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Review

Rational design of complementary peptides to the βAmyloid 29–42 fusion peptide: An application of PepDesign

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Abstract

Peptides in solution currently exist under several conformations; an equilibrium which varies with solvent polarity. Despite or because of this structure versatility, peptides can be selective biological tools: they can adapt to a target, vary conformation with solvents and so on. These capacities are crucial for cargo carriers. One promising way of using peptides in biotechnologies is to decipher their medium–sequence–structure–function relationships and one approach is molecular modelling. Only few "in silico" methods of peptide design are described in the literature. Most are used in support of experimental screening of peptide libraries. However, the way they are made does not teach us much for future researches. In this paper, we describe an "in silico" method (PepDesign) which starts by analysing the native interaction of a peptide with a target molecule in order to define which points are important. From there, a modelling protocol for the design of 'better' peptides is set. The PepDesign procedure calculates new peptides fulfilling the hypothesis, tests the conformational space of these peptides in interaction with the target by angular dynamics and goes up to the selection of the best peptide based on the analysis of complex structure properties. Experimental biological assays are finally used to test the selected peptides, hence to validate the approach. Applications of PepDesign are wide because the procedure will remain similar irrespective of the target which can be a protein, a drug or a nucleic acid. In this paper, we describe the design of peptides which binds to the fusogenic helical form of the C-terminal domain of the Aβ peptide (Aβ29–42).

Keywords: Alzheimer; Amyloid; Angular dynamic; ApoE; Complementary peptide; Computer-aided design; In silico design; Peptide interaction; Tilted peptide

Contents

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1. Introduction

Despite the promising future of peptides in drug discovery, few methods of rational design of peptides are described in the literature. Several groups made peptides which inhibit a target protein with high affinity. They used peptide libraries expressed in phages or synthesized by solid-phase. Notably, Kasher et al. [1] and Katchalski et al. [2] designed peptides with a high affinity for α-Bungarotoxin (α-BTX), a toxic snake neurotoxin; these peptides inhibit the α-BTX binding to acetylcholine receptor (AchR) at the neuromuscular junction. They proposed a general approach named "systematic residue replacement" (SRR): they screened peptide libraries to identify a lead, characterized its interaction with the target by NMR or X-ray and performed a restricted SRR of the lead using residues categorized into 6 groups according to their physico-chemical properties. This method gave very good results but requires numerous biological assays in order to select the best peptides.

Other groups used molecular dynamics in order to simulate the interaction of a peptide with a target. Yang et al. [3] designed a peptide which had potential bioactivity to antagonize the function of human interleukin-6 (hIL-6) using molecular modelling and molecular dynamics trajectory analysis. However, due to the time required for molecular dynamics calculation, this method allows to test only few peptides.

We developed an "in silico" method named "PepDesign" to propose peptides with selected binding patterns. In this paper we used the method to make a binding partner to the helical form of Aβ29–42 peptide. By analogy to the SSR method, new peptides are designed by residue substitution of a template. The procedure is automatic up to the selection of molecules with improved interaction with the target. Experimental assays come as the validation and quantification of the peptide quality.

Aβ29–42 is implicated in the formation of senile plaques of Alzheimer's disease [4–6]. The peptide is known to have several conformations, from random coil to helix and to beta-extended forms. Because of its high hydrophobicity, the latter is responsible for the peptide aggregation often observed in NMR experiments. Beta aggregates are considered as a denaturated stable structural conformation. Before the peptide is aggregating, a transient helical form might have peculiar biological properties because of its hydrophobicity profile [7]. It should be a tilted peptide like the helical conformations of the N-terminal fragments of fusion proteins of several viruses such as SIV (Simian Immunodeficiency Virus) [8] or BLV (Bovine Leukaemia Virus) [9]. Tilted peptides are short fragments (10–20 residues) with an asymmetric hydrophobicity gradient along their helix axis. Their mean hydrophobicity leads them to insert into membranes and the assymmetric profile of this hydrophobicity allows them to insert tilted with an angle ranging from 30° to 60° with respect to the membrane surface. The tilted orientation is thought to destabilize membranes and to induce processes such as fusion [7]. The Aβ29–42 peptide induces liposome fusion in relation with its helix hydrophobicity properties [10].

A relationship exists between the type of ApoE (ε_2, ε_3 and ε_4) allele in human and the risks to develop the Alzheimer's disease. For a while, the debate was whether the ε_2 and ε_3 allele of ApoE prevented from, or whether the ε_4 allele was a risk factor for the Alzheimer's disease. We supported that the ε_2 and ε_3 alleles prevent the disease because we found that their C-terminal parts interact specifically with the C-terminal domain of the amyloid peptide. Interestingly, this interaction partially inhibited Aβ29–42 fusogenic properties on liposomes in vitro [11]. In contrast, the ε_4 allele of apolipoprotein E as well as fragments of apolipoprotein A1 failed to inhibit the amyloid peptide fusogenic properties supporting the specificity of the ε_2 and ε_3 apolipoprotein effects [11–13]. It was then demonstrated that the 200–299 fragment of ApoE can have a direct interaction with the C-part of Aβ in vitro [11]. Parallely, Lins et al. [14] studied the ApoE–Aβ interaction by molecular modelling and suggested that the minimal binding site of apolipoprotein E was in its helix 270–287.

Using the "in silico" PepDesign method, we attempted the rational design of complement peptides to Aβ29–42 by taking the ApoE270–287 fragment as a lead and looking for an improved stability of its interaction with Aβ. First, we reproduced the complex described by Lins et al. [14] and identified the key-residues of interaction. Mutant peptides were then generated by residue substitution. Energies of interaction of mutants with Aβ29–42 were computed. We selected peptides likely to show a stronger interaction with Aβ29–42 than the native apolipoprotein peptide and analysed the reasons for the improvement. Mainly two classes of mutants were

detected, one in which electrostatic interactions were responsible for a better pairing with the Aβ29–42 peptide, another in which hydrophobicity was the main responsible. One peptide of each class was synthesized.

We used the self-ability of Aβ29–42 to induce fusion of liposomes to compare the native ApoE270–287 and the mutant peptides capacities to inhibit this capacity. We assumed that the best the inhibition of fusion, the best the interaction. Experimental inhibition of lipid fusion monitored by fluorescence spectroscopy (fusion of lipid phases and leakage) is in agreement with our calculations emphasizing that it is possible to design peptides whose interactions with Aβ29–42 are stronger than those of the native ApoE270–287 fragment, validating the "in silico" approach.

2. Materials and methods

2.1. In silico

2.1.1. Peptides

Helical peptides (ApoE270–287 and Aβ29–42) were constructed using Hyperchem (release 6.1 for windows-Hypercube) assigning values of phi/psi angles of −58° and −47° corresponding to classical alpha-helical structure [15]. The conformation of backbone and side chains was optimised by a steepest descent procedure completed by a conjugated gradient procedure.

2.1.2. ApoE/Aβ complex

The hypermatrix procedure, derived from the method allowing to surround a drug with lipids [16] was used to build the ApoE270–287/Aβ29–42 complex. The Aβ29–42 helix was maintained still while the ApoE270–287 alpha helix moved around with five degrees of freedom (36 rotations around Aβ29–42, 36 self-rotations by steps of 10°, 10 translations perpendicular and parallel to the Aβ helix axis by steps of 1 and 0.5 Å, respectively, and 20 tilts of 1° with respect to the Aβ helix axis). During the procedure, 2.6×10^6 relative positions of the two peptides were explored. For each position, the energy of interaction was calculated as the sum of Coulomb, Van der Waals and solvation energies. The complex exhibiting the best gain of energy was kept.

2.1.3. Key positions for substitution of ApoE

In order to determine residues of the ApoE270–287 helix in interaction with Aβ, we first optimised the hypermatrix complex by an angular Monte Carlo procedure as described later. Then, we compared the accessible surface area (ASA) of each ApoE and Aβ residue in the complex and in the isolated molecules, analysed the atoms in interaction, their distance, the energy of interaction, etc [17–19]. From there, we defined which residues and what kinds of interaction were involved in the ApoE270–287/Aβ29–42 interaction. Results were used to select residues for substitution.

2.1.4. Generation of ApoE mutants

To generate mutants of the ApoE270–287 peptide, we used the complex of ApoE270–287 and Aβ29–42 helix structures obtained after the hypermatrix procedure as a template. This complex was loose enough to allow residue substitutions without generating harsh steric clashes. All key positions amino acids of the wild type ApoE peptide in interaction with Aβ in the complex were open to substitutions generating 1024 possible peptides by random combination of substitutions.

The energy of each mutant/Aβ complex was minimized by an angular Monte Carlo procedure based on the angular dynamics previously described [20]. This procedure differs from a current energy minimization on several points: valence angles and bond lengths are maintained constant, atom movements are rotations around molecule torsion axes and rotation movements are propagated along the chain; finally, energy of atomic interactions is distributed on rotation axes, and every axis is independently minimized resulting in a local rather than a global energy minimization. During the procedure, one molecule remains still and the other moves around and along. The two molecules move their side chains and the mutant also adapts its backbone architecture. Energy of interaction is minimized at 25 °C for 200 steps of Monte Carlo procedure, each step allowing a maximal 3D translation of 0.25A of the moving molecule centre and a maximal tilt of 1° of its axis. At each step, 7 successive rotations of all axes at 25 °C optimised the side chain structures. The energy of the system is the sum of intra and intermolecular energies of non-bound interactions: for the intramolecular interaction Van der Waals (using Levitt description of soft-atom with 1000 kcal/mol as a limit energy value [21] and electrostatic energy (Coulomb) were summed; for the intermolecular interactions, Van der Waals (Levitt description of soft-atom with 100 kcal as a limit energy value), Coulomb (with a sigmoid description of ε variation and FCPAC atomic charges [22] and, two terms of hydrophobicity (i.e. due to peptide–peptide interactions and to water/peptide interaction [20] were calculated. The first selection step of peptide/Aβ29–42 complexes is at that point on minimal total energy (internal and external atomic interactions).

2.1.5. Complex analysis and final selection

Complexes were characterized by their energy patterns: the residue Mean Force potential was calculated from atomic mean force potential scale [23]. Scale for atomic Mean Force Potential values (MFP) was prepared by computing all atomic interactions in of 500 3D structures [24]. The equation of Lenard Jones was used for Van der Waals energy term. A sigmoid description of the dielectric constant and the FCPAC partial atomic charges [22] were used for the Coulomb energy. Last, the equation developed by Brasseur [20] for inter and molecule/solvent hydrophobicity was used. All energy values are in kcal/mol.

2.2. In vitro assays

2.2.1. Preparation of SUV

Small unilamellar vesicles were made of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), sphingomyelin (SM) and cholesterol (Chol) (30%: 30%: 2,5%: 10%: 5%: 22.5% respectively; w/w).

Lipids were diluted in chloroform/methanol (2/1 vol/vol). After evaporation, the film was dried for 2 h before being hydrated with Tris buffer pH 7.4 (Tris–HCl 10 mM, NaCl 150 mM, EDTA 0.5% and NaN_3 1 mM) incubated at 37 °C with stirring every 10 min. The solution was sonicated at 50W twice for 5 min. Particulate matters and residual multilamellar vesicles were discarded by centrifugation at 2000g for 5 min. Final concentration of phospholipid was determined by the method of Barlett [25].

2.2.2. Fusion of lipid phase

Lipid fusion is monitored by fluorescence measurement using the method previously described by Hoekstra and Klappe [26]. R-18 (octadecyl rhodamine B chloride) labelled SUV are mixed with unlabelled SUV. In the presence of a fusogenic agent, an increase of fluorescence signal is observed due to the dilution of R18 in lipid phase. Labelled and unlabelled liposomes (1:4w/w ratio) and Aβ29–42 peptide (different peptide/lipid molar ratio between 0.01 and 0.2) were mixed at room temperature and the fluorescence signal was recorded (excitation wavelength at 560 nm and emission wavelength at 590 nm).

2.2.3. Leakage of liposomes

The release of a substance encapsulated in liposomes is followed by fluorescence [27]. HPTS (8-aminonaphtalene-1,3,6-trisulfonic acid) and his quencher DPX (p-xylylenebis[pyridinium] bromide) were both encapsulated in the aqueous phase of liposomes. Liposomes were eluted on a Sephadex G75 to remove HPTS and DPX from medium. The HTPS fluorescence was measured at room temperature using excitation and emission wavelengths at 450 nm and 512 nm, respectively. Dequenching of released HPTS followed leakage. The percentage of release is defined as F_t/F_{tot} x100, where F_t is the fluorescence signal at t time and F_{tot} is the signal obtained after vesicle lysis with 0.5% Triton X100.

2.3. Materials

All solvents came from Sigma (St. Louis, USA); ApoE and mutant peptides were synthesized by SYNT:EM (Nimes, F); Aβ29–42 peptide came from Polypeptide (G) and was 80% pure. All peptides are N-acetylated and C-amidated; lipids were from Lipid Products (Surrey, UK), Sigma (St. Louis,

USA) and Aventi Polar lipids (Alabaster, USA). We used a sonicator from Sigma Chemical (USA) and the spectrofluorimeter LS-50B from Perkin Elmer (Norwalk, USA).

3. Results and discussion

The method is a multi-step procedure that can be summarized as follows: First we choose a complex in which the target is in interaction with a molecule. This molecule will become our lead for modelling. If no model of interaction exists, we calculate one based on experimental data describing the interaction of the target with a molecule. The second step is the characterization of the initial complex. This step is crucial because it will define which residues and which type of energies are responsible for the association. Then, residues for substitution are proposed for each key position. Structures corresponding to all combinations of mutations are constructed and tested for their interaction with the target. After optimisation, the best complexes are selected on calculated criteria (energy of interaction, covered surfaces...). The method is here applied to the design of an anti helical form of Aβ peptide. The template complex is the calculated complex between Aβ C-terminal domain and ApoE270–287 amphipathic helix.

3.1. Calculation of the template complex

We started from the molecular modelling approach by Lins et al. [14] and used the 270–287 fragment of apolipoprotein E as template for the design of an anti Aβpeptide. In a first step, we calculated the best ApoE270–287 (**EDMQRQWAGLVEKVQAAV**) and Aβ29–42 (**GAIIGLMVGGVVIA**) complex using a hypermatrix procedure. 2.6×10^6 relative positions were explored and the structure with the lowest energy was saved. Orientation of the two helices is antiparallel as previously reported [14]. The role of hydrophobicity is major in the complex as seen by comparing hydrophobic accessible surface area of residues in the free peptides and in the complex. Hydrophobic accessible surface area of the complex (1760 $Å^2$) is 21% lower than the sum of hydrophobic accessible surface area of the free peptides (2229 $Å^2$). In parallel, the hydrophilic accessible surface area is almost unchanged in the complex as compared to the free peptides. This indicates that formation of the complex hides hydrophobic parts of both molecules. Those results confirm those of Lins et al. [14] and clearly point towards hydrophobicity as a key parameter for the complex stability.

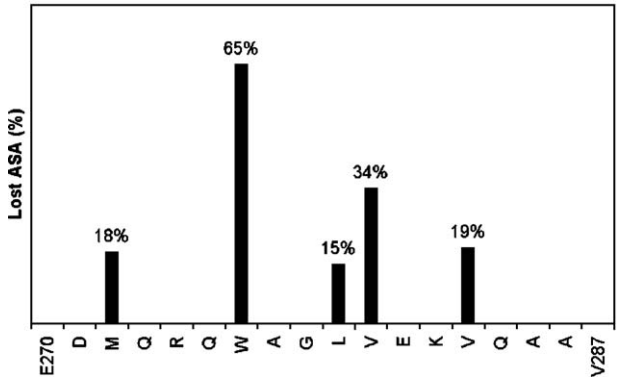

Fig. 1. Lost Accessible Surface Area (ASA) as a function of ApoE270–287 residues in the complex with Aβ and alone (in %).

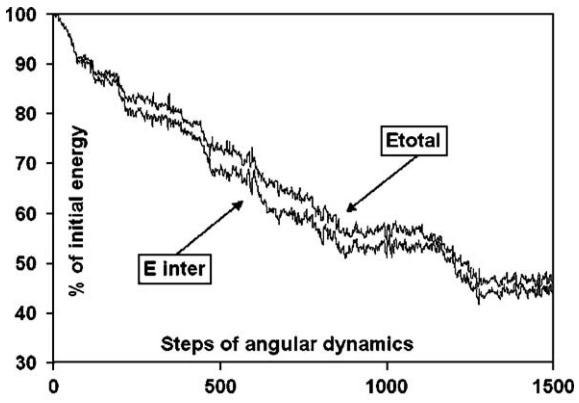

Fig. 2. Evolution of the energy of inter and total (inter plus intra) molecule interactions of the peptide 11 and Aβ complex during the procedure of Monte Carlo and angular dynamics. The procedure lasts for few minutes and does not show significant energy decrease if continued over. Energies are calculated as detailed in Materials and methods.

3.2. Identification of key residues

The ApoE270–287 residues in interaction with Aβ were characterized by analysis of accessible surface (Fig. 1). We assumed that peptide residues of ApoE270–287 with less water-accessible surface in the complex than in the free form were implicated in the interaction. Those residues are located on the same side of the ApoE helix: M272, W276, L279, V280 and V283 and they have lost 18%, 65%, 15%, 34% and 19% of their solvent accessibility in the complex, respectively. These residues were selected for mutation by substitution.

3.3. Residues proposed for substitution

Two criteria were used to define which residues would be used for substitution. First, in the template complex, most residues are involved in apolar interactions, hence residues proposed for substitutions had to be hydrophobic to keep the initial character of the interaction.

Second, although the structure of $Aβ_{29–42}$ is known to be variable, its fusogenic activities are related to helix structure properties. On the other hand, the ApoE270–287 is an amphipathic helix structure in the whole protein. Hence, substituted residues had to induce a high helix propensity for the de novo peptide [28]. Based on these criteria, Met, Trp and Leu were chosen. Despite its low propensity as helix inductor, Val was also selected due to its high hydrophobicity and its presence in the original peptide.

3.4. Mutant complex calculations and initial selection

Using the original ApoE–Aβ complex, we tested the 1024 structures obtained by combinations of residue substitution for M272, W276, L279, V280 and V283. All peptides were

Table 1
Sequences of ApoE mutants selected by the automatic procedure

		1	2	**3**	4	5	6	**7**	8	9	**10**	**11**	12	13	**14**	15	16	17	18
Mutants	**W.T.**	E	D	M	Q	R	Q	W	A	G	L	V	E	K	V	Q	A	A	V
	11	E	D	**M**	Q	R	Q	**L**	A	G	**V**	**V**	E	K	**V**	Q	A	A	V
	12	E	D	**M**	Q	R	Q	**L**	A	G	**L**	**V**	E	K	**W**	Q	A	A	V
	28	E	D	**M**	Q	R	Q	**L**	A	G	**M**	**W**	E	K	**V**	Q	A	A	V
	308	E	D	**V**	Q	R	Q	**L**	A	G	**L**	**V**	E	K	**V**	Q	A	A	V
	413	E	D	**M**	Q	R	Q	**M**	A	G	**L**	**M**	E	K	**M**	Q	A	A	V
	450	E	D	**M**	Q	R	Q	**V**	A	G	**M**	**W**	E	K	**V**	Q	A	A	V

Mutated positions are in bold.
Residues are renumbered from 1 to 18.

energy-minimized in interaction with Aβ29–42 by 1400 steps of a Monte Carlo procedure based on angular dynamics. A primary selection was based on the minimization of the energy of intra plus inter molecule interactions (Fig. 2).

Six complexes were selected which all have gained energy as compared to the initial ApoE–Aβ complex. Analysis of their sequences (Table 1) shows that Met 3 of the original ApoE peptide is frequently conserved, probably because of its side chain flexibility which allows a high ability of interaction. Conversely, Trp residue seems to be excluded from central positions 7 and 10. Residues at the other positions are more variable.

3.5. Final selection of peptides

In the previous steps, valence angles and bond lengths were constant. Now, peptide geometry of complexes is relaxed by a conjugate gradient procedure using HyperChem that converged to 0.1 kcal/step. A close comparison of the 6 relaxed complexes was then carried out based on the analysis of partner interactions (Aβ and the mutant peptide) (Tables 2 and 3). If 6 complexes have a global gain of energy with respect to the ApoE–Aβ complex, only 3 have gained this energy in the interaction between peptides (Table 2). These 3 complexes are with peptides 11, 12 and 413 and were thus selected to go ahead.

From the three complexes, two types can be identified according to the energy gain, either electrostatic gain (complexes 12 and 413) or hydrophobic gain (complex 11).

Complex 11 gains 5 kcal/mol in the hydrophobicity "Epho inter" as compared to the ApoE–Aβ complex. It also shows a significant gain in Mean Force Potential supporting that several atoms have found favourable partners. The Aβ surface initially covered by ApoE is increased by 133 Å^2 further supporting a good Aβ-peptide 11 surface matching (Table 2, Fig. 3).

Main interest of complexes with peptides 413 and 12 results from electrostatic gains and from a good matching of the two peptides surface (+56 Å^2 more of Aβ surface is covered by peptide 12 as compared to ApoE, and +31 Å^2 more by peptide 413) (Table 2). The two peptides have linked their backbone to enable the interaction of the N end of Aβ with the C end of the two peptides (Fig. 3).

Reasons for pairing improvement are further analysed in Table 3. In this table, the closest inter-peptide atomic interactions are listed: the three first for the initial ApoE–Aβ complex only, the first one for the peptides 11 and 413 in complex with Aβ. This highlights the major role of W276 in the initial ApoE–Aβ complex. Tryptophan is a bulky hydrophobic residue hence its role in binding is easy to understand. Its central position in the ApoE peptide attracts apolar atoms of Aβ but also keeps the rigid helix away decreasing by this way the other residue binding possibilities. Mutation of this tryptophan which was frequently noticed in our peptides increases the peptide flexibility (backbone kicking is observed) and enables a more complete surface pairing. Hence, the terminal valine (V18) of peptides (corresponding to V289 in the ApoE) becomes a major partner of Aβ as do residues at positions 3 and 7.

3.6. Biophysical assay

The peptides 11 and 413 were synthesized, each representative of a different kind of complex.

3.6.1. Fusion of lipid phase

Aβ29–42 induces liposome fusion "in vitro" [29]. This is detected by dequenching of R18 fluorescence, a lipophilic probe. In this study, we compared the inhibitory effect of ApoE270–287 and of peptides 11 and 413 on liposome fusion induced by Aβ29–42. Neither ApoE270–287 fragment nor mutants have self-fusogenic properties: none induces the fusion of liposomes. By contrast, in the presence of Aβ29–42

Table 2
Energy gain of the six primary-selected complexes (peptide/Aβ29–42) by reference to the ApoE270–287/Aβ29–42 complex

Aβ partner	δ *E*tot_inter	δ MFP_inter	δ E VdW Lenard Jones	δ E electrostatic	δ E pho intermolecule	δ E pho solvent	δ masked ASA
Apo E	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11	−10.1	−73.3	−6.0	0.9	−5.0	−18.5	−133
12	−16.4	3.8	4.1	−20.7	0.1	22.4	−56
28	5.1	3.9	5.3	−1.3	1.1	46.6	−27
308	32.3	96.7	17.4	−9.0	23.8	93.1	116
413	−15.7	1.1	8.4	−27.3	3.3	104.8	−31
450	27.0	16.0	7.6	−4.5	23.8	151.2	−22

1st column gives the Aβ partner name, other columns give the gain in several terms of energy (kcal/mol) and the loss in solvent-accessible surface (Å^2). E_{tot} is the sum of Van der Waals (Lenard Jones), electrostatic and intermolecular hydrophobicity, the explicit values of which are detailed in the next columns. Values of mean force potential are calculated as described in Materials and methods. The hydrophobicity contribution of the solvent-accessible surface of the complex is also given in the last column and was used as an index of the complex solubility.

Table 3
Pex analysis of complexes

Aβ residues	3 shortest atomic interactions With ApoE												1st interaction with pep 11						1st interaction with pep 413					
	Atom distance	Aβ atom	ApoE atom	ApoE residue nb	ApoE residue	Atom distance	Aβ atom	ApoE atom	ApoE residue nb	ApoE residue	Atom distance	Aβ atom	ApoE atom	ApoE residue nb	ApoE residue	Atom distance	Pep11 atom	Aβ atom	PEP11 residue nb	PEP11 residue	Atom distance	pep413 atom	Aβ atom	PEP413 residue nb	PEP413 residue
29 GLY													2.4	H	HG1	18	VAL	1.8	H	O	18	VAL		
30 ALA													2.5	HB	HG2	18	VAL	3.8	H	HG2	18	VAL		
31 ILE	2.8	HG1	HH2	276	TRP	3.4	HD1	HD2	279	LEU	3.8	HD1	HG1	283	VAL	2.1	H	HG2	18	VAL	2.5	HG1	HE	14	MET
32 ILE	2.3	HA	HH2	276	TRP	2.8	HG2	HG2	280	VAL						2.3	HG2	HG1	14	VAL	2.3	HG2	HA	15	GLN
33 GLY	4.9	N	HZ2	276	TRP											4.7	H	HG1	18	VAL					
34 LEU																4.7	O	HE	3	MET					
35 MET	2.4	HE	HB	276	TRP	3.0	HE	OE1	275	GLN	3.2	SD	HD2	279	LEU	2.4	HE	HB	7	LEU	2.6	HE	HE	7	MET
36 VAL	2.1	HG2	HE1	276	TRP											2.4	HA	HD2	7	LEU	3.7	HB	HE	11	MET
37 GLY																									
38 GLY	3.3	C	HE	272	MET											2.4	HA	HE	3	MET					
39 VAL	2.3	HG2	HA	273	GLN	2.4	HG1	HD1	276	TRP	2.4	HB	HE	272	MET	2.4	HG2	HB	3	MET	2.7	HG2	HE	3	MET
40 VAL																									
41 ILE																									
42 ALA	3.4	HB	SD	272	MET											4.5	HB	SD	3	MET					

In the first column, the Aβ residues are listed and, in the following columns, individual atomic interactions are described with the atom centre to centre distance, the name of the Aβ and partner atoms, the number and name of the partner residue. The three shortest interactions are listed for the Aβ complex with ApoE, the shortest one for the Aβ complex with peptides 11 and 413, respectively.

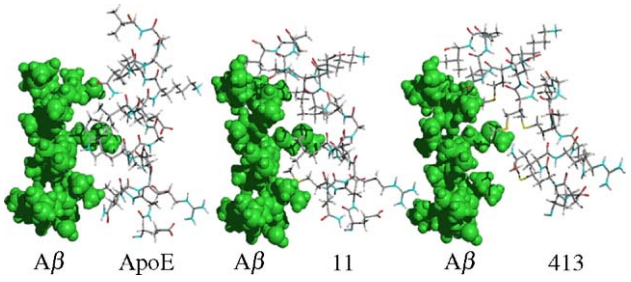

Fig. 3. Complexes of Aβ with ApoE, peptides 11 and 413. The green CPK view is the Aβ peptide whereas the stick structures are ApoE270–287, peptide 11 and peptide 413 as specified on the figure. The N side of Aβ is up and the N side of peptides ApoE, 11 and 413 are down.

(peptide/lipid ratio of 0.4 (mol/mol)), the R18 fluorescence rapidly increases indicating lipid destabilization and liposome fusion. This signal stands for 100% Aβ activity (Table 4) and will be inhibited in case of Aβ binding with the partner peptide (ApoE, peptides 11 and 413). In the inhibition assays, we assumed a 1/1 molar ratio between the Aβ29–42 peptide and the inhibitor peptide in agreement with the pairing hypothesis. When the ApoE270–287 peptide is added to liposomes at the same time as the Aβ29–42 fragment, 11% decrease of the fluorescence signal is observed indicating that the interaction between the two peptides has slightly decreased the fusogenic potential of Aβ29–42 (Table 4). Those results are in agreement with those of Pillot et al. [11]. When peptides 11 and 413 are added, inhibition is more important (−83% and −79%, respectively) (Table 4). This suggests a more potent interactions of peptides 11 and 413 with Aβ29–42 than of ApoE with Aβ in agreement with our calculations.

3.6.2. Liposome leakage

We next checked that the R18 dye response was due to a true perturbation of membranes and not only to its dilution in lipids after vesicle aggregation. In order to assess the destabilization of SUV membranes, HTPS and DPX were both trapped in liposomes. In case of leakage, HTPS fluorescence will increase. Peptides 11 and 413 induce no fluorescence increase when added alone. In the presence of Aβ29–42 (peptide/lipid molar ratio of 0.4), the fluorescence of HTPS immediately increases (28% of the maximal signal). In the presence of peptide 11 or peptide 413 (at a 1:1 molar ratio), the Aβ29–42 signal is close to zero, 0% and 4%, respectively (Table 4). This suggests that both peptides impair the membrane-destabilizing properties of Aβ29–42. The leakage signal remains close to zero when the

Table 4
Lipid fusion and leakage induced by Aβ29–42 and inhibitory effects of ApoE WT, ApoE mutant 11 and ApoE mutant 413 monitored by fluorescence at room temperature at a 1/1 peptide ratio

Peptides added to the liposomes	% of fusion after 15 min	% of leakage after 15 min
Aβ alone	100	28
Aβ+ mutant 413 ($R=1$)	21	4
Aβ+ mutant 11 ($R=1$)	17	0
Aβ+ ApoE WT ($R=1$)	89	ND

Values are in % and are averages of values in the plateau of fluorescence (between 10 and 15 min) of three different experiments.

peptides (11 or 413)/Aβ29–42 molar ratio are varied from 0.4 to 4. This is in agreement with the lipid fusion assays and the modelling calculations.

4. General conclusions

The PepDesign algorithm is a rapid multi-steps procedure based on algorithms that others and we have developed in the past (Soft Levitt approach for the modulation of steric clashes between atoms [21], hypermatrix for calculating the complex structure [16], angular dynamics for the rapid optimisation of the complex structure [20], Pex for the analysis and comparison of structures [17]). The major strength of the method is in its insight in the mechanism of molecule interaction. In this case, we designed a peptide able to bind to the hydrophobic side of the helix structure of Aβ29–42 because previous data supported the hypothesis that hydrophobicity pattern of this helix was involved in Ab fusogenic capacities. The mechanisms of interaction between carrier peptides and their cargoes should go the other way round. The carrier should cover cargo hydrophilic parts since they make hydrophobic complexes. The biological process is inverse but the modelling protocol will remain similar.

Actual capacities of molecular modelling are still a subject of debates. We support the idea that real success in molecular modelling strongly depend upon the pertinence of hypotheses and the adequacy of empirical calculation approaches. Computer-aided design of molecules has been used for many years but often as side-support of experimental approaches. We here suggest that in silico calculations can take a major part in molecule-design processes. If it cannot replace experimental assays it might valuate them by focusing them to a few selected cases. Both experimental and theoretical approaches should gain in that collaboration. The Molecular Recognition Theory has allowed designing peptides that non-covalently bind proteins or peptides. Campbell et al. [30], using the MIMETIC program designed peptides which recognize fragments of HIV-1 reverse transcriptase. Three of the ten peptides they designed were able to inhibit reverse transcription in vitro. Using the same algorithm, Shimomura et al. [31] designed complementary peptides to thrombomodullin. This protein decreases the formation of the thrombomodullin/thrombin complex implicated in the carboxypeptidase R activation. In that case, two of the three computed peptides were efficient.

Recent progresses in molecular modelling seem to result from opportunities opened by increases in computer capacities, by increases in the number of research in the domain and last but not least by the demand urging from biotechnologies. As a consequence, tremendous progresses have been made in the last decade which are evidenced by the emergence of calculation tools. PepDesign is one example. Many factors are combined for PepDesign efficacy, three are major: first is Pex ([17]*). Pex transforms PDB files of structures into worksheets compiling large series of intra and inter molecule geometry and energy parameters. This enables the rapid and complete comparison of structures and leaves no place to subjective appreciations. Pex were already used in several studies [18,19,32,33]. Second is the Soft Levitt's equation for Van der Waals energy. Indeed, one problem in computing structures is atomic steric clashes. A single clash can result in a huge burst of energy which may hide any real energy benefit farther in the structure. This can lead to discard a structure which is however on the right way of folding. Can steric clash be avoided in computing approaches? No because all molecule movements proceed through discrete steps in opposition with the continuity of natural movements. The larger the steps, the most rapid the folding, the most frequent the steric clashes. The smaller the steps, the longest the calculation times, the less perturbing the steric clashes. Hence, a compromise must be found. In its empirical description of Van der Waals energy, M. Levitt introduced a limit value, i.e. a maximal penality for a steric clash. This subterfuge is precious since it allows a few hence transient atomic collisions on the way to a final well-packed structure.

Another factor of PepDesign efficacy is angular dynamics. Angular dynamics has been used for several years in protein folding [20] and has been recently fully described for the de novo design of a non-natural peptide [34]; its main advantage over molecular dynamics is that large molecule movements can result from small energy changes since movements are propagated on the molecule torsion axes. In this study, minimization of peptide complexes can be achieved within an hour in the best conditions. This opens possibilities for comparative series of tests within reasonable delay.

Combination of calculation procedures and of Pex analyses of structures seem crucial to help peptide design at best. For instance, in our assays of liposome leakage, we were unable to efficiently distinguish between peptides 11 and 413. Hence, we should have concluded that both peptides are equivalent. However, the Pex analysis of Aβ-peptide complexes leads to the conclusion that peptide 11 should be more active than peptide 413 when the entire Aβ protein is concerned. Indeed peptide 413 has part of its interaction with the N backbone end of the Aβ peptide. This fragment will not be accessible in the complete protein as it is in the peptide. Thus, the modelling approach suggests that only one of the three selected peptides, peptide 11 will efficiently bind the Aβ 1–42 protein.

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