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Review

Diabetes mellitus due to the toxic misfolding of proinsulin variants

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A R T I C L E I N F O

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A B S T R A C T

Dominant mutations in the human insulin gene can lead to pancreatic β-cell dysfunction and diabetes mellitus due to toxic folding of a mutant proinsulin. Analogous to a classical mouse model (the Akita mouse), this monogenic syndrome highlights the susceptibility of human β-cells to endoreticular stress due to protein misfolding and aberrant aggregation. The clinical mutations directly or indirectly perturb native disulfide pairing. Whereas the majority of mutations introduce or remove a cysteine (leading in either case to an unpaired residue), non-cysteine-related mutations identify key determinants of folding efficiency. Studies of such mutations suggest that the evolution of insulin has been constrained not only by its structure and function, but also by the susceptibility of its single-chain precursor to impaired foldability.

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1. Introduction

Insulin plays a central role in the regulation of vertebrate metabolism. The mature hormone, the post-translational product of a single-chain precursor, is a globular protein containing two chains, A (21 residues) and B (30 residues). Advances in human genetics over the past five years have identified dominant mutations in the insulin gene associated with diabetes mellitus (DM) (for reviews, see [1–3]). This syndrome arises from the toxic misfolding: the clinical mutations impair the folding of proinsulin in the endoplasmic reticulum (ER) of pancreatic β-cells [4,5]. Originally described in relation to neonatal-onset DM [6–9], DM may also present in childhood [10] or early adulthood [11]. Age of onset is proposed to reflect mutational differences in the degree of impaired folding and its cellular consequences [5,12]. Phenotypic variation may also arise due to polygenic differences in β-cell susceptibility to ER stress [13].

Patients with the mutant proinsulin syndrome are heterozygous. Although expression of one wild-type insulin allele would in other circumstances be sufficient to maintain metabolic homeostasis, studies of a corresponding mouse model (the Akita mouse [14–16]) have demonstrated that misfolding of the variant proinsulin perturbs the biosynthesis of wild-type insulin [17,18]. In a survey of human mutations similar perturbations in trans have been demonstrated in β-cell lines [4,5,12]. Impaired β-cell secretion is associated with ER stress, distorted organelle architecture, and eventual cell death [19,20]. These findings have stimulated renewed interest in the biosynthesis of insulin [21–23] and the structural basis of disulfide pairing [24–29]. The foundational importance of this problem and its clinical relevance have motivated construction of novel molecular reagents and animal models [30–33]. Such studies have led in parallel to an enhanced understanding of structure–function relationships [13] and cellular mechanisms of proinsulin folding and insulin biosynthesis [1].

Clinical mutations in the insulin gene provide probes into evolutionary constraints shared by a family of single-chain factors (such as mammalian insulin-like growth factors) and in part by a superfamily of invertebrate insulin-like proteins [34–36]. Such relationships have motivated studies of the mitogenic properties of proinsulin, revealing preferential signaling through the A isoform of the insulin receptor (IR) [35]. Although beyond the scope of this review, these activities may be relevant to carcinogenesis [37]. Therapeutic use of proinsulin in the treatment of human DM has been investigated but would have unclear clinical benefits [38].

2. Biosynthesis of insulin

The insulin gene encodes a single-chain precursor polypeptide, designated *preproinsulin* (Fig. 1A, top). The signal peptide (gray bar in Fig. 1A) is cleaved on translocation into the ER to yield *proinsulin*.

Abbreviations: DM, diabetes mellitus; ER, endoplasmic reticulum; GA, Golgi apparatus; MODY, maturity-onset diabetes of the young; NMR, nuclear magnetic resonance

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Reduced and unfolded, the translocated polypeptide contains a connecting domain (black in Fig. 1A) between the C-terminal residue of the B domain (blue) and N-terminus of the A domain (red) [39]. Its folding in the ER is coupled to the specific pairing of three cystines (Fig. 1A, center). The disulfide bridges (A6–A11, A7–B7, and A20–B19; shown in gold in Fig. 1B) contribute to protein stability and biological activity [27,40–48]. The solution structure of proinsulin as an engineered monomer consists of a folded insulin-like moiety and a flexible C domain (black segment in Fig. 1B) [49]. The overall structure is in accordance with prior biochemical evidence [50–55]. The structure, stability, and receptor-binding activity of insulin (Fig. 1A, bottom) require maintenance of each disulfide bridge [27,40–42,44–48]. Conserved among insulin-related ligands, the cystines provide interior struts (A19–B20 and A6–A11) and an external staple between chains (A7–B7). Insulin disulfide isomers exhibit molten structures of marginal stability and low activity [56–58].

Proinsulin binds only weakly to the insulin receptor; proteolytic processing liberates the mature hormone [22,38]. On transit through the Golgi apparatus (GA) and immature secretory granules [59], the C-peptide is excised by prohormone convertases [60], which cleave conserved dibasic sites flanking the C domain (BC and CA junctions; green in Fig. 1A, B). Insulin is stored as Zn^{2+}-stabilized hexamers as microcrystalline arrays [61–63] within specialized secretory granules [64]. The hexamers dissociate on secretion into the portal circulation, enabling the circulating hormone to function as a Zn^{2+}-free monomer. Because the insulin monomer is exquisitely susceptible to fibrillation at millimolar concentration and body temperature [65], its zinc-mediated assembly within β-cells may represent a defense against toxic misfolding [66] during storage in the secretory granule [23]. Evidence that such assembly contributes to insulin storage and efficient secretion has recently been obtained through studies of a mouse model lacking the β-cell zinc transporter [67].

Although insulin biosynthesis occurs via a single-chain precursor, its chemical synthesis has traditionally employed isolated A- and B-chain peptides (for review see [68]). The success of insulin chain combination implies that chemical information required for folding is contained within the sequences of the A- and B domains [69,70]. A variety of analogs have been prepared by this protocol, facilitating their pharmaceutical development for treatment of DM [71,72]. Despite the general robustness of insulin chain combination, synthesis of certain analogs has been confounded by low yields [26,73–79]. Such synthetic failures provide models of impaired folding, providing insight into structural mechanisms that underlie the mutant proinsulin syndrome.

3. Mechanism of disulfide pairing

Oxidative folding of globular proteins has traditionally been investigated by chemical trapping of populated disulfide intermediates [80]. Such studies of proinsulin and related polypeptides – foreshortened single-chain analogs (mini-proinsulin [81]) and insulin-like growth factors – are notable for the transient accumulation of one- and two-disulfide intermediates [24,25,82]. Successive steps of partial folding define a series of trajectories on a sequence of free-energy landscapes (Fig. 2A). Each landscape underlies the dynamics of an ensemble of accessible polypeptide conformations in the presence of a specific subset of disulfide bridges. The landscapes proceed from shallow to steep, enabling the folding chain to acquire structure stepwise on successive disulfide pairing. The preferred sequence of disulfide intermediates, as defined by chemical trapping, thus corresponds to a progression of multiple folding trajectories on funnel-shaped landscapes. This perspective extends the classical disulfide-centered view of protein-folding intermediates [83] to encompass general biophysical paradigms of protein folding [84–86].

Although refolding studies of proinsulin are limited by aggregation (imposing a requirement for pH conditions >9, confounding interpretation [87]), the disulfide pathways of mini-proinsulin and IGF-I are well characterized near neutral pH [24,25,40,43,82,88,89]. A structural pathway is suggested by spectroscopic studies of equilibrium models (Fig. 2B) [27,40–42,44–48,90]. In this pathway a key role is played by initial formation

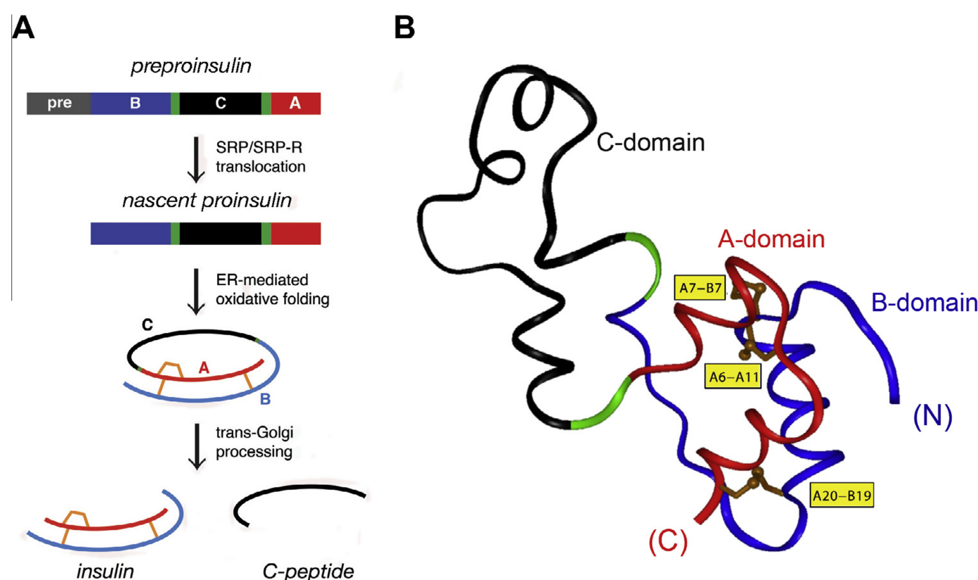

Fig. 1. Biosynthesis of proinsulin. (A) Pathway begins with preproinsulin (top): signal peptide (gray), B-domain (blue), dibasic BC junction (green), C-domain (black), dibasic CA junction (green), and A-domain (red). Specific disulfide pairing in the ER yields native proinsulin (middle panels). BC- and CA cleavage (mediated by prohormone convertases PC1 and PC2) releases insulin and C-peptide (bottom). (B) Solution structure of proinsulin: insulin-like moiety and disordered connecting peptide (dashed line). A- and B-domains are shown in red and blue, respectively; C-domain contains a nascent α-helical turn near the CA junction [49]. Cystines are labeled in yellow boxes. The solution structure exploited an engineered monomer (DKP-proinsulin) as characterized by multi-dimensional $^{1}H–^{13}C–^{15}N$ NMR. Panel A is adapted from Ref [13] panel B depicts a representative member of an ensemble of solution structures (Protein Databank entry 2KQP).

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