Radical-Initiated Calpain-Inhibiting Peptide Configuration Changes. A Theoretical Study.

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Introduction

- The pentapeptide Leu-Ser-Glu-Ala-Leu (LSEAL) was discovered in an oligopeptide screen for calpain-repressive activity.
- Free radicals, such as the hydroxyl radical (•OH), are known contributors to oxidative stress and aging. They may react with amino acids, assisting in converting L-amino acids to D-amino acids.
- This has a negative effect on protein structure, since the inclusion of a D-amino acid in a protein often does not allow for proper folding and function.
- Configuration changes will be made to each of the residues in LSEAL using an MM/QM approach, providing new information regarding the effect of oxidative stress on peptide structure.

Methodology

Initial Structures
- Created initial structure using tleap (AmberTools).
- Manual modification to create 5 more structures, each with one mirrored residue.
- Visual inspection of structures.

Minimisation
- All structures minimised by steepest descent (500 steps) and conjugate gradient (9500 steps) methods.

Simulated Annealing
- Minimised structures used in simulated annealing algorithm.
- 300 K → 1000 K (1 ps), 1000 K (5 ps), 1000 K → 500 K (1 ps), 500 K → 200 K (2 ps), 200 K → 50 K (7 ps).
- For each configuration, 2000 simulations performed (producing 2000 structures per configuration in total).

***Ab Initio* Calculations**
- Lowest-energy structures from simulated annealing used in *ab initio* calculations.
- Calculations performed using Gaussian 09, a Becke three-parameter approximation of density functional theory (DFT-B3LYP) with the 6-31G(d) basis set, and the SMD implicit solvent model (in water).

Structural Analysis
- Pentapeptide hydrogen bonding analysed using criteria that d(A-D) < 3.5 Å and α(A···H-D) > 100°.
- Secondary structure analysed using DSSP algorithm and ptraj (AmberTools).
- Density Ramachandran plots computed for all residues (pseudo-Ramachandran plots for first and last residues).

Energetic Analysis
- Thermodynamic values extracted from *ab initio* calculation results.
- Thermodynamic values for chirality interconversions and radical formation reactions computed.

Results

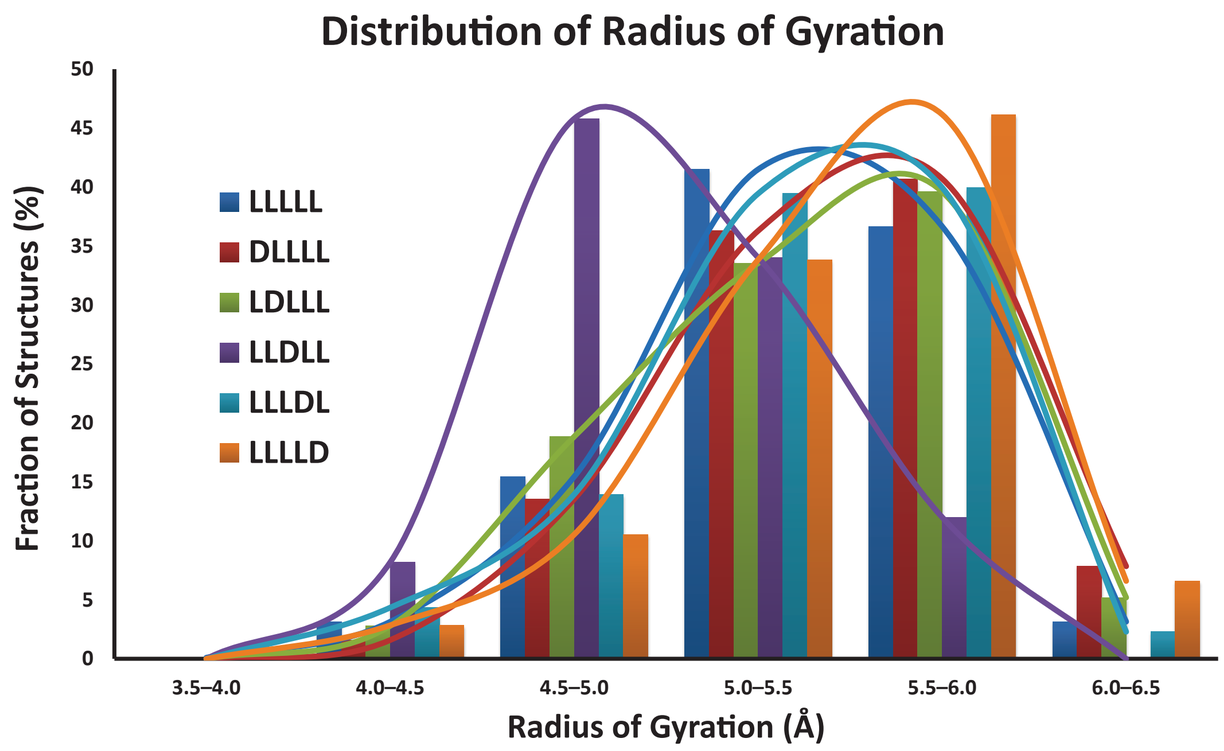

Figure 1 — Graph displaying the distribution of values (among the 2000 structures) of radius of gyration (in ångströms) for the wild-type (LLLLL) configuration of LSEAL, as well as for configurations with one residue of mirrored chirality.

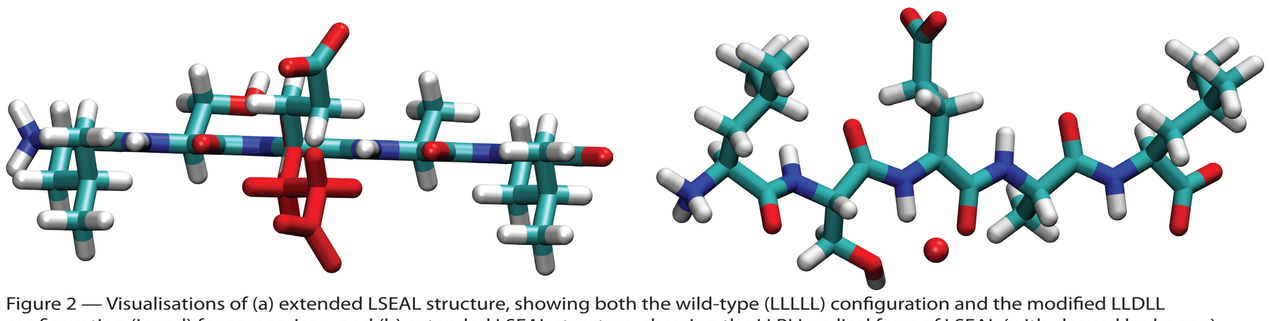

Figure 2 — Visualisations of (a) extended LSEAL structure, showing both the wild-type (LLLLL) configuration and the modified LLDLL configuration (in red) for comparison, and (b) extended LSEAL structure, showing the LLRLL radical form of LSEAL (with cleaved hydrogen).

		3_{10}-Helix		Turn	
	Residue Number	Quantity	Percentage	Quantity	Percentage
LLLLL	Ser_2	15	0.75	72	3.60
	Glu_3	**15**	**0.75**	**126**	**6.30**
	Ala_4	15	0.75	57	2.85
DLLLL	Ser_2	21	1.05	68	3.40
	Glu_3	21	1.05	133	6.65
	Ala_4	21	1.05	65	3.25
LDLLL	Ser_2	24	1.20	161	8.05
	Glu_3	24	1.20	184	9.20
	Ala_4	24	1.20	27	1.35
LLDLL	Ser_2	3	0.15	189	9.45
	Glu_3	**3**	**0.15**	**290**	**14.50**
	Ala_4	3	0.15	113	5.65
LLLDL	Ser_2	2	0.10	40	2.00
	Glu_3	2	0.10	129	6.45
	Ala_4	2	0.10	91	4.55
LLLLD	Ser_2	21	1.05	50	2.50
	Glu_3	21	1.05	104	5.20
	Ala_4	21	1.05	59	2.95

Table 1 — The quantity (out of 2000 total) and percentage of structures displaying a given secondary structure motif at a given residue (note that when the second or third residue is a D-isomer, both the second and third residues exhibit a higher propensity for the turn motif).

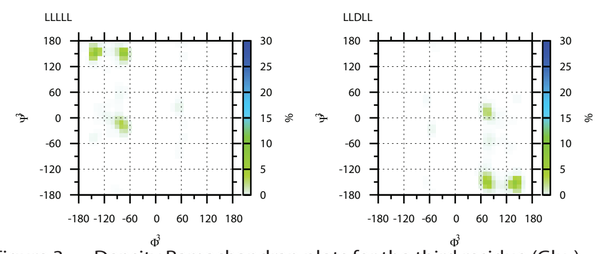

Figure 3 — Density Ramachandran plots for the third residue (Glu_3) displaying the changes to the φ and ψ angles when the residue's chirality is changed from an L-configuration to a D-configuration.

	L-Configuration	Radical Intermediate			D-Configuration		
Residue Number	Conformation	ΔH° (kJ·mol^{-1})	ΔG° (kJ·mol^{-1})	ΔS° (kJ·mol^{-1}·K^{-1})	ΔH° (ΔH°+R) (kJ·mol^{-1})	ΔG° (ΔG°+R) (kJ·mol^{-1})	ΔS° (ΔS°+R) (kJ·mol^{-1}·K^{-1})
1	EXT	-88.4	-94.0	32.1	-49.7 (-138.1)	-51.1 (-145.1)	4.6 (36.7)
	RMSD	-136.2	-145.8	32.1	-10.3 (-146.5)	-5.8 (-151.6)	-15.4 (16.7)
2	EXT	-115.6	-122.7	23.8	-23.3 (-138.9)	-22.0 (-144.7)	-4.3 (19.5)
	RMSD	-100.0	-104.8	16.1	-26.6 (-126.6)	-26.1 (-130.9)	-1.8 (14.3)
3	EXT	-132.2	-135.5	11.3	-8.8 (-141.0)	-11.7 (-147.2)	9.8 (21.1)
	RMSD	-44.5	-59.3	49.5	-32.8 (-77.3)	-31.1 (-90.4)	-5.8 (43.7)
4	EXT	-125.8	-129.4	12.4	-15.4 (-141.2)	-17.8 (-147.2)	8.1 (20.5)
	RMSD	-78.6	-97.2	62.6	-32.5 (-111.1)	-24.6 (-121.8)	-26.8 (35.8)
5	EXT	-126.6	-130.3	12.5	-12.2 (-138.8)	-12.9 (-143.2)	2.4 (14.9)
	RMSD	-56.1	-72.8	55.8	-7.2 (-63.3)	-2.9 (-75.7)	-14.3 (41.5)

Table 2 — The change in enthalpy (ΔH°), Gibbs free energy (ΔG°), and entropy (ΔS°) associated with the reaction between a hydroxyl radical (·OH) and the α-carbon of a given residue in LSEAL. Values given for both extended/linear (EXT; baseline is wild-type extended) and RMSD (RMSD; baseline is wild-type global minimum) conformations. Parenthesised data refers to the full L-D reaction.

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