Association of (+)-catechin and catechin-(4α → 8)-catechin with oligopeptides

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NOE studies on the complexation of (poly)flavanoids with peptides containing proline residues in aqueous solutions reveal site specific approach directed by hydrophobic interaction of the aromatic rings of catechin and its dimer, catechin-(4α → 8)-catechin, to conformationally accessible regions of peptides without strong preference for interaction with proline residues.

Recent findings on pharmacological activities such as antiviral and antitumour properties of tannins and related polyphenols[1] have prompted renewed studies on the fundamental mechanisms of polyphenol interaction with proteins in aqueous solutions.[2,3] Murray[4] showed that, in the interactions of pentagalloylglucose with proline rich peptides, the interaction was selective to proline and neighbouring residues. Changes in NMR chemical shifts were used to predict sites of complexation. In studies of the interactions of polyphenols with some oligopeptides including bradykinin, Haslam[3] stressed the importance of hydrophobic interactions. The pharmacological activities of polyphenols seem to depend on their structures, and some specificity among the polyphenols was shown.[5,6] Our investigations reveal specificity in the locations of association of both oligopeptides and of flavanoids related to condensed tannins.

(+)-Catechin (**1**), a representative of the monomeric constituents of condensed tannins, has been considered too small to cause precipitation of proteins.[7] However, when a small amount of catechin was added to an aqueous solution‡ of poly(-L-proline) (m.w. 10 000–30 000) (9 mmol dm^{-3} for catechin and 120 mmol dm^{-3} for a monomeric proline residue), a white precipitate was formed that showed only the polyproline signals at the same chemical shifts as polyproline alone. This result indicates that even low proportions of catechin to prolyl residues form a complex so insoluble as to obscure NMR resonances due to the complex, although the interaction of catechin with a local portion of the polypeptide chain was proposed.[8]

When catechin was added to more soluble peptides, precipitates did not occur and the changes in chemical shifts[4] that might be due to complex formation were extremely small. Duplication or broadening of ^1H and ^{13}C signals due to equilibration between the free and complexed forms were not observed. This suggests that the association is not strong enough to result in changes in the chemical shifts or that the equilibration is too fast to see changes. Therefore, NOE experiments might offer a better approach to study these complexes.

Catechin showed intermolecular NOEs with either monomeric proline or hydroxyproline in experiments using the NOESYHG pulse sequence.[9] When catechin and L-proline were dissolved in water‡ (50 mmol dm^{-3} each) no precipitate was observed and the NOESYHG experiment showed a cross peak for correlation between catechin B-ring protons and the proline C_γ protons (Fig. 1). The C_β and C_δ protons of proline also have weaker interactions with aromatic protons of both the A- and B-rings of catechin.

The NOESYHG spectrum of the mixture of catechin and *cis*-4-hydroxy-L-proline in water‡ (50 mmol dm^{-3} each) showed significant cross peaks between the C_δ protons of hydroxyproline and B-ring protons of catechin. Correlations between a hydroxyproline C_δ proton (α-oriented proton) to catechin H-6_A and H-8_A also showed cross peaks. However, presence of strong hydrogen bonding effects was not shown by comparison of ^{13}C chemical shifts. In the presence of catechin, none of the carbons of these amino acids (including carbonyl carbons) shifted from their frequency in spectra recorded in the absence of catechin (changes were within 0.02 ppm). Likewise, none of the catechin carbons (including those bearing a hydroxy group) moved. These results pointed to the importance of hydrophobic interaction of the aromatic A and B rings with the aliphatic regions of proline and hydroxyproline.

Although polyphenols have been considered to preferentially attack proline residues in peptides, catechin showed intermolecular NOEs with the other amino acid residues when we used the following dimeric peptides as targets for complexation. Gly-Pro showed two sets of ^1H and ^{13}C signals, which are attributable to the isomers of different configuration at the nitrogen of imino group of proline. The major isomer, with a 2H singlet-like signal for glycine C_α protons, was assigned to the *trans*-isomer. The minor isomer, with two doublets of the corresponding protons are separated due to the neighbouring proline carbonyl carbon, was assigned to the *cis*-isomer. For Gly-Pro, the NOESY spectrum showed significant cross peaks between the catechin H-6_A and H-8_A and the glycine protons. Among them, the *cis*-isomer showed strong cross peaks whereas the *trans*-isomer showed only very weak cross peaks with the B-ring protons of catechin.

In mixtures of catechin with Pro-Gly, the catechin H-8_A proton showed NOE with the glycine C_α protons. Pro-Val showed NOE between methyl signals of the valine residue and the catechin H-8_A as well as the H-2_B proton. Pro-Phe showed NOE between the catechin B-ring and the phenyl ring of the peptide. These results indicate that the two aromatic rings of catechin molecule preferentially associate with hydrophobic substituents other than the proline residues. Catechin also exhibits strong self-association[10] when dissolved in water (50 mmol dm^{-3}), even in competition with oligopeptides, due to hydrophobic interaction as shown by NOEs between H-2_B to H-8_A.

Experiments using the following two oligopeptides showed that not only the hydrophobicity, but also the molecular

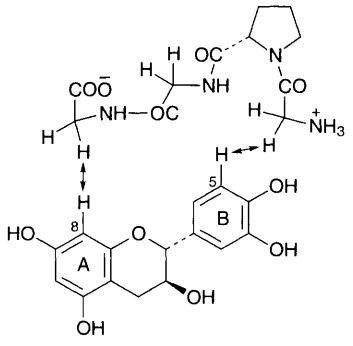

Fig. 1 Intermolecular NOEs suggesting the preferred sites in the association of (+)-catechin and Gly-Pro-Gly-Gly

conformations of both the polyphenol and peptide are important factors for specificity in their complexation. Bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) is a nonapeptide having various physiological activities including regulation of blood pressure. The conformations of this peptide in various solvents[11–13] and its complexation with polyphenols[3] have been studied. Our NOE studies on the association of catechin (20 mmol dm^{-3}) and bradykinin (14 mmol dm^{-3}) showed significant cross peaks between the catechin A-ring protons and the proline C_δ as well as to the Ser C_β methylene protons. Also, the catechin B-ring protons show correlations with the phenyl protons of the phenylalanyl residues. A cross peak due to the correlation of catechin A-ring protons and the phenyl protons was also observed. The amino acid residues in bradykinin showing NOEs with catechin aromatic ring protons are interestingly contained in the sequence Phe_5, Ser_6, Pro_7 and Phe_8, where bradykinin interacts with micelles including sodium dodecyl sulfate,[11] and where the physiological activity of bradykinin is considered to be based.

Gly-Pro-Gly-Gly is a tetrapeptide known as an inhibitor of dipeptidyl peptidase IV. This compound has also been reported to inhibit the entry of HIV into cells.[14] Although the *cis*-isomer (due to the asymmetry of proline nitrogen) is present, its content is low so the mixture can be treated practically as the *trans*-isomer. The NOESYHG spectrum of the mixture of catechin and Gly-Pro-Gly-Gly in water (50 mmol dm^{-3} each) showed correlations between catechin A-ring protons and the C_α methylene protons of the *C*-terminal glycine residue. A cross peak between the catechin H-5_B proton and methylene protons of the *N*-terminal glycine residue was also observed. Differentiation of the three sets of methylene protons of the glycine residues in the peptide was substantiated by ^1H–^1H and ^1H–^{13}C long-range correlation spectroscopy (COSY) measurements. When measured with a mixing time of 1 s, NOEs indicating that the A-ring preferentially associates with the *C*-terminal and the B-ring with the *N*-terminal glycine residues are observed (Fig. 1). The proline residue did not participate directly in the complex. However, proline plays a role of the definition of the relative spatial positions of the two glycine residues.

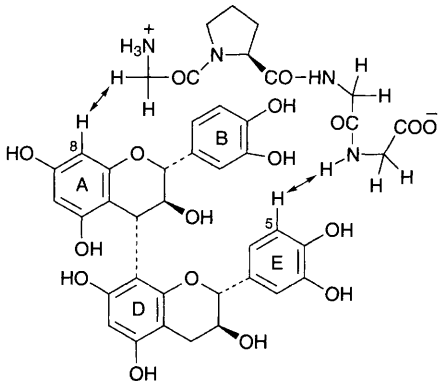

Fig. 2 Intermolecular NOEs suggesting the preferred sites in the association of catechin-(4α → 8)-catechin and Gly-Pro-Gly-Gly

Table 1 Chemical shifts of Gly-Pro-Gly-Gly protons in the presence and absence of polyphenols (300 MHz; in H_2O: [$^2H_4$]methanol, 9 : 1)

^1H Chemical shifts (δ)			
Gly-Pro-Gly-Gly	Gly-Pro-Gly-Gly + **1**[a]	Gly-Pro-Gly-Gly **2**[a]	Assignment
8.59	8.61	8.57	Gly_3 NH
7.91	7.96[b]	7.93[e]	Gly_4 NH
4.47	4.45	4.42	Pro_2 C_α-H
−3.99	−3.98[c]	−3.95[f]	Gly_1 CH_2
−3.98	−3.97	−3.94	Gly_3 CH_2
3.85	3.86[d]	3.85[g]	Gly_4 CH_2
3.78	3.79[d]	3.77[g]	Gly_4 CH_2
3.62	3.60	3.57	Pro_2 C_δ-H
3.56	3.53	3.49	Pro_2 C_δ-H
2.32	2.29	2.26	Pro_2 C_β-H
−2.04	−2.02	−2.00	Pro_2 C_γ-H
2.00	1.99	1.96	Pro_2 C_β-H

[a] The NOESYHG spectra showed correlations of the peptide protons with the following polyphenol protons. [b] H-6_A (weak) and H-8_A (weak). [c] H-5_B. [d] H-6_A and H(8_A. [e] H-5_E, H-2_E and H-6_A (weak). [f] H-6_D and H-8_A (strong). [g] H-2_B.

Catechin-(4α → 8)-catechin (**2**), also showed NOE with Gly-Pro-Gly-Gly, but the combination of the association sites between the polyphenol and the peptide are different from those observed in the case of catechin. When measured with a mixing time of 0.7 s, a cross peak with relatively strong intensity attributable to NOE between H-8_A (the upper unit) and the methylene protons of the *N*-terminal glycine residue was observed. E-ring protons, H-5_E and H-2_E of the dimer showed cross peaks with the amide proton of the *C*-terminal glycine residue (Fig. 2). Changes in ^1H chemical shifts were generally unclear due in part to the multiplicity of the peptide protons (Table 1). The use of NOE experiments is more sensitive for definition of complexation between oligomeric flavanoids and polypeptides.

These results have shown that NOESYHG experiments reveal considerable selectivity in the hydrophobic interactions between the aromatic protons of flavanoids and aliphatic and phenyl protons of oligopeptides. Analysis of the sites of association in these complexes using chemical shift differences were comparatively insensitive. Strong selectivity for prolyl residues is not seen; rather complexation is directed to conformationally accessible hydrophobic regions so the molecular shapes of both the polyphenol and polypeptide are important to selectivity.

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Footnotes

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‡ The solvent contains CD_3OD (H_2O : CD_3OD = 9 : 1).

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