Solvent Accessibility to Aspartyl and Succinimidyl Residues at Positions 7 and 23 in the Amyloid β 1–28 Peptide

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The water accessibilities to aspartyl residues at positions 7 and 23 in the amyloid β 1–28 peptide associated with Alzheimer's Disease have been calculated using different techniques. These accessibilities of water were compared to those of the succinimidyl residues (SUC) replacing the aspartyl ones (ASP). It has been possible to ascertain that these modifications (ASP → SUC) lead to a significant increase in the water accessibility to the backbone and α-carbon atom of the SUC7 and SUC23 residues. It is suggested that the spontaneous transformation of ASP → SUC might lead to an increase of the racemization rates due to the higher accessibility of water at these sites. It is also proposed that the behavior of the adjacent residues in the selectivity of the racemization is to control the water accessibility at the reactive residue.

Introduction

Studies on the possible relationship between the amyloid β peptide and Alzheimer's disease, a degenerative and progressive disease of the central nervous system, have been recently reported (Hilbich *et al.*, 1991; Burdick *et al.*, 1992; Mori *et al.*, 1992; Roher *et al.*, 1993; Iversen *et al.*, 1995; Cintron *et al.*, 1996; Lansbury, 1996). It has been found that the fibrils formed by the amyloid β peptide are present in the brains of Alzheimer's patients (Hilbich *et al.*, 1991; Burdick *et al.*, 1992; Mori *et al.*, 1992; Roher *et al.*, 1993; Iversen *et al.*, 1995). This peptide is produced by proteolysis of an integral membrane protein, namely, the proteinase inhibitor (trypsin). Fragments of several lengths are produced, ranging from 28 amino acid residues, which are mainly hydrophilic, up to more than 50 residues with a hydrophobic portion. Residue racemization, especially at the ASP 23 site of many amyloid β peptide chains, appears to favor or even to induce the formation of fibrils (Iversen *et al.*, 1995). These site-specific racemizations have been studied and seem to be associated with the appearance and/or growth of the senility plaques and cerebral hemorrhage (Iversen *et al.*, 1995).

Racemization of amino acid residues is, in general, a very slow reaction; however, aspartic acid residues in many peptides have racemization rates that can be very large when compared to other amino acid residues or with the free aspartic acid residue (Bada *et al.*, 1973; Bada *et al.*, 1974; Bada and McDonald, 1995; Radkiewicz *et al.*, 1996). The general mechanism of racemization of amino acids occur through a deprotonation of the α-carbon atom followed by its protonation on the either side of the newly generated α-carbanion, which can be schematically represented as

$$\text{L-}(R_1R_2R_3)C_\alpha\text{-H} + H_2O/OH^- \rightarrow (R_1R_2R_3)C_\alpha^- + H_3O^+/H_2O \qquad (1)$$

$$(R_1R_2R_3)C_\alpha^- + H_3O^+/H_2O \rightarrow \text{L/D-}(R_1R_2R_3)C_\alpha\text{-H} + H_2O/OH^- \qquad (2)$$

It has been proposed that Eqn. (1) is the limiting step for the racemization process (Radkiewicz *et al.*, 1996; Stephenson and Clarke, 1989; Capasso, 1996). Thus, the racemization rate can be generally expressed as $v = k_1[\mathrm{AA}][\mathrm{H_2O}]$, where k_1 is the

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rate constant for the first step, with [AA] and [H_2O] representing the amino acid and water concentrations, respectively. For reactions in aqueous solution, the water concentration is considered constant and is incorporated into the rate constant yielding a pseudo-first order reaction. However, in the case of a peptide residue the reaction is usually processed in a cellular medium where the available water molecules cannot be compared to an aqueous solution. In addition, the solvent accessibility of water to the α-carbon hydrogen in a peptide residue is different of that of a free amino acid in aqueous solution. Thus, the approximation of considering the water concentration constant in the rate equation is not appropriate for the racemization of a residue in a peptide. In fact, the water accessibility should be of significant importance. The main concern of this paper is the calculation of the water accessibility to different residues in the amyloid β-(1–28) peptide. To our knowledge this is the first time that the effective solvent concentration, represented by the water accessibility, has been taken into account in the description of the racemization process.

According to the racemization mechanism expressed by Eqns (1) and (2) above, there are two possibilities for altering the racemization rate, namely, changing k_1 and/or [H_2O]; the latter actually means the effective water concentration available and accessible to the residue. With respect to altering k_1 it would imply, mainly, changing the activation energy for the limiting rate step, Eqn. (1). This could be achieved by modifications in the structure of the residue, so as to decrease the activation energy relatively to the direct racemization. Theoretical calculations (Radkiewicz *et al.*, 1996) of model compounds have been performed in order to correlate the pK_a with the racemization rate of the aspartyl/succinimidyl residues, thus addressing, at least partially, the residue structural modification. There is some experimental evidence that the racemization of the L-aspartic residues involves the formation of intermediate species such as L- and D-succinimidyl residues, as shown in Fig. 1, modified from Stephenson and Clarke, (1989).

These intermediates should account for the rates of racemization of aspartic acid in polypeptides, which are significantly larger than for other amino acids. Measurements of these ratios have been used to date paleontological and archeological materials (Bada *et al.*, 1973; Bada *et al.*, 1974; Bada and McDonald, 1995). Racemization is expected to contribute to the loss of function during biological processes in some tissues, including the formation of senility plaques of the amyloid β peptide.

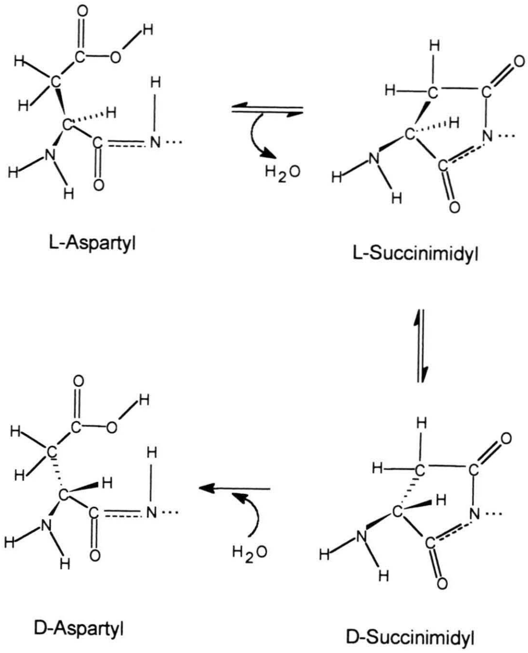


Fig. 1. Racemization pathway of L-aspartic acid residue in peptides. The possible isomers *iso*-D-arpartyl and *iso*-L-aspartyl are not shown.

The transformation from aspartyl to succinimidyl residues (L-ASP → L-SUC) in some peptides is known to occur in the order of minutes (Stephenson and Clarke, 1989; Capasso, 1996). Assuming that the deprotonation step after conversion of ASP → SUC is the limiting step of the whole process, the racemization reaction should be critically dependent upon the hydration. Hence, the accessibility to water of these residues should be an important factor in kinetic studies.

Methodology

The amyloid β (1–28) peptide obtained from the protein data bank (PDB) has several conformational structures determined via NMR in solution (Barrow and Zagorski, 1991; Zagorski and Barrow, 1992; Talafous *et al.*, 1994). These structures have been averaged and the starting coordinates for this peptide were obtained and used to perform the calculations of water accessibility to the aspartyl residues at positions 7 and 23. The 1-aspartyl residue is also known to isomerize, but it seems that this process is not connected with fibril formation (Iversen *et al.*, 1995).

The β-(1–28) peptide was surrounded by a 10 Å layer of water. The whole system had its energy minimized with respect to the atomic coordinates to a gradient norm (RMS) smaller than 0.04 kJ/mol by the steepest descent approach (500 steps) followed by a conjugate gradient refinement. The accessibility of water to those residues was then computed for the minimized amyloid structure. The next step was to replace the aspartyl residues at positions 7 and 23 in the above-minimized structures by succinimidyl residues and then to perform energy minimization as described above, before the new accessibility calculations.

Accessibility of water calculations

All the values of accessibility of water were calculated with the What If v.4.95 program (Vriend, 1993), where the contact surfaces for the water probe used was 1.4 Å. The accessible surface is not the same as the Connolly surface, even though both surface calculations agree very well. The algorithm used to calculate the accessibility of water in the What If v.4.95 program can be briefly described as follows steps:

i) Dots are placed on the surface generated by the sum of the van der Waals radius and the radius of water molecule.

ii) Every dot receives a value 1.

iii) Dots that falls within another sphere receive a value 0 (zero).

iv) The sum of the values of all dots, divided by the total number of dots, multiplied by the surface area of the sphere is the accessibility to water.

v) The dots on the surface are placed as homogeneously as possible onto the surface according to the Fibonacci algorithm.

In order to check the accuracy of the solvent accessibilities calculated with the What If 4.95 program, additional methods and programs, namely, Quanta96 (Quanta, 1996) and Insight II v.95 (Insight II, 1995) were used to compute the total water accessibilities and compared to the What If results.

Geometry optimization

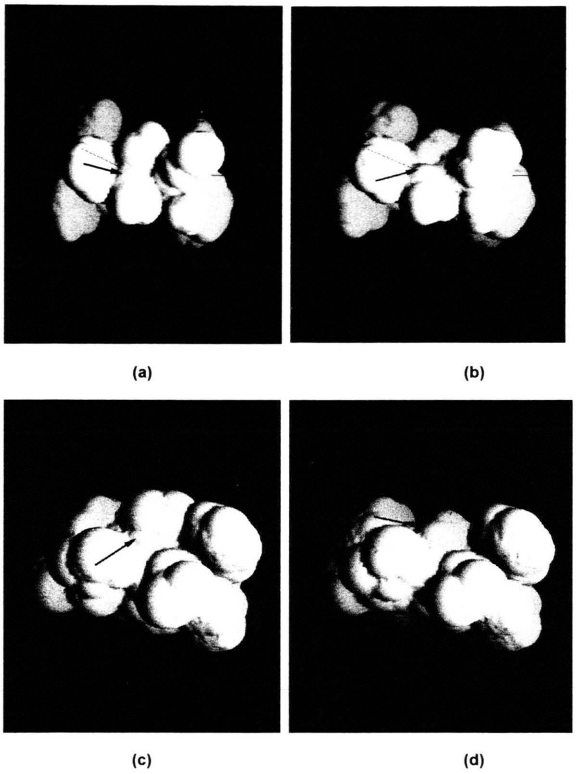
The molecular modeling and geometry optimizations were performed with the Discover 3.2.5 program using the Amber force field, installed in an IBM/RS-6000 computer. The Amber force field has been extensively used in computer simulations of biomolecules and can be briefly described as (Weiner *et al.*, 1984; Weiner *et al.*, 1986),

$$\begin{aligned}E_{\text{total}} = {} & \sum_{bonds} K_R(R-R_0)^2 + \sum_{angles} K_\theta(\theta-\theta_0)^2 \\ & + \sum_{dihedrals} \frac{V_n}{2}[1+\cos(n\phi-y] + \frac{1}{\text{VDW}} \sum_{i>j} \left(\frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^{6}}\right) \\ & + \frac{1}{\text{EEL}} \sum_{i>j} \left(\frac{q_i q_j}{\varepsilon(R) R_{ij}}\right) + \sum_{H\text{-}bonds} \left(\frac{C_{ij}}{R_{ij}^{12}} - \frac{D_{ij}}{R_{ij}^{10}}\right) \\ & \sum_{constraints} K_R(R-R_0)^2 \end{aligned} \tag{3}$$

where all variables, except for the distances, angles and dihedrals, are constants of the force field, obtained via parameterization. The first three terms in this equation are referred as the bonded interactions, the fourth and fifth terms are the so called non-bonded ones, and the last term is included in order to keep a part of the molecular system restrained. The bonded terms are described by a harmonic valence force field for stretch (1st term), bending (2nd term) and torsion or improper dihedral (3rd term). The non-bonded terms are represented by the 4th, 5th and 6th terms of the expression for E_{total} above, where the 6–12 Lennard-Jones potential simulates the van der Waals interactions (attractive London dispersion interactions and the repulsive interactions due to the Pauli exclusion principle), except that when the pairs of atoms are involved in hydrogen-bonds, the van der Waals function is replaced by a 10–12 function (6th term). The electrostatic interactions are described by the Coulomb potential due to point charges (5th term). All geometric parameters were allowed to vary during the optimization, so the last term is null.

Results and Discussions

The qualitative results for the water accessibility surfaces to the peptides containing ASP7, SUC7, ASP23, and SUC23 are displayed in Fig. 2, where the arrow indicates the proton of the α-carbon.



(a) (b) (c) (d)

Fig. 2. Surfaces of water accessibility with the hydrogen of the α-carbon indicated by the arrow. Region of ASP7 (a), SUC7 (b), ASP23 (c), and SUC23 (d).

Quantitative results for the accessibility of water to the ASP7 and ASP23 residues obtained from the averaged NMR structure and from the minimized structure in water are shown in Table I. In addition, the percentage differences between the calculated accessibility of water for these two structures are also shown. It can be argued from these results that the changes in the calculated accessibility of water from the averaged NMR structure to the calculated one are neither qualitative nor quantitatively significant.

It can be inferred that the calculated accessibility of water using the minimized structure reproduces the accessibility of water calculated with the averaged NMR structure is conformed by the fact that the superimposed structures of the amyloid β-(1–28) peptide obtained from the PDB archives (NMR) and from optimization technique used agree very well. The backbone RMS deviations for the minimized wild type, SUC7 and SUC 23 structures, were 0.37, 0.06 and 0.09 Å, respectively, against the NMR determined one.

The NMR structure retrieved from the PDB archives was obtained in an aqueous solution of 1,1,1-trifluoroethanol and it exists in a helical conformation (Barrow and Zagorski, 1991; Zagorski and Barrow, 1992). It has been shown that there is no helical conformation of this peptide in pure water (Lee *et al.*, 1995). It is plausible to mention that the aqueous solution of 1,1,1-trifluoroethanol might be a better representation of the intracellular media than pure water. Thus, it seems appropriate to limit this investigation to the helical conformation obtained from the NMR in 1,1,1-trifluoroethanol aqueous solution (Barrow and Zagorski, 1991; Zagorski and Barrow, 1992) and from molecular dynamics simulations (Szendrei *et al.*, 1996). This validation procedure is necessary due to the fact that when the aspartyl residues are

Table I. Accessibility of water to ASP7 and ASP23 residues in the amyloid β-(1–28) peptide.

Residue	Total	Backbone[a]	C-α	Side Chain[a]
		Averaged structure obtained from NMR		
ASP 7	25.22	1.72 (6.8)	0.00	23.50 (93.2)
ASP 23	24.78	0.79 (3.2)	0.00	23.99 (96.8)
		Optimized structure in water		
ASP 7	25.16	2.21 (8.8)	0.00	22.95 (91.2)
ASP 23	24.90	0.94 (3.8)	0.17	23.96 (96.2)
		Difference between averaged NMR and the calculated structures (%)		
ASP 7	0,2	−28.5	0.00	2.3
ASP 23	−0.5	−19.0	18	0.1

[a] Numbers in parenthesis are the percentage values of the backbone and the side chain accessibilities of water relative to the total accessibility.

changed to the succinimidyl one (ASP → SUC) the peptide structure needs to be relaxed and this relaxation is not available experimentally.

The comparisons between the calculated values for the accessibility of water to aspartyl and/or succinimidyl residues in the 7 and 23 positions of the amyloid β-(1–28) peptide are presented in Table II. The structures used in the accessibility calculations were obtained from a geometry optimization in water after the site specific modifications (ASP → SUC) had been performed.

It can be noted that, in general, the modification of an L-aspartyl to an L-succinimidyl (ASP → SUC) leads to a decrease in the total and side chain accessibility by water, but more importantly it leads to a significant increase in the backbone accessibility at the site modified residue. Even the unmodified site has its accessibility of water changed by a small percentage. In particular, it should be emphasized that the backbone accessibility of water of the modified sites increase by 200–600% relative to the unmodified site. This is a strong indication that the ASP → SUC conversion, which occur spontaneously (Stephenson and Clarke, 1989; Lee *et al.*, 1995), leads to a significant increase in the accessibility to water which should facilitate the racemization assisted by water. This trend can also be observed in the accessibility of water to the α-carbon of the aspartyl and/or succinimidyl residues as presented in Table II.

It is possible to conclude that the aspartyl residue has an insignificant water accessibility compared to the backbone in the unmodified peptide. However, when the aspartyl is modified to a succinimidyl, the α-carbon atom is exposed and a 4–5 fold increase in the water accessibility is calculated. This increase in the water accessibility relative to the unmodified residue is also observed with respect to the backbone, since the contribution of the α-carbon atom corresponds to 65 – 75% of the backbone access. These results are significant since the racemization mechanism involves a deprotonation of the α-carbon, which is mediated, in general, by the solvent (water). Thus an increase in the water accessibility to this carbon atom should be very significant for explaining and rationalizing the site specific racemization observed in peptides. A comparison between the accessibility of the modified aspartyl residues at positions 7 and 23 can also be performed. It can be seen from Table II that both the backbone and the α-carbon accessibility of water are larger for the ASP → SUC transformation at the 23 residue than at the 7 position. The origin of these differences in water accessibility at different residue positions in the peptide is not clear, but it should be due to the adjacent residues. Thus, in addition to a possible alteration of the chemical reactivity of a residue at a given position, the adjacent residues should more importantly change the water accessibility and thus determine the preference for racemization of the same residue in different positions of the peptide. If this supposition is correct ASP23 should be more susceptible to racemization than the ASP7. Unfortunately, there is no experimental

Table II. Accessibility of water to residues in the 7 and 23 positions in the amyloid β-(1–28) peptide after the modification: L-aspartyl → L-succinimidyl. Optimized structure in water.

SITE SPECIFIC MODIFICATION ASP 7 → SUC 7				
Residue	Total	Backbone	C-α	Side Chain
SUC 7	22.93	6.39	4.19	16.54
ASP 23	24.55	1.12	0.35	23.43
Relative changes in the accessibility of water upon modification (%)				
ASP7 → SUC7	−8.7	189	66	−279
ASP23	−1.4	19.1	31	−2.2
SITE SPECIFIC MODIFICATION ASP 23 → SUC 23				
ASP 7	23.89	2.35	0.00	21.45
SUC 23	18.68	6.57	5.07	12.11
Relative changes in the accessibility of water upon modification (%)				
ASP7	5.0	6.3	0	−6.5
ASP23 → SUC23	−25.0	600	77	−49.5

Table III. Accessibility of water to the amyloid β-(1–28) peptide calculated with three different methods and programs.

β-(1–28) peptide	WHAT IF 4.5	QUANTA96	INSIGHTII V.95
Unmodified	843.69 (0.0%)	2910 (0.0%)	774.52 (0.0%)
Minimized	901.04 (+6.8%)	3110 (+6.9%)	835.20 (+7.8%)
SUC at position 7	844.07 (+0.0%)	2901 (−0.3%)	771.05 (−0.4%)
SUC at position 23	839.93 (−0.5%)	2892 (−0.6%)	769.11 (−0.7%)

* Values in parenthesis are the relative (percentage) solvent accessibility.

information about the selectivity of racemization at these sites to compare with this prediction.

Therefore, there is an ongoing work to calculate the water accessibility of some peptides where the selectivity in the rate of residue conversion has been verified quantitatively to be vary drastically (Stephenson and Clarke, 1989). It should also be noted that only the modification L-aspartyl → L-succinimidyl has been studied, but the isomers *iso*-D-arpartyl and *iso*-L-aspartyl should also be investigated.

The results presented in Table III compare the total water accessibility calculated by three different methods and programs.

This comparison shows that the relative values calculated by these programs are in very good agreement, and even the absolute values calculated by the What If (Vriend, 1993) and Insight II (1995) programs agree quite well. It thus gives additional assurance of the reliability of the calculated values by the What If 4.5 program.

Conclusions

The calculated wild type (amber force field minimized) and the average NMR structures for the amyloid β-(1–28) peptide are in very good agreement. It thus supports the assumption that the calculated structures for the modification of an L-aspartyl to an L-succinimidyl (ASP → SUC) are appropriate for the accessibility of water calculations.

The modification ASP → SUC leads to a significant increase of the water accessibility both to the backbone and to the α-carbon atom. These results can be used to explain and rationalize the high racemization rates of the aspartyl residue compared to other residues, since it can undergo the ASP → SUC modification, thus allowing for a more readily access of water at the racemization site.

The differences between the accessibility of water at ASP7 and ASP23 is probably due to the adjacent residues which could explain their role in the relative selectivity of racemization at different sites of the same residue. For instance, the racemization rate for the aspartyl residue at peptides with a different adjacent residue can vary from 40.8 days (...ASP–GLY...) to 168 days (...ASP–SER...) and up to 266 days (...ASP–ALA...). It can be very difficult to explain this large variation of the racemization rate solely on the electronic (reactivity) influence of the adjacent residue at proton of the α-carbon. Thus the differences in the water accessibility at aspartyl residues in different peptide positions can be of great help in explaining the racemization selectivity of these residues and the role of the adjacent residues and the peptide supramolecular structure.

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Bada J. L., Kvenvolden K. A. and Peterson E. (1973), Racemization of amino acids in bones. Nature **245**, 308.

Bada J. L., Schroeder R. A. and Carter G. F. (1974), New evidence for the antiquity of man in north america deduced from aspartic acid racemization. Science **184**, 791–793.

Bada J. L. and McDonald G. D. (1995), Amino acid racemization on mars – implications for the preservation of biomolecules from an extinct martian biota. Icarus **114**, 139.

Barrow C. J. and Zagorski M. G. (1991), Solution structures of β peptide and its constituent fragments – relation to amyloid deposition. Science **253**, 179–182.

Burdick D., Soreghan B., Kwon M., Kosmoski J., Knauer M., Hesschen A., Yates J., Cotman C. and Glabe C. (1992), Assembly and aggregation properties of synthetic Alzheimer's α4/β amyloid peptide analogs. J. Biol. Chem. **267**, 546–554.

Capasso S. (1996), Thermodynamic parameters of the reversible isomerization of aspartic residues via a succinimide derivative. Themochimica Acta **286**, 41–50.

Cintron M., Diehl S., Gordon G., Biere A. L., Seubert P. and Selkoe D. J. (1996), Evidence that the 42- and 40-amino acid forms of amyloid β protein are generated from the β-amyloid precursor protein by different protease activities. Proc. Natl. Acad. Sci. USA **93**, 13170–13175.

Hilbich C., Kisters-Woike B., Reed J., Masters C. L. and Beyreuther K. (1991), Aggregation and secondary structure of synthetic amyloid beta-a4 peptides of Alzheimer's disease. J. Mol. Biol. **218**, 149–163.

Insight II (1995), User Guide, October version. San Diego: Biosym/MSI.

Iversen L. L., Mortishire-Smith R. J., Pollack S. J. and Shearman M. S. (1995), The toxicity *in vitro* of β-amyloid protein. Biochem. J. **311**, 1–16.

Lansbury Jr. P. T. (1996), A reductionist view of Alzheimer's disease. Acc. Chem. Res. **29**, 317–321.

Lee J. P., Stimson E. R., Ghilardi J. R., Mantyh P. W., Lu Yi-An, Felix A. M., Llanos W., Behbin A., Cummings M., Criekinge M. V., Timms W. and Maggio J. E. (1995), H-1 NMR of β amyloid peptide congeners in water solution – conformational changes correlate with plaque competence. Biochemistry **34**, 5191–5200.

Mori H., Ogawara M. and Selkoe D. J. (1992), Mass spectrometry of purified amyloid-beta protein in Alzheimer's disease. J. Biol. Chem. **267**, 17082–17086.

Quanta 96 (1996), User Guide, version 96. San Diego: MSI.

Radkiewicz J. L., Zipse H., Clarke S. and Houk K. N. (1996), Accelerated racemization of aspartic acid and asparagine residues via succinimide intermediates – an *ab initio* theoretical exploration of mechanism. J. Am. Chem. Soc. **118**, 9148–9155.

Roher A., Lowenson J. D., Clarke S., Wolkow C., Wang R., Cotter R. J., Reardon I. M., Zücker-Neely H. A., Heinrikson R. L., Ball M. J. and Greenberg B. D. (1993), Structural alterations in the peptide backbone of β-amyloid core protein may account for its deposition and stability in Alzheimer's disease. J. Biol. Chem. **268**, 3072–3083.

Stephenson R. C. and Clarke S. (1989), Succinimide formation from aspartyl and asparagynyl peptides as a model for the spontaneous degradation of proteins. J. Biol. Chem. **264**, 6164–6170.

Szendrei G. I., Prammer K. V., Vasko M., Lee V. M.-Y. and Otvos Jr. L. (1996), The effects of aspartic acid-bond isomerization on *in vitro* properties of the amyloid β-peptide as modeled with *n*-terminal decapeptide fragments. Int. J. Peptide Protein Res. **47**, 289–296.

Talafous J., Marcinowski K. J., Klopman G. and Zagorski M. G. (1994), Solution structure of residues 1–28 of the amyloid β-peptide. Biochemistry **33**, 7788–7796.

Vriend G. (1993), WHAT IF 4.5 Program Manual. EMBL Meyerhofstrasse 169117 Heidelberg, Germany.

Weiner S. J., Kollman P. A., Case D. A., Singh U. C., Ghio C., Alagona G., Profeta, Jr. S. and Weiner P. (1984), A new force field for molecular mechanics simulation of nucleic acids and proteins. J. Am. Chem. Soc. **106**, 765–784.

Weiner S. J., Kollman P. A., Nguyen D. T. and Case D. A. (1986), An all atom force field for simulations of proteins and nucleic acids. J. Comp. Chem. **7**, 230–252.

Zagorski M. G. and Barrow C. J. (1992), NMR studies of amyloid β-peptides – proton assignments, secondary structure, and mechanism of an α-helix → β-sheet conversion for a homologous, 28-residue, *n*-terminal fragment. Biochemistry **31**, 5621–5631.