Genetic Engineering

Human Recombinant Insulin

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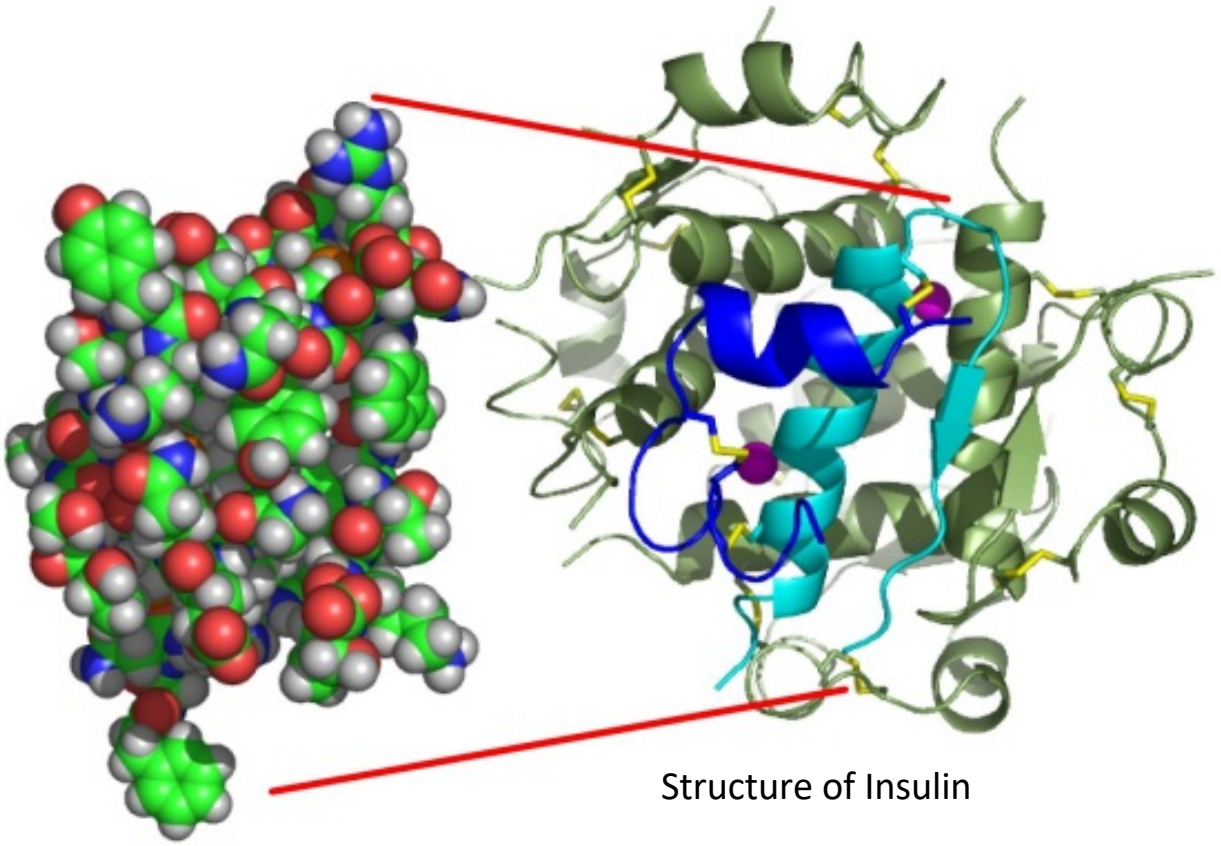


Structure of Insulin

Index

Preface

Dear Reader,

We chose the topic of the production of human insulin, because we are very much interested in human medicine and wanted to learn something about the red part of genetic engineering.

Our questions to this topic are:

- What is the history of insulin?
- How does the production of insulin actually work?
- What are pros and cons of the human recombinant insulin?
- Are there other methods to treat people with diabetes?
- Are there any ethical aspects?

Especially interesting is that one makes use of a bacterium in order to get and reproduce an essential human hormone and how scientists found out how insulin is related with diabetes.

Introduction

Insulin is a hormone produced by the pancreas. It is important for the regulation of carbohydrate and fat metabolism in the body. Insulin makes cells in the liver, muscle and fat tissue able to take up glucose from the blood and store it there as glycogen.

When the body has a problem with the control of the insulin, the person will have diabetes mellitus. As a result the person has to inject the insulin manually in his body.

There are two types of diabetes:

Type 1 This is the juvenile diabetes, which occurs because of the destruction of the islets.

Type 2 This is the adult-onset diabetes. Here the insulin level is high, but the body tissue isn't able to take up and use the insulin. This type has mostly a genetic component.

Until the early 1980s, pharmaceutical insulin was extracted from the pancreas of animals like cows and pigs. But after the 1980s it was possible to produce human insulin by genetic engineering.

In 1869 Paul Langerhans was studying the structure of the pancreas. He identified some previously unnoticed tissue clumps scattered throughout the bulk of pancreas. The function of these cells, we call them islets of Langerhans, was unknown. Edouard Laguesse later affirmed that they might produce secretions that play a regulatory role in digestion.

In 1889 the Polish-German physician Oscar Minkowski in cooperation with Joseph von Mering, removed the pancreas from a healthy dog to test its role in digestion. A few days later, Minkowski's animal keeper noticed that flies are feeding on the urine of the dog. When they had a closer look on the dog's urine they found out that there was sugar in it. In 1901 Eugene Opie established the link between the islets of Langerhans and diabetes. He recognized that diabetes mellitus is caused by the destruction of the islets of Langerhans and it only occurs when these are partly or completely destroyed. Before his researches the link between the pancreas and diabetes was clear, but not the exact role of the islets.

The technique of the production of insulin is used in the pharmacy to produce medical insulin for diabetics.

Another treatment instead of the use of the genetically engineered insulin is the use of animal insulin. But animal insulin isn’t used very often anymore, because of the following reason:

On account of the different amino acid sequence the animal insulin was a potential allergen, so it causes the continuously production of specific antibodies. These antibodies bond after the injection on the animal insulin and abolish its effect.

Description of the Engineering Technique

The human *INS* gene encodes an insulin precursor protein. This precursor protein is post-translationally processed into insulin, which consists of two separate amino acid chains. The so-called “A” chain is composed of 21 amino acids, whereas the “B” chain is composed of 30 amino acids. The two chains are covalently linked by two disulfide bonds.

Synthesis of recombinant human insulin requires cloning of the DNA sequence, which encodes the “A” and “B” peptide chain.

For synthesis of the “A” chain 63 nucleotides have to be cloned. For the “B” chain 90 nucleotides are needed. Additionally, a stop-codon (TGA) is added at the end of each chain (C-terminus). This will stop the protein synthesis. Furthermore, an anti-codon methionine (ATG) is placed at the beginning of each chain (N-terminus). This will be important later for the separation of the insulin protein chains from a fused bacterial peptide.

After these steps the cloned “A” and “B” chains are individually inserted C-terminally of a gene for a bacterial enzyme, β-Galactosidase, into a bacterial plasmid. The fusion of the insulin chains to this bacterial enzyme has been shown to enhance the protein yield 3 times. Here, it is important that the codons of the synthetic insulin chains are compatible with the one of the β-Galactosidase.

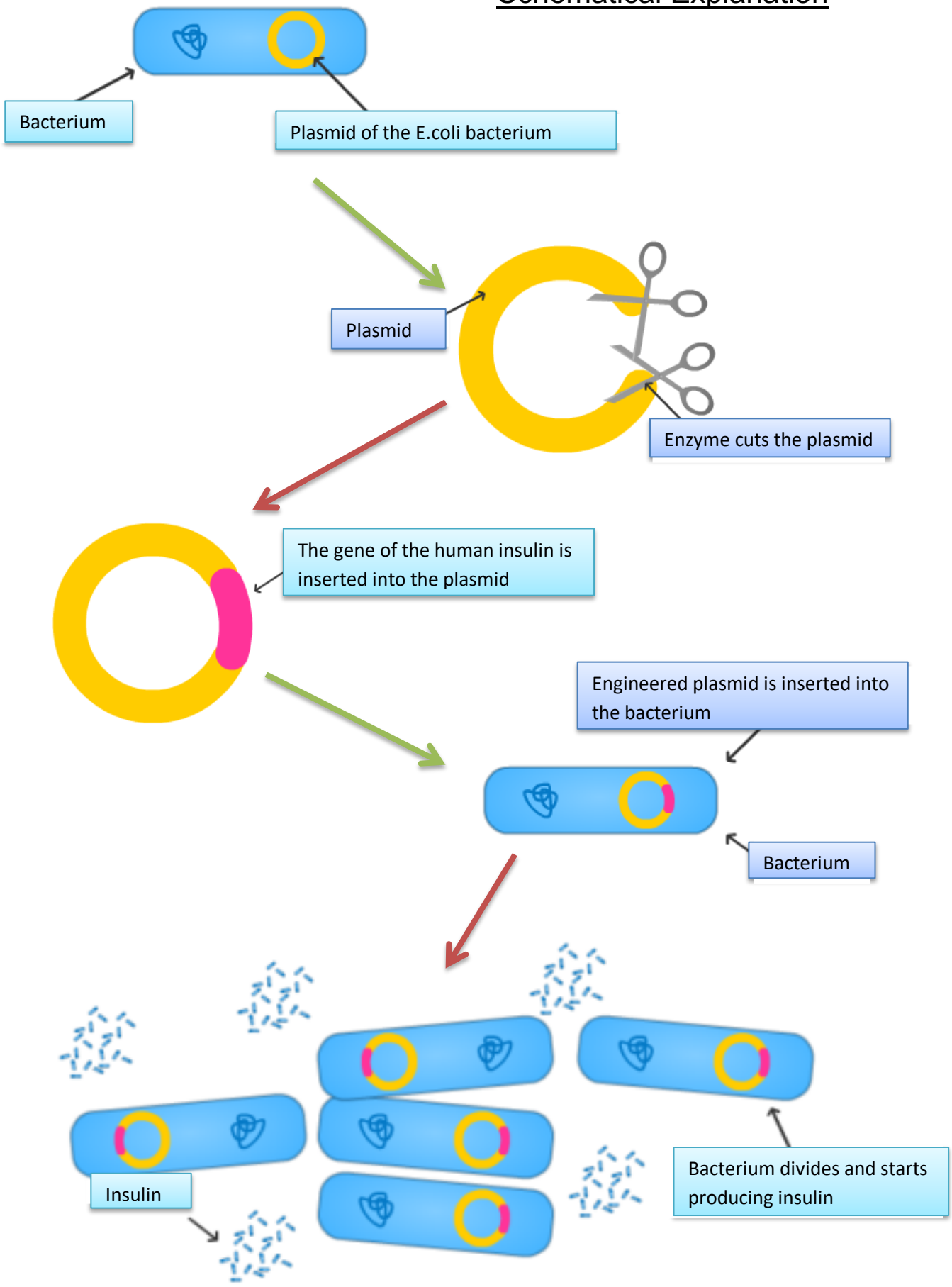
Finally, the recombinant plasmids are inaugurated into Escherichia coli (*E. coli*) cells. The β-Galactosidase DNA and the recombinant insulin DNA on the bacterial plasmid will replicate together during mitosis. It requires millions of copies of the bacterial cells, which carry the plasmid with the insulin gene in order to get enough synthetic human insulin.

The protein, which is now formed, consists partially of β-Galactosidase. It is joined N-terminally to either the “A” or “B” chain of insulin. A chemical that cleaves the C-terminal side of methionine residues can be used to separate the bacterial β-Galactosidase from the recombinant insulin. It will cut at the anti-codon methionine (ATG), which was placed previously at the N-terminus of each recombinant insulin chain. The “A” and “B” chains are then purified. After this procedure the two protein chains (“A” and “B”) are mixed. Under specific chemical conditions the two disulfide bridges will be formed.

Now we have synthetic human insulin.

The technique we have chosen to explain is the old-fashioned method. It is the method how they did it at the beginning of taking use of genetic engineering. Today they have many different methods to make GMO insulin, but they are sometimes more complicated. But they are all based on the method we have explained, that's why we chose this one.

Schematical Explanation

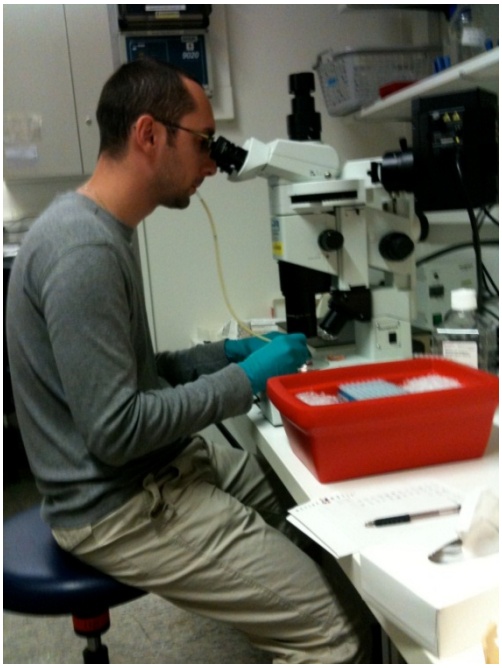


Documentation and Pictures of Research Institutions

In order to demonstrate how scientists work:

The first photo is from the internet. It shows a recombinant bioreactor, wich is used for making human insulin. The bioreactor is located in an insulin manufacture in Indianapolis, USA.

The next four photographs are taken by Rico Kunzmann, a biologist at the Friedrich Miescher Institute here in Basel. The photos show his working environment.



Interview with Dr. med Christof Halter, Pediatrician, Specialist of Endocrinology, 4104 Oberwil

How is the quality of the genetic engineered insulin?

The quality is very good; it is nearly identical to normal human insulin. But there are two ways to create the GMO insulin. The rapid-acting insulin, which is highly identical to the human insulin and the long-acting insulin, which lasts longer, but isn't as identical as the rapid-acting insulin.

How about the animal insulin? We read that it was much polluted. Is that true?

Yes, animal insulin was the normal treatment before the invention of the GMO insulin and it was much polluted because of the other hormones which were there in the pancreas of the slaughter animals.

Is there any intolerance against the genetic engineered insulin?

It is very rare that someone shows intolerance, but it is possible.

How are these people treated?

In these cases it is possible to give them animal insulin instead, but also this is very infrequent. But people who have taken animal insulin for a long time mostly won't take the synthetic insulin, if they were happy with the animal insulin. It's a question of their attitude.

Which therapy methods exist for someone with diabetes?

Because insulin is a protein, a person can't swallow it. If you swallow it, it will be downsized in the stomach and the gut and so it isn't effective anymore.

An adult person cannot take up such a big amino acid in comparison with an infant. Though they can.

Therefore the only way to get the insulin in the body is to inject it directly.

One method that has been used for a long time is the "two syringe method". Here the patient has to inject the insulin twice a day; once in the morning and once in the evening. This needs a regular day to day routine. Because an elderly person normally does not have this, this method is more suitable for young children.

Another method is that the patient injects the insulin in small doses whenever he needs it.

The third method I'm familiar with is the one where the patient carries always a syringe on the body with a needle under the skin. By need he can just press a button and the insulin goes into the body. But this method is often annoying for the patient, because he has to carry a box on his body with the insulin all the time.

Are there therapy methods that are in research?

Yes, there is a method in research. It is a therapy method by which the patient has a measuring probe under the skin. This measures the blood sugar level and whenever it drops, a box will automatically insert insulin in the body. But at the moment this is not possible. The technique exists and the continuously measuring system works, but the human body produces a bio film over the probe so that the measured data is falsified.

What restraint is there for a child with diabetes?

There aren't any big dangers for the child. It can do everything what a normal healthy kid can also do. The biggest problem is probably that the parents are too afraid of letting the child do everything. The only restraint a child has, is, that it hasn't such a big endurance.

Are there any new methods in research to get the insulin?

Yes there are. Scientists are looking for a method to extract the islets of Langerhans directly from the pancreas and inject them in the body. But at the moment the problem is that these islets would not be accepted and would be rejected by the body.

Do you think that there are ethical problems with the human recombinant insulin?

No, definitely not. It is absolutely possible to live with diabetes that's why I think it is acceptable to produce and use the human recombinant insulin.

Another aspect are the costs. But also here I don't see any problem because the costs to produce the genetic engineered insulin aren't that high.

Generally speaking, I think there aren't any ethical aspects for me to be a hindrance to do this.

Interview was taken by Raphael Vogel on Wednesday, April 11, 2012 in Oberwil

Discussion

With the application of the technique of genetic engineering it became possible to produce human insulin (human recombinant insulin) in a high quantity. Before the technique was discovered scientists had to extract the insulin from animals like cows or pigs. At this time the use of animal insulin was the only way to treat people with diabetes mellitus.

To get the Insulin of the animals, they took slaughter-animals and extract the insulin out of their pancreas. The first preparations they got by this means were mostly polluted and caused allergic reactions and inflammations at specific body-parts.

Later they improved the extraction procedure so that they could only isolate directly the insulin from the animals. But the amount of needed slaughter-animals was still extremely high. This method was in financial, as well as in ethic aspects disputable.

These problems lead to the development of another method and so they took use of the genetic engineering.

A new treatment method they are working on, is that the insulin will be automatically injected in the body by means of a small probe, which measures constantly the blood sugar level.

Pro and contra of genetic engineered insulin:

The advantages of GMO insulin is that bacteria reproduce much more rapidly and with less resource than mammals. It is cheaper to produce them, because less space is needed to grow and support the bacteria colonies. Another fact is that the supply of this insulin is nearly unlimited, because no pancreases from living organisms are needed. So we can say that the genetically engineered insulin is almost identical to the insulin produced by the human pancreas.

The disadvantages of GMO insulin is that also the genetic engineered version of insulin might cause allergic reactions, but it occurs much less often than by the use of animal insulin. In situations of human insulin intolerance or if the patient desires it, it is possible to take animal insulin as an alternative treatment. Another negative aspect is that lots of technical auxiliary means are needed for the production and some development countries don’t have the possibility to get these medical treatments.

Ethical aspects:

We have also thought about ethical aspects, but couldn’t find anything that would speak against the production of genetically engineered insulin from our point of view. We have the same opinion as Dr. Halter. The most likely people who think that it’s not ethic are the members of some religious groups or of a special religion.

For example, the Jewish community has a problem with some parts of the genetic engineering in human bodies, because they say, gods work should not be affected or changed. That is mainly the case against genetic engineering in the fetus to change the phenotype, to cure genetic diseases.

In our opinion parents should have the right to judge the extent of the situation and should be able to decide whether they want to do something or not.

Summary

Insulin is a very important hormone. A disorder of the production or the disability of the body tissue to take up this hormone leads to diabetes. With diabetes one has to inject genetic engineered insulin, or in some cases animal insulin as a replacement of the own body insulin, in the body. There are two types of diabetes, the juvenile and the adult-onset diabetes. To live with diabetes is absolutely possible, because of different treatment methods available.

The first scientist who made the link between the islets of Langerhans, which play a big role in the production of insulin, was Eugene Opie. He found out that diabetes mellitus mainly occurs because of the destruction of the islets.

To produce the insulin, two specific nucleotide chains are required. These chains have to be cloned and modified. Then the cloned chains are inserted into a bacterial plasmid. The recombinant plasmids are then introduced into *E. coli* cells. During mitosis it replicates. At last the recombinant insulin has to be separated from the byproduct and the two chains have to be mixed together to get the human recombinant insulin.

Before the discovery of human recombinant insulin people with diabetes have been treated with animal insulin. But this version of insulin was unpurified and it was the cause for allergic reactions. In our days the use of animal insulin is basically replaced by synthetic insulin. The use of the synthetic one is in that case better, because the chance to suffer an allergic reaction is less frequent and the costs of the production process are much lower.

In our society it is mostly accepted to use and produce the synthetic insulin.

Words of thanks

We thank Dr. med. Christof Halter for the interview.

We also thank Rico Kunzmann for the photos of his laboratory and that he gave us some information on the application of the technique of the production of human recombinant insulin.

References

Ref1: http://en.wikipedia.org/wiki/Insulin (cover picture, insulin in general and history)

Ref2: http://www.littletree.com.au/dna.htm (application of the technique)

Ref3: http://www.abpischools.org.uk/page/modules/diabetes/diabetes6.cfm?coSiteNavigation_allTopic=1 (schematical explanation)

Ref4: http://blog.sstrumello.com/2008/01/biodel-announces-manufacturing-plans_04.html#.T4nHMtXheiJ (Bioreactor picture)

Picture 2, 3, 4 and 5 are taken by Rico Kunzmann

Ref5: http://flexikon.doccheck.com/Tierinsulin (animal insulin in general)

Ref6: http://www.livestrong.com/article/231364-the-pros-cons-of-the-use-of-gmo-insulin/ (pro and contra of GMO insulin)

Ref7: Marston Ecoli peptidesynth 1986 (PDF)