INORGANIC CHEMISTRY-3

SECOND SEMESTER 2021-22

ASSIGNMENT-1: REPORT ON INSULIN

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STRUCTURAL PROPERTIES OF INSULIN

Insulin is a protein that consists of two chains, an A chain (21 amino acids) and a B chain (30 amino acids), which are connected by sulfur atoms. Proinsulin, a 74-amino-acid prohormone molecule, is used to make insulin. Under normal circumstances, proinsulin is a relatively inert hormone that is secreted in small levels.

The proinsulin molecule is cleaved in two sites in the endoplasmic reticulum of beta cells, giving the A and B chains of insulin and a physiologically inactive C peptide in between. Two sulphur-sulphur (disulfide) links join the A and B chains together.

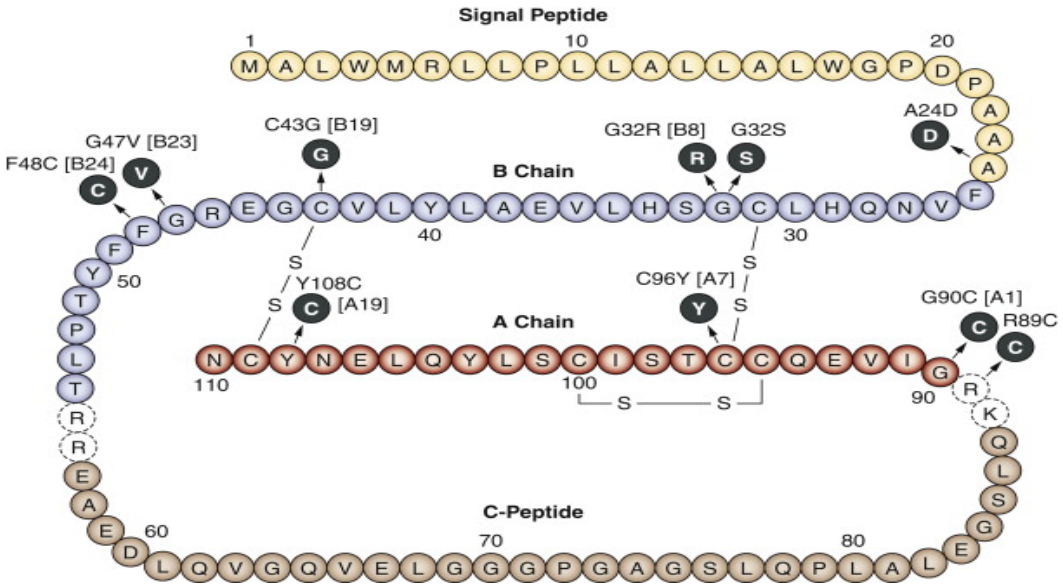

Fig.1 A,B Chains and Peptide structure of insulin

The A and B chains of insulin exhibit a lot of secondary structure despite their small lengths. The A chain is made up of two helical segments that are nearly antiparallel (A1-A8). A non-canonical turn brings the N- and C-chain terminals closer together (residues A9-A12). Disulfide bridges (cystines A7-B7 and A20-B19) and -turns (B7-B10 and B20-B23) flank the B chain's main -helix (residues B9-B19), whereas residues B1-B5 are expanded in the T state. Each of the turns has at least one intact Gly with a positive dihedral angle (residues B8, B20, and B23).

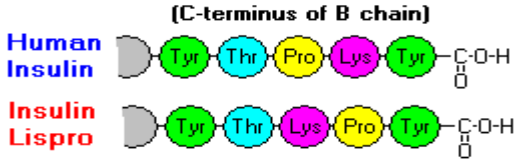

Fig.2 Human insulin peptide linkages

The A- and B-chains of insulin have different architectures. Insulin A-chain (a) and B-chain (b) as determined by three-dimensional X-ray analysis of the T 6 hexamer (2-Zn insulin). Both chains are shown perpendicular to the insulin hexamer's threefold symmetry axis. The following figure shows the A,B chains of insulin.

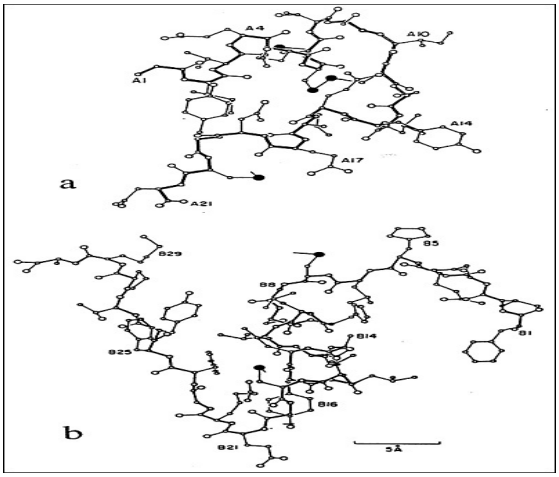

Fig.3 Architecture of A and B insulin chains

Insulins' primary structure provided valuable information regarding the amino-acid composition and size. Insulin's three-dimensional structure has been examined in crystals and in solution using X-ray diffraction and NMR spectroscopy. The folding of proinsulin and the function of insulin have both benefited from such research.

The insulin monomer attaches to the insulin receptor and activates it. The amount to which the monomer changes conformation when it binds to the insulin receptor is a crucial unresolved topic. Advances in structural biology of the insulin receptor are likely to increase our molecular understanding of how insulin binds in the next five years.

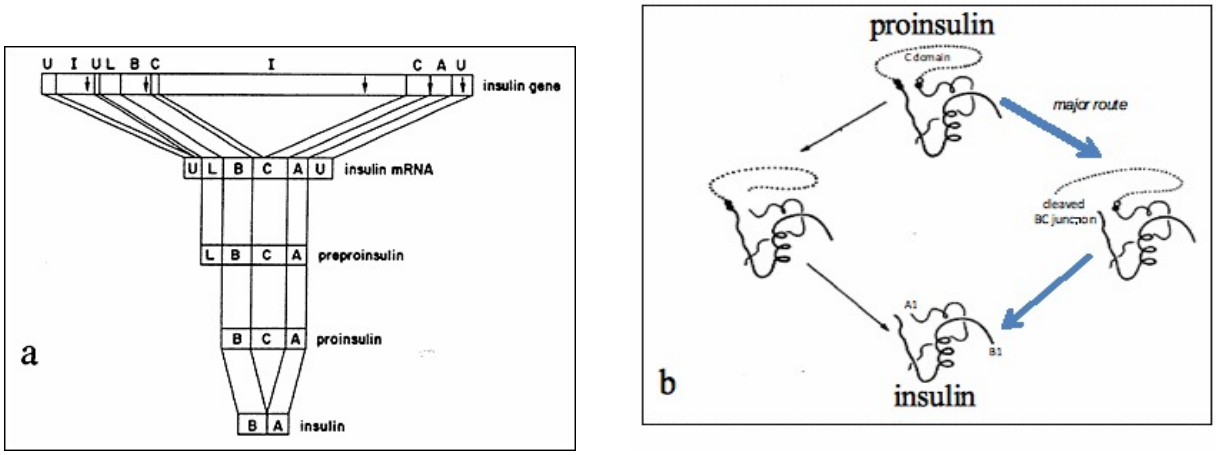

Fig.4 Symmetric insulin receptors and construction of proinsulin

The TR transition's crux occurs at the intersection of the B-N-terminal chain's segment and its central -helix. This junction creates a type II′ -turn with residues B7–B10 in the T-state. This turn's local structure necessitates a glycine at position B8 with main-chain dihedral angles on the right side of the Ramachandran plane (ϕ > 0). L-amino acids are normally "forbidden" from adopting this shape. In the R-state, however, GlyB8 adopts a negative ϕ angle, as one would expect within the interior of a -helix.

Insulin molecules have a tendency to form dimers in solution due to hydrogen-bonding between the C-termini of B chains. Additionally, in the presence of zinc ions, insulin dimers associate into hexamers.

The therapeutic implications of these interactions are significant. Hexamers diffuse poorly, but monomers and dimers rapidly diffuse into blood. As a result, insulin formulations with a large proportion of hexamers are delayed and sluggish to absorb. This characteristic has prompted the development of a number of recombinant insulin analogues, among other things. The first of these compounds, known as insulin lispro, has lysine and proline residues on the C-terminal end of the B chain flipped; this alteration does not affect receptor interaction but reduces the tendency to form dimers and hexamers.

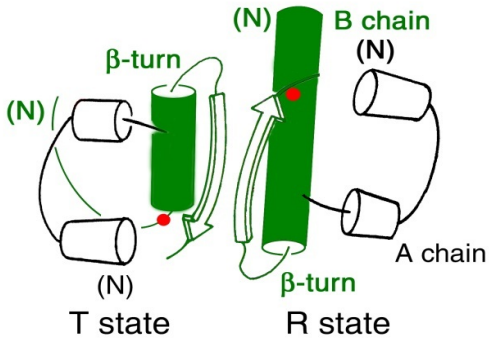

Fig 5. T and R state of insulin

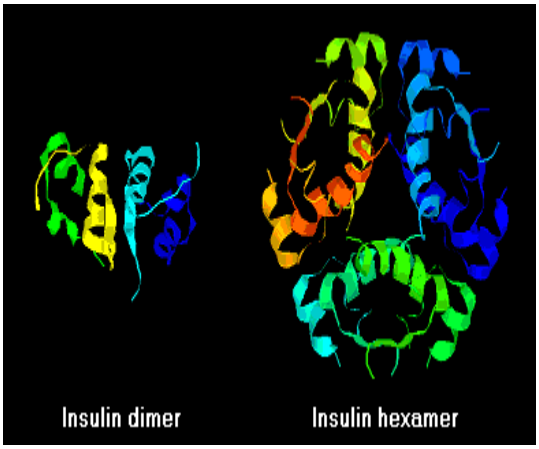

Fig.6 Dimer and Monomer of insulin

Proinsulin is converted to insulin by a fusion of two proteases: one with trypsin-like endoprotease activity that cleaves after the dibasic residue pairs at each end of the C domain, and another with exopeptidase activity similar to carboxypeptidase B that eliminates the basic residues left after tryptic-like cleavage. In vitro conversion of proinsulin to insulin too was established in earlier studies that used a mixture of pancreatic trypsin and carboxypeptidase B.

PURITY TEST AND DISCRIMINATION LIMIT

At position A-21, human insulin is susceptible to deamidation. The USP requires a liquid chromatography purity test for human insulin, as well as a liquid chromatography and capillary electrophoresis analysis of the final degraded product. If the relative concentration of unwanted substance is greater than 3% (USP Limit) after analysis, the test has a 95% chance of detecting deviations. The Discrimination Limit is the term referring to this probability.

SAMPLE PREPARATION AND CAPILLARY ELECTROPHORETIC ANALYSIS

The human insulin sample which will be used as a reference is prepared by storing it in 0.01M HCl at 40 ° C., or illegal model samples were formed by combining human insulin with A-21 DHI solutions in a stoichiometric ratio.

A fused silica capillary and a light diode array detector are both included in the CE instrument. The capillary temperature was kept at room temperature, the detector's wavelength was 214 nm, and a sampling interval was set. Hydrostatic injections are used to introduce the samples, and the mobile phase is made up of Tricine buffer and ethylene glycol at a precise pH, where after voltage is applied. The addition of urea to the mobile phase raises the noise levels while sharpening the output graph's peaks. In any case, ethylene-glycol sharpens the peak shape by increasing the mobile phase viscosity, which inhibits the solute diffusion.

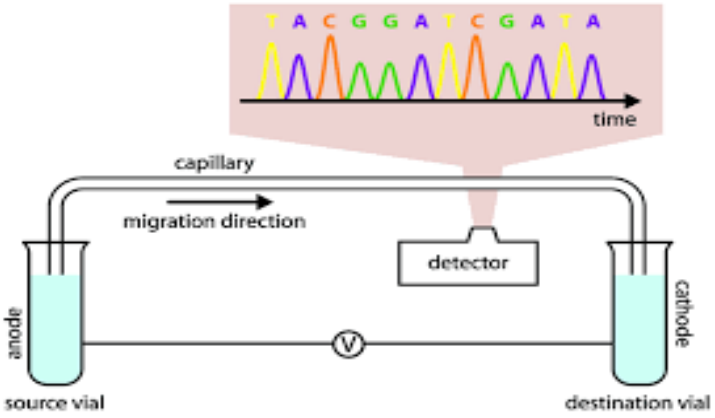

Fig 7. Capillary electrophoretic analysis of insulin

A single electropherogram can theoretically be used to calculate the discrimination limit (single sample analysis). We can employ numerous power spectra to increase our accuracy, and the signal shape that is closest to the average of all the CE signals is used. The discrimination limit is used to create model samples for illegal drugs in just about all cases.

Functioning of insulin and its side effects

Insulin is a hormone produced by the pancreas, an organ located beneath the stomach. The pancreas contains islets of Langerhans, which are specialised regions (the term insulin comes from the Latin insula that means island). The islets of Langerhans are made up of many hormone-producing cells, the most frequent of which are beta cells, which create insulin.

Insulin is subsequently released into the bloodstream by the pancreas, allowing it to reach various sections of the body. Insulin has a variety of functions, but the most important is that it regulates how the body consumes carbs present in particular foods. Carbohydrates are broken down by the human body into glucose, which is a form of sugar.. Extra glucose not used by the cells is transformed and stored as fat, which can be used to supply energy when glucose levels are too low. Insulin also has a number of other metabolic impacts (such as stopping the breakdown of protein and fat).

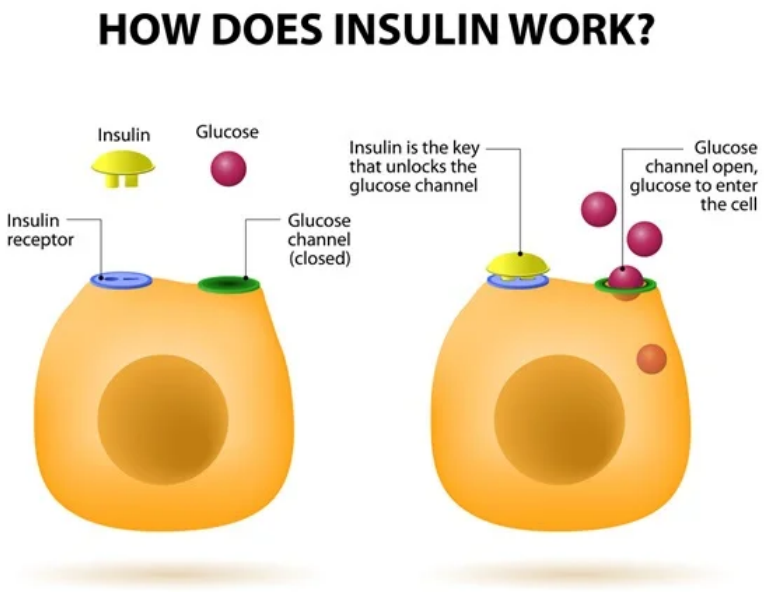

Fig.8 Working of insulin

Cells use glucose as their primary energy source. Insulin enables cells in the muscles, liver, and fat (adipose tissue) to absorb glucose and use it as a source of energy,

allowing them to function normally.Without insulin, cells would be unable to use glucose as a source of energy and will begin to malfunction

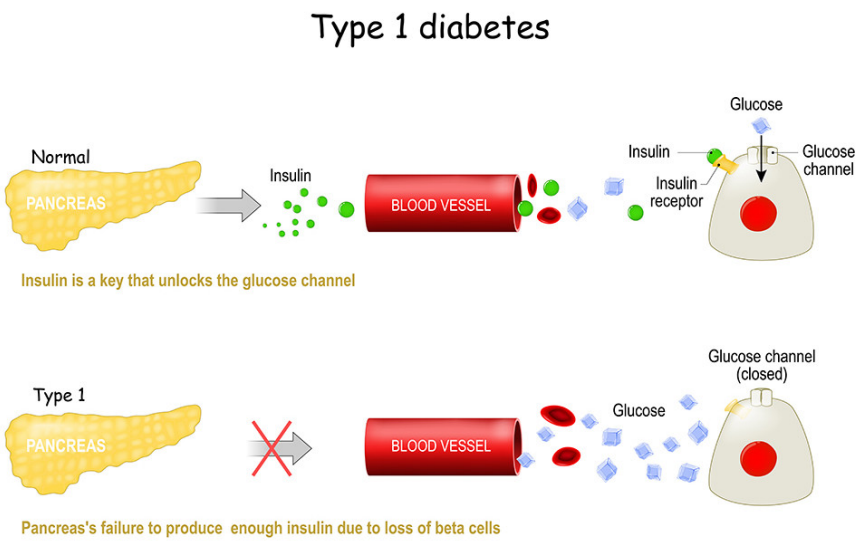

Fig.9 Type 1 Diabetes

Your pancreas does not produce insulin if you have type 1 diabetes.

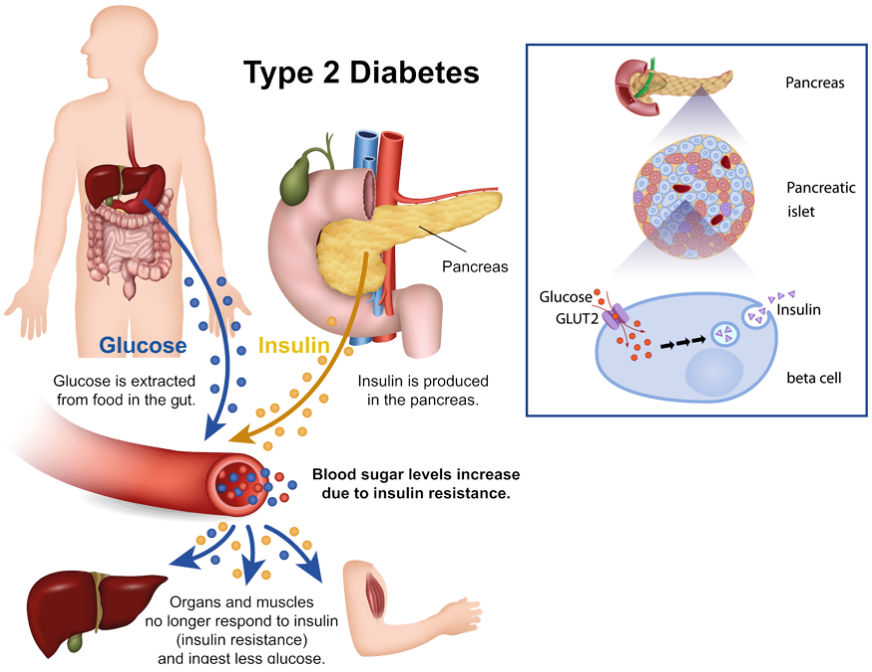

Fig.10 Type 2 Diabetes

If you have type 2 diabetes, your body either doesn't produce enough insulin or can't use the insulin it does produce efficiently. Sugar will linger in your system if you don't have enough insulin, resulting in excessive blood sugar levels (hyperglycemia).

Insulin regular (human) is a man-made, short-acting insulin that mimics the insulin produced by your pancreas. In response to food, it duplicates your body's insulin. This extra insulin aids in blood sugar control and the prevention of diabetes complications.

Insulin resistance, a condition in which muscle, liver, and fat cells do not respond adequately to insulin, is the most common cause of type 2 diabetes. As a result, your body requires extra insulin to assist glucose absorption into cells. To meet the increased demand, the pancreas produces more insulin initially. When the pancreas is unable to produce enough insulin, blood glucose levels rise.

Insulin helps glucose to move into our body cells. Our body uses insulin and glucagon to ensure that blood sugar levels remain in control and cells receive enough glucose for the body.

Glucose levels of a diabetic person keeps on increasing as they eat because there’s not enough insulin to move the glucose into body cells. People with Type1 diabetes do produce little to no insulin whereas people with type 2 diabetes have insulin deficiency and insulin resistance as well.

Since the type 1 diabetic people produce no insulin, insulin therapy is vital for replacing the insulin which their body doesn’t produce. Type 2 diabetic people generally don’t require insulin therapy but if other treatments are not effective in keeping up their insulin levels then they might have to go for insulin therapy as well.

The side effects of insulin therapy depend on type of insulin they are taking but some of the common side effects are:

1. One of the major side effects can be weight gain in initial days as the cells start to take in glucose.

2. Hypoglycemia: Taking insulin causes the cells to absorb more glucose from the bloodstream, due to which there can be excessive drop in blood sugar if not administered properly. If blood sugar levels drop too much, the person might experience dizziness, fatigue, sweating, blurred vision etc.

3. Anxiety or depression.

4. Rashes or swelling at the injection site.

5. One of the serious side effects can be low blood potassium whose symptoms can include weakness, muscle cramps, breathing issues etc.

6. Another serious side effect can be Heart failure.

New research and discovery in Diabetes and Insulin

Potential New agent of diabetes
(Increasing production of insulin by Drug)

Recent studies and trials on the drug name Tirzepatide have been conducted.

In one trial, three doses of Tirzepatide (given subcutaneously once weekly) were compared with placebo in 478 patients, most of whom were not taking other diabetes medications. During 40 weeks of treatment, all three doses lowered mean glycosylated haemoglobin (HbA1c) by about two percentage points (from a baseline of 7.9%) and reduced mean weight by 7 to 9 kg (from a baseline of 86 kg). Neither HbA1c level nor weight changed in the placebo group.

Severe hypoglycemia did not occur.

In the other trial, three doses of Tirzepatide were compared with the GLP-1 agonist semaglutide, injected weekly, in 1900 metformin-treated patients with type 2 diabetes (mean HbA1c, 8.3%; mean weight, 94 kg). At 40 weeks, the mean reduction in HbA1c was significantly more significant with Tirzepatide (2.30% with the highest Tirzepatide dose vs 1.86% with semaglutide). Mean weight reduction was also greater with Tirzepatide (11.2 kg with the highest dose vs 5.7 kg with semaglutide). Severe hypoglycemia was rare in all groups.

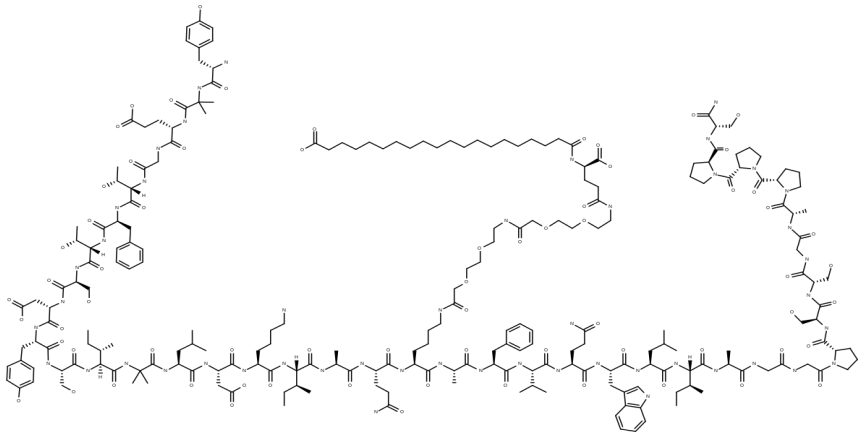

Fig.11 Tirzepatide's Structure

Tirzepatide

Tirzepatide is an analogue of the human GIP(gastric inhibitory polypeptide) hormone present in the human body with a similar C20 fatty-diacid portion attached to it and used to optimise the uptake and metabolism of the compound. The fatty-diacid section (eicosanedioic acid) is attached with a glutamic acid part and two (2-(2-aminoethoxy)ethoxy)acetic acid units to the side chain of the lysine(amino acid) residue. The structural arrangement allows a more substantial base and a much longer half-life, extending the dose uptake time between doses because of its high affinity to albumin.

General Mechanism

Tirzepatide has more affinity to GIP receptors than to Glucagon-like peptide-1 receptors.

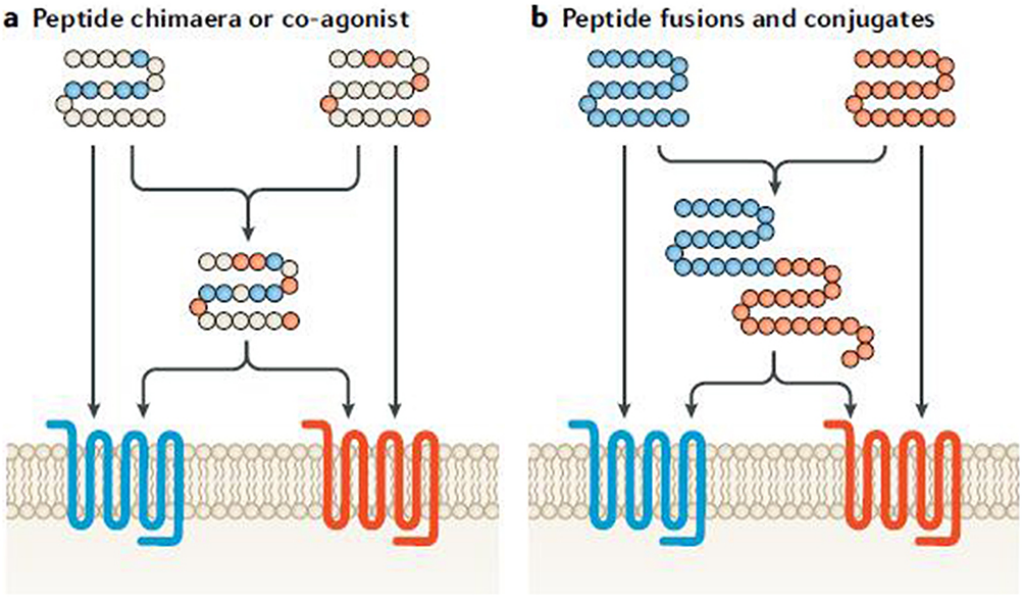

Fig.12 Peptide Fusion schematic

This dual agonist behaviour of Tirzepatide has been shown to produce more significant reductions of hyperglycemia to a selective GLP-1 receptor agonist. It has been proven more effective in reducing blood sugar.

This combination of preference towards GIP receptor & distinct signalling properties at GLP-1 suggests this biased agonism increases insulin secretion. It helps the body to produce insulin which in turn will reduce sugar.

By research, it came to light that novel GIP and GLP-1 agonist Tirzepatide is more effective for reducing liver fat. Many people with type 2-diabetes have shown swelling abdominal adipose and liver fat as their prime symptoms. Tirezepatide helps with liver swelling without showing any adverse effects and increases insulin secretion.
Overall, Tirzepatide can be recommended as an agent for curing diabetes, just like insulin.

Structurally Abnormal Insulin

(Mutant Insulin)

Recently a patient has been identified with fasting hyperinsulinemia. It is a condition where the patient's body becomes insulin resistant and compensates, and the pancreas has to secrete more insulin to compensate for the effect. After researching the patient's insulin, purifying it from the components of the pancreas and after the reverse phase HPLC, it was identified that the patient poses an abnormal insulin addition to the normal insulin.
The first case with a mutant insulin was reported in 1979, 1980, and a few cases (8-12) have been described since that time.

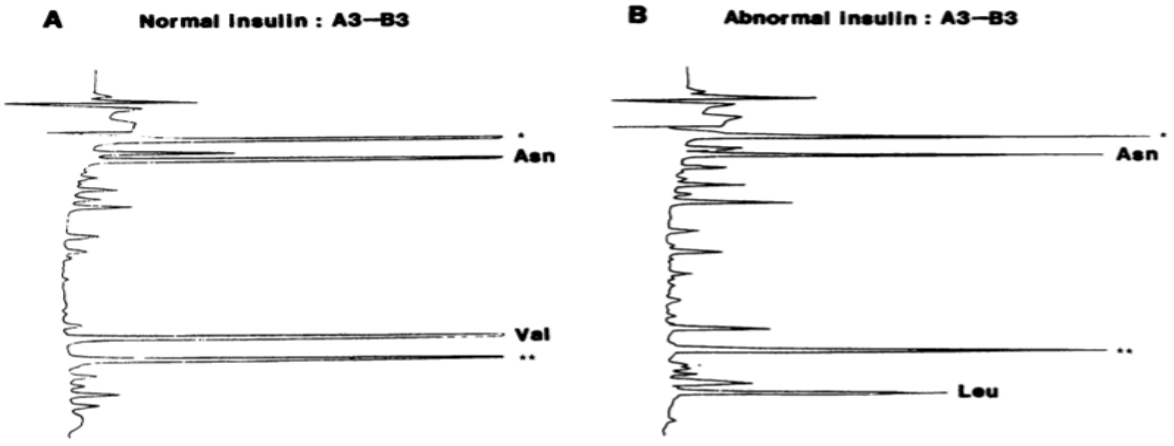

Fig.13 Reverse Phase HPLC

Analysis of the amino acid sequence of the abnormal insulin showed the substitution of the leucine to the valine amino acid of A chain's 3 positions.

We performed amino acid sequencing, both normal and abnormal insulin. As shown in Fig. 13, we found that the third amino acid from the N-terminal of the abnormal insulin consisted of leucine and asparagine. However, that position of the normal insulin was occupied with valine and asparagine. Since we could not find other differences between normal and abnormal insulin (Fig.14), we concluded that the abnormal insulin of this patient contained a leucine for valine substitution at position 3 of the A-chain.

Sample \ cycle		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Human insulin	A chain	G	I	**V**	E	Q	C	C	T	S	I	C	S	L	Y	Q	L	E	N	Y	C	N
	B chain	F	V	N	Q	H	L	C	G	S	H	L	V	E	A	L	Y	L	V	C	G	E
Normal insulin		G	I	**V**	E	Q			T	S	I		S	L	Y	Q	L	E	N	Y		N
		F	V	N	Q	H	L		G		H	L	V	E	A	L	Y	L	V		G	E
Abnormal insulin		G	I	**L**	E	Q			T	S	I		S	L	Y	Q	L	E	N	Y		N
		F	V	N	Q	H	L		G		H	L	V	E	A	L	Y	L	V		G	E

Sample \ cycle	22	23	24	25	26	27	28	29	30
Human insulin B chain	R	G	F	F	Y	T	P	K	T
Normal insulin	R	G	F	F	Y	T	P	K	T
Abnormal insulin	R	G	F	F	Y	T	P	K	T

Fig.14 Differences between normal and abnormal insulin

The ability to bind to the insulin receptors and stimulate glucose oxidation of the patient's abnormal insulin purified by HPLC from her pancreatic insulin was 5% and 8% that of normal human insulin, respectively. The results indicate good parallelism between binding activity and biological activity of the abnormal insulin in the patient.

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Structure

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Potential New agent of diabetes

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Structurally Abnormal Insulin

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Functioning of insulin

https://www.yourhormones.info/hormones/insulin/

https://www.niddk.nih.gov/health-information/diabetes/overview/symptoms-causes#type

Side effects of insulin therapy

https://www.mayoclinic.org/diseases-conditions/diabetes/in-depth/diabetes-treatment/art-20044084

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