Interaction of (+)-Catechin with the Edge of the β Sheet formed by Poly-(*S*-carboxymethyl-L-cysteine)

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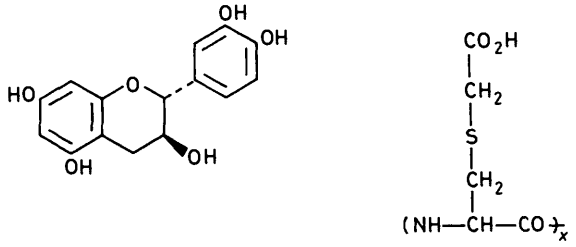
The interaction of (+)-catechin with poly-(*S*-carboxymethyl-L-cysteine) has been studied by circular dichroism spectroscopy. This polypeptide undergoes a pH-induced transition from a random coil to a β sheet under conditions where (+)-catechin is stable. The pH range over which the conformational transition occurs is broadened in the presence of (+)-catechin, but there is little effect on the pH at the midpoint of the transition. Qualitatively the same behaviour of the transition curves is observed in a theoretical model where the (+)-catechin interacts with, and stabilizes, the initial strand in the β sheet. This stabilization could result from the formation of a pair of hydrogen bonds between (+)-catechin and amide units in the initial strand.

Naturally occurring polymers of (+)-catechin and (−)-epicatechin are variously known as condensed tannins and polymeric proanthocyanidins. Their biological role is the protection of the plants that produce them from attack by herbivores and micro-organisms.[1–3] The molecular basis for this protective action is the formation of complexes between condensed tannins and proteins. Complex formation has often been demonstrated by studies of the precipitation of proteins and synthetic polypeptides by condensed tannins.[1,4–8] More recently, a study of the quantum yield for fluorescence has clearly shown that there is a strong, and specific, interaction between condensed tannins and poly-(L-proline).[9] The molecular interactions responsible for the stabilization of these complexes remain obscure. There have been suggestions that the complexes might be stabilized by hydrogen-bond formation[6] or by hydrophobic interaction.[4] Presumably the conformational properties of polymeric proanthocyanidins of high molecular weight also play a role in complex formation. The existence of rotational isomerism at the interflavan bond, which is easily demonstrated for the free phenol forms by time-resolved fluorescence studies,[10] implies that the high polymers have the overall architecture of a random coil with unperturbed dimensions similar to those found with polystyrene chains of the same molecular weight.[11,12]

The research reported here shows that (+)-catechin alters the stability of a β sheet, and does so in a manner that is easily rationalized in terms of a complex in which hydrogen bonds play an important role. There are three simple homopolypeptide models that have been frequently used for the study of β sheet formation in dilute aqueous solution. Two of these models, poly-(L-lysine)[13] and poly-(L-tyrosine),[14] are unacceptable for present purposes because they form β sheets only at a pH so high that (+)-catechin is unstable. The remaining model, poly-(*S*-carboxymethyl-L-cysteine),[15] undergoes the transition from random coil to β sheet at a pH near 5, which is convenient for the study of (+)-catechin. Covalent structures of (+)-catechin and the repeating unit in poly-(*S*-carboxymethyl-L-cysteine) are depicted in Scheme 1. Comparison of the measured random-coil-to-β-sheet transition curves, obtained in the presence and in the absence of (+)-catechin, with theoretical models for the transition shows that (+)-catechin probably interacts with the initial strand in the β sheet.



Scheme 1. Covalent structures of (+)-catechin (left) and the repeating unit of poly-(*S*-carboxymethyl-L-cysteine) (right); for clarity, nine hydrogen atoms have been omitted from the structure of (+)-catechin

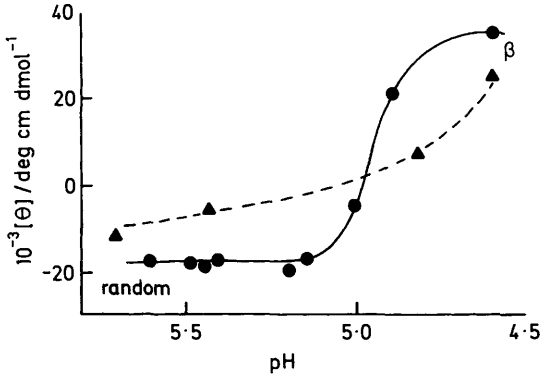


Figure 1. Influence of (+)-catechin on the pH-induced random-coil-to-β sheet transition in poly-(*S*-carboxymethyl-L-cysteine); [poly-(*S*-carboxymethyl-L-cysteine)] 0.015 mg ml^{-1}; [(+)-catechin] 0 (circles) or 3 × 10^{-5} M (triangles); pH increases from right to left

Results and Discussion

Experimental Transition Curves.—Figure 1 depicts two pH-induced transition curves measured with poly-(*S*-carboxymethyl-L-cysteine). The random-coil-to-β-sheet transition is conveniently monitored by measurements of the intensity of the circular dichroism at 200 nm, where there is a large difference in the mean residue ellipticities of the two conformations.[15] The

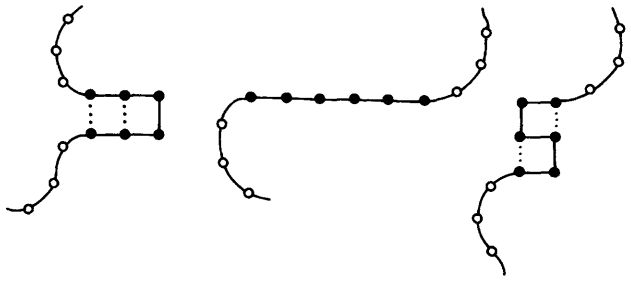


Figure 2. Three small intramolecular antiparallel β sheets with statistical weights $\delta\tau^3t^6$, τ^6t^6, and $\delta^2\tau^2t^6$, reading from left to right

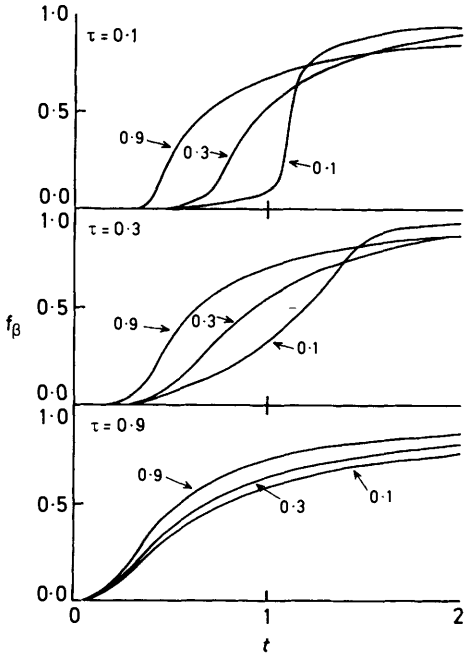


Figure 3. Theoretical curves that depict the transition from random coil to β sheet when a small molecule interacts with the connection between consecutive strands and the difficulty in nucleation of the initial strand is large (top), moderate (middle), or small (bottom); the value of τ is shown in the upper left of each panel, and the value of δ is noted for each curve

transition is achieved by changing the degree of ionization of the carboxy groups in the side chains, with the β sheet being the preferred conformation at sufficiently low charge density and the random coil being the preferred conformation at high charge density.[15] The pH scale increases from right to left in Figure 1 because comparison with theoretical transition curves is more conveniently if conditions that favour β sheet occur at the right side of the Figure.

Comparison of the transition curves obtained in the presence and in the absence of (+)-catechin clearly shows that this molecule exerts an influence on the transition of poly-(*S*-carboxymethyl-L-cysteine) from a random coil to a β sheet. Both transitions are centred near pH 5.0, but the presence of (+)-catechin causes the transition to be spread over a much broader pH range. In the following development of an interpretation of this result, it will be important to remember that the two curves cross one another before the transition from random coil to β sheet has reached its midpoint.

Definitions of Pertinent Statistical Weights.—The theoretical curves that describe the transition from random coil to

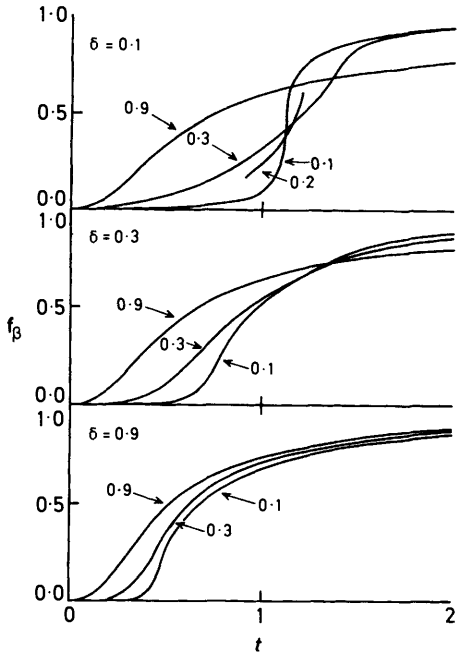
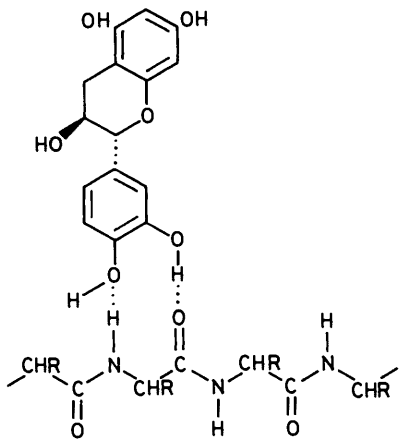


Figure 4. Theoretical curves that depict the transition from random coil to β sheet when a small molecule interacts with the initial strand of the β sheet and the difficulty in forming a bend between consecutive strands is large (top), moderate (middle), or small (bottom); the value of δ is shown in the upper left of each panel, and the value of τ is noted for each curve

intramolecular antiparallel β sheet were computed by the method originally described[16] for the formation of β sheets with tight bends, and subsequently generalized[17] so that consecutive strands in a β sheet might also be connected by disordered loops. The most concise description of the algorithm is that presented by Mattice and Tilstra.[18] Detailed knowledge of the statistical mechanics used in the calculations[16–18] is not required for present purposes, but it is necessary to have an understanding of the molecular interpretation of the statistical weights denoted by δ, τ, and *t*.[16] The necessary understanding is easily developed with the aid of Figure 2, which depicts three small β sheets. Each β sheet contains six amino acid residues, denoted by the filled circles. The second sheet contains a single strand, and the other sketches depict multistranded sheets. Open circles denote residues in the random coil segments at each end of the β sheet. Residues in the random coil segments are taken as the reference state, and they are assigned a statistical weight of one. A factor *t* is included in the contribution made to the statistical weight by every residue that is in the β sheet, *i.e.* by every residue represented by a filled circle. β Sheets are stabilized relative to the random coil as *t* increases. In the absence of any other statistical weights, there would be no discrimination amongst the three β sheets depicted in Figure 2. Each sheet would contribute a weight of t^6. One can, however, easily imagine conditions where the first sheet would be preferred to the second. The first sheet permits interactions between two strands, but these interactions are necessarily absent in the second sheet. This effect is incorporated in the weighting scheme by the use of two additional statistical weights denoted by δ and τ. Each tight bend contributes a factor of δ to the statistical weight, and a factor of τ is contributed by each residue in the β sheet that does not have a partner in the preceding strand. Therefore δ and τ penalize residues located at the edges of the β sheet, with δ being the penalty at the edge that is comprised of the tight bends, and τ being the penalty for residues located in the initial strand. The statistical weights of



Scheme 2. Sketch of the type of interaction between (+)-catechin and a β sheet suggested by comparison of the experimental transition curves in Figure 1 and the theoretical transition curves in Figure 4

the β sheets depicted in Figure 2 are $\delta\tau^3t^6$, τ^6t^6, and $\delta^2\tau^2t^6$, reading from left to right. These statistical weights are in the ratio $\delta\tau:\tau^4:\delta^2$. The single-stranded structure in the middle is preferred if $\delta < \tau^2$, the structure with a very short initial strand (depicted at the right) is preferred if $\tau^2 < \delta$, and the structure at the left is favoured if $\delta < \tau$ and $\delta < \tau^3$. An additional factor is appended to δ if the connection between strands is made by larger loops.[17]

Theoretical Transition Curves.—Several theoretical curves that describe the transition from random coil to intramolecular antiparallel β sheet are depicted in Figures 3 and 4. All curves are calculated for a chain with a degree of polymerization of 300. They are presented as the fraction of the residues in the β sheets as a function of t. Each panel in Figure 3 depicts curves with a specified value of τ and different values of δ. The same curves are depicted in Figure 4, but here each panel employs a specified value of δ and different values of τ.

We now inquire whether the change induced by (+)-catechin in the experimental transition curves can be described by any of the theoretical curves. The effective value of t becomes larger as the pH decreases because the *S*-carboxymethyl-L-cysteine residues with CO_2H groups in the side chains have a greater preference for the β sheet than do residues with CO_2^- side chains. The first model considered is one where (+)-catechin can interact with any residue in the β sheet or with any residue in the random coil. Since every residue in the β sheet has a factor of t in its statistical weight, and every residue in the random coil has a statistical weight of 1, the value of t at every pH value would either increase (if the interaction stabilizes the β sheet) or decrease (if the interaction stabilizes the random coil). In the former case, the entire transition curves would be shifted to higher pH by (+)-catechin, and in the later case it would be shifted to lower pH. In neither case would transition curves in the presence and in the absence of (+)-catechin be found to cross one another. This behaviour is different from that observed in Figure 1. Therefore (+)-catechin must affect the transition by some means other than interaction with all residues in the random coil or all residues in the β sheet. Attention should be directed to potential interactions at the edges of the β sheet.

Figure 3 depicts the effects that should be observed if (+)-catechin were to interact with the bends that occur between consecutive strands of the β sheet. This interaction would modify the value of δ. Each panel in Figure 3 depicts curves where δ is 0.1, 0.3, and 0.9. The value of τ is constant in each panel. There is no evidence that the curves cross one another when τ is 0.9. At the smaller values of τ, the curves do cross one another, but do so only in the late stages of the transition, where f_β is significantly greater than 0.5. In contrast, the experimental curves depicted in Figure 1 cross one another in the early stages of the transition, where f_β is less than 0.5. Consequently the data are not consistent with the model in which (+)-catechin interacts with the β sheet in a manner that modifies the value of δ.

If (+)-catechin were to interact with the initial strand in the β sheet, it would change the value of τ. The data from Figure 3 are replotted in Figure 4, but in a manner where each panel employs the same value of δ. It is now the values ot τ that are assigned as 0.1, 0.3, and 0.9 in each panel. There is no evidence that the curves cross one another in the bottom panel, where $\delta = 0.9$. The curves do cross in the middle panel, but do so near the completion of the transition. In the top panel, the curves clearly cross one another, and they do so in the early stages of the transition if τ is sufficiently small. This panel contains an addition transition curve, calculated with $\tau = 0.2$, which emphasizes that the transition curves cross at $f_\beta < 0.5$ when τ is sufficiently small. The theoretical curves reproduce the features seen in the experimental curves only when δ is small, τ is small, and τ changes in response to the presence of (+)-catechin. The experimental transition curve becomes broader in the presence of (+)-catechin, and the calculated curve becomes broader upon an increase in τ. This combination requires that (+)-catechin interacts with the initial strand in a manner that stabilizes the strand.

Model for the Interaction.—Scheme 2 depicts a model consistent with the conclusions that result from the comparison of the experimental and theoretical transition curves. The side chain in the polypeptide is denoted simply by R. A segment of the polypeptide is extended in the conformation seen in the β sheet. If this strand were in the interior of the β sheet, the NH and CO groups in the polypeptide backbone would participate in hydrogen bonds with CO and NH groups in the adjacent strands. These interstrand hydrogen bonds are clearly unavailable if only a single strand is present. Scheme 2 shows that a single molecule of (+)-catechin can easily form two hydrogen bonds with an isolated β strand. These hydrogen bonds could also be formed with the strands at the edges of a multistranded β sheet, but they cannot be formed with strands in the interior of the sheet. Consequently the interaction depicted in Scheme 2 enters the theoretical analysis of the transition curves by causing an increase in the effective value of τ. This change in τ will cause transition curves to cross one another at $f_\beta < 0.5$ if δ is small and the initial value of τ was also small. It is reasonable to expect that τ and δ should be small because the boundaries of ordered regions are typically of higher energy than the interior of the ordered regions.

Experimental

(+)-Catechin was purchased from Sigma Chemical Company and used without further purification. Freshly prepared solutions were used in order to avoid potential complications that might arise from the discolouration that eventually occurs with aqueous solutions of (+)-catechin. Poly-(*S*-carboxymethyl-L-cysteine) was synthesized, purified, and characterized by the procedure described by Maeda *et al.*[15] The polypeptide sample used had a degree of polymerization of 350. Circular dichroism measurements were performed with a JASCO J-500A spectropolarimeter using 10 mm quartz cells. The transition from the random coil to the β sheet was followed by monitoring the circular dichroism of the poly-(*S*-carboxymethyl-L-cysteine). (+)-Catechin also exhibits circular dichroism in the spectral range used.[19] Spectra measured in the presence of (+)-

catechin and the polypeptide were corrected by subtraction of the spectrum observed with the same concentration of (+)-catechin alone. This correction never exceeded 10% of the signal in the spectral range of interest. The necessity of accurately making this correction places an upper limit on the (+)-catechin concentration that can be studied.

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