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Interaction of catechin with poly(L-proline)

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Summary

Catechin binds strongly to both poly(vinylpyrrolidone) and poly(L-proline) in dilute aqueous solution, inducing a collapse of the more flexible poly(vinylpyrrolidone) chains, but forming a microgel with the more extended poly(L-proline) chains. Low concentrations of poly(L-proline) inhibit the discoloration of aqueous solutions of catechin, thereby implicating the *ortho* hydroxyyl groups in the catechol moiety in the binding process. Modeling shows that the likely binding sites on poly(L-proline) arise from two minor local conformations. These minor conformations are less frequent in poly(γ-hydroxy-L-proline) than in poly(L-proline), which may explain why catechin interacts more strongly with poly(L-proline) than with poly(γ-hydroxy-L-proline).

Introduction

Plants produce condensed tannins which complex with proline-rich proteins in the saliva of animals, thereby providing a defense mechanism for the plant by repelling insects and herbivores with an unpleasant astringent taste and deleterious effects in their digestion (1). In aqueous solutions of poly(L-proline), poly(γ-hydroxy-L-proline), and poly(vinylpyrrolidone), turbidity due to precipitation was detected in the presence of tannins (2). The tannins interact much more strongly with poly(L-proline) than with most other polymers, as judged by the inhibition of the precipitation of a radioactively labeled tracer protein (3).

Fluorescence measurements can be performed in sufficiently dilute solutions so that precipitation is not observed. Fluorescence from the condensed tannins easily detects their interaction with poly(L-proline) and poly(vinylpyrrolidone), but the interactions are qualitatively different, because the fluorescence of the condensed tannins is quenched by poly(L-proline), and it is enhanced by poly(vinylpyrrolidone) (4,5). Viscosity measurements show that small amounts of condensed tannins produce a collapse in the dimensions of poly(vinylpyrrolidone) (4). Collapse of the poly(vinylpyrrolidone) chain about a condensed tannin would increase the intensity of the fluorescence, because these molecules have a higher quantum

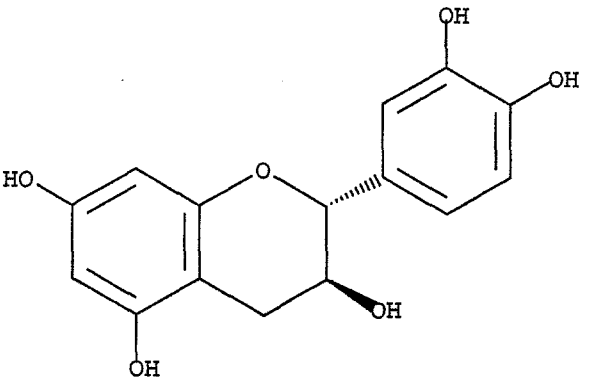

Figure 1. Structure of catechin. Hydrogen atoms bonded to carbon atoms are omitted. The A ring has *meta* hydroxyl groups, and the B ring has *ortho* hydroxyl groups.

yield for fluorescence in typical organic solvents than in water (4,6). Fluorescence measurements also show that the condensed tannins interact differently with poly-(L-proline) and with poly(γ-hydroxy-L-proline), because there is very little change in the fluorescence of the condensed tannins in the present of the latter polymer (5). Therefore poly(L-proline), poly(γ-hydroxy-L-proline), and poly(vinylpyrrolidone) provide useful model systems for understanding how condensed tannins interact with the proline-rich proteins.

One of the most commonly studied monomer units in the condensed tannins is catechin (Figure 1). It contains four phenolic hydroxyl groups, two in an *ortho* arrangement on one ring, and two in a *meta* arrangement on another ring. Removal of one of the hydroxyl groups in the *meta* arrangement has little effect on the interaction of procyanidin monomers with poly(L-proline) and poly(γ-hydroxy-L-proline) (7). Here we report results that implicate the two *ortho* hydroxyl groups in the B ring in the interaction, and describe a model that suggests how these hydroxyl groups could interact with poly(L-proline).

Materials

The catechol, catechin, and poly(vinylpyrrolidone) were purchased from Sigma Chemical Co., and the poly(vinylpyrrolidone) was purchased from Scientific Polymer Products. The suppliers reported average molecular weights of 40,000 for both polymers.

Results and discussion

Viscosity

Aqueous solutions of poly(vinylpyrrolidone) containing catechin are easily filtered, but such is not the case if poly(L-proline) is substituted for poly(vinylpyrrolidone). Dilute aqueous solutions of the sample of poly(L-proline) easily passed through a filter with 0.2 μm pore size, permitting measurement of an intrinsic viscosity of 0.82 dl g^{-1} in water at 30° C. The solution would no longer pass through this filter when catechin was present at a concentration of 4×10^{-5} molar, even though no turbidity was detected by eye. Turbidity was apparent when the concentration of catechin was increased to 7×10^{-4} molar. In contrast, poly(vinylpyrrolidone) easily passed through the filter even when the concentration of catechin was as high as 1.4×10^{-3} molar, and no turbidity was apparent at this concentration. The intrinsic viscosity of the poly(vinylpyrrolidone) at 30° C decreased from 0.209 dl g^{-1} to 0.167 dl g^{-1} as the concentration of catechin increased from 0 to 1.4×10^{-3} molar.

We interpret this result as indicating that catechin acts primarily within a single poly(vinylpyrrolidone) molecule in dilute solution, and produces a collapse in its dimensions, probably by noncovalent intramolecular crosslinks. The increase in the intensity of the fluorescence for the condensed tannins is a consequence of their shielding from contact with water by the collapsed poly(vinylpyrrolidone), which increases the quantum yield for fluorescence (4,6). Poly(L-proline), however, is much more extended than poly(vinylpyrrolidone). At comparable molecular weight (as with the samples used here), the root-mean-square unperturbed radius of gyration of poly(L-proline) is about three times larger than that of poly(vinylpyrrolidone) in water at 30° C (8,9). With this more extended chain, intramolecular crosslinks are disfavored relative to intermolecular crosslinks. The microgel formed by the intermolecular crosslinks prohibits the filtration of the aqueous solutions of poly(L-proline) that contain small amounts of catechin, even though no turbidity may be detected by eye.

Inhibition of color change

Freshly prepared dilute aqueous solutions of catechol and catechin are colorless, but they become yellow after standing for a few days. The discoloration of catechol is the result of its oxidation to an *ortho*-benzoquinone in weakly alkaline or weakly acid solution (10). Two broad peaks, near 440 and 480 nm, are observed in the absorption spectra of the discolored aqueous solutions of catechin. This discoloration is inhibited by small amounts of poly(L-proline), as shown in Figure 2. There is an easily observed inhibition of the increase in absorbance at 480 nm by solutions with poly(L-proline) at a concentration as low as 2.5 mg ml^{-1}. Poly(L-proline) exhibits a similar ability to inhibit the discoloration of dilute aqueous solutions of catechol. This result implicates the catechol ring of the procyanidins in the interaction with poly(L-proline).

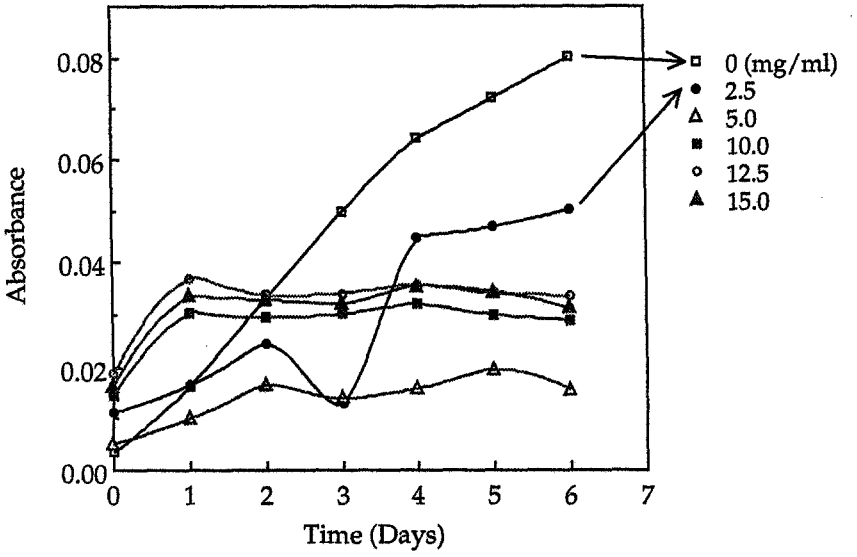

Figure 2. Adsorbance at 480 nm by an aqueous solution that is 4×10^{-5} molar in catechin, and also contains poly(L-proline) at concentrations in the range 0–15 mg ml^{-1}.

Modeling

The conformational energies for poly(L-proline) were computed with CHARMm 22.1, with slight modifications in a few bond lengths and bond angles so that they would match those used previously (11,12). The torsion angles in the main chain are denoted by ϕ, ψ, and ω for N—C^{α}, C^{α}—C′, and C′—N, respectively. The torsion angle ϕ is highly constrained to the vicinity of $-60°$ by the requirement for closure of the pyrrolidine ring. The conformational energy surface as a function of ψ and ω, evaluated when $\phi = -60°$, is dominated by three regions of low conformational energy, as shown in Figure 3. The two most important regions have peptide bonds in the *trans* conformation (ω near 180°), and the least important of the three regions has the peptide bond in the *cis* conformation (ω near 0°). The region that has ω near 0° will be denoted I, because the solid state structure known as poly(L-proline) Form I has peptide bonds in the *cis* conformation (13). Peptide bonds in the *trans* conformation are observed in the solid state structure known as poly(L-proline) Form II (14,15). The region with ϕ, ψ, ω near $-60°, 300°, 180°$ is denoted by II_{α}, because it is close to the conformation that characterizes the α helix. The remaining region is denoted II_{β}. The domination of the conformational energy map of poly(L-proline) by II_{β} has been known for over 30 years (16).

Figure 4 shows that the calculated barrier heights for crossings from II_{β} to II_{α} and from II_{β} to I are near 20 kcal mol^{-1}. The calculation is in good agreement with experimental transition energies, which are reported to be in the range 20–23 kcal

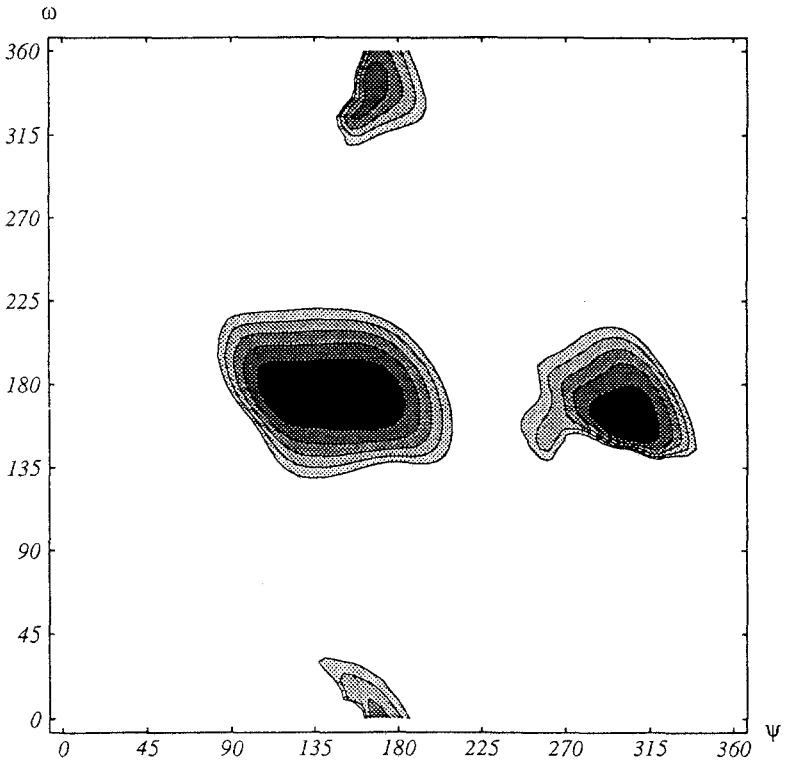

Figure 3. Conformational energy surface for the L-prolyl residue in poly-(L-proline). Contours are at 2, 4, 6, 8, and 10 kcal mol^{-1} relative to the minimum energy. White areas have a conformational energy that is more than 10 kcal mol^{-1} higher than the minimum.

mol^{-1} for the transition between the forms with peptide bonds in the *trans* and *cis* conformations (17,18). A Monte Carlo simulation *in vacuo*, which takes account of the interdependence of the conformations of neighboring L-prolyl residues, finds the populations of the three minima to be 0.73 (II_β), 0.23 (I), and 0.04 (II_α) for a chain with a degree of polymerization of 200. This simulation, the details of which are reported elsewhere (19), takes account of long-range intramolecular interactions and the interdependence of the conformations of successive L-prolyl residues in a more detailed manner than does the calculation of the conformational energy map for a single L-prolyl residue.

Poly(L-proline) contains carbonyl oxygen atoms as hydrogen bond acceptors, but it does not contain any hydrogen bond donors. The chain produced in the Monte Carlo simulation was examined for local conformations in which two successive carbonyl oxygen atoms were oriented so that they could simultaneously serve as hydrogen bond acceptors for the two *ortho* hydroxyl groups in the B ring of catechin. This state is not achieved within sequences of residues that are in the dominant

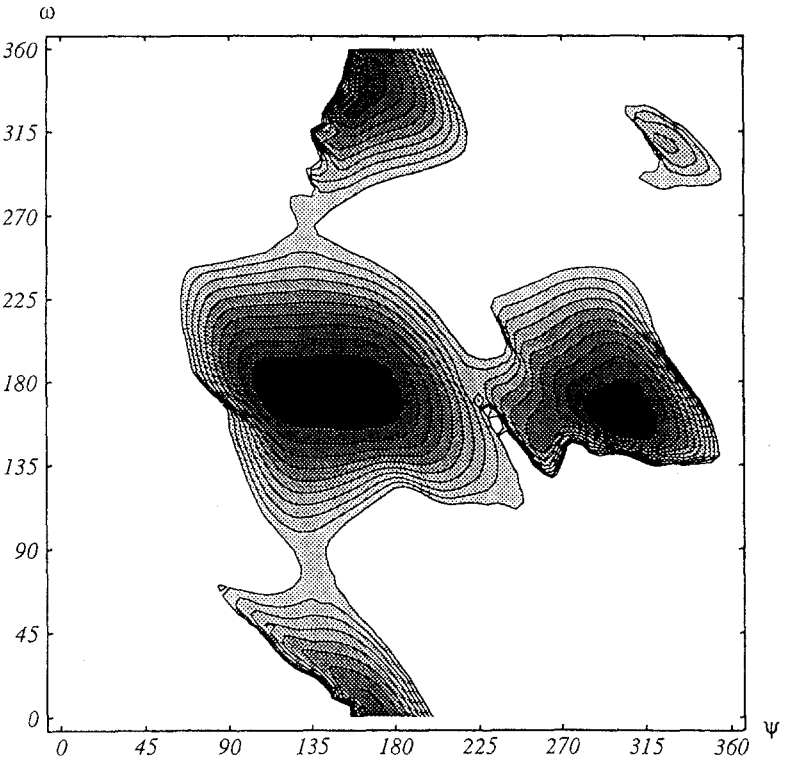

Figure 4. Conformational energy surface for the L-prolyl residue in poly-(L-proline), in which the contours have been extended to 20 kcal mol^{-1} (at intervals of 2 kcal mol^{-1}) to show the passes of lowest energy between the three regions.

II_β conformation, because of the large extension of the chain and the large angle (near 100–120°) between the two C=O bonds. It is also not achieved in sequences where all residues are in the I conformation, because the C=O are then inside the local helical structure, and shielded from the environment by the side chains. The desired conformation of two successive C=O is obtained at the occassional II_β—II_α junctions (but not at the II_α—II_β junctions) and in very short sequences of II_α. Also the desired conformation is produced by next-to-nearest neighbor C=O in the three-residue sequence II_β—I—II_β. Therefore the preferred mode of the binding to poly(L-proline) by the *ortho* hydroxyl groups in catechin must involve sites where the minor conformations, II_α and I, are present.

The implication of the minor conformations in the binding of catechin to poly(L-proline) is consistent with the observation that catechin interacts less strongly with poly(γ-hydroxy-L-proline) than with poly(L-proline). Whereas poly(L-proline) exists in the solid state in a conformation in which all of the peptide bonds adopt the *cis* conformation, no such conformation has been observed in poly(γ-hydroxy-L-

proline). The only solid state conformation observed for poly(γ-hydroxy-L-proline) has all of the peptide bonds in the *trans* conformation (20). Furthermore, poly-(L-proline) can be reversibly interconverted between Form II and Form I in dilute solution, by manipulation of the composition of the solvent (21–23), but no such reversible transformation has been reported for poly(γ-hydroxy-L-proline), which strongly prefers *trans* peptide bonds in dilute solution. Therefore potential binding sites that involve region I are not as common in poly(γ-hydroxy-L-proline) as they are in poly(L-proline). Furthermore, prior conformational energy calculations have demonstrated that the ratio of the populations of II_α to II_β is smaller in poly(γ-hydroxy-L-proline) than in poly(L-proline) (24). The potential binding sites that involve region II_α are also not as common in poly(γ-hydroxy-L-proline) as they are in poly(L-proline). Therefore the suggestion that binding involves the *ortho* hydroxyl groups in catechin, based on modeling of poly(L-proline), simultaneously provides an explanation for why catechin interacts less strongly with poly(γ-hydroxy-L-proline) than with poly(L-proline).

Acknowledgments

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