عنوان المشروع باللغة العربية	إستقرار ووظيفة وتركيب بروتين زيتا كريستالين المرتبط بالسكريات
عنوان المشروع باللغة الإنجليزية	Structure, function and stability of glycated z-crystallin
المشرف الرئيس	Dr. Abdulrahman M. Al-Senaidy
التخصص الدقيق للمشرف الرئيس	Protein engineering
المشرف المساعد	Dr. Ajamaluddin Malik
المدة المتوقعة لإنجاز البحث منذ الحصول على موافقة عمادة الدراسات العليا	9
Abstract or synopsis of the proposal (200 words or less):	Cataract is the major cause of blindness and of visual impairment worldwide. It is estimated that more than 17 million of people are blind due to cataract and more than 1100 new cased reported every hour. Cataracteous lenses, are due to high light scattering little or no light reaches the photoreceptors of the retina is considering to be the major cause of blindness in Sau di Arabia and worldwide. Cataract is caused by change in the conformation of the lens proteins, which is due to the accumulation of glycated, oxidative damage, deamidation, or cleavage with time in the lens proteins. Post-translation modification of proteins leads to unfolding and aggregation of crystallins which decreases lens transparency, therefore resulting in an opaque or cataracteous lens. Camel is well adopted to survive in harsh environmental condition such as high temperature, dryness and solar radiation. It has novel eye lens protein in bulk quantity known as zeta-crystallin. The physiological role of zeta-crystallin in the eye lens is not clear. In this study, zeta crystallin will be recombinantly expressed and purified and glycated. The structural, functional and stability of glycated z-crystallin will be studied in detail.
Hypothesis or scientific justification of the proposal	The eye lens is a specialized structure, which provides flawless transmission of light to reach the retina for proper vision. Crystallins are the major structural proteins in the lens that account for up to 90% of the total soluble protein. There are three distinct families: α-, β- and γ -crystallins, whose structure, stability and short-range interactions are thought to contribute to lens transparency. Cataracts are the leading cause of blindness worldwide, with diabetes and aging the major risk factors that accelerate cataract development

	(Rowe, Mitchell et al. 2000; Hennis, Wu et al. 2004; Abraham, Condon et al. 2006). The glycation reaction occurs between the carbonyl group of sugars and a free amino group within proteins. Amadori products, the first stable product of the reaction, can be consequently transformed into AGEs (advanced glycation end-products). AGEs are generally pigmented or fluorescent adducts on proteins, and participate in the formation of protein cross-links (Monnier, Nagaraj et al. 1996). Formation of AGEs due to glycation may alter the surface charge of the protein, leading to conformational change, which in turn may affect protein–protein and protein–water interactions, and may ultimately lead to a decrease in the transparency of the eye lens (Beswick and Harding 1987). Although all the three major crystallins are susceptible to glycation, differential glycation was observed in in vitro glycation of rat lens soluble fraction, γ -crystallin being more prone to be followed by α-crystallin (Swamy and Abraham 1991). However, preferential glycation of α-crystallin was observed in aging and diabetic human lens (Swamy, Abraham et al. 1992). Camel is well adopted to lives under extreme climatic conditions including intense heat, excessive dryness, and high UV radiation and yet maintains functional and active eye lens proteins. In addition to ubiquitous crystallins (α-, β- and γ), camel lens contain a unique protein in abundance (~10%) known as zeta-crystallin (Huang, Russell et al. 1987) (Rao, Gonzalez et al. 1997). Zeta-crystallin also occurred in human lens but in very little amount. Zeta-crystallin is a NADPH dependent quinone oxidoreductase. The physiological role of zeta-crystallin in the eye lens is not clear. Effect of post-translational modification on the camel lens zeta-crystallin is poorly characterized. To best of our knowledge, this is the first study about effect of glycation on the structure, function and stability of camel lens zeta-crystallin. No literature available about effect of various glycating sugars on zeta-crystallin in terms of degree of glycation, type of AGE that is formed, oxidati ve damage to the protein, secondary and tertiary structure, hydrophobicity and its function. In the present study, we will investigate the effect of glycating agents (hexose sugars, keto sugars, sugar phosphate and dicarbonyls s) on the structure-function and stability of z-crystallin. The level of these glycating agents elevated in various tissues, including the lens in diabetic patients.

Specific objectives	To express recombinant zeta crystallin in E.coli. Purify to homogeneity using chromatographic techniques. Evaluate effect of different glycating agents on the structure-function and stability of z-crystallin.
Methodology & Major Techniques to be used	In this project recombinant Z-crystallin will be overexpressed under optimized condition in E.coli. Z-crystallin will be purified using affinity and gel filtration chromatography. Subsequently, z-crystallin will be treated with different glycating agents. Briefly, stocks of glucose, fructose, G6P and MGO were prepared sodium phosphate buffer (pH 7.4). Zeta-Crystallin (5 mg/ml) well be incubated with glucose, fructose, and MGO for different time period at 3 7 oC. Zeta-Crystallin incubated in the absence of glycating agent under similar conditions served as a control. The extent of glycation will be evaluated by Non-tryptophan AGE fluorescence. Glycation leads to formation high molecular weight mass aggregate due to cross linking which will be monitiored by SDS-PAGE. The glycated z-crystallin will be separated from non-glycated proteins using affinity chromatography. Subsequently, the structure, function and stability of glycated z-crystallin will be studied in detail using various spectroscopic techniques (Spectrophotometer, Spectroflurometer and Circular dichroism).
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	Abraham, A. G., N. G. Condon, et al. (2006). "The new epidemiology of cataract." Ophthalmol Clin North Am 19(4): 415-425. Beswick, H. T. and J. J. Harding (1987). "Conformational changes induced in lens alpha- and gamma-crystallins by

modification with glucose 6-phosphate. Implications for cataract." Biochem J 246(3): 761-769.
Hennis, A., S. Y. Wu, et al. (2004). "Risk factors for incident cortical and posterior subcapsular lens opacities in the Barbados Eye Studies." Arch Ophthalmol 122(4): 525-530.
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.
Monnier, V. M., R. H. Nagaraj, et al. (1996). "Structure of advanced Maillard reaction products and their pathological role." Nephrol Dial Transplant 11 Suppl 5: 20-26.
Rao, P. V., P. Gonzalez, et al. (1997). "Guinea pig and bovine zeta-crystallins have distinct functional characteristics highlighting replacements in otherwise similar structures." Biochemistry 36(18): 5353-5362.
Rowe, N. G., P. G. Mitchell, et al. (2000). "Diabetes, fasting blood glucose and age-related cataract: the Blue Mountains Eye Study." Ophthalmic Epidemiol 7(2): 103-114.
Swamy, M. S., A. Abraham, et al. (1992). "Glycation of human lens proteins: preferential glycation of alpha A subunits." Exp Eye Res 54(3): 337-345.
Swamy, M. S. and E. C. Abraham (1991). "Differential glycation of rat alpha-, beta- and gamma-crystallins." Exp Eye Res 52(4): 439-444.