A Structural Investigation of Squash Aspartic Peptidase Inhibitor (SQAPI) using Nuclear Magnetic Resonance spectroscopy (NMR).

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Ursula Kate MacAskill

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Abstract

Peptidases are enzymes that hydrolyse peptide bonds. This potentially dangerous activity is regulated by post translational modification and peptidase inhibitors. The best characterized of the peptidase inhibitors are the serpins whilst the aspartic peptidase inhibitors are the least characterized. Aspartic peptidase inhibitors are rare with only nine known sources. However, they are of great interest because they play an important part in several human diseases such as metastasis of breast cancer cells, *Candida albicans* infections and HIV.

The aims of this research project were to investigate the structure of Squash Aspartic peptidase inhibitor (SQAPI), using nuclear magnetic resonance spectroscopy (NMR). This required large amounts of relatively pure and isotopically labeled protein, which was achieved by heterologously expressing His-tagged rSQAPI fusion protein in *Escherichia coli* using a rich to minimal media transfer method. The fusion protein was purified with a nickel column and the N-terminal extension containing the His_6-tag was removed by cleavage of the fusion protein with enterokinase followed by nickel column purification.

Preliminary 1 dimensional NMR spectra indicated that SQAPI was folded in solution at pH 3. This was confirmed from the results of a preliminary ^{15}N-edited HSQC. These results combined justified the production of a ^{15}N ^{13}C labeled SQAPI sample for the collection of further NMR spectra. From the spectra produced with double labeled protein the backbone and the side-chain atoms of SQAPI were assigned. The chemical shifts are currently 88.89% complete and have been submitted to the biological magnetic resonance bank (BMRB). A preliminary estimate of the secondary structure of SQAPI has been calculated from the HNHA spectrum suggesting that the SQAPI structure has some similarity to the previously proposed model of the inhibitor's structure. Furthermore, the region corresponding to the putative binding loop on the model of SQAPI was found to be mobile and deuterium exchange experiments indicate that the SQAPI structure is more globular than open.

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Abbreviations

SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide get electrophoresis
NMR	Nuclear magnetic resonance spectroscopy
SQAPI	Squash aspartic peptidase inhibitor
mM	Milli mole
mL	Milli litre
g	Gram
µg	Micro gram
µl	Micro litre
ppm	Parts per million
1D	One dimensional
MHz	Mega hertz
IPAP	In-phase/Anti-phase
LB	Luria broth
OD_{600}	Optical density (at a wavelength of 600 nano-metres)
IPTG	Isopropyl-b-D-thiogalactopyranoside
Da	Dalton
kDa	Kilo-Dalton
HIV	Human immunodeficiency virus
N	Nitrogen
C	Carbon
DNA	Deoxyribonucleic acid
PI	Peptidase inhibitor
SAP	Secreted aspartic peptidase
PCR	Polymerase chain reaction
HSQC	Hetero-nuclear single quantum correlation
EDTA	Ethylenediaminetetraacetic acid

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