GKG BASEL

Insulin producing cells

Term Paper Biology Class 4A

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Preface

We decided to work on this topic because the first thing that came to our mind while thinking about modified cells were bacteria that produce insulin. After further thinking we decided to take this as our topic because we thought that there will be a large interest group to this theme, due to the fact that about 350'000 people in Switzerland suffer from diabetes.

Insulin was the first drug that was synthetically produced with the knowledge of genetic engineering. Therefore we wondered if the oldest technique is also the furthest researched one. We also wanted to find out if there are methods which are modern and outdated and if there is still research going on.

Introduction

Why producing insulin?

In a normal working human body, insulin enables body cells to take up glucose which is resorbed by the blood when food is digested. If there would be no insulin, the glucose would simply stay in the blood and this would lead to several problems in the organism. An abundance of glucose is called hyperglycaemia. Normally, the body produces the right amount of insulin to hold the glucose values within normal limits, but there are some diseases, which can disturb this system. These diseases are called diabetes. There are two generally types of diabetes:

In type 1 diabetes, the body cannot produce enough insulin to enable the cells to take up glucose. People who suffer from this type of diabetes have to inject insulin which is produced externally.

In type 2 diabetes, the body creates a resistance against insulin. The injection of insulin only helps for short times, because the resistance increases over time and the body needs more and more insulin. The only cells in the body which are not resistant against insulin are muscles. Muscles burn glucose independently of insulin during work. Therefore this type of diabetes has to be controlled with physical activity.

In conclusion, insulin production is mainly used for patients with type 1 diabetes.

Where does insulin come from?

Generally the insulin of different mammals are very similar, therefore insulin can be derived from cows or pigs for human use. But after some time, the human body starts to build antibodies against these types of insulin. Therefore, one had to develop new methods to produce human insulin.

There are many ways to produce insulin but we researched about the techniques which are related to genetic engineering. Insulin was gained in the 70's by changing some of the amino acids in the protein structure in insulin of cattle. The donor animal was changed to pigs because porcine insulin was biochemical easier to change. In the year 1979 some Frankfurter scientists succeeded to produce human insulin in great amounts by isolating the human gene for the insulin production and applying this gene into a plasmid. This plasmid is inserted into microorganisms like *E. coli* or *Sacch. cerevisiae* by making use of gene transfer. But due to the discussion about genetic engineering and its effect on the human body the method got no license until 1999. Until then the diabetics have had to make use of modified porcine insulin. This process will be discussed further in the section Engineering Technique.

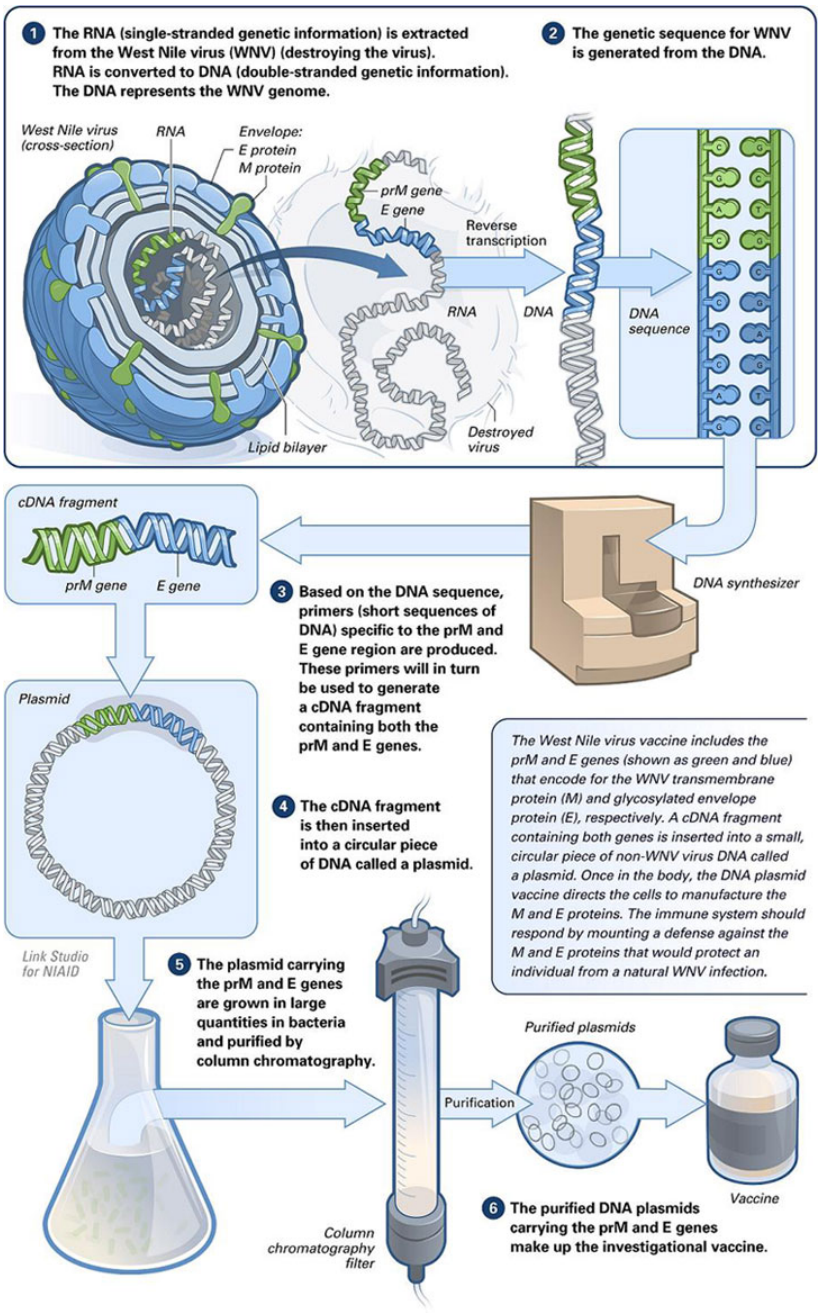
Lately have no further researches been made but in the year 2009 an article of a German scientist was published in which he writes about reprogramming cells in the pancreas by using a gene called Pax4 which changes some of the glucagon producing cells into insulin producing cells. The information about this technique is very vague and still in research. Some tests were already made with diabetic mice which have grown insulin producing cells some days after the injection of Pax4.

Description of the engineering technique

For the production of insulin today all of the major companies use more or less the same method. They use genetically modified organisms (either bacteria or yeast cells) which carry a specific nucleic acid sequence for the production of insulin. This sequence is gained by isolation form a human gene by cutting it out with a restriction enzyme. The cut can have two different forms, either with a sticky end or a blunt end. The advantage of a sticky end is that it is easier to ligate.

The process of ligase happens in a plasmid, which is a circular double-stranded DNA-strand that occurs especially in bacteria. Now for the modification of such a bacterium a plasmid, which has already an antibacterial resistance gene, is being extracted and also cut at the resistance gene with a restriction enzyme into a DNA-strand with sticky ends. After that the nucleic acid sequence for the production of

insulin is added and the plasmid should connect with the sequence and form a ring again. Afterwards the ligase, a specific type of enzyme is added and joins the DNA molecules together and makes the plasmid ready for use. The modified plasmids are now inserted into our bacteria of interest. Half of these bacteria are now grown on agar which was treated with antibacterial before. If everything went correct the bacteria should not grow due to the fact that the antibacterial resistance gene is cut and replaced with the gene for insulin production. If this happens, the other half is now populated on normal agar. The population grows and after a certain period the whole mixture of bacteria and insulin which they have produced is purified by chromatography. The now gained insulin is only an early form of the product sold afterwards. So now the insulin has to be manufactured into a commercial product called insulin analogs. In most of the cases this precursor-insulin is manufactured into an injection solution. There are various products, some work very fast but only over a short time and other preparations are dimensioned for long-time usage. There are also very new products that are inhaled but on several internet forums the customers are very disappointed of this new method due to high costs because 10x the normal dose is used and the fact that smokers and people that have a cold cannot make use of this product.



Similarity between insulin and vaccine production

Interview

Our interview partner was Eva Sägesser of Novo Nordisk. Novo Nordisk is the worldwide leading producer of insulin. The interview was carried out in German and later translated into English. The original version is attached.

1. We read that insulin was the first substance, which was produced with the knowledge of genetic engineering. Is the insulin production also the most developed section of genetic engineering?

   Insulin was the first substance, which was produced by genetic engineering in Switzerland.
   You can find a list of all drugs with active substances, which are produced by genetic engineering on the Swissmedic homepage.

   Due to the fact, that the production of insulin is not very complicated and that there are more complex drugs, which are also produced by genetic engineering, you could say that the production of these more complex drugs is further developed.

2. Why do we have to produce insulin with genetic engineering?

   First you can achieve a higher rate of yield with the genetic engineering techniques than with the production via animals. Secondary you get a pure product.

3. Which methods are used today?

   Today insulin in Europe is only produced by genetic engineering. However, there is one Company left, the CP Pharma in England, which produces insulin with pigs.

4. Is there research going on in the insulin branch?

   Temporary, there is no research going on.

5. Is the method your company uses being further developed?

   Not the technique but the product. We are trying to develop insulin molecules, which have higher physiological active profiles than the ones which are already registered.

6. After reading an article about insulin production with the Fermtec-method, we wondered if in the production with autonomous reproducing bacteria might mutations occur, which could lead to a negative effect on the final product.

   We use yeast cells for our production and are not active in this span.

7. Due to our information your company is producing insulin with the help of yeast fungi; may you portray the manufacturing process more exactly?

Insulin is produced in the following way (i.e. human insulin):

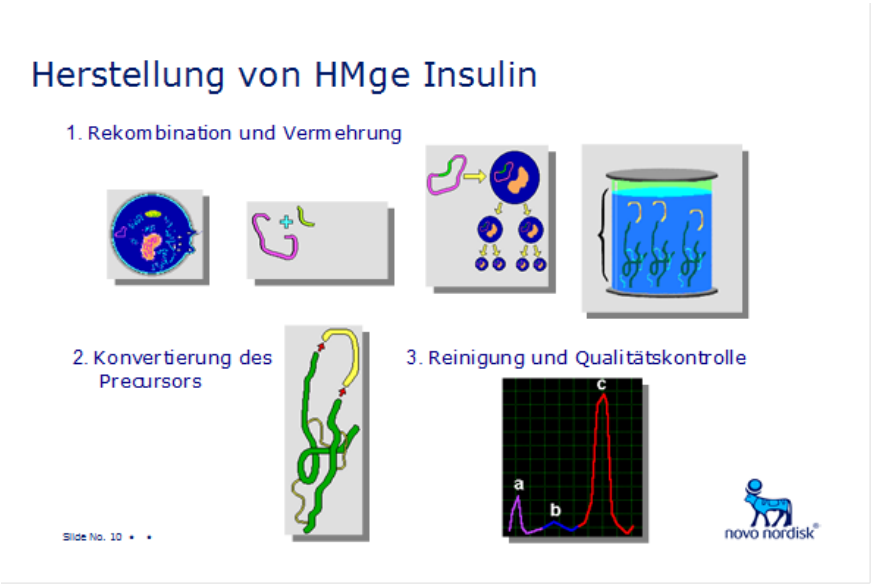
1.1 Production of human insulin

Simplified the production of human insulin via precursor trough yeast cells at Novo Nordisk comprises following steps:

1. Genetic modification of the yeast
2. Treatment with enzymes (“fermentation”) and extraction
3. Conversion of the insulin precursor
4. Purification

1.1.1 Gene technology with yeast

The changed gene gets infiltrated into the yeast cells through a carrier called plasmid. The plasmid is a small circular DNA-strand that once infiltrated remains in the yeast cells. The plasmid persuades the yeast cells to produce insulin precursor.



The human insulin precursor is a “mini-proinsulin”, a modified proinsulin. In contrast to natural proinsulin of humans with A-, B- and C-peptides contains the mini-proinsulin the A- and B-peptides and a very short peptide, which ensures the correct three-dimensional structure after the enzymatic remove.

Novo Nordisk has implemented into the yeast cells an artificial genetic insulin-precursor gene with the code for the insulin production.

Because of the following reasons yeast cells were chosen:

1. Yeast is a well-known organism that was used by men since thousands of years ago for the production of beer and bread.

2. It grows also well on a common culture medium that contains glucose, vitamins and some salts.
3. It is highly developed and more similar to human cells than bacteria.
4. It produces no for human toxic substances.
5. Yeast cells can be persuaded to produce an almost complete molecule of human insulin with perfect three-dimensional structure, which fits into the human insulin receptor.

1.1.2 Fermentation

The special treated yeast cells may ferment three weeks in an 80-cubic-meter container. At the beginning of the fermentation the container encloses only one yeast cell.

Every half hour the cell count doubles. After a half hour has the cell reproduced and two cells are present. After one hour are four cells present etc. Theoretical are after 24 hours 280000 billion cells in the container. During this whole reproduction process produce the all the cells mini-proinsulin, that continually gets pumped off, while simultaneously relative nutrient-solution gets added.

1.1.3 Extraction

Mini-proinsulin (precursor) gets isolated out of the pumped off brew through centrifugation and by use of an absorption-pillar. The still intact yeast cells get deactivated and are discarded.

After the absorption gets the mini-proinsulin dissolved out and through crystallisation and centrifugation isolated.

1.1.4 Transformation of the insulin-precursor

Mini-proinsulin gets changed into human insulin through a process in which the short-chained C-peptide gets separated.

1.1.5 Purificaion

The so produced insulin gets purified through crystallisation and chromatography. Further cleaning steps are gel filtration, ion exchange chromatography and finally high performance liquid chromatography (HPLC).

HPLC is a very effective method for the separation of organic molecules. Originally it was invented for analytic purposes. By Novo Nordisk it got perfected as ideal method for insulin purification.

The resulting human insulin shows only impurities below the detection limit of the finest radioimmunoassay-techniques.

1.1.6 Waste management

After the production of the human insulin the used yeast cells are deaden with the help of heat and lye. Since the start of the production of human insulin by Novo Nordisk, there were never found any genetic changed yeast cells outside of the production.

Discussion

Due to the fact that genetic engineering is a quite young science and therefore has only a few long time studies of the effect on human beings, the broad mass is very ignorant. Therefore most of the population are indecisive if they should support genetic engineering. An exception is the production of insulin because nobody doubts the necessity of insulin. If we used nothing but animal insulin there wouldn’t be the same amount of the product available. Also the costs of insulin would be much higher. So the production of insulin with the help of genetic engineering is great achievement for people who need insulin due to the fact that always insulin is available for a relatively low prize. Also the output of the artificial insulin is very high compared to the output of animal insulin, which is constrained to the amount of animals used for the production.

Summary

In our term paper we informed about the techniques of insulin production. There is one technique used today in which one uses the human gene for insulin and implements this into bacteria or yeast cells which are then producing insulin. Earlier one used insulin form mammals because it is very similar to human insulin, but this technique is not really used anymore due to the facts that there is only a small output and after a while the effect of the animal insulin on the human body decreases. The genetic engineered insulin production a very developed technique and the utility is out of the question. There are many people who are not well informed about this technique so if they hear genetic engineering they automatically are scared. Our final statement: We think that the techniques do not have to be developed anymore but the people have to become familiar with this method.

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Interview

1. Nach unseren Informationen ist Insulin der erste mit Hilfe von Gentechnik hergestellte Stoff. Ist es folglich richtig, das Insulin als Urgestein der Gentechnik anzusehen und falls ja, ist die Insulinproduktion auch die am weitesten entwickelte Sparte der Gentechnik?

   Insulin war in der Schweiz das erste gentechnologisch hergestellte Arzneimittel, das zugelassen wurde. Die Liste der zugelassenen Arzneimittel mit gentechnologisch herstellten Wirkstoffen finden Sie auf der Swissmedic Homepage. Da die gentechnologische Herstellung von Insulin nicht zu den komplexen gentechnologischen Herstellungen zählt, existieren Arzneimitteln, deren gentechnologische Herstellung komplexer ist und deren Herstellung man allenfalls auch als weiter entwickelt bezeichnen kann.

2. Warum muss Insulin überhaupt mit Hilfe von Gentechnik produziert werden?

   Zum einen kann mit Hilfe der Gentechnik die benötigte Insulinmenge garantiert werden (die Ausbeute bei Tieren ist sehr viel geringer). Und zweitens kann auf diese Weise ein reines Produkt produziert werden.

3. Welche Methoden zur Insulinproduktion werden Heutzutage angewendet?

   In Europa wird Insulin heute nur noch gentechnisch hergestellt. Lediglich CP Pharma in England vertreibt noch Schweineinsulin.

4. Warum sucht man nach neuen Methoden?

   Momentan werden keine neuen Methoden gesucht.

5. Wird die von Ihnen angewandte Methode zur Insulinproduktion weiter entwickelt?

   Nicht die Technik wird weiterentwickelt, sondern die Produkte. Man versucht Insulinmoleküle zu entwickeln, welche möglichst noch physiologischere Wirkprofile erzielen als die bereits zugelassenen.

6. Wir fragten uns nach einer Lektüre über die Insulinproduktion im Fermtec-Bereich, ob bei der Produktion mit Bakterien, die sich selbstständig vermehren

keine Mutationen auftreten können, welche das Endprodukt negativ beeinflussen?

Wir verwenden Hefezellen und keine Bakterien für die Produktion und sind in diesem Bereich nicht tätig.

7. Nach unseren Informationen stellt Ihre Firma Insulin mit Hilfe von Hefepilzen her, könnten Sie uns den Produktionsverlauf genauer schildern?

Insulin wir wie folgt hergestellt (Beispiel Humaninsulin):

1.1 Humaninsulinherstellung

Vereinfacht umfasst die Herstellung von Human-insulin via Präkursor mittels der Hefezellen bei Novo Nordisk folgende Schritte:

5. Genetische Modifikation der Hefe
6. Enzymbehandlung ("Fermentation") und Extraktion
7. Umwandlung des Insulin-Präkursors
8. Reinigung

1.1.1 Gentechnologie bei der Hefe

Das veränderte Gen wird durch einen Träger ("carrier") namens Plasmid in die Hefezellen eingeschleust. Das Plasmid ist ein kleiner, kreisförmiger DNA-Strang, der einmal eingeschleust in den Hefezellen verbleibt. Dieser bringt die Hefezellen dazu, Insulin-Präkursor zu produzieren.

Der Humaninsulin-Präkursor ist ein "Mini-Proinsulin", ein modifiziertes Proinsulin. Im Gegensatz zum natürlichen Proinsulin des Menschen mit A-, B- und C-Peptid, enthält das Mini-Proinsulin die A- und B-Peptide und ein sehr kurzes Peptid, das auch nach der enzymatischen Entfernung die richtige dreidimensionale Struktur des Insulins garantiert.

Novo Nordisk hat den Hefezellen ein synthetisches gentechnisches Insulin-Präkursorgen mit dem Code für die Insulinproduktion eingebaut.

Aus den folgenden Gründen wurden Hefezellen ausgewählt:

1. Die Hefe ist ein gut bekannter Organismus, der vom Menschen seit Jahrtausenden zur Bier- und Brotherstellung benutzt wird.

2. Sie wächst auch in einem einfachen Nährmedium gut, das lediglich Glukose, Vitamine und einige Salze enthält.

3. Sie ist hoch entwickelt und den menschlichen Zellen ähnlicher als Bakterien.

4. Sie produziert keine für Menschen toxischen Substanzen

5. Hefezellen können dazu gebracht werden, ein fast komplettes Humaninsulin-Molekül zu produzieren mit perfekter dreidimensionaler Struktur, das in den menschlichen Insulinrezeptor passt.

1.1.2 Fermentation

Die speziell behandelten Hefezellen können in einem 80-Kubikmeter-Tank drei Wochen lang fermentieren. Zu Beginn der Fermentation enthält der Tank nur eine Hefezelle. Alle halbe Stunde verdoppelt sich die Zellzahl. Nach einer halben Stunde hat sich die eine Zelle zu zwei Zellen vermehrt, nach einer Stunde zu vier Zellen usw. Theoretisch sind nach 24 Stunden 280000 Milliarden Zellen im Tank. Während dieser ganzen Vermehrung produzieren die Zellen alle Mini-Proinsulin, das kontinuierlich abgepumpt wird, während gleichzeitig entsprechend Nährlösung zugeführt wird.

1.1.3 Extraktion

Mini-Proinsulin (Präkursor) wird aus dem abgepumpten Sud durch Zentrifugieren und mittels einer Absorptionssäule isoliert. Die noch intakten Hefezellen werden inaktiviert und verworfen.

Nach der Absorption wird das Mini-Proinsulin herausgelöst und durch Kristallisation und Zentrifugieren isoliert.

1.1.4 Umwandlung der Insulin-Vorstufe

Mini-Proinsulin wird durch einen enzymatischen Prozess, bei dem das kurzkettige C-Peptid herausgetrennt wird, zu Humaninsulin umgewandelt.

1.1.5 Reinigung

Das so produzierte Insulin wird durch Kristallisation und Chromatographie gereinigt. Weitere Reinigungsschritte sind Gelfiltration, Ionenaustausch-Chromatographie und schliesslich die Hochdruck-Flüssigkeits-Chromatographie (HPLC = high performance liquid chromatography).

HPLC ist eine sehr effektive Methode zur Trennung organischer Moleküle. Sie wurde ursprünglich für analytische Zwecke entwickelt. Von Novo Nordisk wurde sie als ideale Methode zur Insulinreinigung perfektioniert.

Das resultierende Humaninsulin weist nur noch Verunreinigungen unterhalb der Nachweisgrenze der feinsten Radioimmunoassay-Techniken auf.

1.1.6 Abfallentsorgung

Nach der Herstellung des Humaninsulins werden die benutzten Hefezellen durch Hitze und Lauge abgetötet. Seit Novo Nordisk die Humaninsulinproduktion aufgenommen hat, wurden niemals gentechnisch veränderte Hefezellen ausserhalb der Produktion gefunden.