

## INTERACTIONS OF FLAVANOIDS WITH BRADYKININ IN AQUEOUS SOLUTION

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Complexation with proteins is central to much of the biological and industrial significance of plant polyphenols. Definition of the interaction of these two classes of biopolymers has, therefore, been studied for decades. The most important mechanism seems to involve hydrophobic interactions and also hydrogen bonding but to a smaller extent<sup>1</sup>. Study of specific interactions between polyphenols and peptides has been pursued using Nuclear Magnetic Resonance<sup>2,3</sup>. Hatano<sup>3</sup> showed that information required for conformation and complexation determination can be obtained from nuclear Overhauser effect spectroscopy. NMR experiments can be guided by Macromodel molecular modeling by applying Monte Carlo methods to the conformational searching on complexes<sup>4</sup>. Results show that the interaction is directed to conformationally accessible hydrophobic regions and emphasize the importance of the shape of both the flavanoid and the peptide.

Epidemiological findings have shown that incidence of cardiovascular and neurologic degenerative diseases appears to be lower for populations with regular but moderate drinking of red wine<sup>5</sup>, that contains flavanoids such as catechin and procyanidins. To understand the effects of flavanoids on human health, it is important to obtain an accurate assessment of their interactions with polypeptides involved in the control of blood pressure and cardiovascular functions such as bradykinin<sup>6</sup>. Furthermore, the multiple structural features of bradykinin provide avenues to explore conformational selectivity. In addition to two proline and phenylalanine residues, two arginine residues are present at each end of the peptide, hence our interest in this system.

Our assignments of the <sup>1</sup>H NMR spectra of the nonapeptide bradykinin (Arg<sup>1</sup>Pro<sup>2</sup>Pro<sup>3</sup>Gly<sup>4</sup>Phe<sup>5</sup>Ser<sup>6</sup>Pro<sup>7</sup>Phe<sup>8</sup>Arg<sup>9</sup>) in aqueous solution using a sequential assignment strategy for peptides<sup>7</sup>, are in agreement with a previous study<sup>8</sup> except for some amide protons. Conformational studies also show that BK exists as a family of many different conformers when in aqueous solution. When catechin or procyanidin dimers are added to bradykinin (BK), an emulsion occurs<sup>9</sup>. Nevertheless, <sup>1</sup>H NMR spectra remain sharp and no changes in signal intensity are apparent. NOESYHG<sup>10</sup> experiments are used to define the interactions because changes in <sup>1</sup>H and <sup>13</sup>C chemical shifts due to equilibration between the free and complexed forms are too small to be significant (maximum is Δ 0.07 ppm for Pro<sup>7</sup>). This suggests a loosely held complex. The results obtained from NOESYHG experiments, together with conclusions of previous work<sup>7</sup> on procyanidin B2 and BK interactions based on changes in chemical shift, emphasize hydrophobic interactions. In presence of catechin, BK intramolecular nOes are similar, implying that no relevant conformational change of BK occurs. NOESYHG experiments reveal close contact between both catechin A-ring and B-ring protons with Phe<sup>5</sup>, Ser<sup>6</sup>, Pro<sup>7</sup> and Phe<sup>8</sup> residues where BK has been shown to interact with micelles<sup>8</sup>. Similar intermolecular associations are observed in mixtures of the procyanidin dimers B3 or B4 and BK in water. The preferred binding sites are not only the

prolyl residues and the aromatic rings but interactions are directed to accessible residues of the peptide located at the C-terminal end. Arginine seems to be involved in this interaction because cross-peaks are observed between catechin aromatic protons and the Arg<sup>9</sup> amide proton as well as Arg<sup>9</sup> side chain protons. Experiments are being conducted to determine if interactions take place between the guanidinium group and the phenyl ring of flavanoid. Similar interactions have been observed between basic amino acid and aromatic amino acid<sup>12</sup> and tannin-arginine interactions have been reported<sup>13</sup>. The H<sub>ε</sub> and H<sub>η</sub> protons are unlikely to provide nOe correlations due to the fast rotation of the N<sub>ε</sub>-C<sub>ζ</sub> bond<sup>14</sup>. <sup>15</sup>N resonances could provide evidence of interaction between the flavanoid and guanidinium moieties.

These results are in contrast to the accepted concept that catechin is too low in MW to cause precipitation of proteins". Similar observations have been previously described when catechin is added to poly(-L-proline)<sup>16, 3</sup>. Also, selectivity for prolyl residues seems to have been over-emphasized. The nOe correlations observed in the NOESYHG of catechin (20 mM) speak in favor of self-association of the flavanoid, even though the solution of catechin displays low turbidity. Our results are in agreement with a recent model" for tannin complexation based on the polyphenol self-association prior to or in conjunction with the association with other polymers.

#### REFERENCES AND NOTES

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- BK (1060.2, 10.5 mg, 15 mM) in H<sub>2</sub>O: CD<sub>3</sub>OD/540:60 1 and catechin (290, 3.8 mg, 20 mM). BK (13 mg, 19 mM) in H<sub>2</sub>O: CD<sub>3</sub>OD/540:60 1 and procyanidin dimers (578, 10 mg, 30 mM). The chemical shifts were referenced to external CDCl<sub>3</sub>/tetramethylsilane 1% (coaxial insert). The absolute signal intensities were measured and the spectra were acquired and processed using identical parameters.
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