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The Interaction of Procyanidin and Protein

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Procyanidins or condensed tannins are polymeric phenolics that interact with protein. Reduced digestibility of legume protein due to procyanidins have been observed with several cultivars of common beans (de Lumen and Salamat, 1980). Until 1980, most of the literature suggested hydrogen bonding was the predominant mode of interaction (Synge, 1975 and Van Sumere et al., 1975) However, Oh et al. (1980) found that hydrophobic interactions play an important role in the formation and stabilization of procyanidin-protein complexes.

Tritium labeled dimeric and trimeric catechin procyanidin was synthesized from tritiated sodium borohydride, dihydroquercetin and catechin. Sephadex LH-20 and an aqueous ethanol solvent was used for purification. An Amicon micropartition system was used to quantitate the strength of the binding at different temperatures. Thermodynamic analysis of the temperature dependance of oligomeric catechin binding to bovine serum albumen (BSA) and bean glyco-protein G-1 were completed. Scatchard plots of both the procyanidin dimer and trimer with BSA revealed increased equilibrium binding constants with increased temperature. Van't Hoff plots indicated a reaction with a positive entropy, a positive enthalpy and a negative free energy, i.e. a spontaneous reaction that is totally entropy driven. Therefore, the binding of BSA and procyanidin is predominantly a hydrophobic interaction.

The surface hydrophobicity of a protein can be determined with cis-parinaric acid, a fluorescent probe. Both the procyanidin oligomers decreased the fluorescence of cis-parinaric acid and BSA when added to BSA prior to the addition of cis-parinaric acid. Athough procyanidin may be hydrogen bonding to the protein and sterically hindering the cis-parinaric acid from reaching the site, thermodynamic analysis indicate this is not likely. The procyanidin appears to cover the lipid binding portion of BSA and decrease the amount of cis-parinaric acid able to reach this hydrophobic area.

Scatchard plots of G-1 and the procyanidin trimer indicated decreased binding constants with increased temperature. The Van't Hoff plot showed a reaction with a negative entropy, negative enthalpy and a negative free energy, i.e. a spontaneous reaction that is totally enthalpy driven. This indicated the binding of G-1 and procyanidin is hydrophilic in nature. In addition, the procyanidin did not affect the fluoresence of cis-parinaric and native G-1. These results reveal procyanidins are capable of both hydrophilic and hydrophobic bonding to protein.

Heat denatured G-1 had a surface hydrophobicity eight times that of native G-1. This increase was not unexpected since hydrophobic groups are usually oriented internal to protein in aqueous systems. Heat denaturation of the protein exposes the hydrophobic groups. The fluoresence of heat denatured G-1 and cis-parinaric acid is decreased considerably with prior addition of cis-parinaric acid. Therefore, procyanidin binding to G-1 may be hydrophobic or hydrophillic, depending upon the types of sites available. Removal of procyanidin may be necessary to alleviate problems of reduced protein availability. Any cell disruption will result in the binding of procyanidin to protein. High surface hydrophobicity due to protein denaturation may require an aqueous/organic solvent mixture of sufficient non-polar character to remove bound procyanidins from plant proteins.

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BEAN CONDENSED TANNINS AND GASTROINTESTINAL DISTURBANCES

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Many nutritionally important legumes contain significant quantities of condensed tannins. Deleterious effects of various tannins have included: increased fecal nitrogen excretion (Joslyn & Glick, 1969), increased cation excretion (Mitjavila et al., 1977), decreased feed intake (Glick & Joslyn, 1969) and alterations in the gastrointestinal mucosa (Motilva et al., 1983).

In the following experiments, condensed tannin was purified from black beans, Phaseolus vulgaris L., and found to be 0.75% dry weight of whole bean flour. In a dosage pre-test, 16 male Sprague-Dawley weanling rats were given purified condensed tannin at 0.5% and 5.0% of dry matter. Treatments were via gastric intubation with a saline intubation control and a diet control.

Tannin doses depressed feed intake and growth rates. The 0.5% and 5.0% doses retarded growth 45% and 90% respectively (Fig. 1). The 5.0% tannin dose resulted in one fatality at day 9. The remaining rats given 5.0% condensed tannin were sacrificed at day 20 due to severe dehydration, presumably a result of intestinal osmotic upset. The rats given 0.5% condensed tannin and controls were sacrificed after 30 days.