JOP. J Pancreas (Online) 2005; 6(4):287-302.

HIGHLIGHT ARTICLE

Type 2 Diabetes Mellitus as a Conformational Disease

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Summary

Conformational diseases are conditions that arise from the dysfunctional aggregation of proteins in non-native conformations. Type 2 diabetes mellitus can be defined as a conformational disease because a constituent beta cell protein, islet amyloid polypeptide, undergoes a change in tertiary structure followed by self-association and tissue deposition. Type 2 diabetes mellitus is associated with multiple metabolic derangements that result in the excessive production of reactive oxygen species and oxidative stress. These reactive oxygen species set in motion a host of redox reactions which can result in unstable nitrogen and thiol species that contribute to additional redox stress. The ability of a cell to deal with reactive oxygen species and oxidative stress requires functional chaperones, antioxidant production, protein degradation and a cascade of intracellular events collectively known as the unfolded protein response. It is known that beta cells are particularly susceptible to perturbations in this quality control system and that reactive oxygen species play an important role in the development and/or progression of diabetes mellitus. Oxidative stress and increased insulin production contribute to endoplasmic reticulum stress, protein misfolding, and induction of the unfolded protein response. As the cell's quality control system becomes overwhelmed, conformational changes occur to islet amyloid polypeptide intermediates, generating stable oligomers with an anti-parallel crossed beta-pleated sheet structure that eventually accumulate as space-occupying lesions within the islets. By approaching type 2 diabetes mellitus as a conformational disease in which there is a structural transition from physiological protein to pathological protein, it is possible that the relentless nature of disease progression can be understood in relation to other conformational diseases.

Introduction to Conformational Diseases

Conformational diseases occur when an endogenous protein undergoes a change in shape that leads to self-association of these proteins and tissue deposition [1]. In the course of normal protein biosynthesis, misfolding does occur, and intracellular mechanisms have evolved to shuttle and degrade these aberrant proteins/polypeptides [2]. Although conformational changes occur with normal protein processing, a particular protein's susceptibility to aggregation, and a genetic or environmental predisposition to disease may overwhelm the cell's quality control mechanisms. In the setting of significant and sustained endoplasmic reticulum (ER) stress, these quality control mechanisms prove insufficient. High concentrations of mutant protein lead to

aggregation and slow deposition into tissues over time. This time-requiring sequence of events may partially explain the relatively late clinical presentation of many conformational diseases.

Table 1 is adapted from Carrell and Lomas's original disease classification [1] and illustrates the wide array of recognized conformational diseases. These diseases arise from secondary or tertiary structural changes within constituent proteins, with subsequent aggregation of those altered proteins. For example, in alpha$_1$-antitrypsin deficiency, a single amino acid substitution results in the destruction of a salt bridge that affects the secondary structure of alpha$_1$-antitrypsin [3]. This perturbation leads to a molecular interaction between the A sheet of one

Table 1. Conformational diseases (adapted from [1]).

Protein aggregate	Clinical disease
Serpins	Alpha$_1$-antitrpysin-deficiency C1-inhibitor deficiency angioedema Antithrombin deficiency thromboembolic disease
Prion	Kuru Creutzfeld-Jakob disease/scrapie Bovine spongiform encephalopathy Gerstmann-Straussler-Scheinker disease Fatal familial insomnia
Glutamine repeats	Huntington's disease Spinocerebellar ataxia Machado-Joseph atrophy Dentato-rubro-pallidoluysian atrophy
Tau hemoglobin	Frontotemporal dementia Sickle cell anemia Unstable hemoglobin inclusion-body hemolysis Drug-induced inclusion body hemolysis
Alpha-synuclein	Parkinson's disease
Systemic amyloides	
Immunoglobulin light chain	Systemic AL amyloidosis Nodular AL amyloidosis
Serum amyloid A protein	Systemic AA amyloidosis
Beta$_2$ microglobulin	Prostatic amyloid Hemodialysis amyloidosis
Cystatin C	Hereditary (Icelandic) cerebral angiopathy
Huntingtin	Huntington's disease
Apolipoprotein A1	Familial visceral amyloid Familial amyloid polyneuropathy
Lysozyme	Familial visceral amyloidosis
Transthyretin	Senile systemic amyloidosis Familial amyloid neuropathy Familial cardiac amyloid
Localized amyloidoses	
Abeta	Alzheimer's disease
Beta-amyloid peptide	Down's syndrome
Procalcitonin	Medullary carcinoma thyroid
Islet amyloid polypeptide (IAPP)	Type 2 diabetes mellitus (T2DM)

molecule with the reactive center loop of another [4]. This polymerization results in an accumulation of this enzyme in the ER, activation of the unfolded protein response (UPR) (described below) and ultimately, apoptosis. In the prion diseases Kuru and Creutzfeld-Jakob disease, proteins that have primarily helical structure convert to a beta-pleated sheet configuration [5]. In fact, conformational diseases often feature a protein that aggregates in beta-sheet linkages. Beta-pleated sheets are formed by alternating peptide strands that are linked by hydrogen bonding between their aligned pleated structures. This is a feature of the systemic amyloidoses, neurodegenerative diseases and type 2 diabetes mellitus (T2DM). The diverse clinical presentations of these diseases, as well as the fact that some are almost solely rooted in genetic deficiencies (e.g., Huntington's disease) whereas others such as T2DM can have a relatively strong environmental component (obesity), may seem to controvert the single grouping of them on the basis of conformational abnormalities. However, one utility of the designation 'conformational disease' is that it denotes the mechanisms underlying the sometimes odd and delayed presentation of these diseases. Although fibril formation is a defining feature of these diseases, they are composed of different aggregated proteins, sharing structural properties. Another reason for giving diseases this label is that it suggests common avenues of therapy. It is intriguing to consider that the manipulation of protein structure and/or aggregate assembly can be a platform for the development of novel therapies. For example, processes that result in the tissue deposition of beta-pleated sheets can be inhibited by compounds such as glycosaminoglycan mimetics.

Treating T2DM as a conformational disease does not imply that the disease begins with or can be holistically described as inappropriate protein deposition. Other associated processes, such as the development of insulin resistance, require other models to explain disease pathogenesis. Like Alzheimer's disease, where brain amyloid represents the culmination of multiple previous events and cannot wholly explain cognitive deficits, so in T2DM, islet amyloid is both cause and consequence of several disease processes. The sole purpose of this review is to interpret how protein abnormalities can be understood in the context of other conformational disease processes and specifically how they could arise in T2DM.

Islet Amyloid: The Conformational Problem of T2DM

The contribution of islet amyloidosis to disease pathogenesis has been vigorously debated [6]. Islet amyloid polypeptide (IAPP) oligomers that precede islet amyloid deposition are likely more toxic to beta cells than islet amyloid itself [7]. Islet amyloid is present at autopsy in as many as 96% of patients with T2DM [8]. With accumulations of toxic, misfolded IAPP oligomers and deposition of crossed beta-pleated sheets, T2DM is similar to other protein conformational diseases. It is interesting to note that the human, feline, and non-human primate forms of the IAPP molecule are known to be amyloidogenic, and these are the only members of the animal kingdom that develop spontaneous T2DM. Lower mammals, on the other hand, do not share this feature of having amyloidogenic IAPP due to proline substitutions at positions 25, 28, and 29, and they do not develop spontaneous T2DM [9, 10]. Most animal models of insulin resistance do not feature islet amyloid except for transgenic mice that express human IAPP

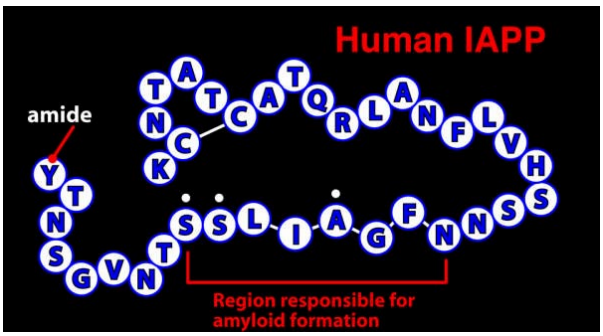

Figure 1. Human islet amyloid polypeptide (IAPP). The amyloidogenic region of IAPP is responsible for providing a toxic conformational structure within islets. Note disulfide bond at position C2 and C7.

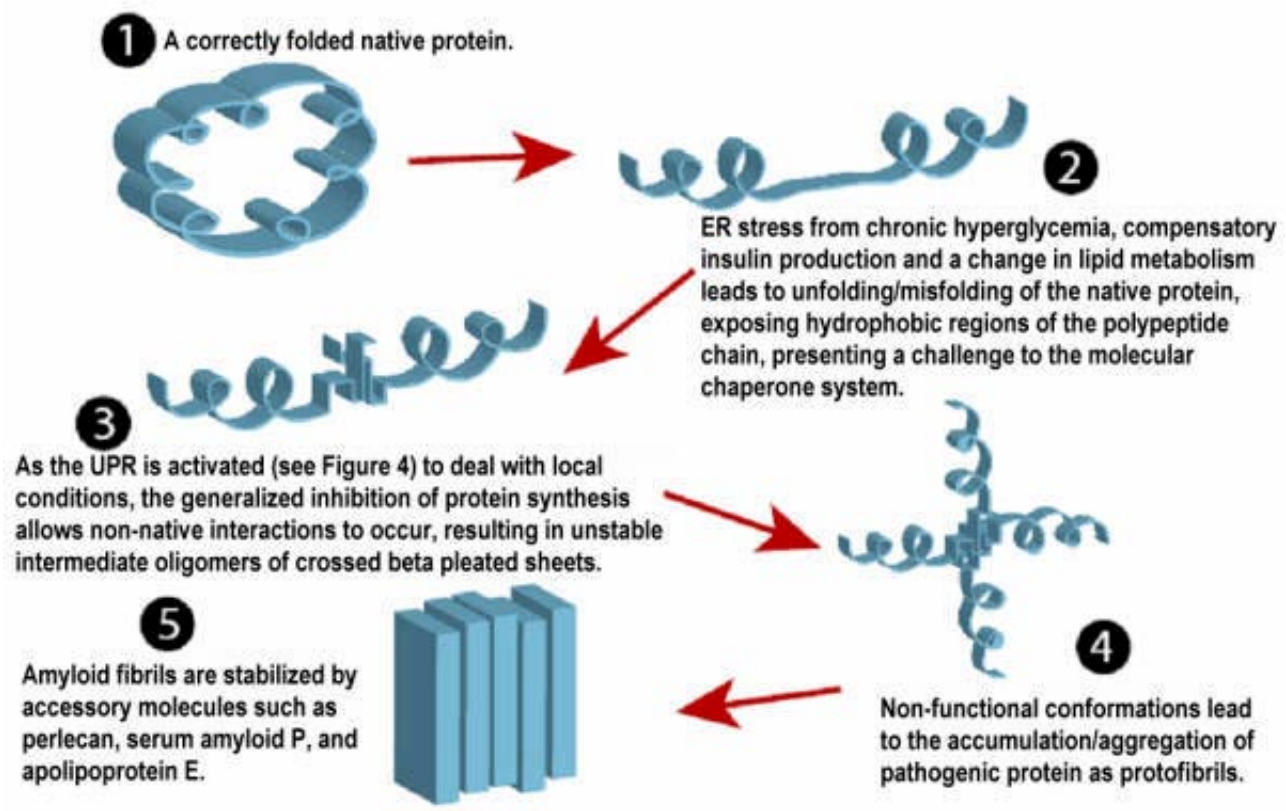

Figure 2. Improper folding of islet amyloid polypeptide (IAPP) results in insoluble fibrils.

[11]. Clinically, it is clear that aggregates of misfolded IAPP are a prominent pathological feature in the development of T2DM (reviewed in [12]). The human IAPP polypeptide is presented in Figure 1.

Proteins must properly fold into three-dimensional structures in order to carry out their proper functions within the cell and organism. One model of dysfunctional protein aggregation represented in Figure 2 involves the following intracellular events: 1) misfolding or unfolding of native protein exposes hydrophobic regions; 2) conformational changes result in unstable intermediates that have a propensity to form oligomers; 3) oligomers form pathogenic subunits and crossed beta-pleated sheets; and 4) in the case of T2DM, amyloid fibrils are formed with subsequent stabilization by accessory molecules, such as serum amyloid P, perlecan, and apolipoprotein E [6]. When precision folding goes awry, the misfolded, soluble oligomeric proteins begin to accumulate, become toxic, and promote apoptosis [7, 13]. Misfolded IAPP stabilizes into crossed beta-pleated formations that are deposited within the adjacent surrounding extracellular matrix, resulting in space-occupying lesions within the islets of the pancreas. The following discussion outlines cellular stressors in T2DM that contribute to protein misfolding and aggregation. One of the most important stressors leading to these protein conformation abnormalities is redox stress (discussed later in the review).

Once unfolded, IAPP may become refolded in the ER-Golgi complex, accompanied by the support of ATP-dependent chaperone proteins. Kinetic refolding experiments using intermediate proteins associated with known conformational diseases have revealed that there is a higher energy requirement to achieve successful refolding due to the increased exposure of hydrophobic regions in unfolded or partially-folded proteins. Therefore, exposed hydrogen ions may cause a folding pathway to produce a relatively stable intermediate form of protein that is 'kinetically trapped' if the ER cannot overcome this higher energy barrier [14].

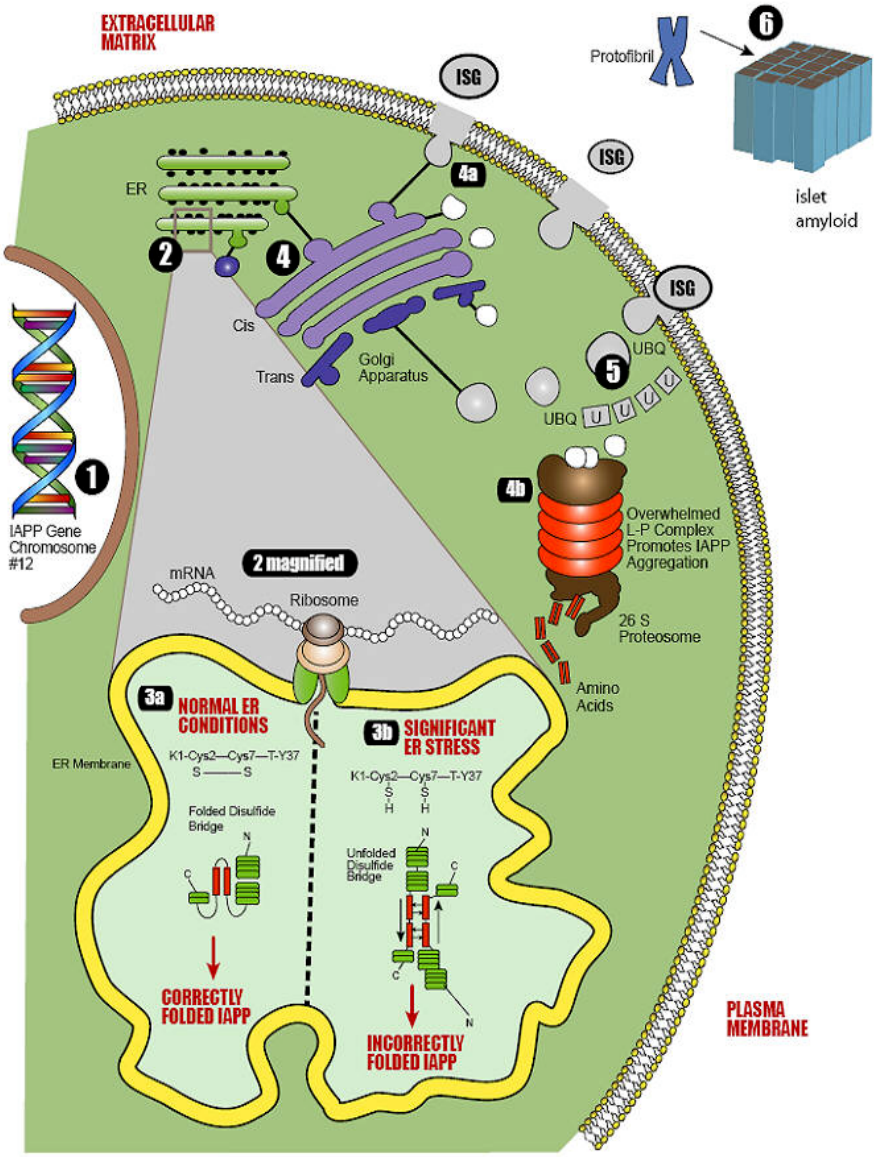

Figure 3. Islet amyloid polypeptide (IAPP) misfolding leads to protein aggregates. This cartoon depicts the endoplasmic reticulum (ER), Golgi apparatus, and the lysosome-proteasome complex in relation to the unfolding and misfolding of IAPP. IAPP is transcribed from chromosome 12 (1). Translation of IAPP gene occurs (2). In the absence of significant ER stress, chaperones are able to properly fold IAPP. Post-translational modifications of IAPP include the formation of a disulfide bond at positions C2 and C7, as well as amide formation at the C-terminal tyrosine. The vulnerability of the disulfide bond may play an important role in the unfolding of IAPP (3a) or in the presence of significant ER stress, IAPP may become unfolded and misfolded (3b). IAPP oligomers may form. IAPP is transported to the Golgi apparatus (4). Normal processing in the absence of significant ER stress results in cosecretion of normal insulin and IAPP in insulin secretory granules (4a). Once unfolded and misfolded, aberrant IAPP is processed initially in the Golgi apparatus, and there is an additional attempt to refold the misfolded protein. If this is unsuccessful, the misfolded protein then goes to the lysosome-proteasome complex for degradation to its constituent amino acids (4b). Ubiquitination pathways are also employed to facilitate trafficking to the lysosome-proteasome complex (5). When these organelles are overwhelmed, as occurs in early T2DM before beta cell failure, the result will be apoptosis of the beta cell and the accumulation and aggregation of protofibrils into beta-pleated sheets. Subsequently, islet amyloid is formed (6).

When this quality control system is overwhelmed and IAPP is not capable of being correctly refolded, this protein can become a soluble toxic monomer due to the innate amyloidogenic properties of the NFGAILSS region of IAPP in amino acid positions 22-29 [15].

Soluble IAPP oligomers have been shown to be cytotoxic and possibly responsible for beta cell apoptosis in T2DM [7, 16, 17]. Additionally, beta cells with a high turnover (replication) rate have been found to be more susceptible to apoptosis by IAPP oligomers [17]. It is the delicate balance between refolding and degradation, maintained by the quality control system that determines the amount of mutant protein allowed to accumulate. The adaptive and apoptotic mechanisms of the quality control system are so selective that even minor perturbations in protein folding efficiency can cause the rejection of nascent IAPP proteins and, consequently, their accumulation or degradation. Accumulation of mature islet amyloid is responsible for the space-occupying lesion with associated secretory and absorptive defects within the islet and is accelerated by free radical polymerization due to reactive oxygen species (ROS). These concepts are summarized in Figure 3.

Table 2. Putative model of stages of type 2 diabetes mellitus (T2DM) considered as a conformational disease.

STAGE I (Latent period)
Increased production of reactive oxygen, nitrogen and thiol species
Beta cell endoplasmic reticulum (ER) stress
Compensatory insulin processing
Protein misfolding/unfolding
Unfolded protein response (UPR) activation/chaperone challenge
Impaired first phase insulin secretory response
Prolific free radical polymerization of islet amyloid polypeptide (IAPP) monomers
STAGE II (Transition period)
Ongoing redox stress
Islet amyloid polypeptide (IAPP) oligomerization/fibril formation
Impaired insulin secretory response
Early beta cell apoptosis
Beta cell protein quality control severely challenged
STAGE III (Impaired glucose tolerance period)
Appearance of advanced glycation endproducts (AGEs)
50-75% amyloid involvement in islet architecture
Impaired beta cell function
STAGE IV (Impaired fasting glucose period)
Increasing global insulin resistance
Increased fasting blood glucose levels
Excess hepatic and renal gluconeogenesis
Progressive amyloid deposition
STAGE V (Overt type 2 diabetes mellitus)
50% loss of beta cell function
75-100% amyloid deposition

Researchers have also addressed whether genetic differences in IAPP could predispose to T2DM development. To date, only one missense mutation has been identified in human IAPP. It is the S20G mutation (glycine is substituted for serine at position 20), and this change in the amino acid sequence results in more rapid amyloid formation and early onset T2DM in Japanese, Korean, Chinese and New Zealand Maori populations (1.9-2.6% of subjects studied thus far) [18, 19, 20, 21, 22, 23, 24, 25]. Although this mutation is restricted to the ethnic groups mentioned, its existence points to the possibility of it being more widespread and that other mutations might contribute to the amyloidogenic properties of IAPP.

Under adverse conditions, and due to its intrinsic conformational instability, the normally soluble IAPP protein quite readily undergoes the structural change to form the crossed beta pleated sheet necessary for aggregation. Due to its propensity to aggregate, IAPP is able to endure free radical polymerization; a process that is further promoted through the cell's decreased ability to clear misfolded proteins. This is the very beginning of the pre-diabetic condition [26]. Once formed, islet amyloid is quite resistant to the normal proteolytic defenses within the body and is therefore allowed to accumulate and undergo an even more rapid free radical polymerization in an islet milieu of increased reactive oxygen species (ROS) [27, 28]. The question arises, how do inherent beta cell characteristics contribute to the development of abnormally folded proteins which culminate as islet amyloid?

Beta Cell and ER Stress

As is true for other cells performing protein synthesis, beta cells regulate the production of their synthesized protein indirectly via glucose sensors and not directly via the levels of insulin which they produce. These same glucose sensors act on molecular pathways in other endocrine cells that maintain glycolysis

and nutrient homeostasis. Thus, transcription in beta cells is not regulated by insulin itself but by translational and post-translational events that are themselves regulated by extracellular glucose levels [29, 30, 31]. Chronic activation of the beta cells' quality control system favors the induction of more apoptotic pathways of the UPR [2] which is described in greater detail later in this review.

The islet beta cell is known to have a highly developed ER, apparently due to an excessive demand for compensatory insulin secretion [32]. An increased demand for insulin secretion may result in beta cell overload ultimately leading to deficient insulin secretion. Beta cell mass (both number and volume of cells) is reduced in the later phases of T2DM as a result of apoptosis, especially in rapidly replicating beta cells [33]. The toxic effects of oligomeric IAPP result in apoptosis of the beta cells, but for a period of time, the more primordial ductal cells of the exocrine pancreas (replicative pool) can replace the damaged, apoptotic beta cells and continue the compensatory hyperinsulinemia causing further beta cell damage. These effects culminate in the development of a defective diffusion barrier within the islet [34]. Table 2 presents a putative model of how accumulations of IAPP-derived protein aggregates may relate to T2DM pathogenesis.

The beta cell ER has unique responses to unfolded or misfolded proteins [32]. The first response is up-regulation of genes encoding antioxidants and ER chaperone proteins, such as BiP/GRP78 and GRP94, to increase protein folding activity and prevent protein aggregation [35, 36, 37]. The second response consists of translational attenuation to reduce the load of new protein synthesis and prevent further accumulation of unfolded proteins. The third is degradation of misfolded proteins in the ER (endoplasmic reticulum-associated degradation, ERAD) [35]. The misfolded proteins are transported from the ER to the cytosol, where most are tagged with ubiquitin-conjugating enzymes for degradation by the 26S proteasome, as well as the lysosome [38]. The fourth is transcriptionally-activated apoptosis, which occurs when the ER is chronically overwhelmed and its function has been severely impaired [35]. These mechanisms will be described in greater detail in a forthcoming section of this review.

Insulin Secretory Granule in a Conformational Disease

Post-translational processing of pro-IAPP in the insulin secretory granule (ISG) yields the soluble, functional IAPP hormone. The same prohormone convertases 1, 2 and 3 process pro-IAPP and pro-insulin, and cosecrete the cleaved forms of both into the circulation [39]. It has been proposed that a balance of these ISG components, including C-peptide, Ca^{2+} and Zn^{2+}, contributes to maintaining IAPP and insulin in their mature native conformations, thereby hindering aggregate formation [40]. The authors of this study conjectured that if these factors are in an inappropriate concentration, conditions could favor IAPP aggregation. Further, in T2DM, alterations in the proportions of insulin and IAPP in granules could favor fibril formation [41]. Preserving a normal physiologic ratio of proinsulin to insulin in ISGs disfavors fibril formation and beta-pleated sheet formation of IAPP [41]. Several studies have demonstrated, in both human and animal models of T2DM and fasting hyperglycemia, a disproportionate ratio of proinsulin to insulin, relative to the overall increase of both in plasma concentrations [42, 43, 44, 45, 46]. Whether this increase in proinsulin is due to chronic hyperglycemia or impaired glucose tolerance, the resulting disturbance to the proper functioning of the ISG could exacerbate protein misfolding. The abnormal processing of proIAPP with incomplete conversion to IAPP could result in increased IAPP-derived islet amyloid deposition, as proIAPP is also amyloidogenic [40, 47, 48]. Thus, incomplete processing of proinsulin and proIAPP could each present a mode for increased aggregation of misfolded proteins.

The glycosaminoglycan called perlecan is a ubiquitous part of beta cell's basement membrane, synthesized in the islets [49].

Although not a structural element, perlecan does provide stability to amyloid fibrils by allowing binding of IAPP to the basement membranes surrounding islet capillaries. This pathologically promiscuous binding decreases the secretory response of the ISG as a result of an adsorptive barrier created by thickened basement membranes. This condition may well be the beginning of structural transformations within the islet which provides a located environment with a predilection toward a disproportionate ratio of IAPP to insulin secretion.

Chaperones and Conformational Disease

Molecular chaperones are ubiquitous, highly conserved small proteins present in all eukaryotic cells (reviewed in [50]). As noted above, their overall purpose is to minimize aggregation by assisting target proteins, such as IAPP, in proper folding and to covalently transport functional proteins across extracellular space. Under conditions of sustained redox stress, the ability of chaperones to regenerate the redox potential of the cell is compromised due to the increased occupation of chaperones by nascent polypeptides (reviewed in [51]). Consequently, protein misfolding occurs, amplifying the chaperone requirements, and in the case of T2DM, causes islets to be more susceptible to the deleterious effects of redox stress.

In most conformational diseases studied, the soluble, partially-folded intermediates contain an area of exposed hydrophobic regions that are, in the protein's native state, buried and protected against non-native interactions. These areas of increased hydrophobicity have been implicated in allowing non-native interactions to occur that result in the crossed beta-pleated sheet structure seen in protein aggregates [14] and Figure 2. Thus, it becomes necessary to overcome a higher energy barrier in order for the folding process to continue to completion. Chaperones assure that the correct stoichiometric amounts of folding co-factors are present so that these non-native isoforms can achieve their functional quaternary structure. As very important components of the quality control repertoire, the cell dedicates a substantial amount of metabolic energy to performing chaperone functions.

The Unfolded Protein Response (UPR) and the Balance of Quality Control Mechanisms

An accumulation of misfolded/unfolded polypeptides in the ER of cells presents a challenge to chaperones in the cell [52, 53]. Due to prolonged interactions with these mutant proteins, chaperones are challenged to fulfill their folding duties in a timely manner. The overall function of molecular chaperones is to minimize protein aggregation by ensuring proper protein folding and providing transport to target proteins through covalent cross-linking [54] (see section below). Under challenge, a process known as the UPR recruits existing proteases and ubiquitination enzymes to help deal with this accumulation [55, 56]. As a point of fact, in normal cells, many of the substrates for proteases 'are' misfolded proteins, reflecting the importance of conformation in determining protein selection for degradation.

After existing proteases have been consumed, the UPR will induce survival and apoptotic pathways in response to the particular stress. Survival responses include transcriptional regulation (antioxidant and chaperone production) and translational regulation (protein synthesis inhibition). Apoptotic responses of the UPR include protease synthesis (26S proteasome), ubiquitin-conjugating enzymes, and caspases (in the case of chronic stress/severe ER impairment) [57, 58, 59]. The integrity of the quality control system is of paramount importance at this step. Maintaining a balance between folding and degradation determines the amount of mutant protein that can accumulate and potentially cause conformational diseases [1]. An imbalance in the quality control system created by, among other things, abnormal temperatures, high/low glucose concentrations, glycosylation inhibitors,

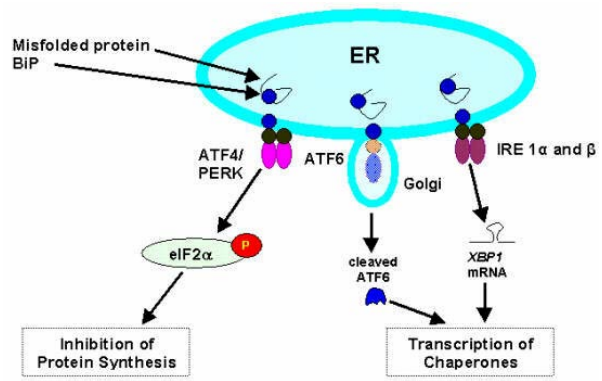

Figure 4. The unfolded protein response (UPR) to endoplasmic reticulum (ER) stress. The UPR is a particularly important cellular response for cells that must accommodate high loads of secretory proteins like the beta cell. The UPR is principally mediated by ATF4/PERK, ATF6 and IRE which are activated by the abundant ER chaperone BiP. Misfolded proteins activate IRE1 and PERK by phosphorylation. IRE1 is a kinase that contains an ER regulatory domain and an RNaseL domain. IRE1 activation leads to upregulation of XBP1 which subsequently activates a family of genes encoding ER chaperones. PERK activation results in the phosphorylation of eIF2alpha that leads to a generalized inhibition of translation initiation. Finally, ATF6, a leucine-zipper transcription factor, transits into the Golgi following activation where it is cleaved into an active transcription factor for chaperones.

influx of Ca^{2+} ions, alterations in local pH, or redox stress will cause further accumulation of misfolded/unfolded proteins.

In the setting of hyperinsulinemia, the beta cell likely has significantly increased ER activity and can become stressed in responding to the demands of the peripheral tissues to prevent hyperglycemia [32]. Previously, this process has been referred to as 'beta cell fatigue', but a more encompassing explanation can invoke ER stress and an overwhelmed UPR. The basic pathways of the UPR have been exhaustively explored in recent years [60]. The significance of these studies is that they can partly explain how a stressed cell becomes committed to survival or to apoptosis [57]. The UPR is initiated under various conditions of stress which compromise protein folding in the ER. Three major survival pathways are employed as the transcriptional response to this ER stress (Figure 4). Each pathway involves an ER-resident transmembrane protein that senses ER stress or the presence of unfolded proteins: IRE1, ATF4/PERK and ATF6. IRE1 and ATF4/PERK, which both have cytoplasmic serine/threonine kinase domains, are activated by ER stress and undergo homodimerization and phosphorylation [61, 62, 63]. The accumulation of inappropriately folded proteins in the ER lumen results in ATF6 translocation to the ER [55]. ATF6 cleaves its cytosolic domain then translocates to the nucleus to activate transcription of the chaperones GRP78 (BiP) and other co-factors of ER stress target genes [64]. The downstream sequelae of the UPR are transcriptional activation of chaperones, antioxidants, co-factors and regulators involved in ER-associated protein degradation (i.e. ERAD) and inhibition of new protein synthesis. These responses presumably conserve cellular resources in the face of increasing stress. Activation of UPR pathways is also intrinsically important in the initiation of proapoptotic responses (protease and caspase synthesis). Chaperone participation in these responses, as well, provides an additional quality control mechanism. However, the means by which cells commit to apoptosis rather than survival are less well understood.

Redox Stress in the Formation of Islet Amyloid

1. Reactive Oxygen Species (ROS)

Multiple biochemical pathways and mechanisms of action have been implicated in the deleterious effects of chronic hyperglycemia and oxidative stress on the function of the kidney, retina, vascular tissues and, to a lesser extent, pancreatic islets in T2DM [65]. There is currently wide acceptance for the destructive potential of oxidative stress on the islets and arterial vessel walls in patients with T2DM. The damaging effects of ROS and other free radicals on proteins, nucleic acids and fats is key to a better understanding of the formation of amyloid within the islets of patients with

T2DM. Excessive redox stress may lead to protein accumulation and aggregation in the ER, with severe consequences for the cell [66].

ROS may impact disulfide bond formation [67] and subsequently influence the development of IAPP misfolding. Disulfide bonds formed in newly synthesized proteins in the ER of cells are important for proper protein folding, protein structure, biological activity, and stability of many secreted and membrane proteins [66, 68, 69]. Protein folding in eukaryotes takes place in the ER with assistance from many redox-sensitive chaperones and oxidoreductases (e.g., protein disulfide isomerase, ERp44, ERp72, ERp57, GRP58, Hsp33) [69]. The effects of excessive ROS on native IAPP within the ER may cause covalent breakage of the disulfide bond at positions Cys_2 and Cys_7 in this 37 amino acid polypeptide, allowing it to unfold or preventing it from properly folding.

ROS may have an effect on both proteins and lipids within the ER by altering the ER bi-lipid membrane [70]. Hyperglycemia may have an additive effect by altering the protein content of the ER membrane through formation of advanced glycation endproducts (AGEs) [71]. Oxidative stress, combined with hyperglycemia, has been shown to alter a protein's susceptibility to glycation (the process of forming irreversible Amadori products from reversible Schiff bases with non-enzymatic rearrangement reactions) [72, 73]. These AGEs, which are prone to cross-linking and aggregation, can modify IAPP through post-translational attachments [74]. Upon proteoglycan binding, there is an observed increase and acceleration in total amyloid fibril formation. Amadori products are currently used as a clinical marker of glucose control because they exist in equilibrium with glucose levels [75]. AGEs, on the other hand, become irreversibly bound to protein. It is this long-term consequence of glycation, leading to the formation of AGEs that can take years to complete and can be detrimental to the patient with diabetes.

These processes (oxidation and glycation) may contribute to a dysfunctional ER membrane, allowing the abnormal leakage of misfolded proteins into the cytosol before they are properly folded into their native 3-dimensional conformation. This same ER membrane leak may also allow the influx of ROS into the ER lumen, disrupting the redox-sensitive milieu within, and allowing for an even greater unfolding and misfolding of proteins to occur.

2. Reactive Nitrogen Species (RNS)

RNS are increased in T2DM [76] and could contribute to protein misfolding. Growing evidence implicates both ROS and RNS (such as the reaction of superoxide anion (O_2^-) with nitric oxide (NO) to form peroxynitrite and other RNS) as important molecules in the development of diabetes [77, 78, 79, 80, 81]. In other conformational diseases, such as Alzheimer's disease and Parkinson's disease, abnormal NO production is involved in protein misfolding leading to aggregates and proteasome dysfunction on ubiquinated material [82]. Peroxynitrite is an RNS important in the evolution of diabetes [76]. Peroxynitrite reacts relatively slowly with most biological molecules and as a result becomes a potent selective oxidant. Peroxynitrite specifically modifies tyrosine in proteins/polypeptides to create nitrotyrosine, which leaves an indelible footprint detectable in vivo. Nitrotyrosine and nitrosylated arginine (nitroarginine), known biomarkers of redox stress, are capable of competing with the natural substrate L-arginine for the production of endothelial nitric oxide (eNO) via the endothelial nitric oxide synthase (eNOS) reaction [83]. The presence of RNS in the plasma of diabetic patients suggests a possible involvement of peroxynitrite in the development of diabetic complications [84]. Could RNS-induced protein modifications also increase the propensity for IAPP to become misfolded? Post-translational events, such as nitrosylation, can affect the 3-dimensional configuration of proteins [85]. Nitrosylation of various amino groups could therefore result in prevention of the proper folding of IAPP.

3. Reactive Thiol Species

Although thiols are traditionally viewed as non-enzymatic antioxidants, reactive thiol species may be yet another consequence of redox stress that promotes conformational disease. In all antioxidant reactions employing thiols, thiyl radicals are simultaneously formed from the reduction of the disulfide bridges and subsequent oxidation of the sulfhydryl groups [86]. Maintaining a balance in the redox state of the cell ensures that thiols can continue their biological action as necessary antioxidants, and, just as importantly, that these thiyl radicals are efficiently reduced to thiols again. An elevated and sustained tension of redox stress in the ER, such as with T2DM, has the potential to disrupt this delicate balance, initiating a process termed 'disulfide reshuffling', in which newly synthesized polypeptides undergo disulfide rearrangement with free thiol groups [86]. These thiol/disulfide exchange reactions promote polymerization of other amyloidogenic proteins such as prion protein PrP found in spongiform encephalopathies [87] and can therefore presumably stabilize aggregates of other proteins, considering the generally reducing environment of the cytoplasm. Hence, not only are IAPP aggregates stabilized by the extensive non-covalent hydrogen bonding of crossed beta-sheet formation, but also this conformational change may provide covalent protection to intermolecular disulfide bonds, hindering any attempt of the UPR at aggregate disassembly. This concept lends support to the widely held belief that amyloid fibrils form via a nucleation-dependent kinetic process [6, 9, 88].

4. Redox Stress in T2DM: Conclusion

The beta cell is poorly equipped to handle redox stress as compared to other cells such as hepatocytes [76, 83], and this very sensitivity has allowed researchers to use the oxidizing agents streptozotocin and alloxan to create diabetic animal models. Not only is the islet inundated with ROS but also the beta cell within is known to be deficient in the classic antioxidants to protect itself from the surrounding redox stress [65, 89]. Additionally, once overt T2DM has developed, the antioxidant reserve is known to be compromised with a systemic deficiency of catalase, superoxide dismutase, and glutathione peroxidase [90, 91, 92, 93].
Redox stress, as manifested by increased ROS, RNS and reactive thiol species, may significantly post-translationally modify IAPP to the extent that protein misfolding is favored. Additionally, these redox stressors can overwhelm the beta cell's ER folding complex, chaperone-induction signaling mechanism, lysosome-proteasome pathway and attenuate the secretory capacity of this cell [94, 95]. These effects likely result in augmented beta cell apoptosis and the accumulation of islet amyloid.

Conclusion

If T2DM is viewed as a conformational disease, it may be possible to rationally design therapies that specifically focus on the forces which lead to protein misfolding and deposition. This can include decreasing the redox stress associated with increased metabolic demand in obesity or promoting plaque destabilization. New small molecule therapeutics can modify the kinetics of amyloid formation or promote their amyloid resorption. For example, small molecule drugs can be used to stabilize the amyloidogenic protein precursor, or to act on the partially folded intermediates in the folding process, or to actually interact with mature amyloid fibrils to weaken their structural stability. Displacing important cofactors of amyloid deposits such as glycosaminoglycans and serum amyloid P component with these small molecules can favor dissolution of the fibril aggregate [96]. Antibodies can also be used to reduce the ability of an amyloidogenic protein to form partly unfolded species and can be an effective method of preventing its aggregation [97]. Thus, it is possible that in the future, as

these therapeutics are developed, it will be possible to slow or prevent the inexorable progression of disease so frequently seen in conformational disease.

Seeing T2DM as another type of conformational disease may facilitate a broader understanding of islet biology beyond the regularly understood parameters of this disease. For example, islet cell stress may lead to a form of T2DM in type 1 diabetic patients bearing islet transplants. It is known that human islets rapidly form amyloid when transplanted into immunodeficient mice [98]. In this relatively successful 'Edmonton era' of islet transplantation, the hope for a cure for type 1 diabetes is diminished by a disappointing loss of function in a significant percentage of recipients [99] which can occur only months after transplantation. It is intriguing that a foundational component of modern era transplant success resides in sustaining an optimal islet mass [100]. Could gradual loss of islet cells due to immune attack be compounded by a conformational disease akin to T2DM in limiting the long term success of islet transplants? This and other questions may be addressed if T2DM and non-autoimmune beta cell dysfunction are viewed as a conformational disease. New avenues of therapy that are directed at minimizing forces leading to deleterious accumulations of proteins may offer hope to patients at risk for T2DM.

Received February 24th, 2005 - Accepted April 6th, 2005

Keywords Amyloid; Diabetes Mellitus, Type 2; Islets of Langerhans; Islets of Langerhans Transplantation; Molecular Chaperones; Protein Conformation

Abbreviations AGE: advanced glycation endproduct; eNO: endothelial nitric oxide; eNOS: endothelial nitric oxide synthase; ER: endoplasmic reticulum; ERAD: endoplasmic reticulum-associated degradation; IAPP: islet amyloid polypeptide; ISG: insulin secretory granule; RNS, reactive nitrogen species; ROS: reactive oxygen species; T2DM: type 2 diabetes mellitus; UPR: unfolded protein response

Acknowledgements This work was supported in part by National Institutes of Health grant DK-03-024 (MRN). The authors would like to thank Mike Cobb for assistance with illustrations and David Stenger for critical review of the manuscript

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