عنوان المشروع باللغة العربية	توضيح لدور عدسة الإبل ألفا البلورية في للطي و استقرار زيتا البلورية
عنوان المشروع باللغة الإنجليزية	Elucidation of the role of camel lens alpha crystalline in the folding and stability of zeta crystalline
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التخصص الدقيق للمشرف الرئيس	Protein engineering
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المدة المتوقعة لإنجاز البحث منذ الحصول على موافقة عمادة الدراسات العليا	9 months
Abstract or synopsis of the proposal (200 words or less):	-crystallin and different spectroscopic (absorption, fluorescence and circular dichroism) techniques.ζcrystallin on the unfolding and refolding of zeta cystallin will be studied by activity assays of –α-crystallin is a NADPH dependent quinone oxidoreductase, abundantly present (~10%) in the camel eye lens. The role of ζcrystallin (a 140 kDa tetrameric protein comprising four identical subunits, each with a molecular weight of 35 kDa) will be studied. –ζ-crystallin subunits on the unfolding and refolding of camel lens αcrystallin will be recombinantly produced in E.coli and purified to homogeneity using different chromatographic techniques. Subsequently, the role of –αB subunits in the prevention of other lens protein aggregation is not studied. In this study, both subunits of αA and αB, named according to their charges. The role of camel lens αA and α -crystallin is the major constituent of the eye lens water soluble proteins. It is oli gomeric in nature, which is constituted with two subunits, αAlpha-crystallin is ubiquitously present in all the vertebrates' eye lens. It's a member of the small heat-shock protein family and is known to assists in the folding and stability of other proteins under conditions of stress.
Hypothesis or scientific justification of the proposal	-crystallin (Huang, Russell et al. 1987; Rao, Gonzalez et al. 1997).ζ), but it also contain a unique protein in abundance (~1 0%) known as γ and β, αCataract is a major cause of blindness in Saudi Arabia and worldwide (al Faran 1990; Truscott and Zhu 2010) which is caused by denaturation and aggregation of eye lens proteins. It has been found in several epidemiological studies that various environmental factors such as high temperature, UV radiation and dehydration enhances the propensities of cataractogenesis (Al-Ghadyan and Cotlier 1986; Minassian, Mehra et al. 1989; Taylor 1999; Neale, Purdie et al. 2003; Heys, Friedrich et al. 2007). Camel adopted to successfully survive under extreme environmental conditions of heat, high UV radiation and excessive dryness, and yet maintains eye lens proteins in soluble and functional state. Like all other vertebrates, camel eye lens had three ubiquitous crystallins (-crystalline is an attractive substrate of protein folding studies because it has enzymatic activity, contains single tryptophan which

	facilitates fluorescence studies and also it has NADPH binding site which allows monitoring changes in protein strucζ-crystalline. α-crystallin in the unfolding and refolding process of other eye lens proteins. To achieve our goal, we have selected another eye lens protein (zeta-crystallin) as substrate of αB-crystallins in the folding and stability of other eye lens proteins are unknown. This study will provide an insight into the molecular aspects of αA- and αB-crystallins and the molar ratio of these subunits in the heteromultimer vary from species to species (Siezen, Bindels et al. 1978). Earlier studies showed that both the polypeptides, either in their homo- or heteromultimeric states, exhibit molecular chaperone like activity in preventing unfolding and aggregation of other proteins (Sun, Das et al. 1997). The ro le camel lens αA- and αB-crystallins are elevated. Two different genes encodes αB-crystallins are also found in other tissues (Bhat and Nagineni 1989; Sax and Piatigorsky 1994) but under stress condition or sickness the level of αA- and α-Crystallins reveals that it is a member of small heat-shock proteins (sHSPs) (Ingolia and Craig 1982; Klemenz, Frohli et al. 1991). Both α -Crystallins, i.e. aA- and aB-crystallins, are abundantly present in the eye lens. Sequence alignment of αB-crystallins). αA- and αEye lens a-crystallin is a large soluble (~800kDa) heteromultimeric protein which is composed of two subunits (ture spectroscopically.
Specific objectives	-crystallineζB and αA, α1- Expression and purification of camel lens 2- Characterization of aA, aB crystalline on the unfolding and refolding of camel ζ-crystallin.
Methodology & Major Techniques to be used	crystallin will be evaluated by Coomassie Blue staining of SDS-PAGE. In the control experiment, untransformed Escherichia coli strain will be cultivated in parallel in identical conditions and analyzed by SDS-PAGE. To optimize expression (if required), a variety of growth (Pre- and post-induction cultivation temperature, type of growth medium, growth rate and aeration) and expression parameters (Inducer concentration, OD600 at the time of induction, duration of post-induction cultivation) will be optimized. Subsequently, all three proteins will be purified to homogeneity using Ni-NTA, ion-exchange and gel filtration chromatography. The purity will be checked on SDS-PAGE.–ζ-crystallin) will be induced by treatment with Isopropyl-β-D-thiogalactoside (IPTG) as described by (Studier, Rosenberg et al. 1990) . Briefly, IPTG will be added in the culture at final concentration of 1 mM when the culture will reach in mid-logrithmic phase of growth at 37 °C, 200 rpm. Harvesting of Es cherichia coli cells will be done after 3 hours of post-induction incubation at 37 °C, 200 rpm. At the end of the incubation, 1 ml of culture will be centrifuged at 13,000 rpm for 5 minutes. Supernatant will be discarded and pellet will be analyzed by SDS-PAGE. Expression level of ζB and αA, α-crystallin) cloned on pET vector under T7 promoter will be transformed into competent Escherichia coli strains. In the preliminary expression experiment, the synthesis of recombinant (ζB and αA, α-crystallin) will be recombinantly produced in E.coli. If required, overexpression conditions will be optimized to increase the specific as well as volumetric yield of these proteins in native state. The open reading frame (ORF) of camel lens (ζB and αA, αThree

	camel lens proteins (B crystallin, respectively.αA and α-crystallin and increasing concentrations of urea, for 1 h. In the second and third sets, zeta-crystallin will incubating with increasing concentrations of urea in the presence of ζ-crystallin will be carried out at room temperature by incubating ζUnfolding studies: This experiment will be done in three sets. In the first set of experiment, unfolding experiments of B crystallin, respectively. The denaturant will be removed by rapid dilution method. Activity and other spectroscopic studies (Intrinsic tryptophan fluorescence, ANS-binding studies, NADPH-binding studies, Circular dichroism) will be done after 1 hours of refolding.αA and α -crystallin will be denatured in the presence of ζRefolding studies: This experiment will also be done in three sets. In the first set, zeta-crystallin will be denartured alone while in the second and third set,
Availability of Samples	YES
If the answer is no, kindly justify	
Availability of Chemicals	YES
If the answer is no, kindly justify	
Availability of Instruments	YES
Availability of Ethical Approval (if needed)	YES
Recent References	Al-Ghadyan, A. A. and E. Cotlier (1986). "Rise in lens temperature on exposure to sunlight or high ambient temperature." Br J Ophthalmol 70(6): 421-426. al Faran, M. F. (1990). "Visual outcome and complications after cataract extraction in Saudi Arabia." Br J Ophthalmol 74(3): 141-143. Bhat, S. P. and C. N. Nagineni (1989). "alpha B subunit of lens-specific protein alpha-crystallin is present in other ocular and non-ocular tissues." Biochem Biophys Res Commun 158(1): 319-325. Heys, K. R., M. G. Friedrich, et al. (2007). "Presbyopia and heat: changes associated with aging of the human lens suggest a functional role for the small heat shock protein, alpha-crystallin, in maintaining lens flexibility." Aging Cell 6(6): 807-815. Huang, Q. L., P. Russell, et al. (1987). "Zeta-crystallin, a novel lens protein from the guinea pig." Curr Eye Res 6(5): 725-732. Ingolia, T. D. and E. A. Craig (1982). "Four small Drosophila heat shock proteins are related to each other and to mammalian alpha-crystallin." Proc Natl Acad Sci U S A 79(7): 2360-2364. Klemenz, R., E. Frohli, et al. (1991). "Alpha B-crystallin is a small heat shock protein." Proc Natl Acad Sci U S A 88(9): 3652-3656. Minassian, D. C., V. Mehra, et al. (1989). "Dehydrational crises: a major risk factor in blinding cataract." Br J Ophthalmol 73(2): 100-

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