African Journal of Biotechnology Vol. 11(50), pp. 11110-11115, 21 June, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.769
ISSN 1684-5315 ©2012 Academic Journals

Full Length Research Paper

Influence of different concentrations of two chemical chaperones on human islet amyloid polypeptide folding under experimental conditions

Seyyed Mehdi Mirhashemi* and Samira Mehrabi

Biochemistry Department, Faculty of Medicine, Kashan University of Medical Sciences, Kashan, I.R. Iran.

Accepted 23 April, 2012

It has been implicated that type 2 diabetes mellitus is a conformational disease because amylin, a peptide produced by beta cell, undergoes an alteration in the native formation followed by self-aggregation and deposition. Amyloidogenesis causes destruction of pancreatic beta-cells. The subsequent lack of insulin leads to increased blood glucose. The main aim of this study was to investigate whether two chemical chaperones named glycerol and spermine vary islet amyloid polypeptide folding under near-physiological circumstances. For this purpose, fluorescent method was used with LS55 spectrofluorometer instrument. Results obtained from *in vitro* study show that after 240 h incubation by shaker incubator in 37°C, glycerol had contradictory effects on amylin folding and these effects were glycerol concentration dependent. Glycerol with concentration of 24% had the most inhibitory effect but 40 to 50% promoted amylin misfolding significantly ($p<0.05$). The obtained data also demonstrate that spermine with concentrations of 40, 50 and 60 µM had stimulatory effects on formation of beta-amyloid sheet significantly ($p<0.05$). It is concluded that amylin misfolding and cytotoxicity to beta-cells might be glycerol dose-dependent in diabetic patients.

Key words: Chemical chaperones, islet amyloid polypeptide, diabetes mellitus, conformational disease.

INTRODUCTION

Diabetes mellitus is characterized by hyperglycemia. Hyperglycemia occurs as a result of the lack of insulin discharge from pancreatic beta-cell and/or its wrong functioning in tissues which may lead to diabetic complications, for instance neuropathy, retinopathy, nephropathy, atherosclerosis and food disease (Naqshbandi et al., 2008). Type 2 diabetes mellitus result from a change in the protein conformation that directs the protein to misfolding and aggregation (Carrell and Lomas, 1997; Hayden et al., 2005). Recent studies have shown that the main component of beta-amyloid sheet in pancreas of diabetic patients is a peptide named amylin (Zheng et al., 2010). Amylin is a 37-amino-acid peptide that is normally secreted from the beta-cells together with insulin into blood circulation. Amylin hormone decreases food intake and inhibits pancreatic glucagon hormone discharge and contributes to glycemic control (Cummings and Overduin, 2007; Lee et al., 2011; Lutz, 2010). The relationship between amylin deposition and the development of type 2 diabetes has been known. Recent biophysical studies have demonstrated that the islet amyloid polypeptide is unstructured in solution and aggregates due to misfolding to form toxic oligomers and further aggregates to form amyloid fibers so that this amyloid deposition can be toxic to beta-cells and induce the cell-death (Brender et al., 2012; DeToma et al., 2012; Soong et al., 2009; Rhoades et al., 2000). In recent years, small molecule chemical chaperones have been shown to reverse misfolding or mislocalization of several mutant plasma membrane, lysosomal and secretory proteins (Perlmutter, 2002; Cohen and Kelly, 2003). These chemical chaperones are of two kinds. The first, the specific chemical chaperones, which binds to the active site of the mutant proteins and assists in protein

*Corresponding author. E-mail: mirhashemism@gmail.com.

folding. The second class of chaperones is called nonspecific chaperones, some of which are also known as osmolytes. The natural osmolytes such as glycerol are thought to enhance correct folding of the mutant proteins (Bonapace et al., 2004). The aim of this study was to investigate the possible roles of different concentrations of two chemical chaperones named glycerol and spermine on human islet amyloid polypeptide folding under near physiological conditions.

MATERIALS AND METHODS

Human islet amyloid polypeptide and other materials were purchased from Sigma-Aldrich Company. Human amylin used in this project had the following characteristics: (1-37)(Lys- Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Ser-Ser-Asn-Asn-Phe-Gly-Ala-Ile-Leu-Ser-Ser-Thr-Asn-Val-Gly-Ser-Asn-Thr-Tyr-NH2, intra-molecular disulfide bridge: between Cys2 and Cys7). Its purity was 97% and the lyophilized salt included 70% peptide by weight. Amylin stock solution was prepared by adding 1.0 ml dimethylsulfoxide (DMSO) to dry purified peptide, sonicated at room temperature for 15 min.

Groups designing

In order to assay the effects of different concentrations of glycerol and spermine on islet amyloid polypeptide folding and aggregation, control and treated groups were considered. The peptide stock solution was diluted by modified Krebs-Hensleit (KH) buffer (NaCl: 123.5 mM, glucose: 11.0 mM, $CaCl_2$:1.4 mM, NaN_3: 0.05% w/v), at pH 7.4, to the final concentration of 0.8 µM. Different concentrations of glycerol (8, 16, 24, 32, 40 and 50%) and spermine (10, 20, 30, 40, 50 and 60 µM) were prepared in KH- buffer containing 0.8 µM amylin as treated groups, separately. The samples without glycerol and spermine were selected as the control group.

Incubating manner

All studied groups were incubated at 37°C for 240 h with shaking by a shaker incubator (GFL 3031, Germany).

Folding monitoring

To identify the conformational changes and formation of beta-pleated sheets of amyloid, intrinsic and thioflavin T (ThT) fluorescent assay were used.

ThT assay

Thioflavin T assay was performed by adding 40 µl of each incubated solution to 700 µl of 10 µM ThT solution. Fluorescence measurements were recorded in a Perkin-Elmer LS55 fluorescence spectrometer (Perkin-Elmer LS55, USA) at room temperature using a 1-cm path length quartz cell. The ThT signal was quantified by averaging the fluorescence emission at 485 nm (slit width = 10 nm) when excited at 440 nm (slit width = 5 nm).

IF assay

The intrinsic fluorescence of the peptide tyrosine residue was measured for the studied groups after 168 h, averaging the fluorescence emission at 304 nm when excited at 270 nm.

Statistical analysis

Descriptive statistics was accomplished to obtain means and standard deviations. Statistic significance level was established at $p<0.05$. Analysis of data was performed using SPSS statistical software package.

RESULTS AND DISCUSSION

Amylin itself readily folded and formed a ThT-positive material in the control group. Data indicate that at zero time, ThT-fluorescence mean value for control group was 82.12 which at 240 h had increased to mean value of 105.33 ($p<0.05$). In glycerol treated groups, ThT fluorescence assay showed that 8 and 16% of glycerol had no significant effect on amylin conformation ($P>0.05$), whereas 24 and 32% concentrations of glycerol significantly ($p<0.05$) inhibited amylin misfolding by 19.8 and 11.3%, respectively, after 240 h incubation at 37°C (Figure 1). By increasing glycerol concentration, inhibitory effect of this element was inversed so that 40 and 50% of glycerol elevated the ThT- fluorescence by 3.7 and 11%, respectively, at the end of incubation time, significantly ($P<0.05$) (Figure 1). Figure 2 presents different concentrations effects of spermine (10, 20, 30, 40, 50 and 60 µM) on amylin folding. These data indicate that as compared to control group, 10 and 20 µM of spermine had little effects on ThT-fluorescence of amylin, but these effects were not statistically significant ($p>0.05$), whereas by increasing spermine concentration, ThT-fluorescence decreased significantly in a dose dependent manner ($p<0.05$). 30, 40, 50, and 60 µM of spermine inhibited amylin aggregation and misfolding by 4.97, 12.9, 18.6 and 20.4% as compared to the control group ($p<0.05$) (Figure 2). Intrinsic fluorescence (IF) of human amylin hormone was measured for different groups. Glycerol had dual effects on IF so that 24% of this agent had the most reducing property ($p<0.05$), while 50% concentration of glycerol increased IF significantly ($p<0.05$) (Figure 3). IF decreased in spermine treated group which is proportional to spermine concentration. Spermine with concentration of 60 µM had the highest effect on human islet amyloid polypeptide structure just as measured by IF test ($p<0.05$) (Figure 4).

Due to the rising prevalence of diabetes, multi-disciplinary study intended at preventing and treating it is one of the world-wide research priorities. It is shown that lack of insulin secreting cells in pancreatic islet of type 2 diabetic patients is related to amyloid depositions resulting from amylin misfolding (Zheng et al., 2010; Wang et al., 2011). Although, the amyloid formation is well shown in the diabetic patients, the factors affecting this process remain elusive. Our previous publications shows that a number of elements and herbal extracts

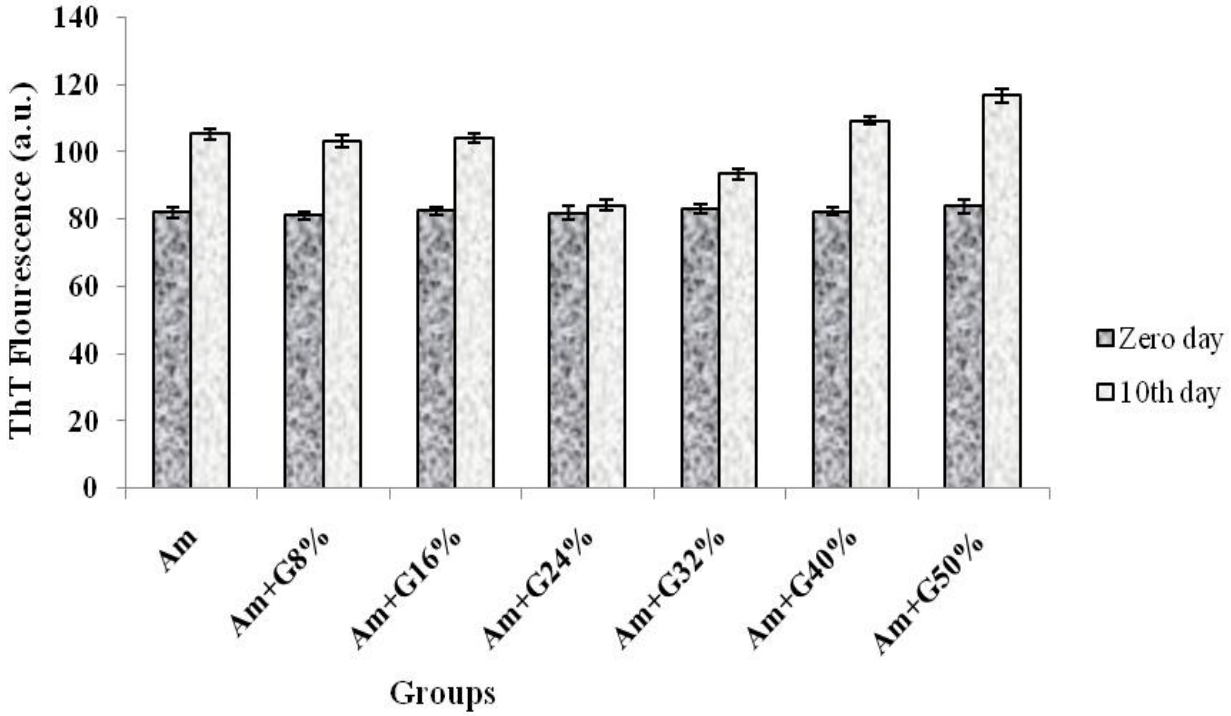

Figure 1. Influence of different concentrations of glycerol on human islet amyloid polypeptide folding. Changes in folding were monitored by ThT fluorescence in the absence and presence of different concentrations of glycerol for 240 h at 37°C. Glycerol with concentration of 24% had the highest inhibitory effect, whereas 40 to 50% promoted amylin misfolding significantly (Am, amylin; G, glycerol).

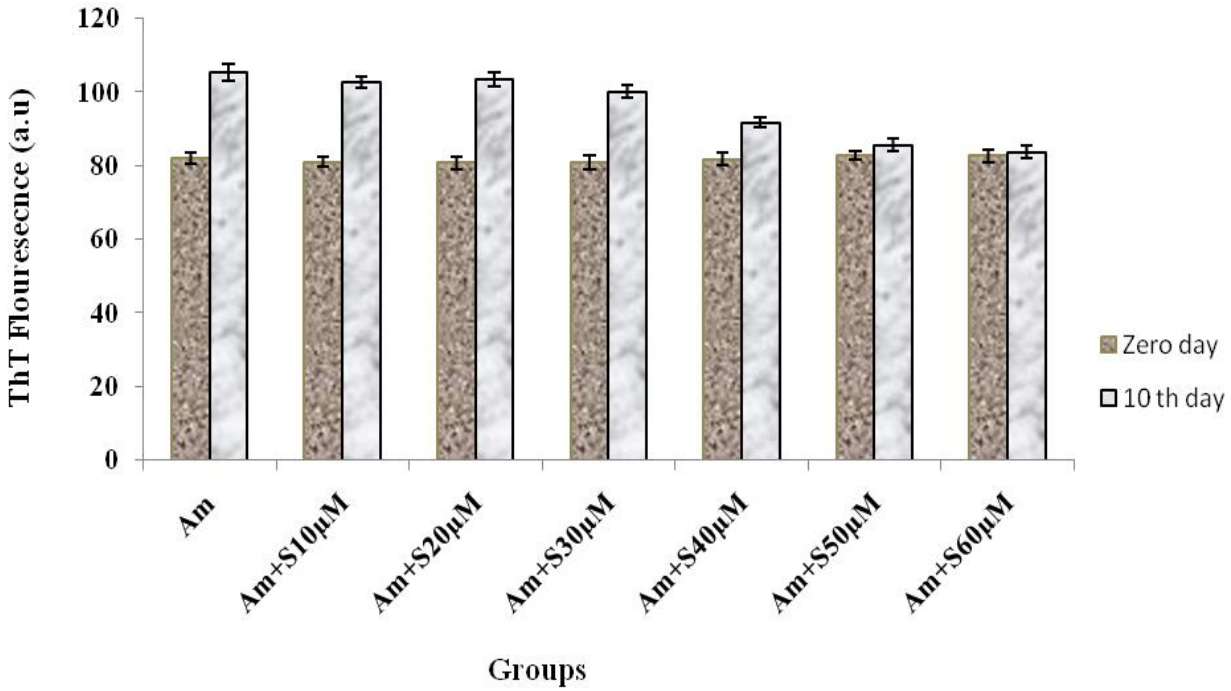

Figure 2. Influence of different concentrations of spermine on human islet amyloid polypeptide folding. Changes in folding were monitored by ThT fluorescence in the absence and presence of different concentrations of aluminium for 240 h at 37°C. Spermine stimulated amylin misfolding and this effect was dose dependent (Am, amylin; S, spermine); spermine: 10, 20, 30, 40, 50 and 60 µM.

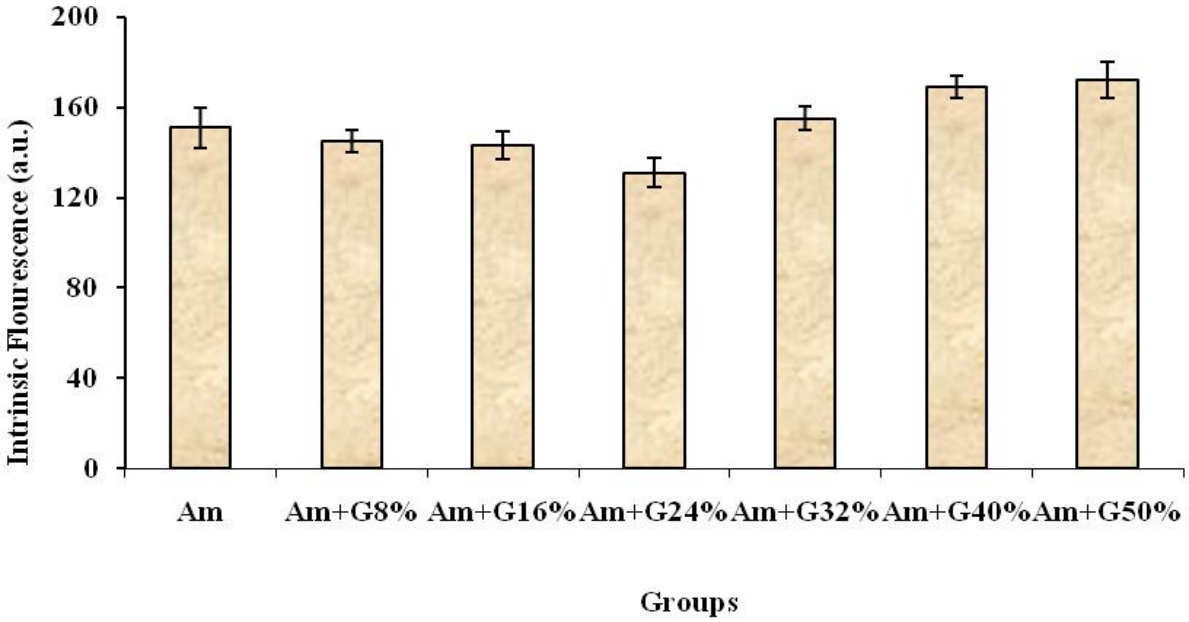

Figure 3. Intrinsic fluorescence of the control and glycerol treated groups. Tyrosine intrinsic fluorescence of amylin solutions in the absence and presence of the different concentrations of glycerol (8, 16, 24, 32, 40 and 50%) was measured after 168 h incubation in 37°C. Glycerol showed dual effect on islet amyloid polypeptide folding. Data is shown as mean ± SD; n = 6.

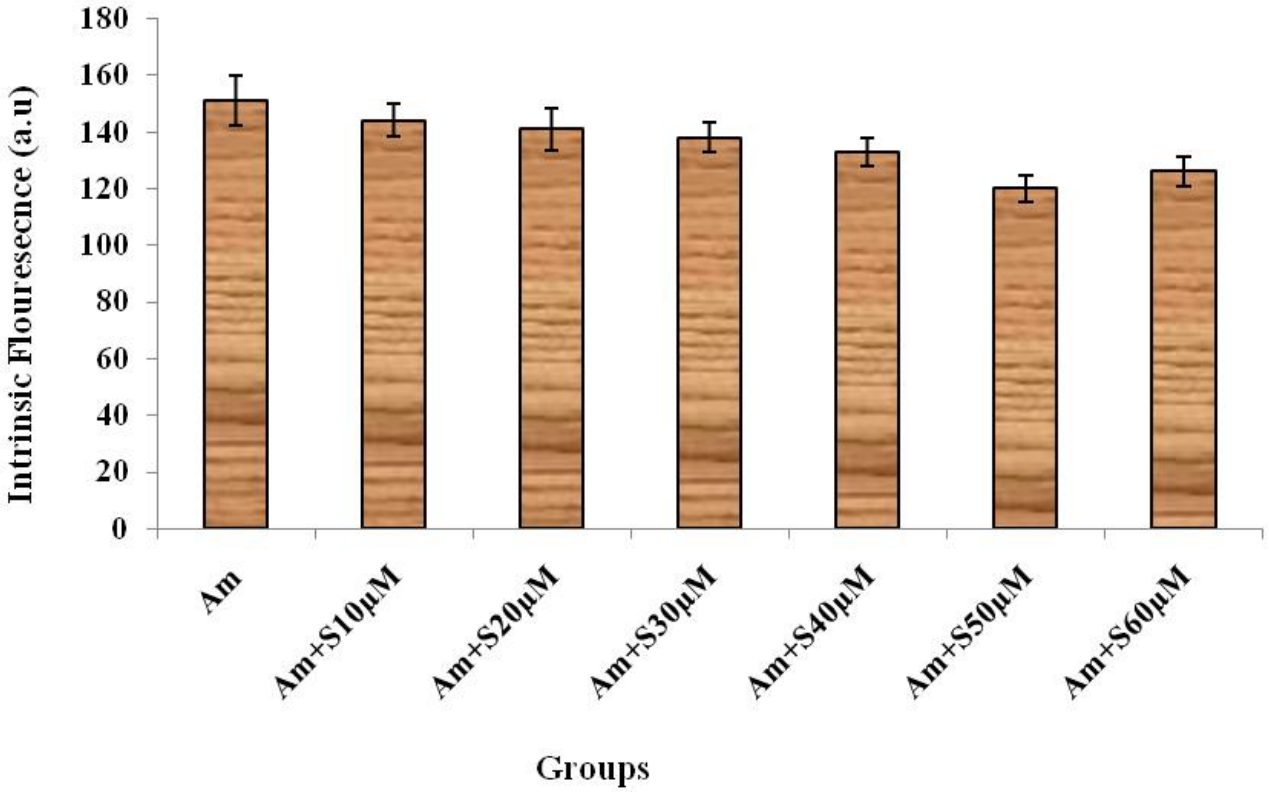

Figure 4. Intrinsic fluorescence of the control and aluminium treated groups. Tyrosine intrinsic fluorescence of amylin solutions in the absence and presence of the different concentrations of spermine (10, 20, 30, 40, 50 and 60 µM) was measured after 168 h incubation in 37°C. Spermine showed one type effect on islet amyloid polypeptide folding. Data is shown as mean ± SD; n = 6.

influence the folding of amylin *in vitro* (Mirhashemi and Aarabi, 2011a, 2012c; Mirhashemi and Shahabaddin, 2011b). A number of small molecules, known as chemical chaperones such as glycerol and spermine have important role in protein conformation against thermal and chemically induced denaturation (Bathaie et al., 2011). Since there is no data concerning effect of glycerol and spermine as chemical chaperones on amylin folding, thus, the present study was designed. These findings show that different concentrations of glycerol had dual effects on amylin conformation and folding but spermine only inhibited misfolding in a dose dependent manner ($p<0.05$), (Figures 1 and 2). As mentioned in the method section of this article, the peptide had an intra-molecular disulfide bridge between Cys2 and Cys7. Disulfide bonds are important for proper protein folding and biological activity (Kopito and Ron, 2000; Anelli et al., 2002; Fassio and Sitia, 2002). It may be assumed that low level of glycerol inhibited amylin misfolding by the following possible mechanism: 1, affecting the intra molecular disulphide bond; 2, increasing the lag-time for fiber formation and 3, decreasing the amylin addition rate to preformed fibers. The opposite effects might occur at higher concentrations of glycerol. Previous investigations have shown that fibrillization of several polypeptides such as amylin is accompanied by formation of free radicals, mainly reactive oxygen species (ROS). ROS accelerate fibril formation, possibly via oxidation reactions, so that the free radicals formed during amyloid fibrillization enhance fibrillization (Schoneich, 2005; Shoval et al., 2007). ROS may have impact on the disulfide bond (Cumming et al., 2004) and subsequently influence the development of amylin misfolding.

Although, the exact mechanism by which spermine inhibit amyloid formation remains unclear, it may be suggested that the antiamyloidogenic power of this compound is due to its antioxidant property. Several lines of evidence have shown that spermine act as an antioxidant due to its ability to scavenge free radicals (Sava et al., 2006; Das and Misra, 2004; Ha et al., 1998; Fujisawa and Kadoma, 2005). Further study is required to elucidate the exact mechanism of these two chemical chaperones.

Conclusion

In summary, this experimental study established that glycerol and spermine could induce conformational changes in human islet amyloid polypeptide *in vitro*. We have shown that glycerol has a dual effect on islet amyloid polypeptide folding. Glycerol had inhibitory and stimulatory roles on the peptide folding and this effect was dose dependent.

REFERENCES

Anelli T, Alessio M, Mezghrani A, Simmen T, Talamo F, Bachi A, Sitia R (2002). ERp44, a novel endoplasmic reticulum folding assistant of the thioredoxin family. EMBO. J., 21: 835-844.

Bathaie SZ, Nobakht BB, Mirmiranpour H, Jafarnejad A, Moosavi-Nejad SZ (2011). Effect of chemical chaperones on glucose-induced lysozyme modifications. Protein J., 30(7): 480-489.

Brender JR, Salamekh S, Ramamoorthy A (2012). Membrane Disruption and Early Events in the Aggregation of the Diabetes Related Peptide IAPP from a Molecular Perspective. Acc. Chem. Res., 45(3): 454-462.

Carrell RW, Lomas DA (1997). Conformational disease. Lancet, 350: 134-138.

Cummings DE, Overduin J (2007). Gastrointestinal regulation of food intake. J. Clin. Invest., 117: 13-22.

Cumming RC, Andon NL, Haynes PA, Park M, Fischer WH, Schubert D (2004). Protein disulfide bond formation in the cytoplasm during oxidative stress. J. Biol. Chem., 279: 21749-21758.

Das KC, Misra HP (2004). Hydroxyl radical scavenging and singlet oxygen quenching properties of polyamines. Mol. Cell. Biochem., 262(1-2): 127-133.

DeToma AS, Salamekh S, Ramamoorthy A, Lim MH (2012). Misfolded proteins in Alzheimer's disease and type II diabetes. Chem. Soc. Rev., 41(2): 608-621.

Fujisawa S, Kadoma Y (2005). Kinetic evaluation of polyamines as radical scavengers. Anticancer Res., 25(2A): 965-969.

Fassio A, Sitia R (2002). Formation, isomerisation and reduction of disulphide bonds during protein quality control in the endoplasmic reticulum. Histochem. Cell. Biol., 117: 151-157.

Ha HC, Sirisoma NS, Kuppusamy P, Zweier JL, Woster PM, Casero Jr. RA (1998). The natural polyamine spermine functions directly as a free radical scavenger. Proc. Natl. Acad. Sci. USA., 95(19): 11140-11145.

Hayden MR, Tyagi SC, Kerklo MM, Nicolls MR (2005). Type 2 diabetes mellitus as a conformational disease. JOP., 6(4): 287-302.

Kopito RR, Ron D (2000). Conformational disease. Nat. Cell. Biol., 2(11): E207-E209.

Lee HJ, Choe YH, Lee JH, Sohn YB , Kim SJ (2011). Delayed response of amylin levels after an oral glucose challenge in children with Prader-Willi syndrome, 52(2): 257-262.

Lutz TA (2010). Roles of amylin in satiation, adiposity and brain development**.** Forum. Nutr., 63: 64-74.

Mirhashemi SM, Aarabi MH (2011a). To determine the possible roles of two essential trace elements and ascorbic acid concerning amyloidal beta-sheet formation in diabetes mellitus. Sci. Res. Essays, 6(26): 5507-5512.

Mirhashemi SM, Shahabaddin ME (2011b). Evaluation of aluminium, copper, and selenium effects on human islet amyloid polypeptide hormone aggregation. Pak. J. Biol. Sci., 14(4): 288-292.

Mirhashemi SM, Aarabi MH (2012c). To Evaluate Likely Antiamyloidogenic Property of Ferulic acid and Baicalein against Human Islet Amyloid Polypeptide Aggregation, *in vitro* Study. Afr. J. Pharm. Pharmacol., 6(9): 671-676.

Naqshbandi M, Harris SB, Esler JG, Antwi-Nsiah F (2008). Global complication rates of type 2 diabetes in Indigenous peoples: a comprehensive review. Diabetes Res. Clin. Pract., 82: 1-17.

Rhoades E, Agarwal J, Gafni A (2000). Aggregation of an amyloidogenic fragment of human islet amyloid polypeptide. Biochim. Biophys. Acta., 1476: 230-238.

Ryu S, Ornoy A, Samuni A, Zangen S , Kohen R (2008). Oxidative stress in Cohen diabetic rat model by high-sucrose, low-copper diet: Inducing pancreatic damage and diabetes. Metabolism, 57: 1253-1261.

Sava IG, Battaglia V, Rossi C A, Salvi M, Toninello A (2006). Free radical scavenging action of the natural polyamine spermine in rat liver mitochondria. Free Radic. Biol. Med., 41: 1272-1281.

Schoneich C (2005). Methionine oxidation by reactive oxygen species: reaction mechanisms and relevance to Alzheimer's disease, Biochim. Biophys. Acta, 1703: 111-119.

Shoval H, Lichtenberg D, Gazit E (2007). The molecular mechanisms of the anti-amyloid effects of phenols. Amyloid, 14: 73-87.

Soong R, Brender JR, Macdonald PM, Ramamoorthy A (2009). Association of highly compact type II diabetes related islet amyloid polypeptide intermediate species at physiological temperature

revealed by diffusion NMR spectroscopy. Am. Chem. Soc., 131(20): 7079-7085.
Wiltzius JJW, Sievers SA, Sawaya MR , Eisenberg D (2009). Atomic structures of IAPP (amylin) fusions suggest a mechanism for fibrillation and the role of insulin in the process. Protein Sci., 18(7): 1521-1530.
Zheng X, Ren W, Zhang S, Liu J, Li S (2010). Serum levels of proamylin and amylin in normal subjects and patients with impaired glucose regulation and type 2 diabetes mellitus. Acta. Diabetol., 47(3): 265-270.