(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) **EP 1 520 859 B9**

(12) **CORRECTED EUROPEAN PATENT SPECIFICATION**

(15) Correction information:
Corrected version no 1 (W1 B1)
Corrections, see
Description Paragraph(s) 2-5, 9-14, 18-20
Numerous spelling errors of minor importance

(48) Corrigendum issued on:
29.12.2010 Bulletin 2010/52

(45) Date of publication and mention of the grant of the patent:
14.04.2010 Bulletin 2010/15

(21) Application number: **04030374.5**

(22) Date of filing: **12.01.1995**

(51) Int Cl.:
C07K 14/00 *(2006.01)* ***A61K 38/00*** *(2006.01)*
C12N 9/04 *(2006.01)*

(54) **Hydrophilic signal oligopeptides and methods of therapeutic use**

Hydrophile Signal-Oligopeptide und Verfahren der therapeutischen Anwendung

Oligopeptides signaux hydrophiles et leurs procédés d'utilisation à des fins thérapeutiques

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE

(30) Priority: **14.01.1994 US 182248**

(43) Date of publication of application:
06.04.2005 Bulletin 2005/14

(62) Document number(s) of the earlier application(s) in accordance with Art. 76 EPC:
95908522.6 / 0 744 027

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(56) References cited:
US-A- 4 554 101

- **CHIN, DANIEL J. ET AL: "Nucleotide sequence of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase, a glycoprotein of endoplasmic reticulum" NATURE (LONDON, UNITED KINGDOM) , 308, 613-17 CODEN: NATUAS; ISSN: 0028-0836, 1984, XP008094019**
- **LUSKEY, KENNETH L. ET AL: "Human 3-hydroxy-3-methylglutaryl coenzyme A reductase. Conserved domains responsible for catalytic activity and sterol-regulated degradation" JOURNAL OF BIOLOGICAL CHEMISTRY , 260 (18), 10271-7 CODEN: JBCHA3; ISSN: 0021-9258, 1985, XP002981007**
- **SKALNIK, DAVID G. ET AL: "The nucleotide sequence of Syrian hamster HMG-CoA reductase cDNA" DNA , 4(6), 439-44 CODEN: DNAADR; ISSN: 0198-0238, 1985, XP008093880**
- **19000101, 1 January 1900 (1900-01-01), XP008093979**

Remarks:

The file contains technical information submitted after the application was filed and not included in this specification

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EP 1 520 859 B9

Description

Field of Invention

[0001] The present patent claims further specific signal oligopeptides and their therapeutic use.

References

[0002]

1. Watson JD, Crick FHC. 1953. Nature 171:737
2. Rath. M. 1993. J. Orthom. Medicine 8:11
3. Rath, M. 1994. J Appl. Nutr. (in Press)

Background

[0003] The discovery of the genetic code four decades ago defined the principles by which genes encode for proteins (1). The protein code, however, the biological language by which protein act and interact has remained obscure. Now the protein code is discovered (2,3). The interaction of hormones and other ligands with their respective receptors, of enzymes with protein substrates, of adhesive proteins with integrins, and of antibodies with antigens as well as other protein actions and interactions are determined by the same structure/function principles and the same biological language. For a very limited number of proteins the hydrophobic interior of the protein forms the active center which also determines their function. In the vast majority of proteins, however, biological activity is primarily mediated via specific surface structures which mediate their specific functions. The structure/function principles determining these protein actions are discovered (1) and they are hereafter referred to as the protein code.

Summary of the invention

[0004] Like the human language the protein code consists of letters words and sentences. The letters (amino acids) and sentences (complete three dimensional protein) had been known before. The new discovery are the protein words or verbs. These protein verbs are represented by signal oligopeptides which are localized on the surface of the protein and are represented by the hydrophyticity maxima of the protein. These signal oligopeptides are enriched in charged amino acids in a versatile arrangement with neutral spacer ammo acids The specific signal character of these oligopeptides is determined by a characteristic combination of conformation and charge within the same signal sequence.

[0005] Like in the human language the whole sentence (complete three dimensional protein) is needed to determine the specific and complete action of any given protein. In the human language eliminating or changing the verb of a sentence renders the whole sentence meaningless. Similarly, blocking the protein code verbs (signal oligopeptides) can be therapeutically used to block the undesired action or interaction of an entire protein.

[0006] The discovery of the protein code provides the rationale for deciphering the communication code of diseases. Infectious diseases, cancer, cardiovascular and other diseases develop by means of one or more pathogenicity-mediating proteins. Blocking the signal oligopeptides of these proteins allows the specific therapeutic interception of a pathological communication and thereby blocks disease propagation.

[0007] From Luskey and Stevens, 1985, The journal of Biological Chemistry 260: 10271-10277, it is known the full length cDNA for human 3-hydroxy-3-methylglutaryl coenzyme A reductase, the membrane bound glycoprotein that regulates cholesterol synthesis.

[0008] Synthetic analogs to signal oligopeptides can be used therapeutically in several ways. First, synthetic analogues to signal oligopeptides can be used as competitive inhibitors of pathological communication. Second, synthetic analogs to signal oligopeptides can be used as vaccines. This second therapeutic approach makes use of the fact that signal oligopeptides on the surface of the protein are identical with the antigenicity determining epitopes of this protein. Thus, antibodies are interceptors of metabolic communication. By binding to the signal oligopeptide of a protein antibodies and other mediators of immune response reduce or block its metabolic interaction. If synthetic analogs to signal oligopeptides are used as vaccines it is necessary to render these peptides antigenic and to allow their discrimination as 'non-self'. Synthetic analogs to signal oligopeptides can be rendered immunogenic by coupling them to haptens and by other conventional methods.

[0009] The invention is based on methods by which oligopeptide sequences can be rendered immunogenic. This method is based on the discovery of the primary structural principles determining immunogenicity. The discrimination between self and non-self between species and between individuals is primarily based on amino acid residue substitutions or other residue variations within the signal oligopeptide sequences of a protein. By making use of this discovery effective

therapeutic signal oligopeptide can be rapidly produced in the following way: signal oligopeptides of a given protein in one species are the antigenicity determinants of this protein in another species. To block the action of a pathogenicity-mediating protein in the treatment of a human disease the synthetic signal oligopeptide vaccines are designed by copying corresponding amino acid signal sequences from another species. A glucagon signal oligopeptide vaccine for the treatment of diabetic patients would be based on glucagon signal sequences from rabbits, sheep, mice or other species. A titration of the therapeutic efficiency is possible: The greater the genetic and evolutionary distance of the selected species to humans the greater its antigenicity and consequently the greater its therapeutic efficiency as a vaccine.

Detailed Description of the Figures

[0010]

Figure 1. This figure illustrates several of the protein code principles. *Legibility:* Within the amino acid sequence of a protein one or more oligopeptides represent the words - or verbs - of the protein code which mediates its action and interaction. *Accessibility*: The signal sequences of a protein are enriched in charged amino acids and represent the hydrophylicity maxima within the potential sequence (Figure 1A). *Variability*: An infinite number of possible combinations between amino acids with different charges as well as neutral resides provide the variability for differentiated metabolic communication. *Specificity:* The specificity of a signal sequence is the result of a characteristic combination of charge distribution and structural conformation within the signal oligopeptide sequence. Oppositely charged amino acids can attract each other thereby enhancing confirmational specificity. Signal oligopeptides mediate specific information transfer to their metabolic counterparts (Figure 1 B). *Differentiation*: Substitution, deletion or other amino acid residue variations within the signal sequence of a protein enable differentiation between 'self' and 'non-self'. Signal sequences are the antigenic epitopes of a protein and are responsible for potential immune responses when exposed to other organisms. *Interception:* Direct and indirect Peptide Interception Therapy (PIT) can be used to intercept undesired or pathological communication and thereby block the disease. In direct PIT synthetic analogs to signal oligopeptides can be used to competitively inhibit the interaction of proteins (Figure 1C). Indirect PIT makes use of synthetic oligopeptides rendered immunogenic. These vaccines stimulating the production of antibodies which block pathological communication pathways not only in the prevention and treatment of infectious diseases but also in the therapy of neoplastic diseases, metabolic disorders and other diseases (Figure 1D).

Figure 2. Fundamentals of Peptide interception Therapy (PIT). Proteins are essential carriers of specific metabolic information: moreover, proteins are frequently mobile which makes them ideal and versatile communication molecules. This figure illustrates several key elements of PIT: PIT is an interception therapy. To *promote protein action*, the entire three dimensional protein structure (protein sentence) is required. To *intercept protein action,* only the signal oligopeptide (verb) has to be blocked by direct or indirect PIT.

Figure 2 shows the principles of Direct Peptide interception Therapy (Direct PIT) including the methods for identification, design, development and therapeutic use of synthetic analogs to signal oligopeptides in Peptide Interception Therapy as direct competitive inhibitors of selected protein actions.

Figure 3. This figure shows the principles of Indirect Peptide Interception Therapy (Indirect PIT) including the methods for identification, design, development and therapeutic use of synthetic analogs to signal oligopeptides in Peptide Interception Therapy as vaccines to stimulate a specific immune response with the aim to decrease or block selected protein actions.

Figure 4. This figure shows the principles of Peptide Regulation Therapy (PRT) including the methods for identification, design, development and therapeutic use of synthetic analogs to signal oligopeptides in Peptide Regulation Therapy as negative feed-back regulators for the synthesis rate of selected proteins.

Figure 5. Amino acid sequence selection and therapeutic design of peptide vaccines for Indirect Peptide interception Therapy, exemplified for the development of glucagon vaccines in clinical therapy of diabetes mellitus.

Figure 6. Peptide therapy in diabetes. This figure shows the potential signal sequences of proglucagon and therapeutic alternatives for peptide therapy in diabetic patients. Conventional treatment in diabetes focuses on an increased availability of insulin. Peptide therapy enables an alternative approach. Synthetic analogs to the signal sequences of glucagon can be therapeutically used to attenuate the effect of this insulin antagonist. Note the three hydrophilicity peaks in the glucagon precursor sequence (A,B,C). The mature glucagon hormone is activated by contact and charge redistribution within the peaks A and C. PIT therapy could have two distinct targets: Blocking

charge redistribution of precursor molecule (PIT for Peak A or C) prevents activation of precursor to mature hormone. PIT targeting peak B prevents the action of the glucagon hormone. Vaccination with synthetic glucagon signal oligopeptides rendered immunogenic could become a baseline treatment for diabetic patients.

I. Determining Principles of the Protein Code

[0011] The protein code is determined by the following principles:

1. Universality. The protein code is universally valid. Proteins are important carriers of metabolic information in living organisms. The protein code is the biological language for protein-mediated information transfer during health and disease. The protein code is the underlying communication principle for the interaction of antigens with antibodies, enzymes with substrates, receptors with ligands, adhesion molecules with integrins and other forms of protein communication.

2. Compatibility. The protein code is compatible with other elements of biological communication. The protein code and the genetic code are closely connected. The genetic code determines the structure of proteins and, thereby, their function. The protein code determines the relation between structure and function within a protein sequence and, thereby, the specific biological action. Specific and efficient action of a protein are responsible for the advantage of a gene and, therefore, for the survival of the genetic information encoding for the protein.

3. Legibility. The protein code is composed of letters, sentences and words. The protein code *letters* are represented by the amino acid residues of a protein. The protein code sentence is represented by the complete three dimensional structure of the protein which ultimately determines its action. The decisive signal of protein communication is mediated by the protein code words. These protein code words are represented by one or more signal oligopeptides within a protein sequence. In the human language verbs are the action words within a sentence. Signal oligopeptides are the verbs of the protein code and determine the action of the protein. Interference with these signal oligopeptides can lead to substantial modification or loss of the biological message of a protein.

4. Accessibility. The protein code has to be accessible. Protein information transfer and protein interaction is dependent on the accessibility of the signal sequence. Signal oligopeptides are generally represented by the regions of maximum hydrophilicity on the surface of the protein molecule. Critical determinants for these hydrophilicity maxima is an enrichment of the cationic amino acid residues arginine and lysine and/or the anionic amino acid residues glutamate and aspartate.

5. Variability. The protein code requires a sufficient reservoir of structural variations encoding for a large number of diverse signals. The protein code is based on optimum variability. The protein code is based on an almost infinite number of possible signal oligopeptide structures. A specific arrangement of charged amino acid residues in combination with a variable number of uncharged residues within signal tripeptides, tetrapeptides, pentapeptides, hexapetides or larger polypeptides provide the necessary number of possible residue combinations for a giant signal reservoir.

[0012] A new type of signals is represented by oligopeptides which obtain their characteristic conformation by a specific arrangement of oppositely charged amino acid residues within this oligopeptide sequence. These residues with opposite charge can attract each other thereby modulating a characteristic folding of this signal sequence. In the signal sequence RGD the cationic residue arginine and the anionic residue aspartate attract each other leading to a characteristic folding of this tripeptide around the 'spacer' residue glycine.

6. Specificity. The protein code is specific. The specific and reliable metabolic function of a protein is dependent on the specificity of its biological signal sequences. The signal character of a specific oligopeptide is determined by a characteristic combination of electrical charge with structural conformation. Within a protein, RGD and analogous tripeptides can serve as strong primary anchors while the specific biological message is mediated by additional longer and more complex signal oligopeptides.

7. Differentiation. The protein code provides the basis for the immunological differentiation between individuals and species. Substitutions, omissions, and other variations of one or more amino acid residues within the signal sequence of the protein enable an organism to differentiate between 'self' and 'non-self'. Thus, the protein code comprises the basic language of immunology.

8. Diversification. The protein code is a key for individual development within a species as well as for the evolutionary diversification of species. The effectiveness of signal oligopeptides to mediate specific biological messages were the ultimate criterion for the evolutionary advantage of a protein and, thus, for the evolutionary survival of the gene encoding for it. Genetic mutations leading to the substitution of one or more amino acid residues within a signal oligopeptide sequence were an economic and therefore frequent mechanism to modulate and differentiate protein action and thereby promoting evolutionary diversification.

9. Interception. The protein code is interceptable. The signal oligopeptides of a protein are identical with the potential antigenic determinants. Antibodies and other mediators of immune response are interceptors of specific biological communication. Signal oligopeptides as promoters of differentiated protein communication and immune response mediators as interceptors can form a sophisticated network of biological communication. Decoding the physiologic aspects of this communication network will lead to a precise understanding of millions of metabolic interactions including the principles for development and differentiation of the body. Decoding the pathophysiological aspects of this communication network will lead to the therapeutic control of many diseases and eventually to their eradication as causes of human mortality.

10. Regulation. The protein code could also provide a missing link in the regulation of protein synthesis. The signal sequences of a protein may have important feed-back functions directly or indirectly modulating the synthesis rate of this protein.

II. Principles of Peptide Interception Therapy (PIT) and of Peptide Regulation Therapy (PRT)

[0013]

1. The metabolic interaction of proteins is primarily modulated by one or more oligopeptide signal sequences which determine the metabolic interaction of this protein. Peptide Interception Therapy (PIT) is defined as the specific therapeutic interception of pathological or undesired protein actions and interactions by the therapeutic use of synthetic analogs to the signal oligopeptide sequences of this protein. Peptide Regulation Therapy (PRT) makes therapeutic use of synthetic analogs to signal oligopeptide sequences to decrease the synthesis rate of an undesired protein via feed-back mechanisms.

2. Peptide interception Therapy uses the discovery of the protein code: To *promote* protein action, the entire three dimensional protein structure (protein sentence) is required. To *intercept* protein action, only the signal oligopeptide (protein verb) has to be therapeutically blocked.

3. These signal oligopeptide sequences are located on the surface of the protein and are represented by the hydrophilicity maxima within the amino acid sequence of the protein. The signal oligopeptide sequences of a protein can be identified from its primary structure by use of a protein data base in combination with a suitable algorithm, such as for hydrophilicity or surface probability. In this algorithm the highest hydrophilicity or probability values have to be assigned to the charged amino acids lysine, arginine, aspartate and glutamate followed by asparagine and glutamine.

4. Synthetic analogs to signal oligopeptide sequences can be therapeutically used. An unlimited number of signal oligopeptide analogs can be synthesized covering the entire sequence of a selected hydrophylicity peak or parts of it.

5. Signal tetrapeptides, pentapeptides, hexapeptides and longer peptides represent primary candidates for specific peptide therapy. Shorter peptides, such as the tripeptide RGD, are less specific and ubiquitous side effects limit their broad therapeutic use.

6. Peptide Interception Therapy (PIT) can be used in two principal ways: Direct PIT uses synthetic analogs to signal oligopeptides as competitive inhibitors of pathological or undesired metabolic interaction. Indirect PIT uses these synthetic analogs as vaccines to stimulate the production of specific antibodies. In indirect PIT the antibodies, not the therapeutic peptide itself, function as interceptors of protein communication.

7. Direct Peptide Interception Therapy (Direct PIT) uses synthetic analogs to Signal oligopeptides as direct competitive inhibitors for undesired protein communication. Direct blocking of pathogenicity mediating protein communication leads to the control of the related disease or clinical condition. This therapeutic approach would be preferentially used in acute conditions, e.g. antithrombotic or fibrinolytic therapy. Direct PIT would be preferentially used

intravenously in higher therapeutic dosages of the synthetic peptide. Poly-oligopeptide analogs, time release delivery systems and other modifications of the peptide delivery mechanism can be used to extend the range of possible therapeutic applications.

8. Indirect Peptide Interception Therapy (Indirect PIT). Today vaccines are essentially limited to prophylaxis and therapy of infectious diseases. Indirect PIT enables the extension of the preventive and therapeutic use of vaccines to all areas of medicine. The great advantage of oligopeptide vaccines, compared to conventional vaccines, is that not the entire protein is used as a vaccine but only synthetic analogs to one or more of the signal oligopeptides of the selected protein.

9. Indirect PIT is based on the therapeutic use of antibodies produced by the patient's own immune system against the signal sequences of proteins mediating pathogenicity for undesired metabolic action. Indirect PIT is preferentially used for preventive therapy, for the treatment of chronic conditions or as adjuncts to direct PIT or other forms of acute therapy.

10. Indirect PIT makes use of two newly discovered principles of immunology: First, the signal oligopeptides of a given protein are identical with its potential antigenicity determining regions (antigenic epitopes). Second, the primary mechanism determining antigenicity between different individuals and different species are amino acid residue substitutions, omissions and other residue variations within the signal oligopeptide sequence(s) of a protein.

11. Therapeutic peptide design for indirect PIT is based on the following principle: Signal oligopeptides of a given protein in one species are the antigenic epitopes of this protein for the immune system of another species. To block the action of a pathogenicity-mediating protein in the treatment of a human disease, the amino acid residue sequence of the oligopeptide vaccines should be homologous to the signal sequences of the same protein - but from another species. A glucagon signal oligopeptide vaccine for the treatment of diabetic patients would be based on the glucagon signal sequences from rabbits, sheep, mice or other species. The aim of this residue manipulation is to create an antigenic epitope without compromising the ability of the antibodies produced to effectively block the metabolic interaction of the protein. This therapeutic approach mimics nature's way to discriminate between 'self' and non-self and make therapeutic use of it.

12. The designer of therapeutic compounds for indirect PIT can also make use of the evolutionary clock. The further apart two species are in evolution, the more amino acid residue mutations occurred, including mutations in the signal oligopeptide sequences, and the more antigenic is the therapeutic peptide composed of this sequence. A titration of the therapeutic efficiency of indirect PIT is possible: Indirect PIT therapy with synthetic analogs to glucagon signal oligopeptides from a *fish* species are more effective than those from a *mammalian* species in blocking *human* glucagon action.

13. Antigenicity required for effective indirect PIT therapy can also be stimulated in conventional ways, e.g. by coupling the therapeutic peptides to defined haptens or other immunogenic compounds enhancing 'non-self' recognition.

14. A third therapeutic mechanism using synthetic analogs to signal oligopeptides is as feed-back regulators for protein synthesis. This form of therapy is designated Peptide Regulation Therapy (PRT). The synthesis rate of many proteins is determined by the amount of protein end-product available. The signal sequences of pathogenicity mediating proteins can be used to decrease the synthesis of this protein via direct or indirect feedback mechanisms.

III. Advantages of Peptide Therapy

[0014]

1. Efficacy. Peptide therapy is a highly effective form of treatment. The discovery of the peptide code provides the rationale for deciphering the communication code of human diseases and allows the interception of pathological interactions with maximum effectiveness.

2. Specificity. Peptide therapy enables maximum therapeutic specificity. Based on the precise understanding of the structure/function relation of specific protein signals, peptide therapy allows therapeutic targeting with unprecedented specificity.

3. Safety. Peptide therapy is extremely safe. The use of synthetic analogs to physiologic compounds essentially eliminates the problem of toxicity. Possible undesired biological side-effects should be controllable by optimizing the length and composition of the therapeutic peptide.

4. Savings in therapeutic research and development. Time and expenses for the development of therapeutic peptides is a fraction of conventional therapeutic research and development. Identification of potential therapeutic peptides takes minutes; in vitro screening of potential peptides is a matter of weeks: animal studies should provide first in vivo results within a few months. Most importantly, the unprecedented specificity and safety background of peptide therapy will allow clinical studies without delay.

5. Advantage of peptide therapy compared to conventional therapies. The advantages of peptide therapy become even more obvious when this novel therapeutic approach is compared to conventional therapies or alternative future therapies. *Conventional drug therapy* is frequently compromised by an unknown therapeutic mechanism and by a wide range of side effects and considerable toxicity. *Gene therapy* is compromised by limited availability, its technological requirements and its high costs, which restricts its therapeutic application to exclusive areas in the foreseeable future. *Heterologous or synthetic antibody therapy,* the therapeutic application of antibodies produced outside of the patient's body, can cause incalculable adverse reactions by the patient's immune system against these 'foreign' antibodies. In contrast, peptide therapy makes elegant use of the patient's own immune system thereby excluding adverse immunological reactions.

IV. Therapeutic Applications of Peptide Therapy

[0015] ***Metabolic disorders***. A novel therapeutic area for peptide therapy is the treatment of metabolic disorders. The potential of peptide therapy is exemplified here for the treatment of diabetes and hypertension.

[0016] *Diabetes.* Conventional diabetic therapy aims at an increased availability of insulin, Peptide therapy allows a novel and alternative approach by inhibiting the action of glucagon, the insulin antagonist. The inhibition of glucagon can be accomplished by using the therapeutic peptides analogous to the glucagon signal sequence for direct competitive inhibition or as a vaccine (Figure 5).

[0017] The following oligopeptide signal sequences of Hydroxyl-methyl-glutaryl coenzyme A reductase as well as their use for therapeutic puposes are claimed in this patent:

HYOROXY-METHYL-GLUTARYL COENZYME A REDUCTASE

INFORMATION FOR SEQUENCE ID NO 42

[0018]

(A) LENGTH
(B) TYPE amino acid
(D) TOPOLOGY linear
-S-Q-D-E-V-R-E-N-

INFORMATION FOR SEQUENCE ID NO 44

[0019]

(A) LENGTH:
(B) TYPE: amino acid
(D) TOPOLOGY linear
-E-L-S-R-E-S-R-E-G-R-

Table 1: The Maxima of the Following Algorithms -or Modifications Thereof-Can Be Used to Determine Potential Signal Oligopeptides in the Primary Structure of a Protein From a Protein Sequence Database

Amino Acid Residue	A. Hydrophylicity Algorithm*	B. Surface Probability Algorithm**
Arginine	3.0	9.5

(continued)

Table 1: The Maxima of the Following Algorithms -or Modifications Thereof-Can Be Used to Determine Potential Signal Oligopeptides in the Primary Structure of a Protein From a Protein Sequence Database

Amino Acid Residue	A. Hydrophylicity Algorithm*	B. Surface Probability Algorithm**
Aspartate	3.0	8.1
Glutamate	3.0	8.4
Lysine	3.0	9.7
Aspartate or Asparagine	1.6	8.0
Glutamate or Glutamine	1.6	8.4
Serine	0.3	6.5
Asparagine	0.2	7.8
Glutamine	0.2	8.4
Glycine	0.0	4.8
Proline	0.0	7.5
Threonine	-0.4	7.0
Alanine	-0.5	4.9
Histidine	-0.5	6.6
Cysteine	-1.0	2.6
Methionine	-1.3	4.8
Valine	-1.5	3.6
Isoleucine	-1.8	3.4
Leucine	-1.8	4.0
Tyrosine	-2.3	7.6
Phenylalanine	-2.5	4.2
Tryptophan	-3.4	5.1

C. Algorithm Based on the Following Amino Acid Categories:

1. Highest Values Assigned to Charged Amino Acids: Aspartate, Glutamate, Lysine, Arginine, Histidine
2. Medium Values Assigned to Uncharged Polar Amino Acids: Asparagine, Glutamine, Glycine, Cysteine, Serine, Threonine, Tyrosine
3. Lowest Values Assigned to Non Polar Amino Acids: Alanine, Valine, Leucine, Isoleucine, Proline, Phenylalanine, Methionine, Tryptophan

* According to Hopp TP, Woods. KR. 1981. Proc. Natl. Acad. Sci. USA. 78: 3824- 3828.

** Boger. Proceedings of the 1988 Miami Bio/Technology Winter Symposium. 10 Oxford and Washington. IRL Press.

Table 2

Methods of Use disclosed For the Signal Sequences in This Patent And For Those in Preceding Patents With Serial No. 07/997,734; 07/997,727; and 08/045,394

A. Synthetic Analogs to Human Signal Sequences Can Be Used	B. Synthetic Analogs to Protein Signal Sequences From *Other Species* Can Be Used
1. As Therapeutic Agents for the Direct Competitive Inhibition of Selected Protein Interaction (Direct PIT).	1. As Therapeutic Agents (Vaccines)in the Prevention and Treatment of Human Diseases. Stimulating a Specific Immune Response Which Blocks or Decreases the Action of the Selected Protein in the Human Body.
2. As Therapeutic Agents in Feed-Back Regulation With the Therapeutic Aim to Decrease the Synthesis Rate of the Selected Protein (PRT).	2. As Therapeutic Agents According to 1 and 2 in Column A in the Treatment of Diseases in the Respective Animal Species.

(continued)

Table 2

Methods of Use disclosed For the Signal Sequences in This Patent And For Those in Preceding Patents With Serial No. 07/997,734; 07/997,727; and 08/045,394

A. Synthetic Analogs to Human Signal Sequences Can Be Used	B. Synthetic Analogs to Protein Signal Sequences From *Other Species* Can Be Used
3. As Therapeutic Agents in Combination With Haptens or other Conventional Immunogens or Adjuvants as Vaccines Stimulating a Specific Immune Response Which Blocks or Decreases the Action of the Selected Protein (Indirect PIT).	3. As Antigens to Produce Antibodies Against the Selected Protein for *In Vitro* Diagnostic Purposes in the Respective Animal Species.
4. As Antigens to Produce Antibodies Against the Selected Human Protein for *In Vitro* Diagnostic Purposes.	

Table 3

The Therapeutic Use of Synthetic Signal Sequences as Vaccines (Indirect Peptide Interception Therapy)

A. Diseases Mediated by *Xenologous* Proteins (Infectious Diseases)	B. Diseases Mediated by *Human* Proteins (e. g. Metabolic Disorders. Chronic Human Diseases)
1. Choose Signal Sequence From Xenologous Protein (e.g. Toxin) as Basis For Vaccine in Humans	1. Choose from Different Species the Signal Sequences Corresponding to the Selected Human Proteins (Xenologous Signal Sequence) as Basis For Vaccine
2. Use Vaccines in the Prevention and Treatment of Infectious Diseases	2. Use Vaccines in the Prevention and Treatment of Metabolic Disorders, Cardiovascular Diseases and other Human Diseases

Claims

1. An oligopeptide consisting of an amino acid sequence Ser-Gln-Asp-Glu-Val-Arg-Glu-Asn as shown in SEQ ID No. 42 or Glu-Leu-Ser-Arg-Glu-Ser-Arg-Glu-Gly-Arg as shown in SEQ ID No. 44.

2. A pharmaceutical composition comprising an oligopeptide of claim 1.

3. The oligopeptide of claim 1 for use in therapy.

Patentansprüche

1. Ein Oligopeptid, das aus der in der SEQ ID No. 42 gezeigten Aminosäuresequenz Ser-Gln-Asp-Glu-Val-Arg-Glu-Asn oder der in der SEQ ID No. 44 gezeigten Aminosäuresequenz Glu-Leu-Ser-Arg-Glu-Ser-Arg-Glu-Gly-Arg besteht.

2. Eine pharmazeutische Zusammensetzung, die das Oligopeptid nach Anspruch 1 umfasst.

3. Das Oligopeptid nach Anspruch 1 für die Verwendung in der Therapie.

Revendications

1. Oligopeptide consistant en une séquence d'acides aminés Ser-Gln-Asp-Glu-Val-Arg-Glu-Asn telle que représentée dans SEQ ID n° 42 ou Glu-Leu-Ser-Arg-Glu-Ser-Arg-Glu-Gly-Arg telle que représentée dans SEQ ID n° 44.

2. Composition pharmaceutique comprenant un oligopeptide selon la revendication 1.

3. Oligopeptide selon la revendication 1 destiné à être utilisé en thérapie.

Figure 1

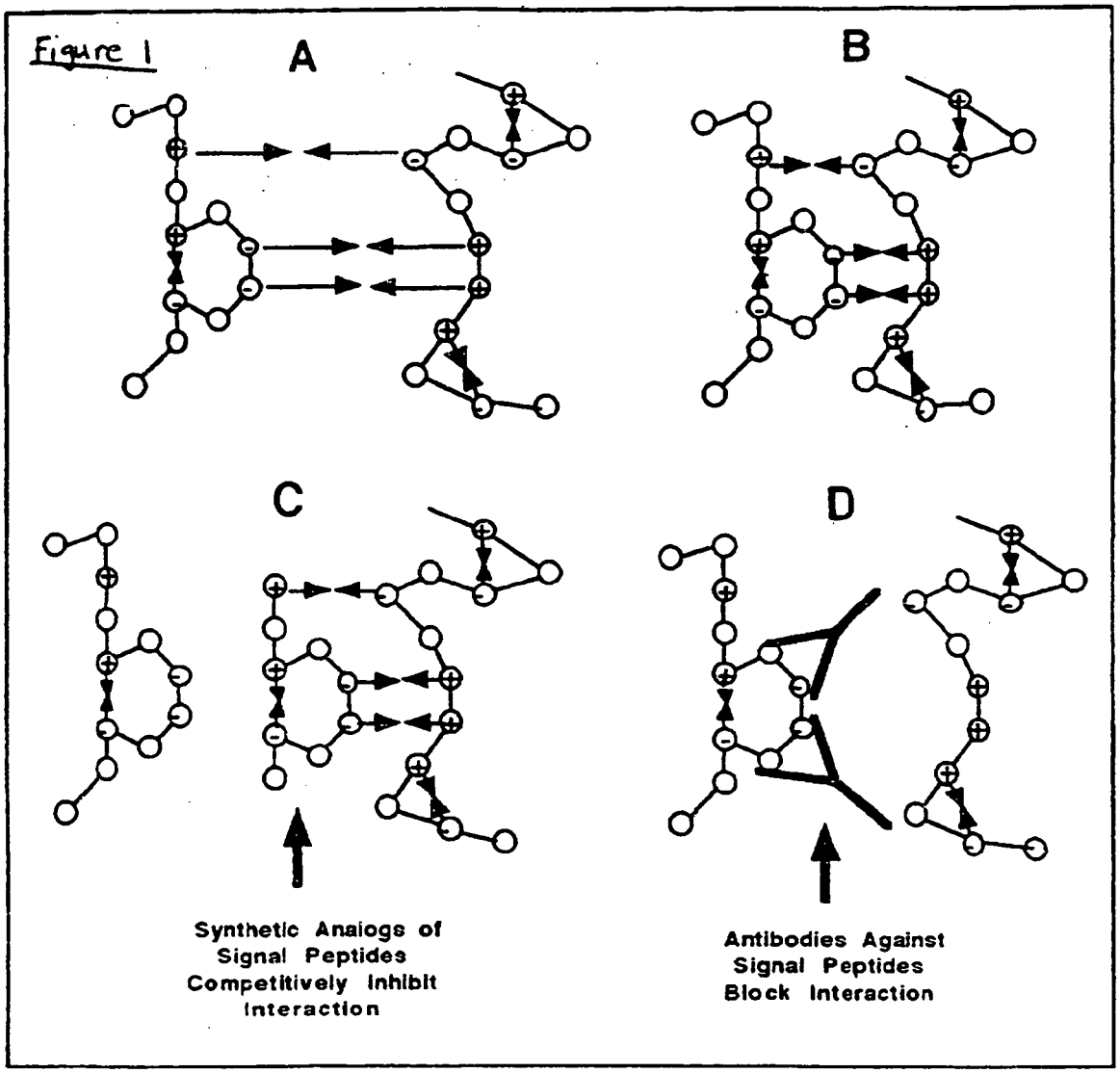

A
B
C
D
Synthetic Analogs of Signal Peptides Competitively Inhibit Interaction
Antibodies Against Signal Peptides Block Interaction

Figure 2: Direct Peptide Interception Therapy (Direct PIT)

Methods For Identification, Design, Development and Therapeutic Use of Synthetic Analogs to Signal Oligopeptides in Peptide Interception Therapy as Competitive Inhibitors Decreasing or Blocking Selected Protein Action

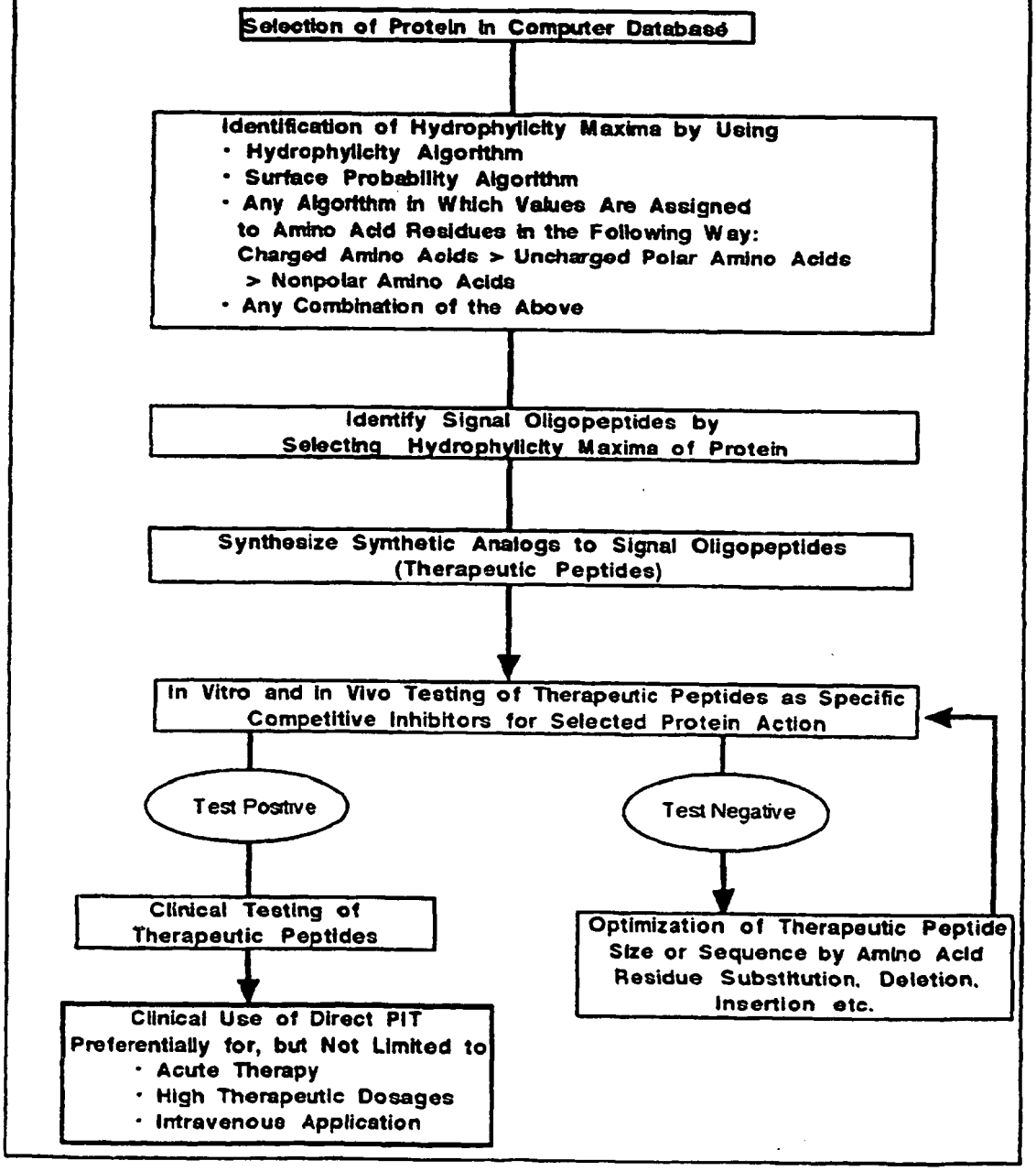


Figure 3: Indirect Peptide Interception Therapy (Indirect PIT)

Methods for Identification, Design, Development and Therapeutic Use of Synthetic Analogs to Signal Oligopeptides in Peptide Interception Therapy as Vaccines to Stimulate a Specific Immune Response Which Decreases or Blocks Selected Protein Action

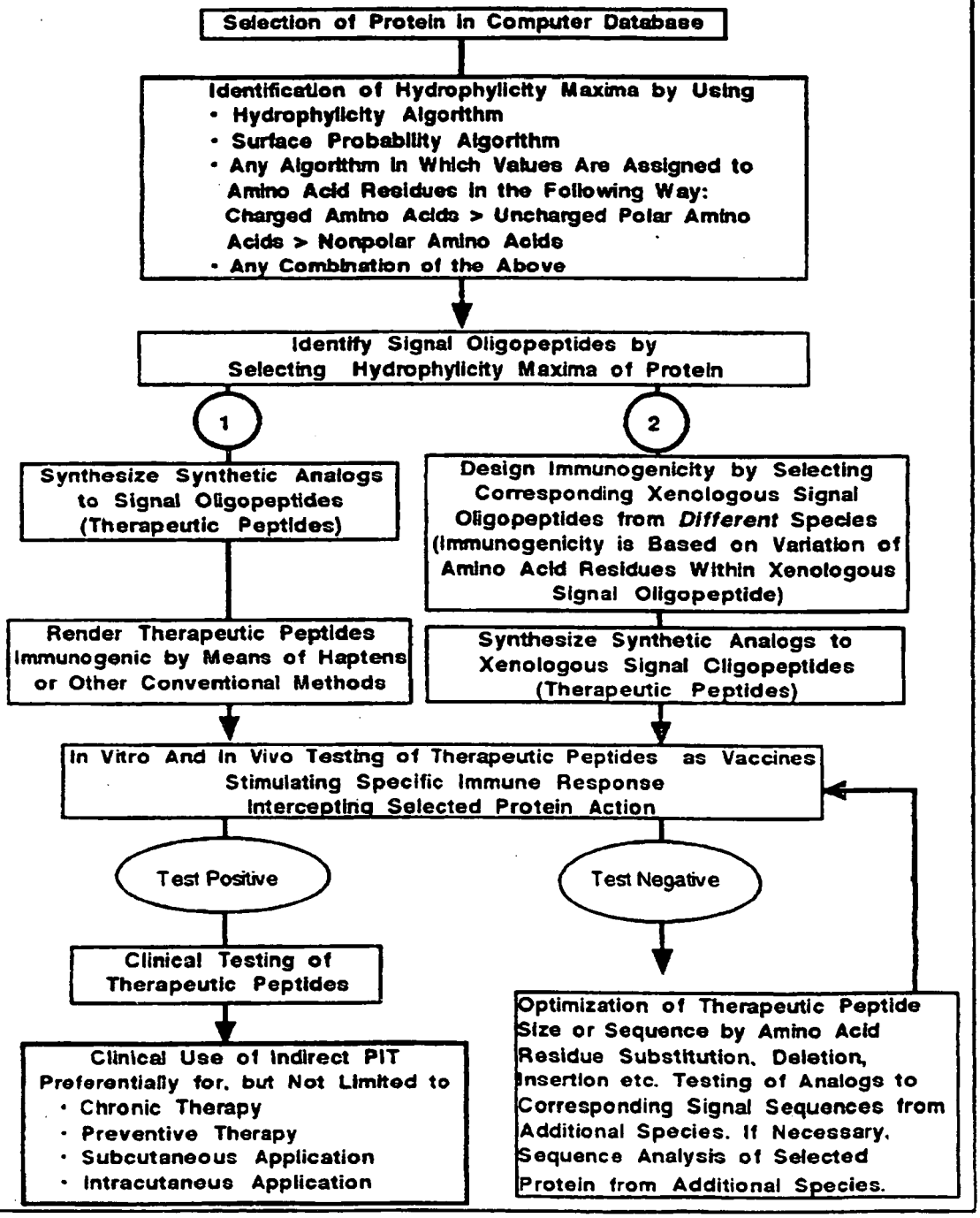


Figure 4: Peptide Regulation Therapy (PRT)

Methods For Identification, Design, Development and Therapeutic Use of Synthetic Analogs to Signal Oligopeptides in Peptide Regulation Therapy as Negative Feed-Back Regulators for the Synthesis Rate of Selected Proteins

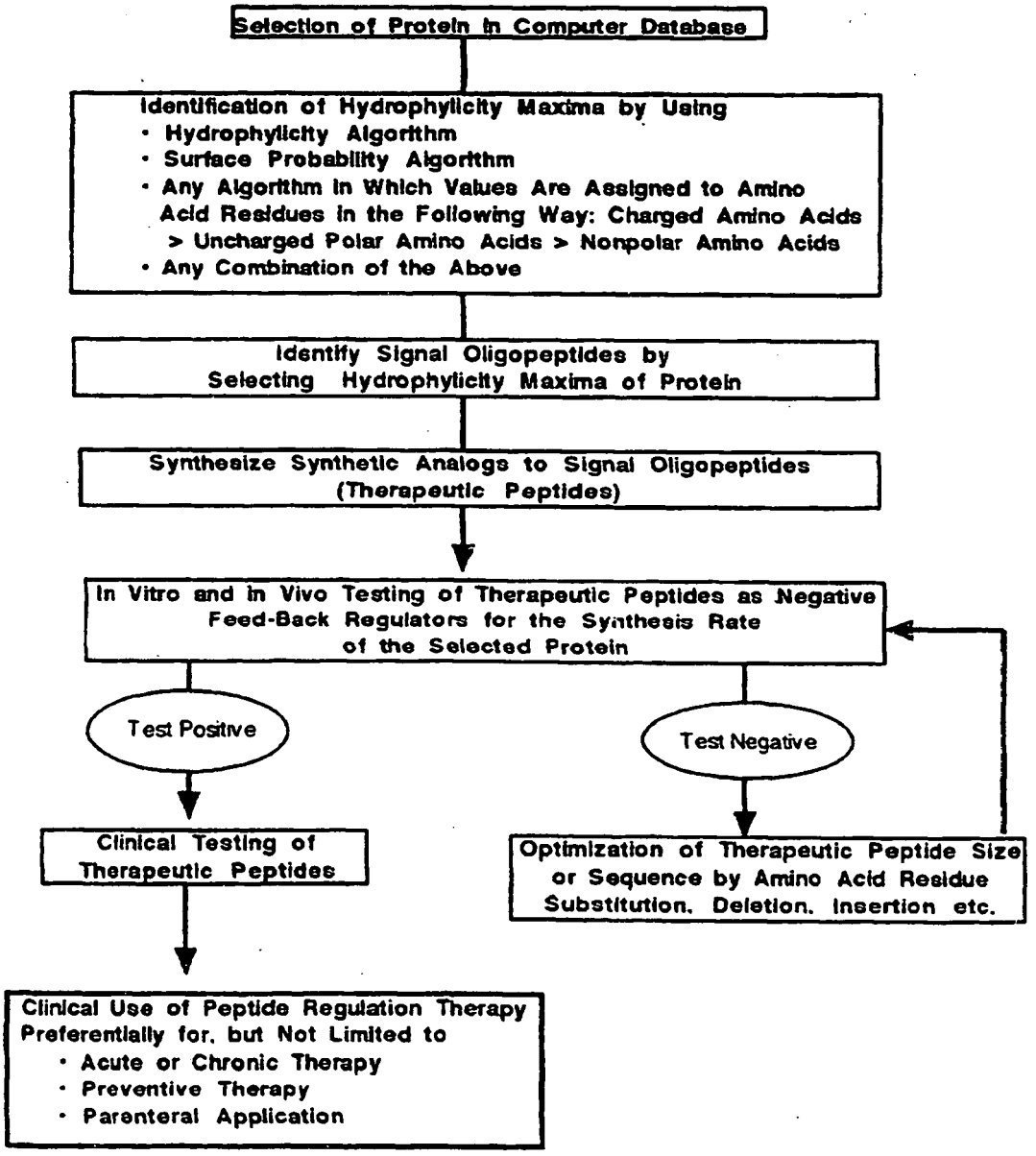
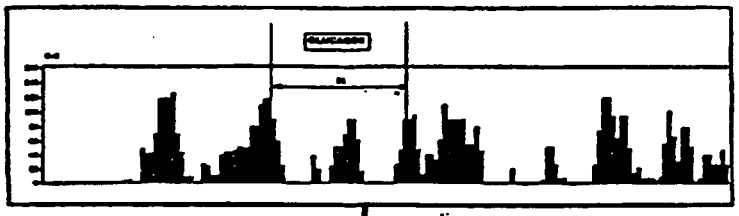


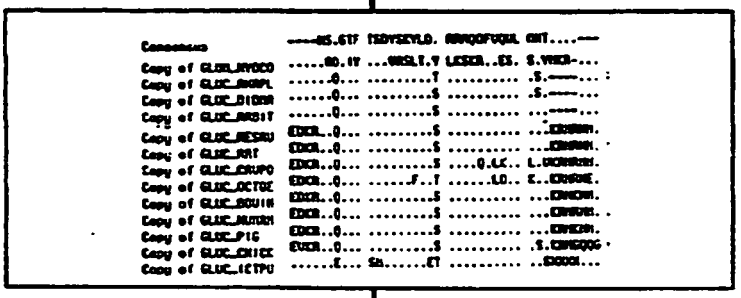
Figure 5: Amino Acid Sequence Selection and Therapeutic Design of Peptide Vaccines For Indirect Peptide Interception Therapy

Exemplified for the the Development of Glucagon Vaccines for Indirect Peptide Interception Therapy of Diabetes Mellitus

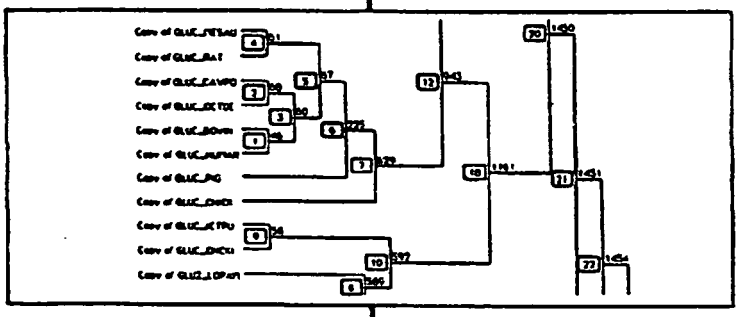
Identify Signal Oligopeptides From Hydrophylicity Maxima From Human Glucagon Precursor Sequence



Identify Corresponding Signal Oligopeptide from Glucagon Precursor Sequences in *Different Species*



Use Evolutionary Tree to Determine Relative Distance of Available Glucagon Sequences to Human Sequence. The Evolutionary Distance is Positively Correlated with Degree of Amino Acid Variation and, thus, with the Antigenicity of the Selected Protein

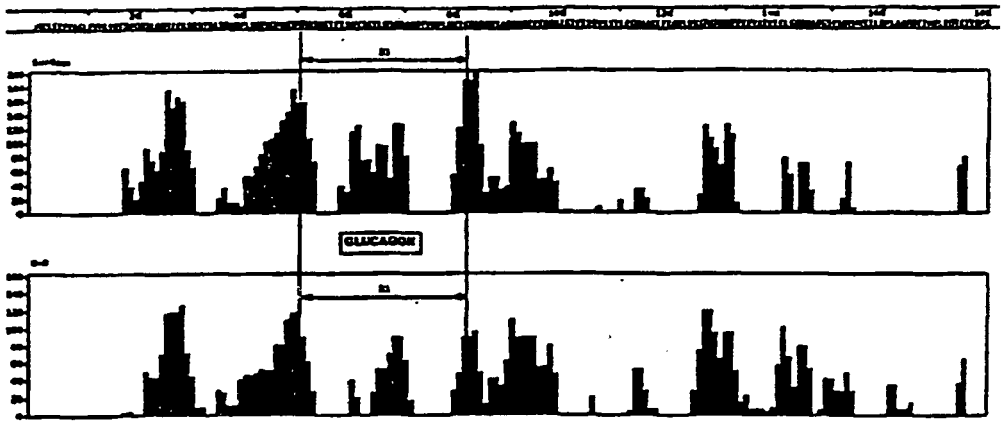


Select Therapeutic Peptide Sequence Among The Corresponding Signal Oligopeptide Sequences According to the Following Criteria

1. Moderate Therapeutic Immune Response Desired: Design of Therapeutic Peptides Analogous to Signal Sequences From Species Genetically Close to Humans (e.g. Mammals)
   -> Amino Acid Residue Variation Within Therapeutic Peptide (Vaccine) Must Be Sufficient to Cause Immune Response
   -> Therapeutic Use of Immune Response

2. Strong Therapeutic Immune Response Desired: Design of Therapeutic Peptides Analogous to Signal Sequences From Species Genetically More Distant to Humans (e.g. Fish, Yeast)
   -> More Amino Acid Residue Variation Within Signal Sequence
   -> Greater Antigenicity of Therap.Peptide
   -> Higher Therapeutic Efficiency

Figure 6: Glucagon Signal Sequences And Therapeutic Use in Direct And Indirect Peptide Interception Therapy

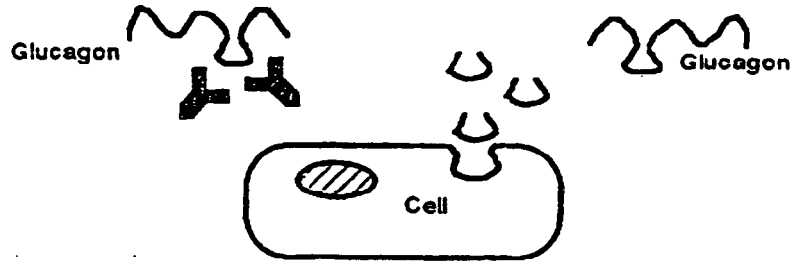


Direct And Indirect Peptide Interception Therapy in Diabetes

- *Decrease* Effect of Glucagon
- *Increase* Relative Effect of Insulin

1. Antibodies Against Glucagon Vaccine (Synthetic Signal Oligopeptide) Decrease Action of Glucagon

2. Synthetic Analogs to Glucagon Signal Oligopeptides Competitively Inhibit Action of Glucagon



REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US 07997734 B **[0019]**
- US 07997727 B **[0019]**
- US 08045394 B **[0019]**

Non-patent literature cited in the description

- **Watson JD ; Crick FHC.** *Nature,* 1953, vol. 171, 737 **[0002]**
- **Rath. M.** *J. Orthom. Medicine,* 1993, vol. 8, 11 **[0002]**
- **Rath, M.** *J Appl. Nutr.,* 1994 **[0002]**
- **Luskey ; Stevens.** *The journal of Biological Chemistry,* 1985, vol. 260, 10271-10277 **[0007]**
- **Hopp TP ; Woods. KR.** *Proc. Natl. Acad. Sci. USA.,* 1981, vol. 78, 3824-3828 **[0019]**
- **Boger.** Proceedings of the 1988 Miami Bio/Technology Winter Symposium. IRL Press **[0019]**