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

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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

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