

Disruption of disulfide bonds as a cause of insulin resistance in D.M 2

Abstract:

Background:

Many theories have been put forward to explain insulin resistance in D.M-2, still it remained an enigma till date. Disruption of the insulin ligand initiated receptor signal transduction is currently the cause espoused. For insulin signal transduction to occur downstream, the insulin should be in tetrameric, holo-enzyme form for the conformational changes and auto- phosphorylation steps to take place. A prerequisite to this is, linkage of the two A sub- units and, A, B sub- units by disulfide bonds. Without this, the receptor is in the A, B dimer half enzyme form, devoid of any binding affinity to the ligand or auto-phosphorylating activity.

Aim:

To explore, disruption of the disulfide bond formation, as a possible cause of insulin resistance in DM 2.

Conclusion:

Under normal physiological conditions of carbohydrate metabolism as a source of energy, disulfide bond formation occurs normally. But when energy metabolism switches to beta oxidation of fats, disruption of disulfide bond formation of the insulin receptor occurs, leading to insulin resistance in DM 2. The exogenously administered insulin restores the normal carbohydrate metabolism, by reversing the conditions that resulted in switch over to Beta oxidation, thus restoring insulin sensitivity in treated diabetics.

Key words: Tetramer, ligand, auto phosphorylation, disulfide bond, B oxidation.

Introduction:

Hyperglycemia, in presence of high levels of insulin in blood, is the hall mark of insulin resistance in DM2. Despite many mechanisms suggested, the issue of insulin resistance in DM2 is yet to be resolved. While resistance observed is to endogenous insulin, the exogenously administered insulin is still effective. This suggests that the insulin in DM2 resistance is relative and reversible. The defect in signal transduction of insulin is believed to hold the key to insulin resistance. The role played by the disruption of disulfide bond formation of the insulin receptor as a cause of Insulin resistance in DM 2 is explored.

The insulin receptor:

The insulin receptor (IR) is a trans-membrane protein having intrinsic tyrosinase activity. It's two dimensional structure has been elucidated since long. THE IR has two A and two B subunits. The A sub unit is extracellular and has the ligand (insulin) binding site/sites as detected by affinity labeling protocols (1, 2, 3). Besides it has a cysteine rich region. The B sub unit has three compartmental regions - the extracellular, the transmembrane and cytosolic domains. The cytosolic Tyrosine Kinase domain has ATP binding consensus sequence and three clusters of Tyrosine residues that can be phosphorylated in response to insulin action. The three clusters are found in juxta-membrane domain residues, the "tri-tyrosine" residues in the tyrosine kinase domain and the carboxy-terminal residues. The insulin ligand binds to one A sub unit in such a way that it precludes attachment of a second ligand to the other A sub unit. And also the second A sub unit shows negative co operability. The physiologically active form of the receptor is the tetramer which is a functional dimeric protein complex. This is the holo-enzyme which has high affinity to the insulin ligand and has the full phosphorylating activity. (4, 5) The A B heteromear is devoid of full ligand affinity or has any phosphorylating activity. The potential receptor- hormone contact sites are – residues 20-120 (6) the disulfide rich region (7, 8) and around the residue- 390 (9) region just to the carboxy side of the disulfide -rich region.

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The disulfide bonds:

The tetrameric form of IR is formed by the disulfide bonds between the two A sub units and B and B sub units. The conformational changes and auto phosphorylation that follow the docking of the insulin to the insulin receptor are dependent on the disulfide bridges. It is observed that the insulin treated receptor generated more cross linked B-B complexes than the receptor without insulin. (9) Though the ligand binds to one A sub unit only, both the B units are auto phosphorylated. The disulfide bonds are formed between the cysteine residues of the two A sub units (class 1 disulfide bonds) and the A B sub units (class 2 disulfide bonds).The class 1 bonds are between the cysteine residues 435, 468 and 524, According to another study A-A linkage is between residues 122-270, part of

cysteine rich domain. The A –B linkage is between CYS 583 and CYS 199 and between residues 724-917 {10}. When it is mutated to serine, no A2B2 dimers could be detected by the affinity labeling, only A2 receptors are formed. Thus the disulfide bonds are essential for auto phosphorylation of the receptor as well as for the insulin signaling transduction.

The role of protein disulfide isomerase (PDI) in disulfide bond exchange:-

The protein disulfide isomerase is an enzyme in the endoplasmic reticulum (ER). It catalyzes the formation of disulfide bonds between the cysteine residues within a protein, in this case, the insulin receptor. It also acts as a chaperon catalyzing proper protein folding. PDI has 4 thioredoxine-like domains, two of which have the canonical C X C motif. In the oxidized form it catalyzes the formation of the disulfide bonds, of the general structure R-SS-R. It is also called S-S bond or Disulfide Bridge.

The thiol- disulfide exchange:

In the reduced state the PDI is a dithiol. The thiol – disulfide exchange is depicted by the following equation.



The reaction proceeds through a SN 2 type mechanism. The nucleophile is the deprotonated thiol anion, which attacks the reacting sulfur of the disulfide bond making a S-S-S like transition state, with negative charge being delocalized but more abundant on attacking and leaving sulfurs.

The role of Endoplasmic oxido-reductin 4:

Endoplasmic oxido-reductin (ERO) is a protein tightly bound to the inner membrane of ER. It transfers the preformed disulfide bonds in the endoplasmic reticulum (ER) to the insulin receptor (IR) through the PDI by way of thiol- disulfide exchange as seen above. It catalyzes the oxidation of Protein Disulfide Isomerase (PDI), by coupling the de novo disulfide bond formation to the reduction of oxygen to H₂O₂. It oxidizes the PDI by accepting electrons from the sulfur and it self-get reduced. Thus the SH HS thiol bond is acquired in exchange of the S-S disulfide bond to PDI. The ERO can oxidize next PDI molecule only if it loses its electrons to the Electron Transport Chain (ETC).

86 The reverse electron transport (RET):

87 The mitochondrial ETC consist of multi sub unit 4 complexes (complex 1 – 1V which along with
88 F0-F1-ATP synthase, (complex V), are encoded by either mitochondrial or DNA. The complexes
89 normally transfer electrons to the final acceptor, the molecular oxygen which is reduced by 4
90 electrons to water at complex 1V. Premature single electron reduction of molecular oxygen,
91 earlier in the chain forms the super oxide radical. RET is a set of reactions that allow electrons
92 to be transported against the gradient of redox potential of electron carriers from reduced
93 coenzyme Q to NAD⁺ instead of oxygen. The reduction of Q enzyme requires FADH₂ linked
94 oxidizable substrates like glycerophosphate or succinate.

95 The redox carriers and the centers of ECT can potentially leak single electrons to oxygen and
96 convert it to super oxide anion, a progenitor ROS.

97 Given a moderate redox potential of the super oxide oxygen couple, ($E_{1/2} =$
98 0.16 V, the reaction of one electron reduction of oxygen is thermodynamically favorable
99 to many oxido-reductases.

100 Reactive oxygen species production (ROS):

101 To maintain the oxidative state of ERO I activity, the transfer of disulfide bond by ERO to PDI is
102 coupled to reduction of molecular oxygen in the ETC by the electrons acquired from PDI. The
103 reduction of oxygen is not complete since the end product is not water but H₂O₂. Thus ERO 1
104 activity constitutes an important source of ER derived oxidative stress, due to production of
105 ROS. ROS includes super oxide (O₂⁻), Hydroxyl radical, (OH⁻) and hydrogen peroxide (H₂O₂).
106 (11) MN SOD catalyzes the dis mutation of O₂⁻ to H₂O₂. (12) and the divalent reduction form,
107 H₂O₂. ROS production implies that there is reverse electron transport. Among the ETC
108 complexes, complex 1 and complex 3 are the major sites of super oxide production, the former
109 generating super oxide within the mitochondrial matrix only whereas the later generate them in
110 the intermembrane space also.(5,6) Complex 1 oxidizes NADH with enzyme Q as electron
111 acceptor coupled with proton pump generating trans-membrane potential. Complex 1 can
112 generate super oxides in presence of NADH. Since the electrons derived from sulfur are at a
113 higher redox potential than complex 1, RET occurs to reduce the NAD Both NAD reduction and
114 Reactive Oxygen Species (ROS) production require high membrane potential provided by ATP
115 hydrolysis.

116 **Role of Peroxy-Redoxin (PROX) 4:**

117 Hydrogen Peroxide (H₂O₂) formed in ERO 1 reaction is harmful, so it is removed by another
 118 protein, peroxy-redoxin (PROX 4). These are group of enzymes present in ER which remove
 119 H₂O₂ and also form the disulfide bond. The peroxidatic cysteines in the PROX 4, take an
 120 oxygen from H₂O₂ and form water and a –SOH group which reacts with the adjacent SH group
 121 to form a disulfide bond. The disulfide bond so formed is transferred to PDI by ERO1 which by
 122 thiol-disulfide exchange forms disulfide bond between the two A and A, B sub units of the
 123 insulin receptor.

124 **The proposed theory:**

125 Under the conditions of normal carbohydrate metabolism, the intermediate products of
 126 glycolysis and T.C.A. enter ETC through the Complex 1 through NADH and complex 11
 127 through FADH₂. The electrons passed by ERO 1 to complex 1 of the ECT, result in RET and
 128 which generates ROS from which the disulfide bonds are generated in the ER for ERO 1 to
 129 initiate oxidation of the reduced PDI. When the metabolism shifts to beta oxidation, the
 130 intermediate products of TCA cycle enter through complex -2, no RET occurs from succinate to
 131 complex 1. Hence no ROS production and hence no disulfide bonds are formed which could be
 132 transferred to the insulin receptor. The tetramer or holo -enzyme which can initiate
 133 conformational change and auto phosphorylation of the two B sub units as well as consequent
 134 IRS and cascade of auto phosphorylation downstream, is not formed. The FFA especially the
 135 LCFA, inhibit RET from succinate to complex 1 thus decreasing the SUCC-dependent ROS
 136 production, in spite of increased FADH₂ production due to beta oxidation of FFA (13). This
 137 situation continues until the B oxidation pathway of energy metabolism prevailing over the
 138 normal glycolysis is overcome. The exogenously administered insulin restores the carbohydrate
 139 metabolism in place of B oxidation of Fatty acids, by reversing the factors that lead to switch
 140 over of the energy metabolism, thus restoring the insulin sensitivity.

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