ELSEVIER

Journal of Molecular Structure: THEOCHEM 757 (2005) 125–136

THEO CHEM

www.elsevier.com/locate/theochem

Study of peptide oligomer derived from HIV-1 integrase molecular modelling

Christopher K. Jankowski [a,*], Simon Foucher [a], Serge Fermandjian [b], Richard G. Maroun [b,c]

[a] *Département de chimie et biochimie, Université de Moncton, Moncton, NB, Canada E1A 3E9*
[b] *Département de biologie et pharmacologie structurales, CNRS UMR 8532, Institut G. Roussy, 94805 Villejuif, France*
[c] *Département des Sciences de la Vie et de la Terre, Faculté des sciences, Université Saint-Joseph, CST-Mar Roukos, B.P. 1514, Beyrouth, Liban*

Received 15 June 2005; received in revised form 21 September 2005; accepted 21 September 2005
Available online 14 November 2005

Abstract

Three synthetic oligopeptides (EAA26, K156 and E156) mutant of the α-4 helix of the HIV-1 integrase and having, respectively, 26, 24 and 24 aminoacids showed variable oligomerisation trend as detected by the ESI-MS (electrospray mass spectrometry) or CD (circular dichroism) methodologies. These peptides oligomers were modelled as potential inhibitors of this enzyme through coiled–coil interactions. The dimer, trimer and tetramer aggregate energies for these synthetic peptides were calculated and their stability discussed. One of these peptides, EAA26 shows some propensity to form a tetrameric structure when attached to the calix[4]arene frame through a succinic linker; the possibility to form the quadruple peptide helix, an entity which was not obtained from peptide systems, was evaluated.
© 2005 Elsevier B.V. All rights reserved.

Keywords: Modelling of peptide oligomers; HIV-integrase inhibitors; Triple peptide helix; Quadruple peptide helix

1. Introduction

The development of new antiretroviral integrase-targeting drugs is strongly dependent on a greater knowledge of the virus's function, structure, and interactions. As of 2001, no integrase inhibitors have been clinically tested, due to their strong cytotoxicity [1]. One approach to overcoming this difficulty is the study of polypeptides as integrase inhibitors, because of their low toxicity and their general similarity to cellular material.

Integrase (IN) is a 32-kDa protein which catalyzes the insertion of the retroviral genome into the host chromosomes. IN is divided into three functional domains: a catalytic domain from residues 50–212, a C-terminal DNA-binding domain from residues 212–280, and an N-terminal domain composed of residues 1–47 [1]. The three functional domains treated separately are able to form oligomers, although the oligomerization order of the total protein is not yet known. X-ray studies show that the central core dimers are bound together via interactions of various types, including hydrophobic. Each monomer is built with five beta-sheets and six α-helices, including the well-defined amphipathic helix α-4 (147–175).

The side chains of the amino acids on the hydrophobic area of the amphipathic α-4 helix alternate according to the so-called heptad (abcdefg) distribution. This large hydrophobic area near the surface of the integrase is thought to favour helix coiled–coil formation [2], generating oligomers. As such, it has much potential as a target for new integrase inhibitors acting at the protein interfaces [1]. Within this new family of peptide-based drugs, non-cytotoxic substances could be developed which would have the capacity to bond to the α-4 helix of IN, blocking off the helix like a plug (Fig. 1).

The synthetic mutant α-4 peptides (EAA26, K156, and E156—Scheme 1) could inhibit IN via this mechanism. We expected them to form coiled–coil interactions with IN via hydrophobic interactions with the α-4 helix—note that α-4's 29 amino acids make it compatible in length with the three aforementioned peptides.

ESI-mass spectrometry (positive ion, PI) revealed the presence of different oligomers, as well as a variable tendency towards oligomerisation [3] for the mutant peptide in water, then, to enhance their helicity and help induce their oligomerisation, in a combination of water and trifluoroethanol (TFE), this latter being an α-helix inducer. The 26-AA test peptide (EAA26) was present as a dimer; tetrameric structures were also found (3–7). E156, the 24-AA peptide, was tested in the

* Corresponding author. Tel.: +1 506 858 4331, fax: +506 858 4541.
E-mail address: jankowc@umoncton.ca (C.K. Jankowski).

0166-1280/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.theochem.2005.09.020

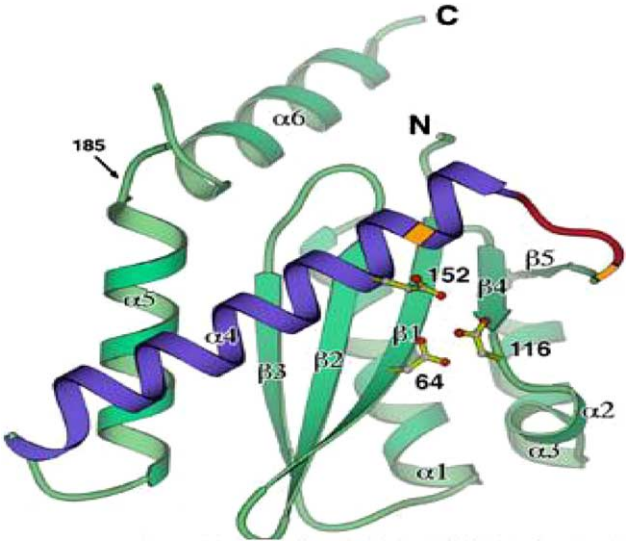


Fig. 1. Integrase general scheme and α-4 helix fragment (THT-turn-helix-turn).

presence of higher concentrations of TFE; it showed only dimers. The 24-AA peptide, K156, has the same sequence as E156, except at locus 10, where Glu10 was replaced by Lys10, and the terminal Trp–Gly of E156 was replaced with tyrosine (Tyr), in order to increase the peptide's UV absorbance and thereby facilitate its measurement. This relatively small modification of the structure led to an unexpected increase in the concentration of the K156 trimer. This article seeks to explain the oligomerisation observed—in particular, the trimerisation of K156—and to justify in detail why the Glu/Lys substitution at locus 10 led to this trimerisation. Finally, we would like to explore modifications of the primary sequence of these peptides, so as to obtain more stable oligomers. This will help to better understand the mechanism of IN inhibition by these peptides via their coiled–coil action on the α-4 helix. Finally, the presence of tri- or tetrameric structures for these peptides (either self-supporting or TFE-initiated) reopens the possibility of construction of triple- and quadruple-helix peptides which are not reinforced by S–S interchain bonds. Such structures have never been reported and are thought to not exist in nature; modelling of these structures is interesting; especially since both Lys156 trimers and EAA26 tetramers were detected by mass spectrometry. However, despite several attempts, no non-disulfide-reinforced triple helices were detected. We chose to model such a quadruple helix on the basis of the EAA26 peptide chains 'dangling' from a rigid tetramino-calix[4]arene support structure. The resulting structure took an 'octopus'-like conformation, with four long arms oriented in the *cis* configuration, and bound additionally via interchain coiled–coil interactions.

Peptide Inhibitor Sequences

Name	Sequence
EAA26	VESMNEELKKIIAQVRAQAEHLKTAY
E156	SQAKLEEMNEELKKLLAQVRAQWG
K156	SQAKLEEMNKELKKLLAQVRAQY
Helix 4	SQGVVESMNKELKKIIGQVRDQAEHLKTA*

*) helix alpha-4 fragment

Scheme 1. Peptide inhibitor sequences. The one letter symbol of amino acids is used. The three letter equivalent symbols are as follows: S, Ser (Serine); Q, Gln (Glutamine); G, Gly (Glycine); V, Val (Valine); E, Glu (Glutamic acid); M, Met (Methionine); N, Asn (Asparagine); K, Lys (Lysine); L, Leu (Leucine); I, Ile (Isoleucine); R, Arg (Arginine); D, Asp (Aspartic acid); A, Ala (Alanine); H, His (Histidine); T, Thr (Threonine); Y, Tyr (Tyrosine); W, Trp (Tryptophan) . *helix alpha-4 fragment.

2. Modelling methodology

This section presents the modelling in four parts: computational data used in this study, and the evaluation of the most probable conformers of dimers, trimers, and tetramers.

All modelled structures and detailed calculations are available on request from the corresponding author at Université de Moncton address or via email jankowc@umoncton.ca.

3. Computational

3.1. Amino acid chain initial model

Using HyperChem Pro 6 Mm+ molecular modelling software, we modelled all three α-helices as in previous works (3–5). Whenever resonance occurred on a given lateral chain (for example, NH_2 in arginine), we assigned a partial charge of 0.5 to each nitrogen. The force field used in this simulation for peptide structures was that as in the Hypercube package (AMBER 4 series software using the all-atom parameters and the potential energy as by the Weiner equation [7]).

The calix[4]arene frame was modelled using the X-ray data for the 1,3-alternate and cone conformations, respectively. The model was built with Sybyl software and submitted to a minimisation [7]. The total energy was then minimized, by moving each individual atom until the molecule reached a stable position. This was defined as a conformation in which no movement could reduce the system's energy by more than 0.01 kcal. This gave the initial peptide a conformation to be used in future oligomer modellings.

3.2. Finding the most likely oligomer conformation of peptide dimers.

The peptide chain initial model of all three peptide helices (single strand) was built, then placed at a physiological pH [8]. To create the dimer, a second peptide strand was introduced, identical in sequence and conformation, aligned at the same height, and in the same plane. The second peptide was oriented in a coiled–coil conformation, so that the hydrophobic section of the α-4 helix pointed outwards, an orientation which encourages dimerisation. The hydrophobic sections of the peptides were aligned, to promote hydrophobic binding in a polar solvent. The coiled–coil conformation is build by

the interactions between lateral chains of some amino acid pairs from two parallel peptide strands. The second, third or fourth stand is attached in a similar manner to the first. The lateral chain functional group interactions are responsible for the oligomerisation and the stability of peptide aggregates, especially in parallel configurations of two or more strands.

From that position, the program moved every atom to reach the most stable conformation of the dimer, as previously defined. Then, a water box ($35\times30\times50$ Å) was constructed around the dimer (roughly, $24\times18\times41$ Å), and the system was restabilised. Although an even greater degree of accuracy and precision could be obtained by optimizing the peptide's structure within the solvent, instead of first optimizing the peptide ex nihilo, then adding the solvent box and re-optimizing, the calculations required for such a procedure would be so lengthy as to be impractical.

The resulting dimer is in its most stable conformation; this takes into account the structure of single strands, the double parallel alignment, and the presence of solvent. To ensure that the final position found was as accurate as possible, many different starting positions were tested, to expose different sections of each peptide to their counterparts. The initial positions tested were interpeptide rotations of 0° (the peptides' hydrophobic chains are aligned with each other), $+45°$, and $-45°$. An interpeptide rotation of 180° was not performed, because the peptides' hydrophobic regions need to be close

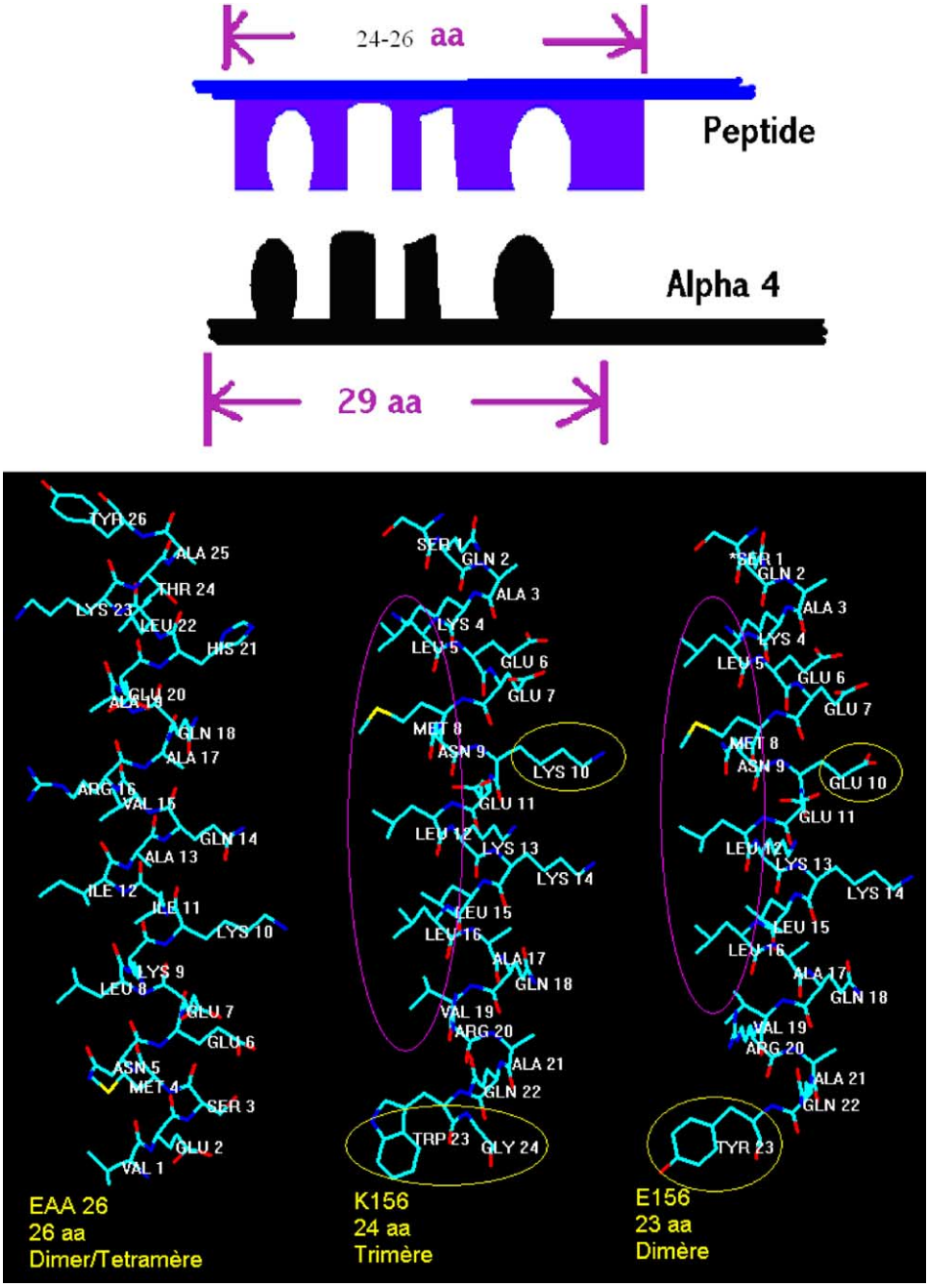


Fig. 2. Coiled–coil 'plug' 24–26 synthetic peptide (EAA26, E156, K156) head–head oligomers $n=2$ dimer, $n=3$ trimer, $n=4$ tetramer.

enough to each other to ensure the formation of the hydrophobic interstrand bond holding the dimer together. Next, the peptides were translated along their major axis. A translation distance of 2.7 Å (half the height of an α-helix) was used for each test: that is, each AA's lateral chain was moved from its initial placement, directly in front of the counterpart AA on the other peptide, to a new position between the two chains of its counterpart's neighbours. These translations were tested from −10.8 Å to +10.8 Å: beyond these boundaries, the hydrophobic region becomes exposed to the polar solvent, which destabilizes the system. For each translational position, the three aforementioned rotational positions (+45°, 0°, and −45°) were also tested, for a total of eight translations × nine rotations (three possible rotations for each of two peptides) = 72 models (Fig. 2).

All models were made in a head–head, tail–tail parallel orientation, in accordance with the results of previous NMR and CD experiments. The investigation of dimers in an antiparallel conformation (head–tail), which would require another 72 models, was therefore deemed unnecessary [9–12].

3.3. Evaluation of the trimers found for K156

Considering the exceedingly high number of potential initial conformations for a trimer, we decided to seek the most probable peptide conformation by focusing on the small differences between the sequence of E156, which dimerizes, and K156, which trimerizes. Since there is only one major amino acid difference between the peptides (locus 10 is either Glu or Lys), obviously this sequence modification is related to the formation of trimers [13]. We also considered the thermodynamic factors which influence the large hydrophobic region of the peptide. When such peptides are dissolved in polar solvent (water or TFE, for example), the hydrophobic region should restructure itself and hide within the core of the trimer, so as to minimize the energy of the system.

3.4. Tetramers as a starting model for the quadruple helix

Since the modelling of a tetramer would be an even greater challenge, we chose to approach it instead as the formation of a quadruple helix of four strands of the EAA26 peptide. Since quadruple peptide helices have not yet been found in nature, we sought to develop a method of artificially creating such a helix. The EAA26 peptide, which oligomerises itself to form both dimers and tetramers, therefore represents an excellent candidate for such a construction [14].

The starting point for such a model should be a rigid frame, built (for example) with a calix[4]arene. Since the α-helix has a considerably larger diameter than the calixarene frame, a linker can be placed on the N-terminus of each peptide. The linker will restrict the α-helices' topological freedom, thereby enhancing the probability that the peptides will self-organize into the desired helix and its stable conformation (Fig. 3).

The choice of the proper linker is important: if the linker is too short, it could denature the peptides, dragging them

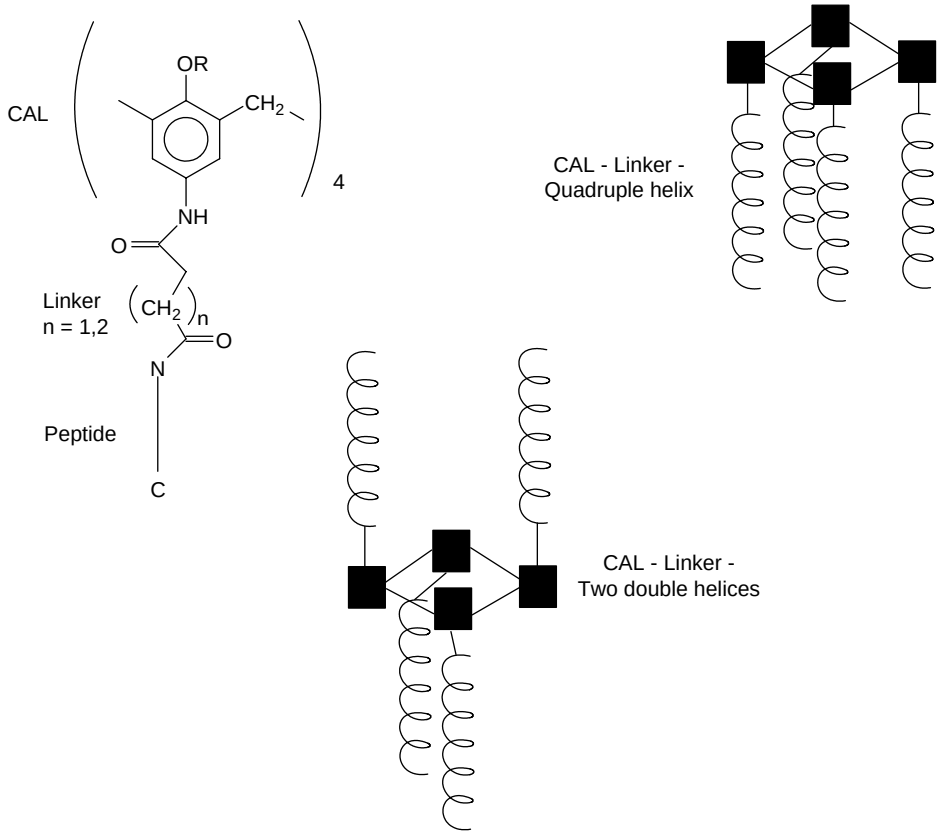


Fig. 3. Quadruple helix—tetramer of EAA26 coupled to calix[4]arene through linker. Quadruple helix-two double helices.

Table 1
EAA26 and E156 dimers

Peptide	Rotation of chain		Translation (Å)	Energy w/solvent (Kcal/mol) (#AA/chain)	Size	Relative mean stability (Kcal/mol/AA)
	Left	Right				
E156	−45	0	0	−566.74	24	−11.81
EAA26	+45	45	10.8	−610.04	26	−11.73

The energy values presented in this table are relative and do not reflect the total energy of the aggregates.

together and inducing electrostatic repulsion, whereas if it is too long, the peptide chains will not be held together closely enough to form the H-bonds necessary to a quadruple α-helix. The linker must also be able to covalently join the N-terminus of a peptide chain to the NH2 group of the calixarene. We chose to calculate two different linkers: succinic and adipic, formed by a 4- and 6-carbon chain, respectively. *To build a quadruple helix structure, the calixarene should be in an all -*cis* (cone) conformation. For that frame, to which were attached four EAA26 strands, three structures were modelled: one with four succinic linkers, one with four adipic linkers, and one with two adipic linkers in 1,3-positions and two succinic linkers in 2,4-positions (this allowed a slight translation of the peptide chains).

We also modeled a double dimer using a calixarene frame in a 1,3-alternate conformation, where the two peptides attached to the 1,3-positions are pointed upwards and the two peptides attached to the 2,4-positions are pointed downwards. Even though this does not actually constitute a quadruple helix, we chose to calculate its stability nonetheless, so as to evaluate the merits of this potential alternate approach.

4. Results and discussion

The results of computational evaluation of peptides are described in three following sections: in order, for dimers, quadruple helix mers, trimers then followed by computer assisted analysis of the results, using specific projections ('cuts') for chosen oligomeric structures of dimers of E156 and EAA26, the trimer of K156, and the quadruple helix built with the EAA26 units.

*Eventually obtained from the aminocalixarene with the acid's anhydride and then coupling the resulting the resulting N-succinylic acid mono amide to the peptide, via the activated ester method [15]

4.1. Computational results

4.1.1. Dimers

First, we found that peptides in a dimer tend to twist around each other, similarly to a DNA helix, or to the dimeric coiled–coils peptide chains found in nature. Such a helical arrangement is the most stable that can be built without reinforcement from covalent or interstrand bonds. This was an indication that the models were adequate. Of all the 45 initial positions tested for both EAA26 and E156, the most stable are presented in Table 1 and Appendices 1 (E156) and 2 (EAA26).

To better compare the energy values obtained, we calculated the mean stability per amino acid in the most stable model: the structural energy of the most stable model (as measured in Kcal/mol), divided by the number of amino acids per chain, and by the number of chains. For example, the most stable EAA26 model had an energy of −610.04 Kcal/mol, divided by 26 AA per chain, divided by two chains, for a mean stability per amino acid of −11.73 Kcal/mol.

4.2. Quadruple helix

As mentioned earlier, a quadruple helix was modelled to further investigate the tetramer structure of EAA26. Using a calix[4]arene frame in a cone, all -*cis* conformation with linkers and four peptide chains attached, the energy values found for such aggregates were as presented in Table 2.

We also created a model based on calix[4]arene with peptides attached in a *trans*−1,3-dialternate conformation. This led to the formation of two double helices with EAA26 peptides, oriented in opposite directions, and with the energy values shown in Table 3.

The most stable structure was that which used the succinic linker: its relative mean stability was −9.10 kcal/mol. This indicates that two double helices are more stable than one quadruple helix. The relative stability of the most stable dimer of EAA26 was −11.73 kcal/mol, indicating a greater stability than the −9.10 kcal/mol value found here (Fig. 3) [16].

4.2.1. Trimers of K156

As was mentioned earlier, the sheer number of possible conformations made it impractical to directly calculate the structural energy of the trimer. Instead, a stable model was engineered based on the differences found between E156

Table 2
Relative stability for quadruple helix aggregates (in kcal/mol)

Linker	Energy	Relative mean stability (per AA)
Succinic	−749.5863	−8.14
Adipic	−753.9238	−8.19

Table 3
Two double helix EAA-26 aggregate on calixarene (in kcal/mol)

Linker	Energy	Relative mean stability (in kcal/mol per AA)
Succinic	−837.8897	−9.10
Adipic	−816.6576	−8.80

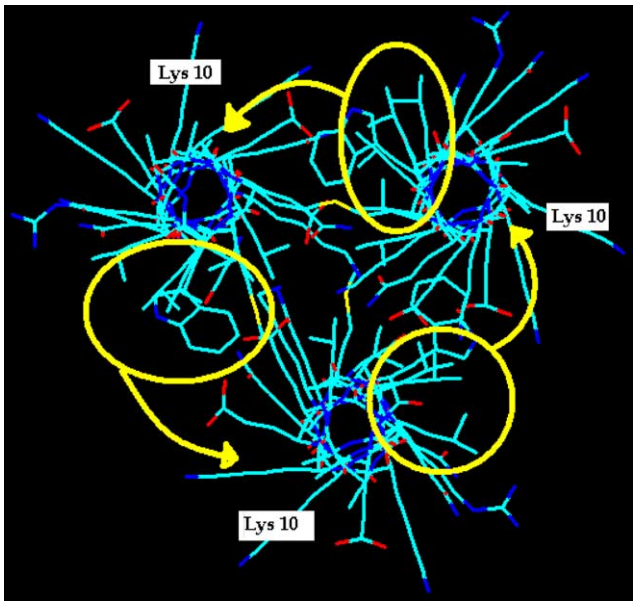


Fig. 4. Trimer K156: lysine 10 points outwards when hydrophobic regions were closely aligned.

and Lys156, namely the locus-10 substitution of glutamic acid Glu-10 with lysine Lys-10.

To construct this model, the hydrophobic regions of the three chains were aligned in the middle of the trimer, to isolate them from the influence of the polar solvent. In this conformation, the 10th amino acid, which is believed to be responsible for the trimerisation, is pointed outwards, and therefore does not take part in the oligomerisation interactions. Therefore, when the trimer was rotated clockwise (relative to the N-terminus), this enabled the 10th amino acid to participate in the trimerisation, and further shielded the peptide's hydrophobic region [17] (Fig. 4).

5. Discussion

To better understand the specific interaction during the oligomerisation, the peptide aggregate was 'cut' into four parts, following the helical axis. These four quarters were then analysed individually to evaluate the proximity and the potential interactions between all lateral chains of the structure.

5.1. Dimer formation for E156

The stabilizing interactions present in the most stable model are described via the following methodology, beginning with two peptide strands in identical -45 degree rotations of the peptides A and B (leading to symmetrical interaction). As a result of this, any given interaction between, for example, amino acid #1 of peptide A and amino acid #2 in peptide B, is reciprocally present between amino acid #1 on peptide B and amino acid #2 in peptide A (Appendix 3).

The first important interaction found was between the amine group of the serine (Ser-1) of peptide A and the carboxyl group of the second amino acid on peptide B, Glutamine (Gln-2). By pointing its carbonyl group towards the lateral chain of the adjacent Serine, the NH group of this Gln interacted with the carboxyl group present on the helix frame of peptide A, therefore participating twice in the energy reduction. Further along, both peptides have leucine (Leu) at locus 5. These are both pointed inwards, forming a hydrophobic interaction, which is already stable since the Leu are not in contact with the solvent.

The 9th AA of each peptide is asparagine (Asn). Since there is no translation between these two peptides, the two Asn are directly facing each other. This allows the formation of a reciprocal, electrostatic hydrogen bond between the amine groups of the Asn on peptide A and the carboxyl group of the corresponding Asn on peptide B, analogous to a salt bond.

At locus 13, the lateral chain of the lysine (Lys) extends far enough towards the α-helix frame of the second peptide strand that its amine can interact with its counterpart's carboxyl group.

The dimer's hydrophobic core, which begins with the lateral chains of Lys-13, also contains Leu-16 and Val-19. The rotation of peptide A allows Ala-17 to participate in this large hydrophobic core, because its methyl group is completely hidden under the carbon arm of Arg-20 on peptide B. The amino group of R-20 is pointed outwards towards the solvent, and helps to shield the hydrophobic core by providing it with a charged shell. By pointing its amine group outward, Arg-20 can also interact with the carboxyl end of Tyr-23 [18] (Fig. 5).

5.1.1. Dimers of EAA26

The energies found for the dimer structures for EAA26 are of the same order as those for Glu156; if one considers the number of amino acids involved, the Glu156 dimer is slightly more stable. The most stable of the 45 dimer structures is that one with a translation of $+45°$ (anticlockwise) between two peptide strands. When four quarter cuts were performed (Appendix 4), the evolution of the helicity axis is easily

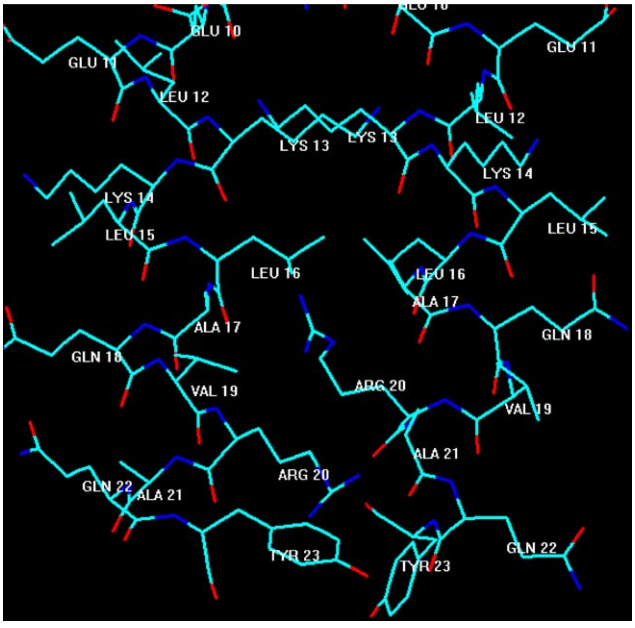


Fig. 5. Dimer E156: hydrophobic core.

observed—the axis of the first quarter is of +15°, and in the fourth quarter is horizontal. This is also interesting in that it demonstrates that HyperChem is fully able to follow such slight variation, especially important in studying the dimers in a non-aqueous, helicity-generating solvent such as TFE.

Several stabilising interactions should be mentioned. The methionines with the 45° rotation were oriented toward the exterior of the dimer, decreasing the energy of the system as a result of their relative separation. Further down the dimeric structure, the two oxygens of the glutamic acid, with partial charges of −0.5 each, are closer to the asparagine Asn-5, as well as to glutamic acid Glu-7 and lysine Lys-9 from two different peptide strands which interact in the same manner. The hydrophobic area—Leu-8, isoleucines Ile-11 and Ile-12, valines Val-15 and Ala-19 together with Leu-22—is involved in the bonding of lysine Lys-23 and tyrosine Tyr-26, such that the Lys-23 can react with the carbonyl of glutamine Gln-18 of the opposite peptide strand. The lateral chain of the arginine Arg-16 is forced in the direction of the opposite peptide, one of this chain's partial charges approaching the Gln-18; however, the second partial charge interacts with the carbonyls of the peptide bonds [19].

5.1.2. Trimer of K156

As already mentioned, the number of trimeric structures is very high. To deal with this difficulty, the preliminary evaluation was based on the hydrophobic areas being as close together as possible. This conformation will, however, lead to the difficulty associated with the 10th amino acid (lysine instead of glutamic acid in E156). This modification is then a logical cause of the trimer's presence. According to the models built, the Lys-10 is on the opposite side of the hydrophobic area and does not participate in the formation of the trimer (Fig. 6). To solve this problem a 'rolled spaghetti' model was built: three peptide strands were placed in parallel, then moved together in a clockwise direction, preserving parallel orientation of the strands [17].

Such a model allowed us to increase the size of the hydrophobic area (Leu-15 and Val-19, three leucines on positions 5, 12 and 16 of every strand). Then, the lysines Lys-10 and Lys-14 oriented by approximately 30°, can form the H-bonds via NH3+ lateral chain group with the carbonyl oxygens of the peptide. Finally the asparagine Asn-9 could be stabilised in arrangement with Asp-11 of next peptide strand [19].

5.1.3. Quadruple helix, built from the EAA26 tetramer

Four strands of EAA26 were attached to the already-suggested calixarene frame, to 'dangle' by succinic linkers. Since the functional group of valine allows it to act as an hydrophobic 'plug', its presence on the linker side of each inward oriented peptide helps to stabilize the quadruple structure. A similar effect results from the methionines and asparagines (Asn-4), whose lateral chains are oriented toward the neighbouring strand (Fig. 7).

Next, two pairs of Lysine–Glutamates serve the same purpose. The large hydrophobic area of the quadruple helix in the centre of the strand shows a possible problem with this entity's stability. The Arg-16 and Lys-23 have their charged chains oriented toward the interior of the quadruple helix. In this way, there are eight charged amino acids (four lysines and four arginines) in the area, which should be neutral in order to enhance the helix formation. Despite this difficulty, the EAA26 tetramer is nonetheless observed. To enhance the formation of the quadruple helix, the amino acid substitution in the newly designed peptides in this series should be performed while taking into account these observations. As was already mentioned, the succinic-linked model is more accurate than the longer, adipic-linked model [20].

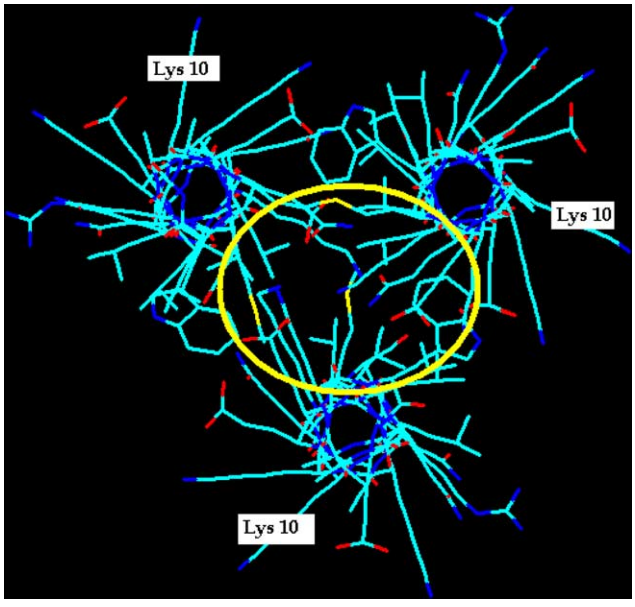


Fig. 6. Trimer K156: three strands are rolled together and enlarged the hydrophobic area.

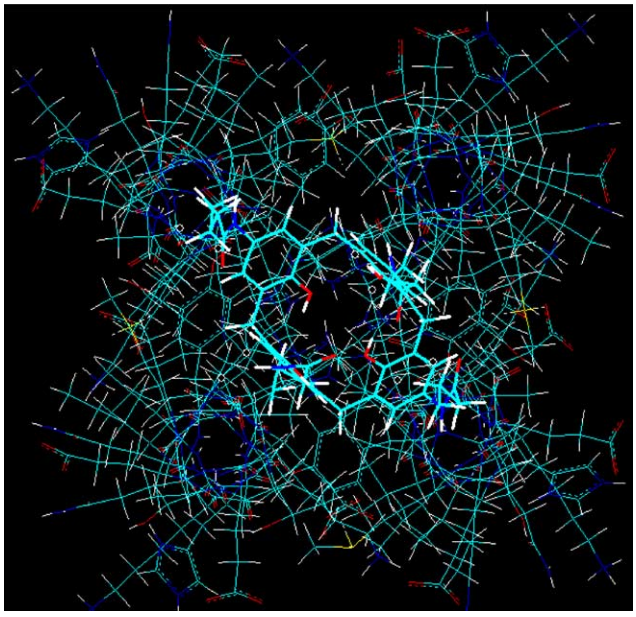


Fig. 7. Quadruple EAA26 helix–calix [4] arene (with succinic linker) top view.

6. Conclusion

During molecular modelling of dimers, the helix structures were easily obtained as a primary result of HyperChem-assisted optimisation. This is a very promising result as far as the further oligomerisation attempts are concerned, and was not induced by HyperChem modulation. Both E156 and EAA26 dimers are stable. Their strong propensity towards dimerisation is also interesting as a potential use for this peptide family in direct interaction with the α-4 helix of IN, where it is conceivable that the inhibition could take place. In order to achieve that, the hydrophobic lateral chains should rather be oriented toward the exterior of such heterodimers; then improved sequences of more efficiently fitting α-4 peptides could be designed. For the trimer K-156 study it will be interesting to substitute Asn-9 by Arg in this position. Such an alteration will confirm the 'spaghetti'-type model proposed, because the arginine will eventually interact with both Glu-7 and Glu-11. It is also expected that such a modification will increase the trimer population (as detected by ESI-MS).

It was also concluded that, in order to improve the chances of formation of a quadruple helix on the calix[4]arene frame, using the EAA26-like sequence peptides connected via the succinic linkers, it will be necessary to rotate each peptide by +45° (clockwise). This will enable the positive charges to be oriented toward the exterior of the aggregate, and thus interact with water. In this conformation the hydrophobic chains of Val-15, Ile-11, Ile-12 and Leu-22 have the necessary orientation relative to the hydrophobic center of the helix. Finally, as was already mentioned, it is necessary to avoid having many positive charges near the hydrophobic center of the molecule. It is expected that the +45° rotation will help to reach this goal [21–23].

As can be noticed, the molecular modelling helped to established a close-to-ideal peptide sequence, in order to achieve the quadruple helix structure based on the rigid calix[4]arene frame. The modelings in this study also allowed us to better rationalise the ESI-MS results previously observed for these three peptides and their oligomerisation pattern.

Acknowledgements

The authors thank G. LeClair for helpful hand with the primary modelling of these peptides as well as to Dr R. Stocklin (Asteris, Switzerland) for some mass spectrometric measurements in this series. We would like to thank Drs M. Delaforge CEA, Saclay) and J.-C. Tabet (PMC, Paris) for helpful comments as well as Mrs N. Boudreau and S. Arseneau for technical help. The project was supported by the U. de Moncton and the CEA Saclay, thank to their research facilities.

Appendix 1. E156 dimer

Dimer structure code:

- first digit: translation number (see following)
- second letter code G (left strand)
- third digit rotation of peptide (left handed) e.g. -45° means rotation of 45° clockwise, hydrophobic area pushed down
- fourth letter code D (right strand)
- fifth digit rotation of peptide (right handed) e.g. +45° means rotation of 45° clockwise, hydrophobic area pushed up.

Translation	Helix turn	Interpeptide distance (A)
0	0	0
1	0.5	2.7
2	1.0	5.2
3	1.5	7.7
4	2.0	10.2

All energies are in kcal/mol.

Position	Ef peptide	Ef peptide + interface	Order of stability
0 G0 D0	−407.7804	−539.1276	8
0 G0 D45	−295.4458	−473.2451	9
0 G0 D−45	−391.9406	−554.0168	4
0 G45 D0	−394.795	−548.604	7
0 G45 D45[a]	−384.2321	−558.1242	2
0 G45 D-45	−388.4249	−552.2216	5
0 G–45 D0[a]	−393.6707	−566.84	1
0 G–45 D45	−382.9251	−550.3419	6
0 G-45 D-45[a]	−387.9493	−555.0587	3
1 G0D0	−388.9197	−552.3882	4
1 G0 D45[a]	−387.8624	−557.0729	2
1 G0 D-45[a]	−375.8951	−554.7819	3
1 G45 D0[a]	−386.526	−563.8138	1
1 G45 D45	−371.5372	−546.7501	6
1 G45 D-45	−388.0903	−552.2216	5
1 G-45 D0	−381.5121	−544.4942	8
1 G-45 D45	−389.0766	−545.5051	7
1 G-45 D-45	−388.1705	−541.4424	9
2 G0D0[a]	−390.2463	−570.3836	1
2 G0 D45[a]	−388.5527	−561.6993	3
2 G0 D-45	−381.61	−541.1475	9
2 G45 D0	−392.1812	−556.457	5
2 G45 D45[a]	−380.9966	−562.9581	2
2 G45 D-45	−381.3131	−554.6827	6
2 G-45 D0	−380.4482	−552.7876	7
2 G-45 D45	−390.368	−556.5215	4
2 G-45 D-45	−376.3174	−541.4837	8
3 G0D0[a]	−374.0728	−564.6984	1
3 G0 D45	−384.4175	−551.7036	5
3 G0 D-45	−376.9233	−555.5991	4
3 G45 D0[a]	−392.7626	−556.0592	3
3 G45 D45	−289.306	−457.046	9
3 G45 D-45	−364.4665	−532.9649	6
3 G-45 D0[a]	−384.8166	−563.2147	2
3 G-45 D45	−379.254	−506.6403	8

Position	Ef peptide	Ef peptide + interface	Order of stability
3 G-45 D-45	−351.4477	−517.1763	7
4 G0D0	−390.2114	−559.0941	6
4 G0 D45	−385.0249	−552.7331	8
4 G0 D-45	−366.1268	−550.15	9
4 G45 D0	−383.94	−554.4495	7
4 G45 D45[a]	−382.0715	−572.7587	2
4 G45 D-45[a]	−391.4804	−573.915	1
4 G-45 D0[a]	−380.6045	−567.8166	3
4 G-45 D45	−376.9875	−560.7435	4
4 G-45 D-45	−387.7054	−559.1816	5

[a] The most stable model at given translation.

Appendix 2. EAA26 dimer

Dimer structure code:

- first digit: translation number (see following)
- second letter code G (left strand)
- third digit rotation of peptide (left handed) e.g. -45° means rotation of 45° clockwise, hydrophobic area pushed down
- fourth letter code D (right strand)
- fifth digit rotation of peptide (right handed) e.g. +45° means rotation of 45° clockwise, hydrophobic area pushed up.

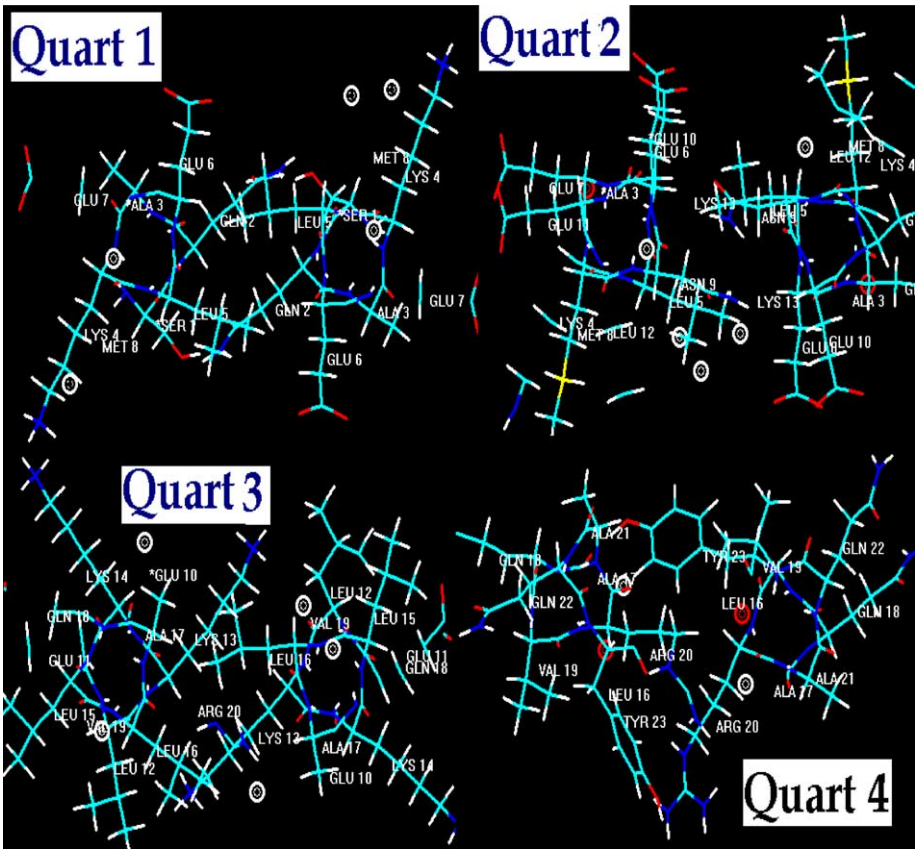
Translation	Helix turn	Interpeptide distance (A)
0	0	0
1	0.5	2.7
2	1.0	5.2
3	1.5	7.7
4	2.0	10.2

All energies are in kcal/mol.

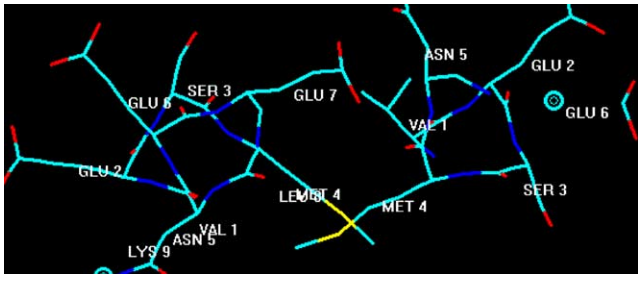
Position	Ef peptide	Ef peptide + interface	Order of stability
0,G0D0	−426.329	−514.7809	7
0, G0, D45	−418.7218	−529.7974	4
0, G0, D-45	−412.9392	−515.0769	6
0,G+45D0	−405.7946	−527.2643	5
0,G45,D45[a]	−404.403	−532.5086	3
0,G45,D-45	−402.6512	−514.5111	8
0,G-45D0[a]	−426.535	−544.783	1
0,G-45,D45[a]	−413.895	−542.1446	2
0,G-45,D-45	−327.967	−443.0065	5
1,G0D0	−404.2744	−517.929	6
1, G0, D45	−402.3572	−523.0179	4
1, G0, D-45	−393.3012	−520.3308	5
1,G+45D0	−406.3347	−472.4136	9
1,G45,D45[a]	−407.5509	−607.7664	1
1,G45,D-45[a]	−400.0197	−530.3621	3
1,G-45D0	−424.0941	−507.8055	8
1,G-45,D45[a]	−411.6575	−534.53	2
1,G-45,D-45	−407.5303	−508.9198	7
2 G0D0	−408.8736	−602.9424	4
2 G0 D45	−416.2488	−591.8558	7
2 G0 D-45[a]	−406.9817	−603.2219	3
2 G45 D0	−394.1852	−597.7599	5
2 G45 D45	−409.678	−586.9025	8
2 G45 D-45[a]	−403.0736	−607.9752	1
2 G-45 D0	−392.8938	−578.5747	9
2 G-45 D45	−407.7969	−592.7674	6
2 G-45 D-45[a]	−403.9297	−607.7643	2
3 G0D0	−391.2136	−570.3132	8
3 G0 D45[a]	−401.0433	−604.2217	2
3 G0 D-45	−404.2036	−592.5397	4
3 G45 D0	−418.5997	−4.4856	9
3 G45 D45	−396.4174	−579.3117	6
3 G45 D-45	−404.2036	−591.6107	5
3 G-45 D0[a]		−606.0486	1
3 G-45 D45	−393.0763	−577.3177	7
3 G-45 D-45[a]	−403.1365	−599.6871	3
4 G0D0	−389.3904	−600.4476	4
4 G0 D45	−396.3226	−591.0458	6
4 G0 D-45	−404.8907	−583.9728	7
4 G45 D0	−406.6207	−572.56	9
4 G45 D45	−401.7633	−610.0456	1
4 G45 D-45	−408.3946	−606.5986	2
4 G-45 D0	−406.6896	−603.6745	3
4 G-45 D45	−403.8971	−600.204	5
4 G-45 D-45	−392.2032	−573.3199	8

[a] The most stable model at given translation.

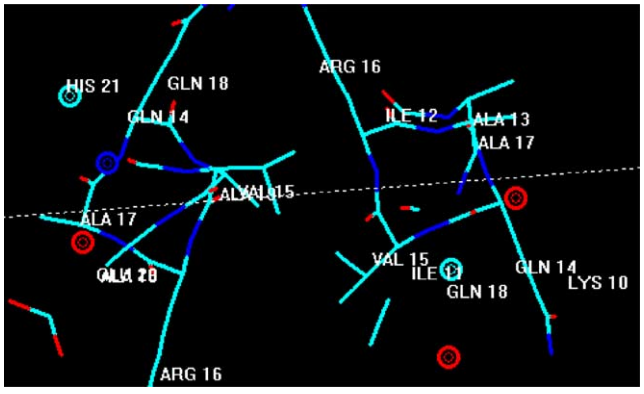
Appendix 3. E156 dimer model OG−45DD: four quarter cuts (Quarts 1–4)



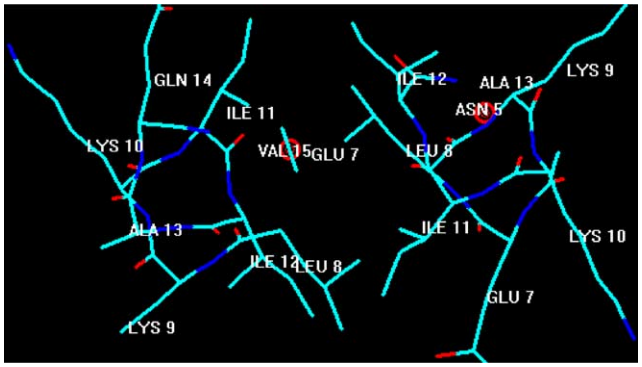
Appendix 4. EAA26 dimer model 2, G45, D45: four quarter cuts.



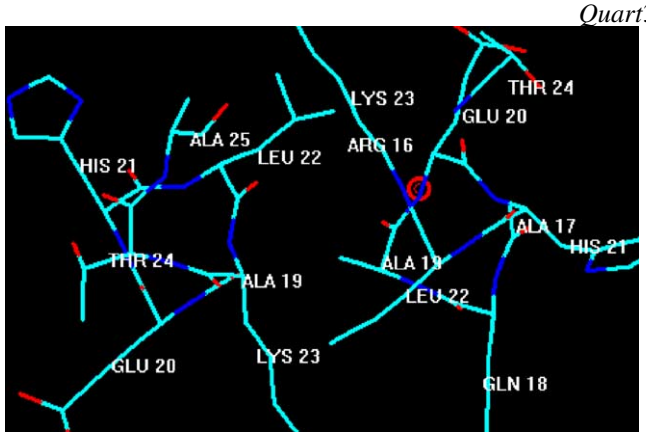
*Quart*1



*Quart*3



*Quart*2



*Quart*4

References

[1] J. d'Angelo, J.F. Mouscadet, D. Desmaie, F. Zouhiri, H. Leh, HIV-1 integrase: the next target for AIDS therapy? Pathol. Biol. 49 (2001) 237–246.

[2] A. Lupas, Predicting coiled-coil regions in proteins, Curr. Opin. Struct. Bio 7 (1997) 388–393.

[3] Y. Pommier, A. Johnson, C. Marchand, Integrase inhibitors to treat HIV/AIDS, Nat. Rev. 4 (2005) 236–248.

[4] R. Lins, T.P. Straatsma, J.M. Briggs, Similarities in the HIV-1 and ASV integrase active sites upon metal cofactor bonding, Biopolymers 35 (2000) 308–315; T. Tasara, G. Maga, M.O. Hottiger, U. Hbscher, HIV-1 reverse transcriptase and intergrase enzymes physically interact and inhibit each other, FEBS Lett. 507 (2001) 39–44; Briggs J., Briggs Group Image Gallery, 2002, http://adrik.b.chs.uh.edu/gallery.html; D. Esposito, R. Caraigie, Sequence specificity of viral end DNA binding by HIV-1 integrase reveals critical regions for protein-DNA interaction, EMBO J. 17 (1998) 5832–5843.

[5] S. Fermandjian, R.G. Maroun, B. Amekraz, C.K. Jankowski, Self-association of an amphipathic helix peptide inhibitor of HIV-1 integrase assessed by electro spray ionization mass spectrometry in trifluoroethanol/water mixtures, Rapid Commun. Mass Spectrom. 15 (2001) 320–324; R.G. Maroun, S. Fermandjian, R. Stocklin, C.K. Jankowski, Rapid Commun. Mass Spectrom. 19 (2005) 2539–2548.

[6] F. Sourgen, R.G. Maroun, V. Frère, M. Bouziane, C. Auclair, F. Troalen, S. Fermandjian, A synthetic peptide from the human immunodeficiency virus type-1 integrase exhibits coiled-coil properties and interferes with the in vitro integration activity of the enzyme, Eur. J. Biochem. 240 (1996) 765–773.

[7] R.G. Maroun, D. Krebs, S. El Antri, A. Deroussent, E. Lescot, F. Troalen, H. Porumb, M.E. Goldberg, S. Fermandjian, Self-association and domains of interactions of an amphipathic helix peptide inhibitor of HIV-1 integrase assessed by analytical ultracentrifugation and NMR experiments in trifluoroethanol/H2O mixtures, J. Biol. Chem. 274 (1999) 34174–34185.

[8] R.G. Maroun, S. Gayet, S. Benleulmi, H. Porumb, L. Zargarian, H. Merad, H. Leh, J.F. Mouscadet, F. Troalen, S. Fermandjian, Peptide inhibitors of HIV-1 integrase dissociate the enzyme oligomers, Biochemistry 40 (2001) 13840–13848.

[9] D. Krebs, R.G. Maroun, F. Sourgen, F. Troalen, D. Davoust, S. Fermandjian, Helical and coiled-coil forming properties of peptides derived from and inhibiting HIV-1 integrase assessed by 1H-NMR. Use of NH temperature coefficients to probe coiled-coil structures, Eur. J. Biochem. 253 (1998) 236–244; D.A. Perlman, D.A. Case, J.A. Caldwell, W.S. Ross, T.E. Cheatham, D.M. Ferguson, G.L. Seibel, U.C. Singh, S.J. Weiser, P.A. Kollman. Amber 4, University of California, San Francisco, US, 1995; S.J. Weiner, P.A. Kollman, D.T. Nguyen, D.A. Case, J. Comput. Chem. 7 (1997) 230; SYBYL 6 series, Molecular Modelling System Software, Tripos Inc., USA, 1994. Forcalixarene modelling see S.J. Weiner, P. Thuery, M. Nierlich, J.C. Bayer, V. Lamare, J-F. Dozol, Z. Asfari, J. Vicens, Crown ether conformations in 1,3-calixarene bis crown ethers, J. Chem. Soc. Dalton Trans. 3 (1997) 4191–4202; P. Thuery, M. Nierlich, Z. Asfari, J. Vicens, J. Inclusion Phenom. Macrocyclic Chem. 27 (1997) 169.

[10] B. Wagner, N. Stojanovic, G. LeClair, C.K. Jankowski, A spectroscopic and molecular modelling study of the nature of the association complexes, J. Inclusion Phenom. Macrocyclic Chem. 45 (2003) 275–283; A. Jankowska, C.K. Jankowski, J.B. Chiasson, On chloralose-cyclodextrin complexes by ESI-mass spectrometry, J. Inclusion Phenom. Macrocyclic Chem. 52 (2005) 213–221; HyperChem, Hypercube, US, 2001. For methodology see references quoted there and reference 21

[11] W.D. Kohn, O.D. Monera, C.M. Kay, R.S. Hodges, The effects of interhelical electrostatic repulsions between glutamic acid residues in controlling the dimerization and stability of two-stranded alpha-helical coiled-coils, J. Biol. Chem. 270 (1995) 25495–25506; J.F. Conway, D.A.D. Parry, Three-stranded alpha-fibrous proteins: the heptad repeat and its implications for structure, Int. J. Biol. Macromol. 13 (1991) 14; P.K. Sorger, H.C. Nelson, Trimerization of a yeast transcriptional activator via a coiled-coil motif, Cell 59 (1989) 807–813; R. Peteranderi, H.C. Nelson, Trimerization of the heat shock transcription factor by a triple-stranded alpha-helical coiled-coil, Biochemistry 31 (1992) 12272–12276; B. Lovejoy, S. Choe, D. Cascio, D.K. McRorie, W.F. degrade, D. Eisenberg, Crystal structure of a synthetic triple-stranded alpha-helical bundle, Science 259 (1993) 1288–1293.

[12] I.A. Wilson, J.J. Skehel, D.C. Wiley, Structure of the haemagglutinin membrane glycoprotein of influenza virus at 3 A resolution, Nature 289 (1981) 366–373; W.I. Weis, A.T. Brunger, J.J. Skehel, D.C. Wiley, Refinement of the influenza virus hemagglutinin by simulated annealing, J. Mol. Biol. 212 (1990) 737–761; E. Brin, J. Yi, A.M. Skalaka, J. Leis, Modelling the late steps in HIV-1 retroviral intergrase-catalyzed DNA integration, J. Biol. Chem. 275 (2002) 39287–39295.

[13] D.J. Hazuda, P. Felock, M. Witmer, A. Wolfe, K. Stillmock, J.A. Grobler, A. Espeseth, L. Gabryelski, W. Schleif, C. Blau, M.D. Miller, Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells, Science 287 (2000) 646–650; G.C. Pais, X. Zhang, C. Marchand, N. Neamati, K. Cowansage, E.S. Svarovskaia, V.K. Pathak, Y. Tang, M. Nicklaus, Y. Pommier, T.R. Burke Jr., Structure activity of 3-aryl-1,3-diketo-containing compounds as HIV-1 integrase inhibitors, J. Med. Chem. 45 (2002) 3184–3194.

[14] L. Zargarian, M.S. Benleulmi, J.-G. Renisio, H. Merad, R.G. Maroun, F. Wieber, O. Mauffret, H. Porumb, F. Troalen, S. Fermandjian, Strategy to discriminate between high and low affinity bindings of human immunodeficiency virus, type I integrase to viral DNA, J. Biol. Chem. 278 (2003) 19966–19973.

[15] D.N. Marti, H.R. Bosshard, Electrostatic interaction in leucine zippers: thermodynamic analysis of the contributions of Glu and His residues and the effect of mutating salt bridges, J. Mol. Biol. 330 (2003) 621–637; P.B. Harbury, P.S. Kim, T. Alber, Crystal structure of an isoleucine-zipper trimer, Nature 371 (1994) 80–83; P.B. Harbury, T. Zhang, P.S. Kim, T. Alber, A switch between two-, three-, and four-stranded coiled-coils in GCN4 leucine zipper mutants, Science 262 (1993) 1401–1407; P. Burkhard, J. Stetefed, S.V. Strelkov, Coiled coils: A highly versatile protein folding motif, Trends Cell Biol. 11 (2001) 82–88.

[16] I. Nishimura, Y. Muragaki, M. Hayashi, Y. Ninomiya, D.R. Olsen, Tissue-specific expression of type IX collagen, Ann. N.Y. Acad. Sci. 580 (1990) 112–119; Foucher S., Modélisation des peptides, Thèse, Université de Moncton, 2003, pp. 87

[17] C.K. Jankowski, S. Arseneau, J. Blu, N. Aychet, L. Mauclaire, Can. J. Chem., accepted F. Djedaiwi-Pilard, F. Désalos, B. Perly, Tetrahedron Lett. 34 (1993) 2457.

[18] P. Dolz, J. Engel, Structure, molecular biology and pathology, Ann. N.Y. Acad. Sci. 580 (1990) 421.

[19] D.W. Banner, M. Kokkinidis, D. Tsemoglou, Structure of the colE1 rop protein at 1.7 Å resolution, J. Mol. Biol. 196 (1987) 657–675; W.H. Landshultz, P.F. Johnson, S.L. McKnight, The leucine zipper: A hypothetical structure common to a new class of DNA binding proteins, Science 240 (1988) 1759–1764.

[20] J.C. Hu, E.K. O'Shea, P.S. Kim, R.T. Sauer, Sequence requirements for coiled-coils: analysis with lambda repressor-GCN4 leucine zipper fusions, Science 250 (1990) 1400–1403; T. Kouzarides, E. Ziff, The role of the leucine zipper in the fos-jun interaction, Nature 336 (1988) 646–651; M. Neuberg, M. Schuerman, J.B. Hunter, R. Muller, Two functionally different regions in fos are required for the sequence-specific DNA interaction of the fos/jun protein complex, Nature 338 (1989) 589–590; R. Gentz, F. Rauscher, C. Abate, T. Curran, Parallel association of fos and jun leucine zippers juxtaposes DNA binding domains, Science 243 (1989) 1695–1699; R. Turner, R. Tjian, Leucine Repeats And An Adjacent DNA binding domain mediate the formation of functional cFos-cJun heterodimers, Science 243 (1989) 1689–1694; L.J. Ransone, J. Visvader, C.P. Sassone, I.M. Verma, Fos-jun interaction: Mutational analysis of the leucine zipper domain of both proteins, Genes Dev. 3 (1989) 770–781; V.J. Dwarki, M. Montiminy, I.M. Verma, Both the basic region and the 'leucine zipper' domain of the cyclic AMP response element binding (CREB) protein are essential for transcriptional activation, EMBO J. 9 (1990) 225–232; C. Augé-Gouillon,

H. Notareschi-Leroy, P. Abad, G. Periquet, Y. Bigot, Phylogenetic analysis of the functional domains of mariner-like element (MLE) transposases, Mol. Gen. Genet. 264 (2000) 506–513.

[21] D.J. Kim, Y.T. Oh, S. Lee, C.G. Shin, Analysis of integration activity of human immunodeficiency virus type-1 intergrase, Mol. Cells 9 (1999) 446–451.

[22] J.L. Martel, Étude Conformationelle Des Adduits Glutariques De Peptides Inhibiteurs De La HIV-1 Integrase. Thesis, Université de Moncton, Moncton, Canada, April 2004; P. Monsan, G. Puzo, H. Mazarguil, Étude du mécanisme d'établissement des liaisons glutaraldehyde-protéine, Biochimie 57 (1975) 1281–1292; K. Okuda, I. Urabe, Y. Yamada, H. Okada, Reaction of glutaraldehyde with amino and thiol compounds, J. Ferment. Bioeng. 71 (1991) 100–105.

[23] C.K. Jankowski, J.-L. Martel, S. Fermandjian, R.G. Maroun, Study of potential HIV-1 inhibition. Glutaric dialdehyde adducts, J. Mol. Struct. (Theochem) 731 (2005) 83–87; for the methodology also see V. Lamare, Apport De La Dynamique Moléculaire A L'étude Des Complexes, rapport Tech. DESD 98/172, CEA (France), pp. 54