عنوان المشروع باللغة العربية	تأثير الجلوكوز ومركباته الأيضية على بروتين الكريستالين في عدسة عين الإبل: محاكات تأثير مرض السكري
عنوان المشروع باللغة الإنجليزية	Effect of glucose and its metabolites on camel lens crystallins: Simulation of diabetic lens conditions
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Abstract or synopsis of the proposal (200 words or less):	These studies are designed to simulate the hyperglycemic conditions encountered in the diabetes lens α-, β- and γ-Crystallins from the cortex of the camel lens are purified and their biochemical properties are characterized in their native state. Effect of glucose and its metabolites such as glyoxal, methylglyoxal, glyceraldehyde and 2-deoxyglucosone is studied by incubating them with the lens homogenate, α-, β- and γ-crystallins. Effect of glycation on these proteins are characterized by determining free ε-amino groups of lysine and arginine residues; carbonyl content, free sulfhydryl groups, glycated products and amyloid formation (fibrillation state) in these incubated proteins. α-, β- and γ-crystallins are further characterized structurally by fluorescence and circular dichorism spectroscopy with respect to their secondary and tertiary structures relating to α-helix and β-sheet contents as well as hydrophobicity, intrinsic fluorescence, ability to bind nucleotides (ATP, NADH and NADPH). Lens homogenate and α-, β- and γ-crystallin (glycated vs. non-glycated) in the lens are compared. Under these simulated conditions, to what extent the chaperone activity of α-crystallin is affected. Lastly, effects of drugs, anti-glycating agents and polyphenols are studied, which can inhibit glycation and protect structural integrity of lens crystallins from incapacitating effects of glucose and its metabolites by interfering in delaying or inhibiting glycation.
Hypothesis or scientific justification of the proposal	The α-, β- and γ-crystallins are the major protein components of all vertebrate eye lens, α-crystallin as a molecular chaperone as well as a structural protein, β-and γ-

	crystallins as structural proteins. For the lens to be able to retain life-long transparency in the absence of protein turnover and tolerate the stress they undergo, the crystallins being at high cellular concentrations, must long-term retain their native structure and be soluble at all times in order to keep the lens environment transparent. The factors in-turn are dependent on intrinsic stability, efficient capture and refolding of proteins by chaperones. Understanding the specific interactions that confer intrinsic stability of the protein fold are combined with the stabilizing effect of protein assembly, and how the non -specific interactions under stressful conditions may force the integrity of lens crystallins resulting in loss of transparency of the lens with age, is thus of important events to understand. Posttranslational modifications can have a major effect on protein stability but an emerging theme of the few studies of the effect of post-translational modification of the crystallins is one of solubility and assembly. This proposal is related to study the structure, assembly, interactions, stability and post-translational modifications of the crystallins, not only in isolation form but also as part of a multi-component system (at homogenate level). Understanding the structural basis of protein stability and interactions in the healthy eye lens as well as in diseased state, is the route to solve the enormous medical and economical problem of cataract.
Specific objectives	1. Objectives of the research proposal is to design the conditions that can simulate the hyperglycemic conditions encountered in the diabetes lens, which may lead to cataract formation. 2. Effects of glucose and its metabolites such as glyoxal, methylglyoxal, glyceraldehyde and 2-deoxyglucosone are studied by monitoring structural and functional changes in the lens homogenate, α-, β- and γ-crystallins. 3. α-, β-γ-Crystallins are purified from the cortex of the camel lens and their biochemical properties are characterized in their native state. 4. Effects of glycation on the lens homogenate and α-, β-γ-crystallins are characterized by determining free epsilon-amino groups of lysine and arginine residues; determination of carbonyl content, free sulfhydryl groups, glycated products and amyloid formation (fibrillation state) in the incubated

	proteins, 5. α-, β- and γ-crystallins are further characterized structurally by fluorescence and circular dichorism spectroscopy with respect to their secondary and tertiary structures relating to α-helix and β-sheet contents as well as hydrophobicity, intrinsic fluorescence, ability to bind nucleotides (ATP, NADH and NADPH). 6. Effects of drugs, anti-glycating agents and polyphenols are studied, those can interfere with glycation and protect structural integrity of lens crystallins.
Methodology & Major Techniques to be used	Size exclusion chromatography Ultraviolet and fluorescence spectroscopy Circular dichorism spectroscopy
Availability of Samples	YES
If the answer is no, kindly justify	
Availability of Chemicals	NO
If the answer is no, kindly justify	Most of the required chemicals are available in the lab, but some fine chemicals will be purchased
Availability of Instruments	YES
Avail ability of Ethical Approval (if needed)	NO
Recent References	1. Ageing and vision: structure, stability and function of lens crystallins Hans Bloemendala, Wilfried de Jonga, Rainer Jaenickeb, Nicolette H. Lubsena, Christine Slingsby, Annette Tardieud. Progress in Biophysics & Molecular Biology 86 (2004) 407–485. 2. Crystallins in the eye: Function and pathology Usha P. Andley, Progress in Retinal and Eye Research 26 (2007) 78–98 3. Effect of glycation on α-crystallin structure and chaperone-like Function. P. Anil Kumar, M. Satish Kumanri and G. Bhanuprakash Reddy

	Biochem. J. (2007) 408, 251–258. 4. Genetics of crystallins: Cataract and beyond. Jochen Graw, Experimental Eye Research 88 (2009) 173–189 5. Nonenzymatic Glycation Alters Protein Structure and Stability – A study of two eye lens crystallins Manni Luthra and Dorairajan Balasubramanians, The Journal of Biological Chemistry, vol. 268, (24), 18119-18127 (1993).