Biophysical Journal, Volume 99

Supporting Material

NMR structures of the histidine-rich peptide LAH4 in micellar environments: membrane insertion, pH-dependent mode of antimicrobial action and DNA transfection

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SUPPLEMENTARY INFORMATION

NMR structures of the histidine-rich peptide LAH4 in micellar environments: membrane insertion, pH-dependent mode of antimicrobial action and DNA transfection

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RESULTS

Table S1: Chemical shifts of the C-terminal carboxyamides of LAH4

sample condition	pH	temperature (K)	chemical shifts (ppm)
TFE/PBS	5.8	317	6.88 / 7.31
DPC/PBS	6.1	300	7.22 / 7.39
DPC/PBS	6.1	317	7.13 / 7.36
DPC/PBS	4.1	317	7.13 / 7.37
DPC/PBS	7.8	317	7.07 / 7.24

Simulated annealing using CNS and quality assessment of the resulting structures

Additional structures were calculated using the NOE constraints at pH 4.1 and 6.1 (317 K) and the CNS Version 1.1 protocols for dynamical simulated annealing (1), with little changes. During the high temperature dynamics, the first cooling was set to 10000 steps per cycle. During the second cooling, the steps were set to 6000 and the final Powell minimisation increased to 100 steps per cycle with k_{NOE} of 50 kcal / $Å^2$ (e.g. (2)).

At pH 6.8 and pH 4.3, respectively, a total of 238 and 262 NOE-derived internuclear constraints were used in the calculations. While the quality of the spectra allowed assignment of both resonances and NOEs, *J*-coupling constants using the $^1H/^1H$ DQF-COSY experiments remained inaccessible due to the intrinsic line widths of peptides associated with large complexes such as detergent micelles. Therefore, the majority of constraints for the structure calculations were derived from NOEs with additional hydrogen bonding constraints.

Starting from an extended conformation a family of twenty structures was generated. The covalent geometry of the conformers was analysed using PROCHECK-NMR and AQUA (3). The criteria for selection of the best structures representative for LAH4 were based for both the ensemble and each individual structure, on Ramachandran plots and the number of violations (4). The mean structures over the corresponding ensemble and the best structures at pH 6.1 and at pH 4.1 in the presence of DPC are accessible through the PDB Database (2KJO and 2KJN, respectively).

Conformers were accepted based on the "accept.inp" routine of the CNS V1.1 software package including the criterium "no NOE violations" (1). Therefore the ensemble of accepted structures had no NOE violations greater then 0.5 Å, no bond angle violations greater then 5°, and no bonds length violations greater then 0.05 Å. Backbone and heavy atom RMSDs for the ranges of residues 4-9 and 14-24 at pH 6.8 and from 9-24 at pH 4.3, were obtained from the pair-wise comparison of structures rather then with the mean structure by using the "accept" protocol and MOLMOL2K (5). The statistics of the structure calculations are shown in Table S2. Notably, similar outlines of the helices were obtained using the Xplor protocol although, as obtained under less restrained conditions, with elevated RMSD.

Once the structure calculations had been performed, the stereochemical quality of the polypeptide can be determined independently from the experimental data. For this purpose, geometrical parameters like dihedral angles, bond lengths and bond angles, are compared to known standard protein geometries which have previously been resolved by X-ray crystallography and deposited in the PDB databank (3). The resulting averaged data (e.g. circular variance, G-factor etc.) allow one to assess the quality of the stereochemical properties of the structure. When the average G-factors of residues H10, H11, L12 and A13 for the structure obtained at pH 4.1 are compared to those at pH 6.1 higher values are obtained. This correlates with the number of NOEs found characteristic for extended α-helical conformations.

Moreover, when the G-factors characterizing the χ_1 frequency distributions are compared either between histidine residues at constant pH, or between high and low pH, nearly constant G-values were observed with the exception of H11. During these considerations the number of NOEs was not taken into account and all histidines were in their most favorable conformation. The G-factor values for H11 are 0.44 and 0.17 at pH 4.1 and 6.1, respectively, with average values for the dihedral angles near zero. The increased G-factor suggests a more flexible side-chain at pH 6.1.

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Table S2: The statistics of the structure calculations of LAH4 in DPC micellar solution is shown for the ensemble of low energy structures obtained by applying the CNS protocol to the multidimensional solution NMR data obtained at pH 4.1 and pH 6.1, respectively.

Structural restraints from NMR spectroscopy	***pH 4.1***	***pH 6.1***	
Total NOE	262	238	
Intra-residue	84	81	
Inter-residue	178	157	
Hydrogen bonds	14	11	
Average energies (kcal/mol)			
E_{over}	260.1±5.2	208.7±7.3	
E_{bond}	21.8±0.4	12.4±0.5	
E_{angle}	76.4±1.7	57.8±2.1	
E_{improp}	7.2±0.3	6.9±0.5	
E_{vdW}	18.8±1.6	26.4±1.9	
E_{NOE}	135.8±1.9	105.3±5.0	
RMSD from idealized covalent geometry			
Bonds (Å)	0.0071±0.00006	0.0053±0.00011	
Bond angles (°)	0.788±0.009	0.686±0.012	
Improper torsions (°)	0.475±0.009	0.463±0.018	
RMSD from experimental constraints			
Distances (Å)	0.0810±0.0006	0.0751±0.0018	
Ramachandran Analysis			
Residues in most favored regions	70.8%	73.3%	
Residues in additionally allowed regions	22.9%	22.5%	
Residues in generously allowed regions	2.1%	2.5%	
Residues in disallowed regions	4.2%	1.7%	
Coordinate Precision for selection (residue numbers)	*8-24*	*3-9*	*14-24*
Pairwise RMSD of the backbone (Å)	0.12 ± 0.07	0.07 ± 0.03	0.19 ± 0.07
Pairwise RMSD for all heavy atoms(Å)	0.79 ± 0.15	0.73 ± 0.25	0-93 ± 0.20