عنوان المشروع باللغة العربية - Title of the proposed project in Arabic	أثر ارتباط الجلوكوز على فعالية وتركيب بروتين الفا - كرستالين من عدسة عين الجمل.
Title of the proposed project in English	Effect of glycation on the structure and chaperone-like function of camel lens α-crystallin
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التخصص الدقيق للمشرف الرئيس - Specialty of PI	Protein folding and engineering
المشرف المساعد - Co-PI	Dr. Mona Al-Onazi
المدة المتوقعة لإنجاز البحث منذ الحصول على موافقة عمادة الدراسات العليا (بالشهور) - Expected time in month to finish	8
Abstract of the proposal (No more than 200 words)	Cataract, caused by protein misfolding and aggregation of the lens proteins, is one of the major cause of blindness in Saudi Arabia and worldwide (al Faran 1990; Abraham, Condon et al. 2006; Sharma and Santhoshkumar 2009; Moreau and King 2012; Stevens, White et al. 2013; Khairallah, Kahloun et al. 2015). The chaperone-like activity of alpha-crystallin is considered to play an important role in the maintenance of the transparency of the eye lens((Reddy, Kumar et al. 2006; Kumar and Reddy 2009)). However, in the case of aging and diabetes,

	the chaperone function of alpha-crystallin is compromized, resulting in cataract formation (Kumar, Kumar et al. 2007; Mukhopadhyay, Kar et al. 2010). Several post-translational modifications, including non-enzymatic glycation, have been shown to affect the chaperone function of alpha-crystallin in aging and diabetes (Thampi, Zarina et al. 2002). Camel adopted to successfully live in harsh environmental stress condition of heat, dryness, solar radiation and low nutrition. The eye lens proteins are the oldest protein in the body synthesized embryonically and retained structure-function lifelong in all the organisms (Bloemendal, de Jong et al. 2004). It's interesting to note that how camel lens proteins retain its native structure and solubility throughout life-span? Primary sequence analysis of camel alpha crystallin showed that alphaB-crystallin contains unique domain at the N-terminal. Our results showed very high chaperone activity of camel alpha crystallin against thermal denaturation of lens protein. In this study, we plan to investigate the role of non-enzymatic glycation on the structure and function of alpha crystallin. Alpha crystallin will be purified from camel lens using different chromatographic techniques. Subsequently, structure and function of glycated alpha crystallin will be analyzed using various techniques (Spectrophotometer, Spectroflurometer and Circular dichroism).
Hypothesis of the proposal	Glycation of lens proteins has been considered to be one of the mechanisms responsible cataract in old-age and in diabetic patients (van Boekel 1991; van Boekel and Hoenders 1992; Ahmed 2005). Cataracts are the leading cause of visual impairment worldwide. Prolonged uncontrolled higher blood sugar level and aging are the major risk factors that accelerate cataract development (Rowe, Mitchell et al. 2000; Abraham, Condon et al. 2006). The glycation reaction is a condensation reaction where carbonyl (aldehyde) group of the reducing sugars covalently attached to free amino group of the proteins. The first stable product of the reaction is called Amadori products which subsequently transformed into AGEs (advanced glycation end-products). AGEs are generally pigmented or fluorescent adducts on proteins, and makes intermolecular cross-links of proteins (Monnier, Nagaraj et al. 1996). Formation of AGEs may change the overall charge on the protein surface which will alter protein conformation. The change in conformation may affect protein–protein and protein–water interactions, and may result into unfolding and reduced in solubility, thus leads to aggregation of lens proteins. Aggregation of lens proteins will reduce the transparency of the eye lens and result in cataract formation (Monnier, Nagaraj et al. 1996). 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. All the major class of crystallins (alpha, beta and gamma) are susceptible to glycation. The rate and extent of glycation in each family are different (Swamy and Abraham 1991). In an earlier study,

	preferential glycation of α-crystallin in aging and the diabetic human lens was observed (Swamy and Abraham 1991; Swamy, Abraham et al. 1992). Glycation of alpha crystalline resulted into loss of chaperone function in diabetic rat and human lens (Cherian and Abraham 1995; Cherian and Abraham 1995; Plater, Goode et al. 1997; Derham and Harding 2002; Thampi, Zarina et al. 2002) which emphasize that the α-crystallin chaperone function is the prime target of glycation. The loss of chaperone function results in the development of cataracts. Camels have exceptional carbohydrate metabolism as their plasma glucose level is high and have low whole body insulin sensitivity, similar to that observed in type 2 diabetes patients (Al-Ali, Husayni et al. 1988; Abdel-Fattah, Amer et al. 1999; Kaske, Elmahdi et al. 2001). It leads to several questions, yet to answer. Weather alpha crystalline is less or not susceptible to glycation, or glycated alpha crystalline retain structure and chaperoning activity? To my best of knowledge, this is the first study to investigate the effect of various glycating sugars on the camel lens α-crystallin in terms of degree of glycation, type of AGE that is formed, oxidative damage to the protein, secondary and tertiary structure, hydrophobicity and its chaperone-like function.
Specific objectives	1- Purify alpha crystallin to homogeneity using different chromatographic techniques. 2- Analyze the effect of glycation on the structure and function of alpha crystallin.
Methodology & Major Techniques to be used	Alpha crystalline will be extracted from camel eye lens. Alpha -crystallin will be purified using different size exclusion column. The purity of the alpha crystalline will be analyzed on the SDS-PAGE. Next, alpha-crystallin will be treated with different glycating agents. Briefly, stocks of glucose, fructose and MGO will be prepared in sodium phosphate buffer (pH 7.4). Alpha-Crystallin at high concentration (mg/ml) will be incubated with glucose, fructose, and MGO for different time period at 37 oC. In the control experiment, alpha-Crystallin will be incubated in the absence of glycating agent under similar conditions. The extent of glycation will be monitored by Non-tryptophan AGE fluorescence. The formation high molecular weight aggregate due to glycation will be monitiored by SDS-PAGE. If required, the glycated alpha-crystallin will be separated from non-glycated proteins using affinity chromatography. Subsequently, the structure and function of glycated alpha-crystallin will be studied in detail using Spectrophotometer, Spectroflurometer and Circular dichroism.

Availability of Samples	Yes
Availability of Chemicals	Yes
Availability of Instruments	Yes
Ethical Approval	Not needed
Recent References	Abdel-Fattah, M., H. Amer, et al. (1999). "Response of one-humped camel (Camelus dromedarius) to intravenous glucagon injection and to infusion of glucose and volatile fatty acids, and the kinetics of glucagon disappearance from the blood." Zentralbl Veterinarmed A 46(8): 473-481. Abraham, A. G., N. G. Condon, et al. (2006). "The new epidemiology of cataract." Ophthalmol Clin North Am 19(4): 415-425. Ahmed, N. (2005). "Advanced glycation endproducts--role in pathology of diabetic complications." Diabetes Res Clin Pract 67(1): 3-21. Al-Ali, A. K., H. A. Husayni, et al. (1988). "A comprehensive biochemical analysis of the blood of the camel (Camelus dromedarius)." Comp Biochem Physiol B 89(1): 35-37. al Faran, M. F. (1990). "Visual outcome and complications after cataract extraction in Saudi Arabia." Br J Ophthalmol 74(3): 141-143. Bloemendal, H., W. de Jong, et al. (2004). "Ageing and vision: structure, stability and function of lens crystallins." Prog Biophys Mol Biol 86(3): 407-485. Cherian, M. and E. C. Abraham (1995). "Decreased molecular chaperone property of alpha-crystallins due to posttranslational modifications." Biochem Biophys Res Commun 208(2): 675-679.

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