Peptides as Model Systems, Antimicrobial Agents, and a Means for Protein Superassembly

by

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AMPs	Antimicrobial peptides
ANS	1-Anilino-8-naphthalene sulfonate
ATP	Adenosine triphosphate
C.D.	Circular dichroism
CT	Cholera toxin holoenzyme
CTXA	Cholera toxin A subunit
CTXB	Cholera toxin B subunit
cVHP	Chicken villin headpiece subdomain
DMSO	Dimethylsulfoxide
DTNB	Dithio-bis(2-nitrobenzoic acid)
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
ESI	Electrospray ionization
FRET	Fluorescence resonance energy transfer
GuHCl	Guanidinium hydrogen chloride
HBTU	2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HFIP	Hexafluoroisopropanol
hFLeu	Hexafluoroleucine
HPLC	High pressure liquid chromatography
ITC	Isothermal titration caloimetry
MAD	Multi-wavelength anomalous diffraction
Maldi-TOF	Matrix assisted laser desorption ionization-time of flight
MBHA	4 - Methylbenzhydrylamine
MIC	Minimum inhibitory concentration
MS	Mass spectrometry
NMR	Nuclear magnetic resonance
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
pFPhe	Pentafluorophenylalanine
PG-1	Protegrin-1
POPC	1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphotidylcholine
POPG	1-Palmitoyl-2-Oleoyl-*sn*-Glycero-3-[Phospho-*rac*-(1-glycerol)]

ROP	Repressor of primer
SDS	Sodiumdodecylsulfate
SUVs	Small unilamelar vesicles
TCEP	Tris(2-carboxyethyl)phosphine
TEM	Transmission electron microscopy
TFE	Trifluoroethanol

Abstract

Proteins are the most diverse class of biomolecules, both structurally and functionally, and have evolved to accomplish many tasks in living systems. The features of proteins and peptides that contribute to their structure, stability and activity have been elucidated through an enormous body of experimental work. Central to this research have been protein engineering and design studies. Knowledge of the basic principles underlying protein folding, stability, and activity provide insight into the fundamental processes *in vivo* and offer the potential of designing protein/peptide based materials and therapeutic agents. The extensive research on protein design and incorporation of non-natural amino acids into peptides and proteins forms the basis of the research presented.

A *de novo* designed 4-α-helix bundle was employed to study the increased stability and potential self-segregating properties imparted by incorporation of the highly fluorinated amino acid, L-5,5,5,5',5',5'-hexafluoroleucine (hFLeu). The fluorinated peptide was shown to have increased biological stability against proteolytic degradation and greater stability toward denaturation by organic solvents in comparison to the non-fluorous peptide. Contrary to the predictions of the "fluorous" effect the fluorinated and non-fluorous peptides showed no tendency to self-segregate.

A series of antimicrobial peptides were studied to examine the affects of fluorination on the stability and antimicrobial activity of the α-helical MSI-78 peptide and β-hairpin PG-1 peptide. The fluorinated α-helical analogs of MSI-78 exhibited broad spectrum antibacterial activity, and importantly increased stability against proteolysis. However, hFLeu did not improve the antimicrobial activity of the β-hairpin PG-1 antimicrobial peptide that was also investigated. The results suggest fluorination may improve the efficacy of AMPs.

Finally, a α-helical coiled-coil peptide was investigated as a means of mediating higher order assembly of the 5-fold symmetric cholera toxin B protein (CTXB). When the α-helical peptide was genetically fused to the N-terminus of CTXB the fusion protein self-assembled into higher order assemblies of CTXB through dimerization of the N-terminal α-helical peptide domain. Further development of the system could be useful for the development of protein-based biomaterials.