (*62*) and other binding assays are being conducted to compare binding between αB crystallin and different target proteins.

Chaperone Assays. The effect of full-length αB crystallin and synthetic αB crystallin peptides on the thermal unfolding and aggregation of FGF-2 was measured as described previously (*12*). Briefly, 3.12 μM FGF-2 was incubated in the absence and presence of 3.12 μM full-length αB crystallin or synthetic αB crystallin peptides $_{73}$DRFSVNLDVKHFS$_{85}$, $_{113}$FISREFHR$_{120}$, and $_{131}$LTITSSLSDGV$_{142}$ in 200 μL of 10 mM PBS buffer (pH 7.2). The optical density at 360 nm (OD_{360}) was measured at 10 min intervals for 90 min at 45 °C using a Pharmacia Biotech Ultrospec 3000 spectrophotometer. The OD_{360} of FGF alone after 90 min was set to 100% aggregation. The effect of full-length αB crystallin

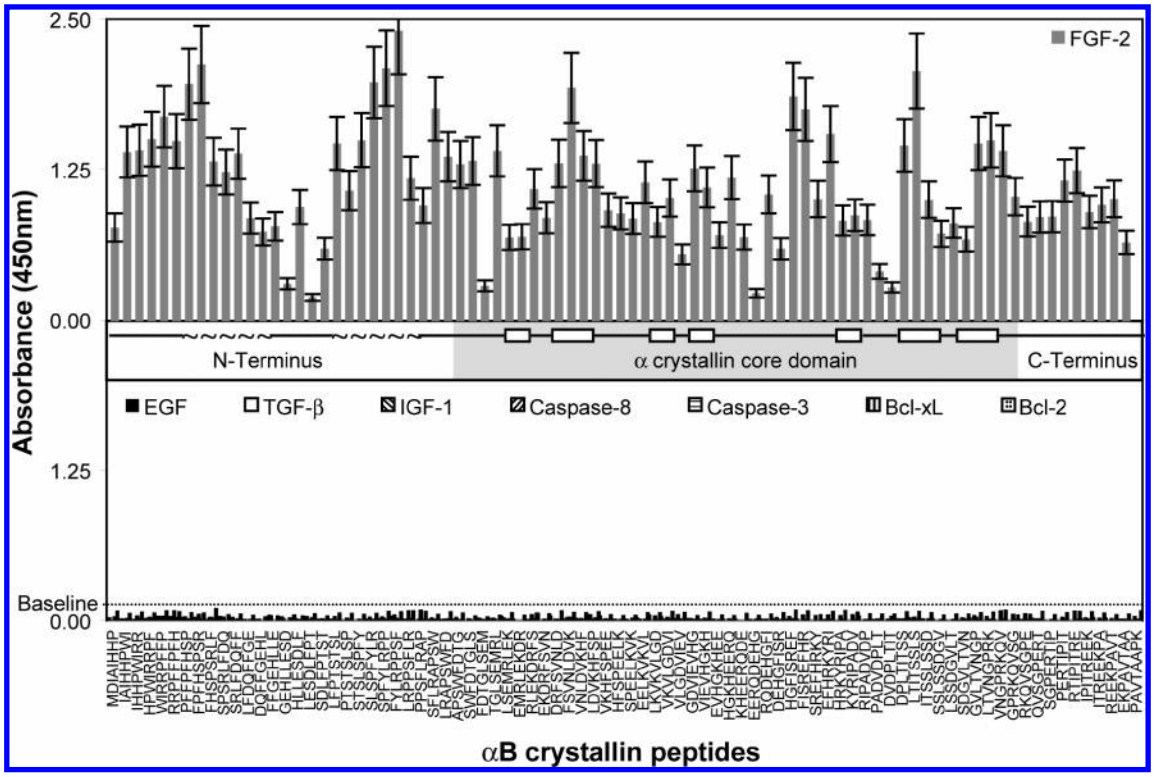

FIGURE 1: Interactions between αB crystallin peptides and EGF, TGF-β, IGF-1, caspase-3, caspase-8, Bcl-2, Bcl-x_L, and FGF-2. The amino acid sequences of the overlapping αB crystallin peptides are plotted on the *X*-axis. The absorbances of the αB crystallin peptides in the presence of EGF (black bars), TGF-β (white bars), IGF-1 (left diagonally striped bars), caspase-3 (horizontally striped bars), caspase-8 (right diagonally striped bars), Bcl-2 (dotted bars), Bcl-x_L (vertically striped bars), and FGF-2 (gray bars) are plotted on the *Y*-axis. The predicted secondary structure of αB crystallin based on homology modeling and electron spin resonance studies is shown in the plot (~ for helix and □ for β strand). The three structural domains of αB crystallin, the N-terminal domain, the α crystallin core domain, and the C-terminal domain, are shown below the secondary structure. In the presence of FGF-2, 83 of 84 αB crystallin peptides had absorbances higher than the baseline ($Abs_{450} = 0.10$). In the presence of EGF, TGF-β, IGF-1, caspase-3, caspase-8, Bcl-2, and Bcl-x_L, none of the 84 αB crystallin peptides had absorbances higher than the baseline. FGF-2 interacts with αB crystallin, while EGF, TGF-β, IGF-1, caspase-3, caspase-8, Bcl-2, and Bcl-x_L have no measurable interactions with αB crystallin.

and synthetic αB crystallin peptides on the dithiothreitol (DTT)-induced unfolding and aggregation of VEGF was measured as described below. VEGF (1.48 μM) was incubated in the absence and presence of 1.48 μM full-length αB crystallin or synthetic αB crystallin peptides $_{113}$FISREFHR$_{120}$ and $_{156}$ERTIPITRE$_{164}$ in 200 μL of 10 mM PBS buffer (pH 7.2) and 75 μM DTT. The optical density at 360 nm (OD_{360}) was measured at 10 min intervals for 90 min at 37 °C using a Pharmacia Biotech Ultrospec 3000 spectrophotometer. The OD_{360} of the VEGF alone after 90 min was set to 100% aggregation.

The aggregation of FGF and VEGF in the presence of full-length αB crystallin and the synthetic αB crystallin peptides was assessed as a percentage of the aggregation of FGF or VEGF alone as follows:

$$\% \text{ aggregation} = \frac{OD_{360}\ (\text{VEGF/FGF} + \alpha\text{B crystallin peptide})}{OD_{360}\ (\text{VEGF/FGF alone})}$$

RESULTS

Interactions between peptides of the αB crystallin protein pin array and 12 proteins (EGF, IGF-1, TGF-β, Bcl-2, Bcl-x_L, caspase-3, caspase-8, FGF-2, NGF-β, VEGF, insulin, and β-catenin) were assessed using a pin array assay. In the presence of human FGF-2 (Figure 1, gray bars), 83 of the 84 αB crystallin peptides had absorbances higher than the baseline ($Abs_{450} = 0.10$), with the peptide $_{47}$FYLRPPSF$_{54}$ having the highest absorbance ($Abs_{450} = 2.42$). These 83 αB crystallin peptides formed a pattern of five absorbance peaks where the maximum absorbance was recorded as a central peptide flanked by peptides with lower absorbances. The central peptides $_{15}$FPFHSPSR$_{22}$, $_{47}$FYLRPPSF$_{54}$, $_{75}$FSVNLDVK$_{82}$, $_{111}$HGFISREF$_{120}$, and $_{131}$LTITSSLS$_{138}$ from each peak were identified as interactive peptides in αB crystallin that mediated interactions with FGF-2. In contrast, when EGF, TGF-β, Bcl-2, Bcl-x_L, caspase-3, and caspase-8 were assayed for interactions with the αB crystallin pin array, no absorbances higher than the baseline ($Abs_{450} = 0.10$) were measured. The absence of absorbances higher than the baseline absorbance indicated that these proteins did not interact with the αB crystallin peptides.

The absorbances of 64 of the 84 αB crystallin peptides were higher than the baseline ($Abs_{450} = 0.10$) when NGF-β was the ligand in the pin array assay (Figure 2, gray bars). The 64 αB crystallin peptides that interacted with NGF-β formed a pattern of four absorbance maxima for peptides that had the highest absorbance flanked by peptides with lower absorbances. Absorbance maxima were recorded for the peptides $_{53}$SFLRAPSW$_{60}$, $_{113}$FISREFHR$_{120}$, $_{131}$LTITSSLS$_{138}$, and $_{143}$LTVNGPRK$_{150}$. These peptides were identified as NGF-β interactive peptides in human αB crystallin. All four interactive sequences were localized in the N-terminal domain and the α crystallin core domain. When VEGF was the ligand in the pin array assay

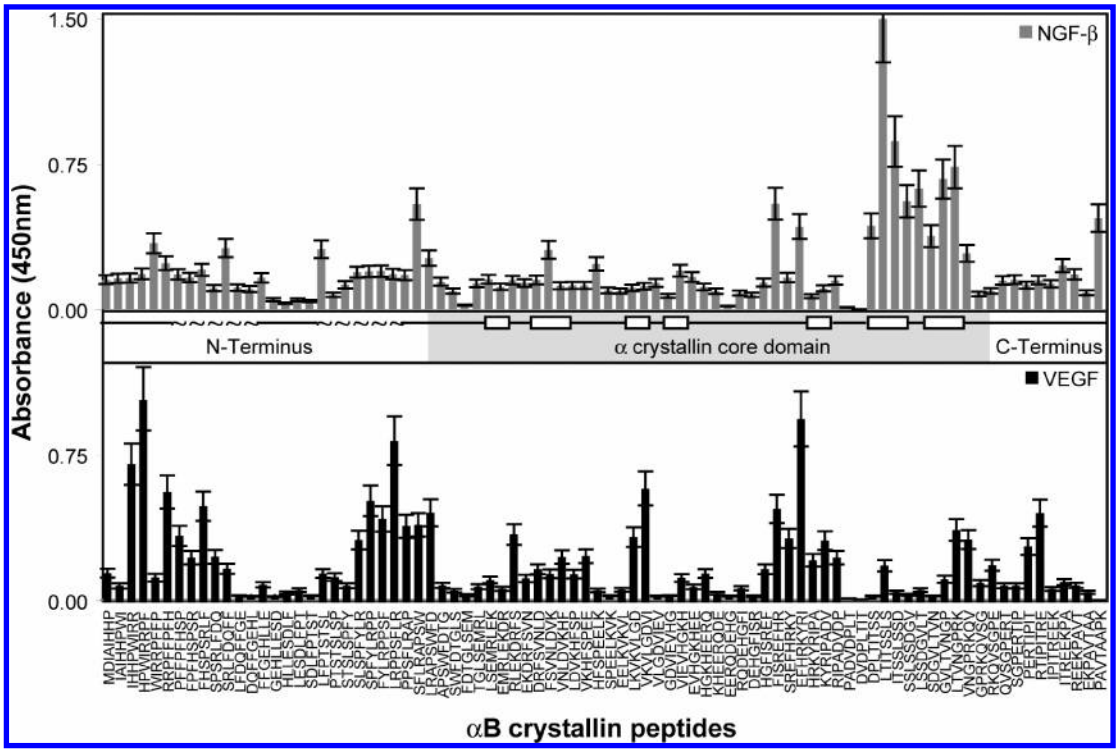

FIGURE 2: Interactions between αB crystallin peptides and NGF-β and VEGF. The amino acid sequences of the overlapping αB crystallin peptides are plotted on the *X*-axis. The absorbances of the αB crystallin peptides in the presence of NGF-β (gray bars) and VEGF (black bars) are plotted on the *Y*-axis. The predicted secondary structure of αB crystallin based on homology modeling and electron spin resonance studies is shown in the plot (~ for helix and □ for β strand). The three structural domains of αB crystallin, the N-terminal domain, the α crystallin core domain, and the C-terminal domain, are shown below the secondary structure. In the presence of NGF-β, 64 of 84 αB crystallin peptides had absorbances higher than the baseline (Abs_{450} = 0.10), and in the presence of VEGF, 47 of 84 αB crystallin peptides had absorbances higher than the baseline.

(Figure 2,- black bars), 47 of the 84 αB crystallin peptides had absorbances higher than the baseline, with the peptide $_{7}$HPWIRRPF$_{14}$ having the highest absorbance (Abs_{450} = 1.04). The 47 αB crystallin peptides that interacted with VEGF formed a pattern of six absorbance maxima for peptides that had the highest absorbance flanked by peptides with lower absorbances. Absorbance maxima were recorded for the peptides $_{7}$HPWIRRPF$_{14}$, $_{49}$LRPPSFLR$_{56}$, $_{91}$VKVLGDVI$_{98}$, $_{117}$EFHRKYRI$_{124}$, $_{143}$LTVNGPRK$_{150}$, and $_{157}$RTIPITRE$_{164}$, which were identified as VEGF interactive peptides in human αB crystallin.

Similar to those of NGF-β and VEGF, interactions between αB crystallin peptides and two signaling molecules, human insulin (Figure 3, gray bars) and human β-catenin (Figure 3, black bars), were assessed using the pin array assay. When human insulin was the ligand in the pin array assay, all 84 αB crystallin peptides had absorbances higher than the baseline and the maximum absorbance (Abs_{450} = 1.25) was recorded for the peptide $_{165}$EKPAVTAA$_{172}$. A pattern of nine absorbance peaks was observed with maximum absorbances being recorded for the peptides $_{11}$RRPFFPFH$_{18}$, $_{33}$LESDLFPT$_{40}$, $_{49}$LRPPSFLR$_{56}$, $_{75}$FSVNLDVK$_{82}$, $_{93}$VLGDVIEV$_{100}$, $_{125}$PADVDPLT$_{132}$, $_{137}$LSSDGVLT$_{144}$, $_{151}$QVSGPERT$_{158}$, and $_{165}$EKPAVTAA$_{172}$, which were identified as insulin interactive peptides in human αB crystallin. When human β-catenin was the ligand in the pin array assay, 76 of the 84 αB crystallin peptides had absorbances higher than the baseline (Abs_{450} = 0.10), and the maximum absorbance (Abs_{450} = 0.79) was recorded for the peptide $_{47}$FYLRPPSF$_{54}$. A pattern of seven absorbance peaks was observed with maximum absorbances being recorded for the peptides $_{13}$PFFPFHSP$_{20}$, $_{47}$FYLRPPSF$_{54}$, $_{75}$FSVNLDVK$_{82}$, $_{89}$LKVKVLGD$_{96}$, $_{113}$FISREFHR$_{120}$, $_{131}$LTITSSLS$_{138}$, and $_{145}$VNGPRKQV$_{152}$, which were identified as β-catenin interactive peptides in human αB crystallin.

The αB crystallin sequences $_{73}$DRFSVNLDVKHFS$_{85}$, $_{113}$FISREFHR$_{120}$, and $_{131}$LTITSSLSDGV$_{142}$ that had strong interactions with FGF-2 were previously identified as chaperone sequences in human αB crystallin. Consequently, these three peptides were synthesized and tested for their individual effect on the thermal aggregation of FGF-2 (Figure 4, gray bars). In the presence of full-length wt human αB crystallin, the extent of thermal aggregation of FGF-2 was approximately 70% of the extent of aggregation in the absence of wt αB crystallin. In the presence of the peptides DRFSVNLDVKHFS and LTITSSLSDGV, the level of aggregation of FGF-2 was 39 and 28%, respectively, a much stronger effect on aggregation than that for full-length wt αB crystallin. In the presence of the FISREFHR peptide, the level of FGF-2 aggregation increased approximately 26%, although in the presence of a 10-fold excess of FISREFHR (31 μM), the level of FGF-2 aggregation decreased to 42% (data not shown). Similarly, the ability of full-length human αB crystallin and two synthetic αB crystallin peptides, $_{113}$FISREFHR$_{120}$ and $_{156}$ERTIPITRE$_{164}$, to inhibit the DTT-induced aggregation of VEGF was assessed spectroscopically (Figure 4, black bars). Full-length wt human αB crystallin decreased the level of VEGF aggregation by approximately 57%, while the FISREFHR and ERTIPITRE peptides decreased the level of VEGF aggregation by approximately 43%. Together, these results confirmed the importance of the interactive sequences in the α crystallin core domain and

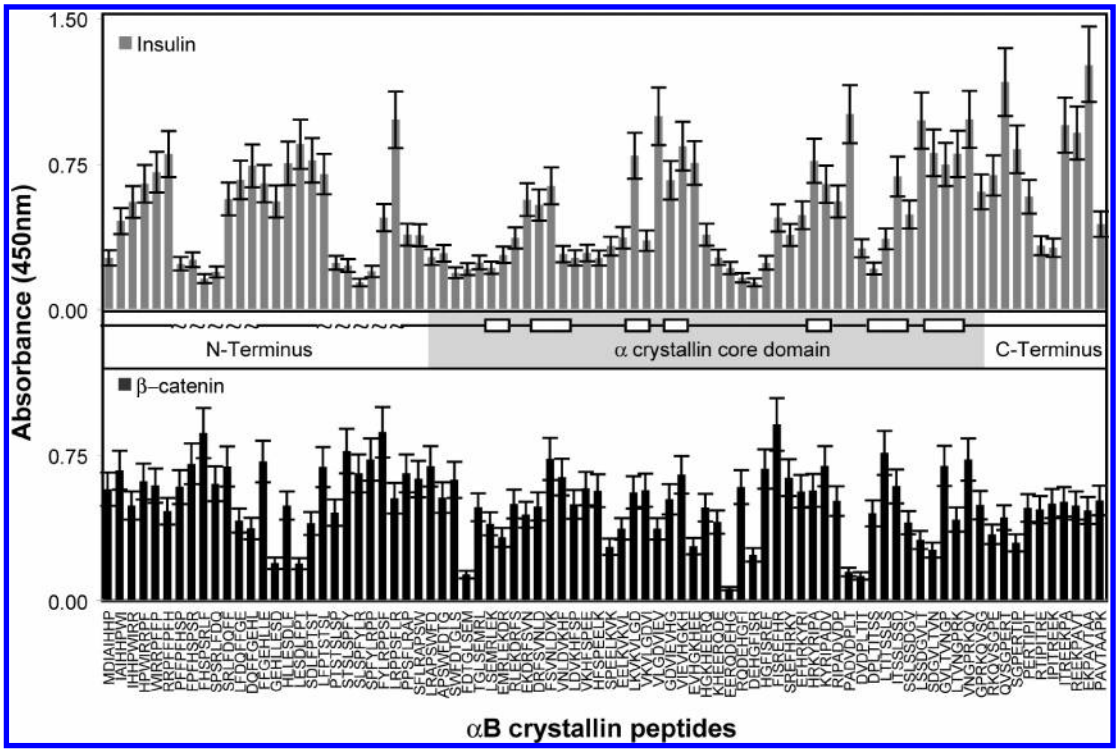

FIGURE 3: Interactions between αB crystallin peptides and β-catenin and insulin. The amino acid sequences of the overlapping αB crystallin peptides are plotted on the *X*-axis. The absorbances of the αB crystallin peptides in the presence of insulin (gray bars) and β-catenin (black bars) are plotted on the *Y*-axis. The predicted secondary structure of αB crystallin based on homology modeling and electron spin resonance studies is shown in the plot (∼ for helix and □ for β strand). The three structural domains of αB crystallin, the N-terminal domain, the α crystallin core domain, and the C-terminal domain, are shown below the secondary structure. In the presence of insulin, all 84 αB crystallin peptides had absorbances higher than the baseline (Abs_{450} = 0.10), while in the presence of β-catenin, 76 of 84 αB crystallin peptides had absorbances higher than the baseline.

C-terminal extension for chaperone activity of αB crystallin with the growth factors FGF-2 and VEGF.

The αB crystallin interactive sequences for subunit−subunit interactions, chaperone activity, filament interactions, and interactions with growth factors, insulin, and β-catenin were compared for similarities in secondary structure and mapped to the surface of the three-dimensional model of human αB crystallin (Figure 5). Overlap was observed in four regions of αB crystallin, including the β3, β8, and β9 sequences in the α crystallin core domain, and the C-terminal extension containing the conserved Ile-X-Ile motif. The β3, β8, and β9 sequences were localized in the same region of the α crystallin core domain and formed a surface for interactions with various types of substrate proteins. Although αB crystallin contains conserved surfaces that interact with a wide variety of proteins, there appear to be additional surfaces that are selective for interactions with specific protein targets. The collective response of these interactive surfaces results in the recognition, stabilization, solubility, and refolding of structurally compromised substrates by full-length αB crystallin.

DISCUSSION

Twelve proteins important for cell differentiation and development were evaluated in this study, and FGF-2, NGF-β, VEGF, insulin, and β-catenin were determined to interact positively with specific sequences in human αB crystallin. These results were consistent with previous reports in which αB crystallin was shown to be involved in pathways involving FGF-2, NGF-β, VEGF, β-catenin, and insulin (*12*, *31*, *37*, *50*, *56*−*58*). αB crystallin sequences that had strong interactions with NGF-β, VEGF, insulin, β-catenin, and FGF-2 overlapped with sequences that were previously identified as chaperone sequences in αB crystallin (*60*, *63*, *64*) (Figure 5). The overlap between the interactive sequences for FGF-2, NGF-β, VEGF, insulin, and β-catenin and the chaperone sequences of αB crystallin suggested a functional relationship among key elements of signaling pathways for cell differentiation, stabilization of cytoplasmic filaments, and the chaperone activity of αB crystallin (*60*). This hypothesis was confirmed by chaperone assays comparing the effectiveness of full-length human αB crystallin and synthetic αB crystallin peptides in protecting against the aggregation of FGF and VEGF. In addition, previous studies found that αB crystallin inhibits the DTT and heat-induced aggregation of insulin (*65*−*68*). The results demonstrated that specific αB crystallin interactive peptides were capable of protecting key regulatory proteins from unfolding and aggregation. The direct functional demonstration of αB crystallin sequences provides support for the hypothesis that the chaperone function of αB crystallin is not limited to stress conditions and has an important function in regulating normal cellular growth and development.

Antibodies, growth factors, enzymes, and receptors have very high substrate specificities and affinities consistent with their functional role. In contrast, sHSPs make up a class of proteins whose cytoprotective functions involve a range of substrates and cellular pathways. Interactions of sHSP with unfolding or misfolding proteins are selective and not specific. The dynamic functional mechanism of sHSP

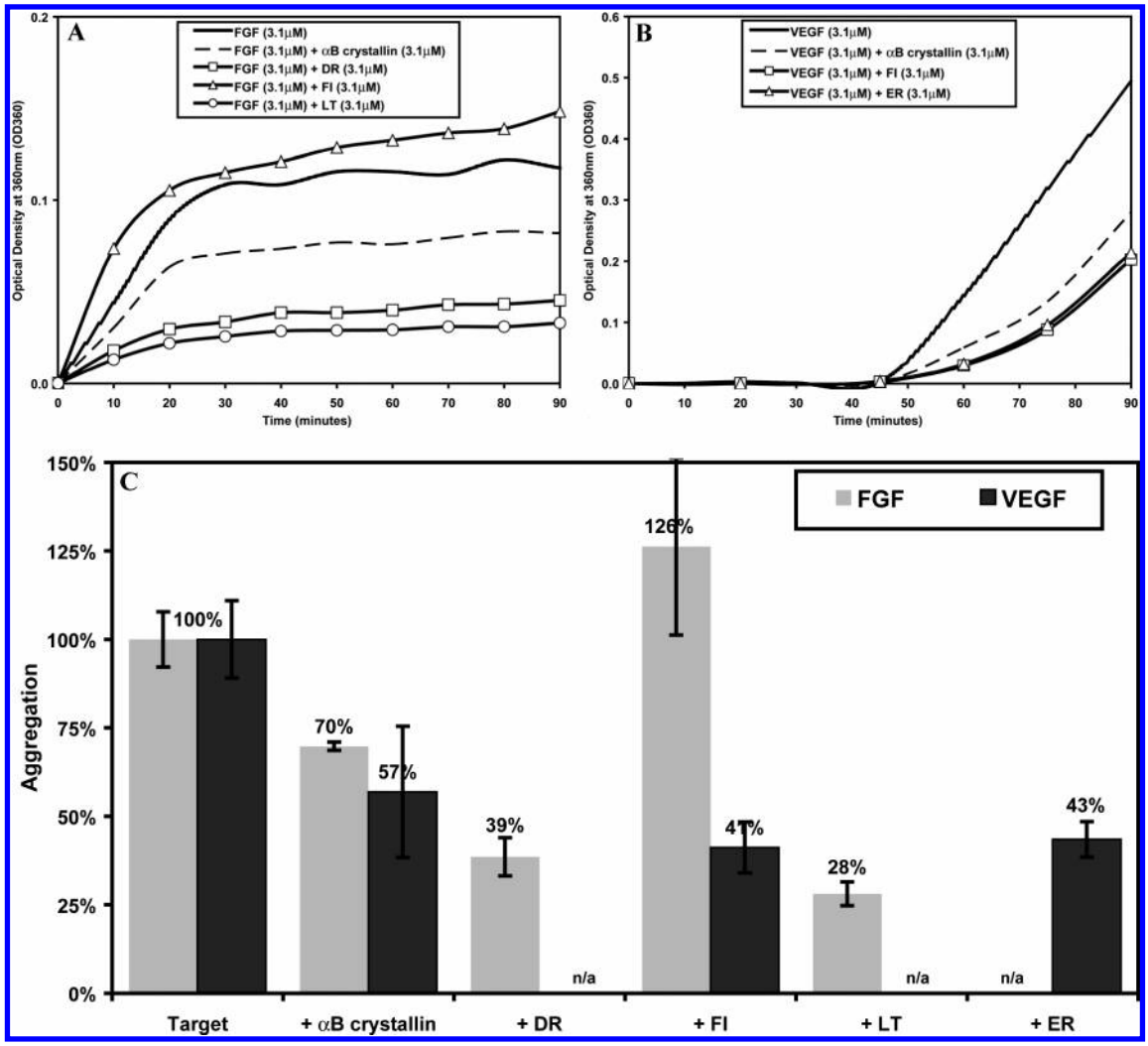

FIGURE 4: Effect of full-length wt human αB crystallin and four synthetic αB crystallin peptides on the aggregation of FGF-2 and VEGF. The thermal aggregation of FGF-2 at 45 °C (A) and the DTT-induced aggregation of VEGF at 37 °C (B) were assessed spectroscopically as the optical density at 360 nm (OD_{360}) for 90 mins. The chaperone activities of full-length wt αB crystallin and the αB crystallin peptides were calculated using the OD_{360} measured at the end of the assay (C). The OD_{360} of the target proteins FGF and VEGF at the end of 90 mins was set to 100% aggregation. Wt αB crystallin decreased FGF and VEGF aggregation by 30% and 43% respectively. The DR (DRFSVNLDVKHFS) peptide decreased FGF aggregation by 61%. The FI (FISREFHR) increased FGF aggregation by 26%, but decreased VEGF aggregation by 59%. The LT (LTITSSLSSDGV) peptide decreased FGF aggregation by 72% which was the strongest protective effect observed for the synthetic peptides. The ER (ERTIPITRE) peptide decreased VEGF aggregation by 57%. Synthetic peptides of the interactive domains of human αB crystallin were effective inhibitors of aggregation.

interaction appears to be mediated by the coordination of multiple interactive sequences on the surface of assembled sHSP complexes and individual sHSP subunits (*69*).

Sequences involved in mediating the αB crystallin−FGF interaction overlapped with previously identified αB crystallin chaperone sequences (Figure 5), which was consistent with a previous report in which αB crystallin was shown to promote the refolding of partially unfolded FGF at 25 °C (*12*). FGF is labile and structurally unstable at physiological temperatures, and the identification of FGF chaperone sequences in αB crystallin suggests a biological role for the chaperone activity of αB crystallin in FGF signaling (*70*−*72*). This hypothesis was supported by chaperone assays in which full-length wt αB crystallin inhibited the thermal aggregation of FGF. The overlap between interactive sequences in αB crystallin for VEGF and for chaperone activity was consistent with the upregulation of both VEGF and αB crystallin in response to stress following myocardial infarction (*56*, *73*, *74*). The identification of chaperone sequences that mediate interactions between αB crystallin and VEGF suggests that in addition to protecting cardiac cytoskeletal proteins from unfolding and aggregation, αB crystallin has a protective role in VEGF signaling during myocardial infarction (*27*−*30*, *32*, *36*, *56*, *75*−*77*). This hypothesis was supported by chaperone assays in which full-length αB crystallin and two synthetic αB crystallin chaperone sequences inhibited the aggregation of VEGF under reducing conditions. The αB crystallin peptides that protect FGF and VEGF against unfolding and aggregation have the potential for use as FGF and VEGF stabilizers in therapeutic formulations for the treatment of advanced coronary artery disease (CAD) (*78*, *79*).

Like the FGF interactive sequences in αB crystallin, all four αB crystallin sequences that interacted with NGF-β overlapped with previously identified αB crystallin chaperone sequences, suggesting that αB crystallin may chaperone NGF-β in vivo. These results are consistent with the role of NGF-β in neuronal signaling and the close association of αB crystallin with microtubules and microtubule-associated proteins (*40*, *41*, *58*, *80*). The identification of chaperone

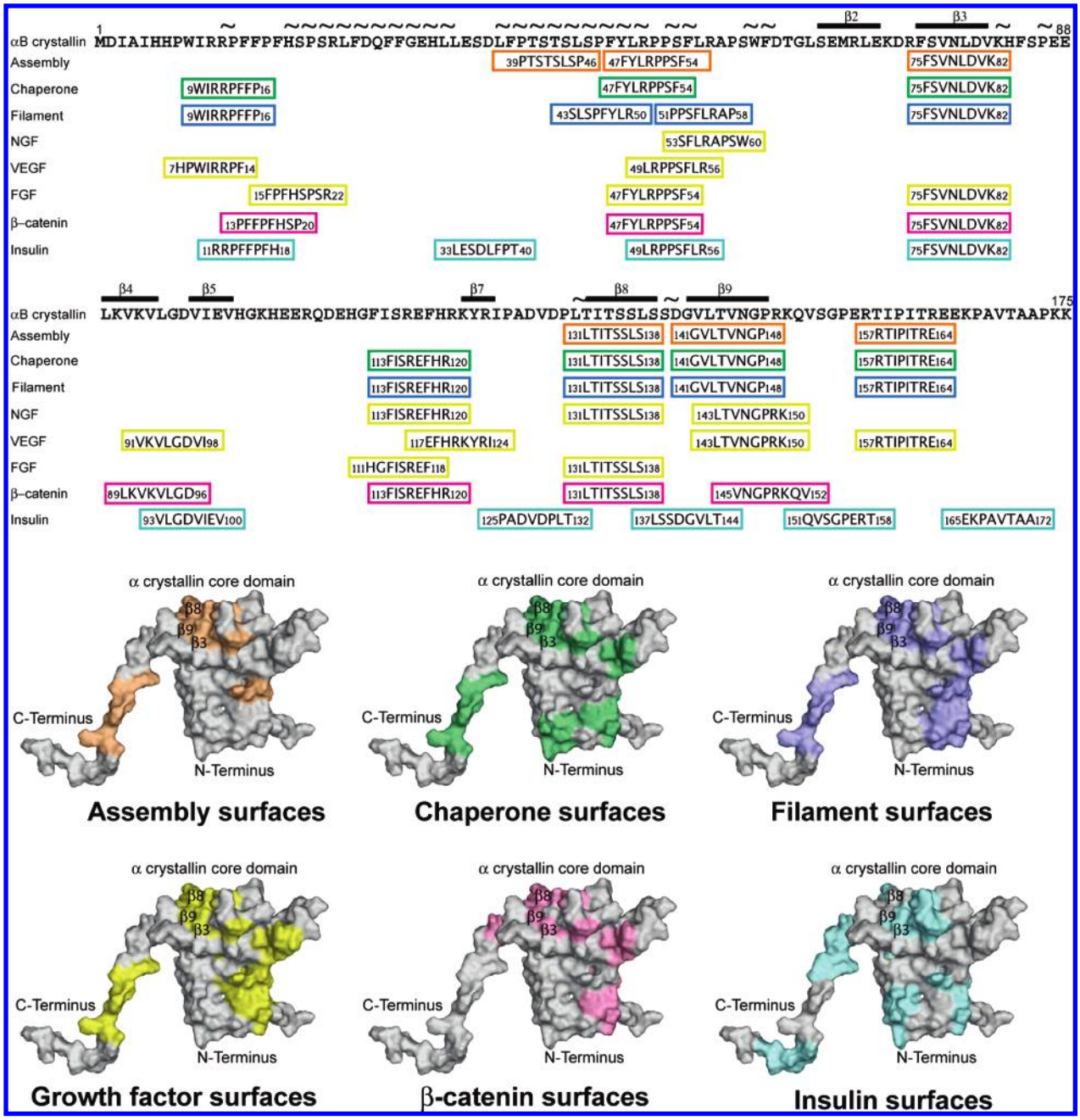

FIGURE 5: Comparison of the αB crystallin interactive sequences and surfaces for subunit−subunit interactions, chaperone activity, filament interactions, growth factor interactions, *β*-catenin interactions, and insulin interactions. The secondary structure of human αB crystallin is shown above its primary sequence (∼ represents helix and − represents *β* strand). The interactive sequences for subunit assembly (orange), chaperone activity (green), filament interactions (dark blue), growth factor interactions (NGF-*β*, VEGF, and FGF-2) (yellow), interactions with *β*-catenin (pink), and interactions with insulin (light blue) are enclosed in boxes below the primary sequence of αB crystallin (top). Sequences containing *β* strands *β*3, *β*8, and *β*9 as well as helical sequences in the N-terminal domain had the strongest interactions with most ligands. The interactive sequences were mapped to the surface of a three-dimensional homology model for human αB crystallin, and the *β*3−*β*8−*β*9 surface appears to be a common surface that mediates interactions with multiple proteins.

sequences for NGF-*β* on the surface of αB crystallin, the archetype of sHSPs, suggests that the chaperone activity of αB crystallin and other sHSPs may selectively protect key growth factors and signaling molecules that are important for normal cellular processes post-stress. αB crystallin colocalizes with *β*-catenin in actin networks, and like *β*-catenin, αB crystallin migrates into the nucleus under conditions of cell stress in culture (*34*, *35*, *50*, *81*−*83*). The identification of chaperone sequences in αB crystallin that mediate interactions with *β*-catenin is consistent with the role of αB crystallin in stabilization of cytoskeletal networks (*13*, *60*, *61*). By protecting *β*-catenin during cellular stress, αB crystallin may inhibit *β*-catenin degradation and facilitate *β*-catenin signaling through the Wnt pathway, which regulates cell proliferation and differentiation (*84*, *85*). This hypothesis is consistent with the role of αB crystallin as an anti-apoptotic and oncogenic protein (*86*, *87*).

This is the first report of specific sequences in the sHSP and molecular chaperone, αB crystallin, that interact with FGF-2, NGF-*β*, VEGF, insulin, and *β*-catenin. Synthetic peptides based on the interactive sequences of αB crystallin were observed to protect against the aggregation of FGF-2 and VEGF in vitro. Taken together, the results suggest the potential importance of sHSPs and molecular chaperones in

the regulation of key components of signaling pathways and cell differentiation under stress and nonstress conditions. A potential role for αB crystallin in regulation of proliferation, differentiation, and structural stabilization during normal development as well as in response to stress associated with myocardial and neurodegenerative disease would be an important advance in understanding the normal function of sHSPs.

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