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Scientific Paper

167 Research by phage display of peptides with specific affinity for the amyloid peptide A-beta 1-42 for the MRI detection of amyloid plaques in Alzheimer disease

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Purpose

Alzheimer disease:

- principal cause of dementia in elderly (before the cerebral stroke and Parkinson disease) and the fourth cause of mortality in the developed countries (after cardiac diseases, cancer, and the cerebral stroke) [1].

Neurodegenerative disease :

- Loose of cognition abilities (memory, computing, etc)
- Intracellular lesions : neurofibrillary tangles (NFTs)
- Extracellular lesions : senile plaques composed of **beta-amyloid peptide** (A-beta) aggregated in parallel and anti-parallel beta-sheets

Beta-amyloid peptide: hydrophobic (the 42-AA fragment is more hydrophobic than the 40-AA one) characterized by [2 - 5]:

- Neurotoxicity (Figure 1 *1) [6, 7]:
  - Oxidative stress (H_2O_2)
  - Modifies neuronal calcemia
  - Inflammatory reaction

Actual diagnostic of AD :

- Cognitive tests (less rigorous)
- Post mortem detection of brain lesions

Great interest to find adequate techniques of molecular imaging [8 - 12]:

- Detection of senile plaques by micro-MRI
- A vectorized contrast agent is essential for clinical diagnosis: (1) reduces the time window for MRI scanning; (2) may allow the specific diagnisis of this neurodegenerative pathology.

Detection of AD by MRI:

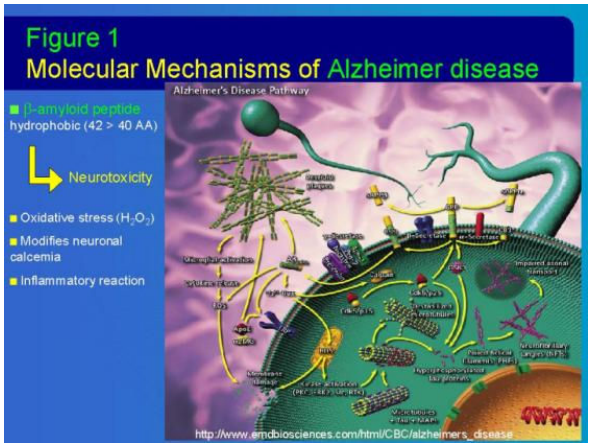
- Without contrast agent è too long time acquisition

Purpose of the present experiment:

Screening by phage display for low molecular weight vectors, specific for beta-amyloid peptide 1-42 :

- Grafted to contrast agents
- Diagnostic of AD by MRI

Linked images in Purpose:



*1:

Methods and Materials

The technology of phage display

Principle (Figure 2 [*2] and Figure 3 [*3]):

- relies on the **insertion of a foreign DNA** in the structural gene of a virus (bacteriophage), which leads to the **expression of a wide diversity of peptides** on the surface of the viral particle
- **selection of peptides specific to a target** molecule:
  - ensemble of phages (the "library") incubated with the targeted receptor
  - phages with highest affinity are retained
  - phage DNA sequence is analyzed and the peptide synthesized and grafted to the magnetic reporter

Method:

1) Rounds of selection :

- Target (A-beta$_{1-42}$, Bachem, Switzerland) immobilized by hydrophobic interactions on an ELISA plate
- Disulfide constrained phage display heptapeptide library (New England Biolabs, The Netherlands)
- Four rounds of selection of the phage display library:

- Pre-selection of the phage display library on BSA-coated plastic surface to remove the non-specific phages

- Selection on A-beta $_{1-42}$

- Augmentation of the selective pressure during each round of selection :

  - time of interaction: progressively increased (60, 45, 30 min)

  - concentration of the detergent Tween-20: progressively decreased (0.1, 0.3, 0.5 %)

- Non-specific elution by diminishing the pH in the presence of a solution of Glycine-HCl (pH 2.2, 0.2M)

2) Isolation of the phage clones

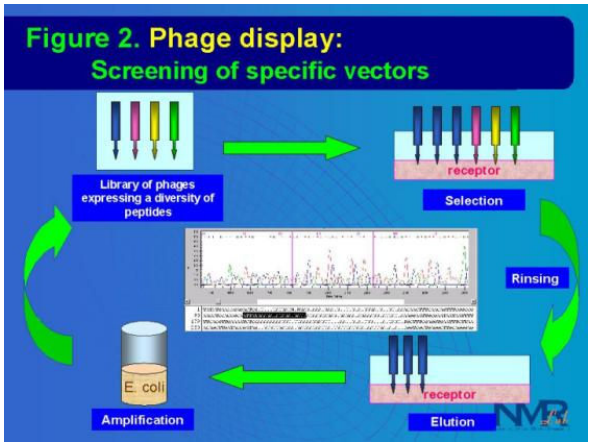
3) Global test of affinity

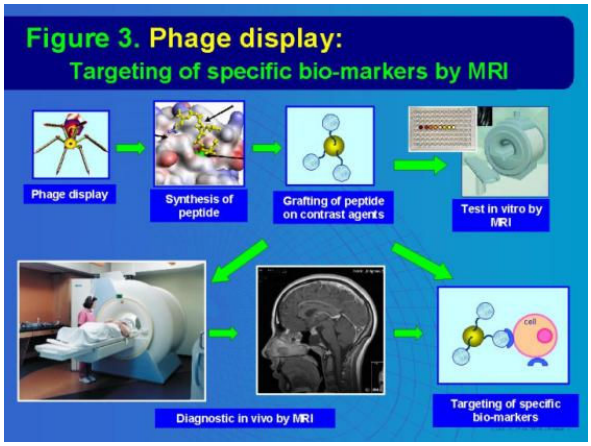
4) Sequencing (ELISA, phage detection with HRP-conjugated anti-M13 antibody)

5) Determination of the dissociation constant (K_d) of the selected phage clones

6) Synthesis of the selected peptide (NeoMPS, Strasbourg, France), biotinylation, and estimation of the apparent dissociation constant (K^*_d) (peptide detection with HRP-conjugated streptavidin) of the phage DNA

Linked images in Methods and Materials:





*2:

*3:

Results

Rounds of selection

The titer of phages obtained after each round of selection was estimated (**Figure 4** *4):

The effect of the selective pressure (coefficient of the selective pressure) (**Figure 4** *4):

Sequencing of the 23 selected phage clones:

The amino acid position in the peptides structure (**Figure 7** *5):

Determination of Kd:

Determination of the Kd of the selected peptide (clone 22):

- The apparent affinity is in the nM range, but is lower than of the corresponding phage clone (**Figure 10** *6)

- Possibly the biotinylation is responsible for the lower affinity, or the system of detection is not sensitive enough.
    - The K_d of the 23 selected phage clones ranged from $2.2x10^{-10}$ M (**Figure 8** *7) to $2.0x10^{-9}$ M
    - Peptide exposed by the clone 22 (K_d = 2.20 10^{-10} M) chosen to be synthesized and grafted to a contrast agent (**Figure 9** *8)
    - Positions 1 3 (>25%) shared by hydrophobic AA: Ile, Phe, Pro, Leu
    - Leu in the 3rd position: 41%
    - The 5th position (36%) : Thr
    - The 7th position is shared by Asn (27%) and Gln (41%)
    - the peptide structure of the 23 phage clones was determined by analyzing the DNA sequence of the fusion insert
    - 12 different peptide sequences were identified
    - the most abundant AA (**Figure 6** *9):
        - Leu, Pro, Phe è hydrophobic
        - His è basic

- is maximal at the 4th round, equal to 35% at the 3rd round and less than 10% during the first 2 rounds

- this suggests an increasing affinity for the target and explains the diminished phage titer during the last two rounds

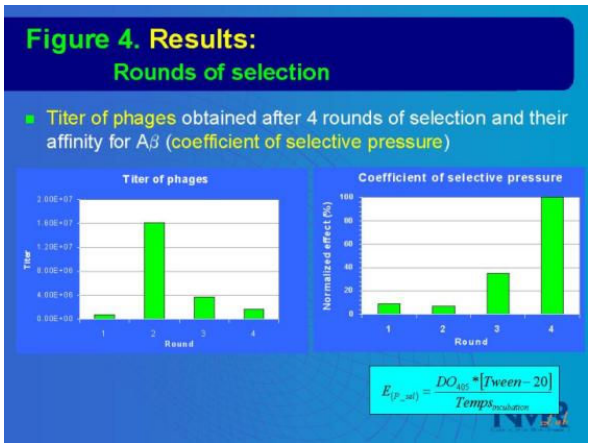
Isolation and characterization of the phage clones (**Figure 5** *10):

- 72 candidate phage clones arbitrarily isolated for further screening of affinity for the target
- the ELISA tests of affinity highlighted 23 phage clones with an optimal affinity for A-beta$_{1-42}$ è about 5 times higher than for BSA-coated plastic surface

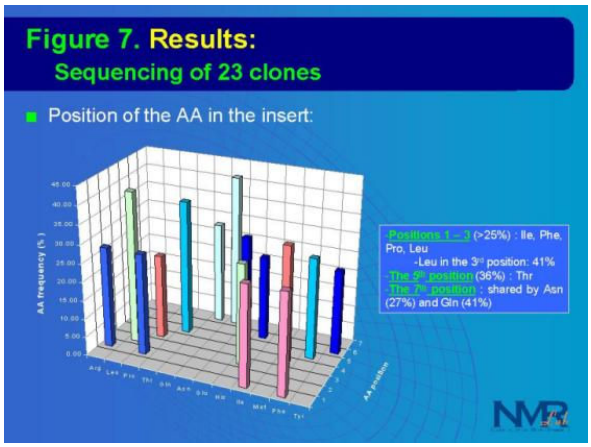
- increases after two rounds of selection by preserving the same conditions of the selective pressure

- decreases during the 3rd and the 4th rounds of selection as a result of increasing the selective pressure

Linked images in Results:



*4:



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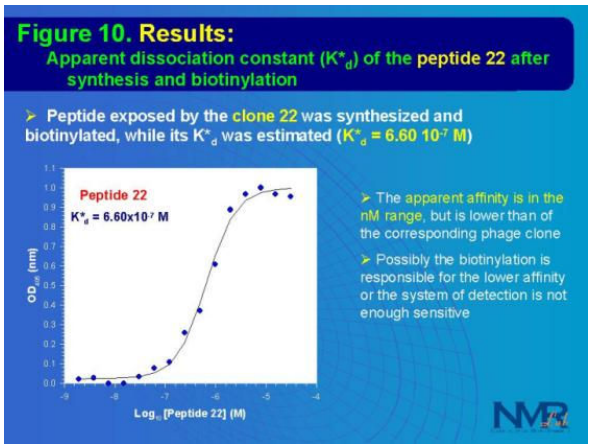
Figure 10. Results:
Apparent dissociation constant (K*d) of the peptide 22 after synthesis and biotinylation
Peptide exposed by the clone 22 was synthesized and biotinylated, while its K*d was estimated (K*d = 6.60 10-7 M)
Peptide 22
K*d = 6.60x10-7 M
ODnm (nm)
Log10 [Peptide 22] (M)
The apparent affinity is in the nM range, but is lower than of the corresponding phage clone
Possibly the biotinylation is responsible for the lower affinity or the system of detection is not enough sensitive

*6:

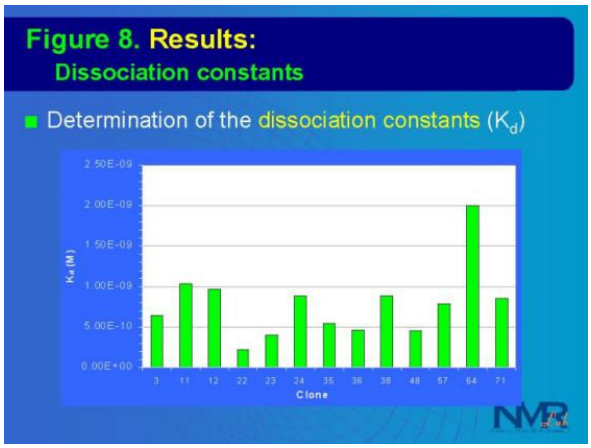
Figure 8. Results:
Dissociation constants
Determination of the dissociation constants (Kd)
Kd (M)
Clone

*7:

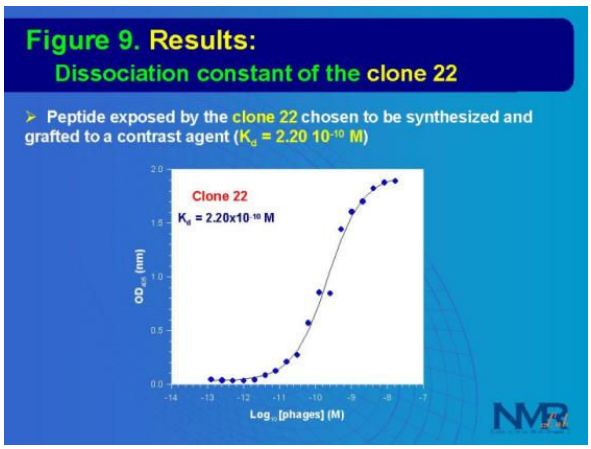
Figure 9. Results:
Dissociation constant of the clone 22
Peptide exposed by the clone 22 chosen to be synthesized and grafted to a contrast agent (Kd = 2.20 10-10 M)
Clone 22
Kd = 2.20x10-10 M
ODnm (nm)
Log10 [phages] (M)

*8:

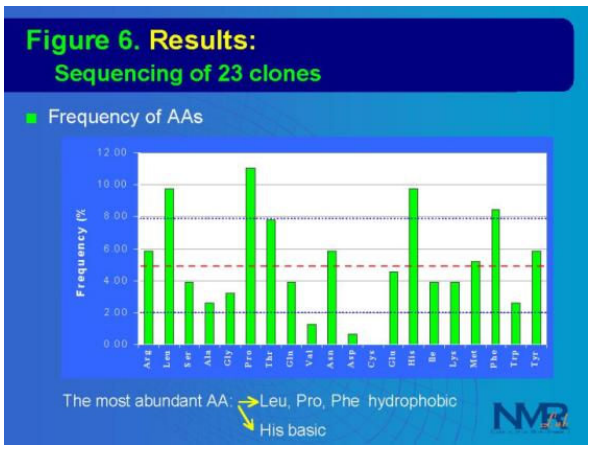
Figure 6. Results:
Sequencing of 23 clones
Frequency of AAs
Frequency (%
Arg Leu Ser Ala Gly Pro Thr Gln Val Asn Asp Cys Glu His Ile Lys Met Phe Trp Tyr
The most abundant AA: Leu, Pro, Phe hydrophobic
His basic

*9:

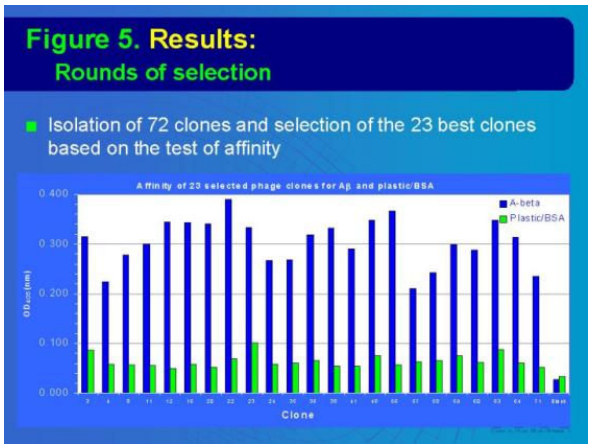
Figure 5. Results:
Rounds of selection
Isolation of 72 clones and selection of the 23 best clones based on the test of affinity
Affinity of 23 selected phage clones for Aβ and plastic/BSA
A-beta
Plastic/BSA
0.400
0.300
0.200
0.100
0.000
Clone

*10:

Conclusion

The results of this experiment emphasize the following main features:

- **Enrichment of the phage pool in peptides specific to A-beta 42**
- **The selection has favored 2 sequences rich in hydrophobic and basic AA**
- **The clone 22 is characterized by the best K_d (2.2×10^{-10} M)**
- **The corresponding peptide of the clone 22 has an apparent affinity in the nM range ($K^*_d = 6.60\ 10^{-7}$ M)**

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Keywords

Own Keywords:
phage display, Alzheimer disease, senile plaques, MRI, molecular imaging, contrast agent

U.S. National Library of Medicine is the creator, maintainer, and provider of all MeSH 2004 data.
