A novel method to evaluate the relative affinity of protein to tannins under physiological conditions

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Abstract: The method is aimed both at facilitating the identification of tannin-binding proteins and to investigate their relative tannin binding at pH 8.2, thus providing physiologically relevant information.

Introduction: Many herbivore species developed a strategy to circumvent the protein precipitating effects of dietary tannin intake by secreting tannin-binding proteins in their saliva (Levin, 1976). To characterize these proteins and to evaluate their binding potential, methods are needed which allow the identification of these proteins based on their biological effects (Hagerman and Butler, 1978; Tempel, 1982) .

Method : *Binding assay:* The assay was carried out according to Bergmeyer (1989) and modified as follows: per well a 100 µ1 preincubation-mix was prepared. It consisted of: 90mM Tris-HCL, pH 8.2, 18 µM CaCI2, 100 µg/ml trypsin, 10-100 µg/ml tannin and 10-40 µg/ml protein. Its components were added in the following order: H2O, buffer, tracer enzyme, tannin, test protein. After 15 min of preincubation at room temperature and subsequent addition of 100 µ1 1 mM N(-benzoyl-DL-arginine-p-nitroanilide, the mixture was incubated for 20 min at 37°C. Trypsin was then completely inhibited by adding 50 µ1 of 3% acetic acid. All microtiterplates were routinely measured 3 times at 405 nm. Two controls were utilized, one lacking both tannin and binding proteins, the other one lacking the test protein to evaluate remaining trypsin activity and/or absorption of all components without any enzymatic reaction. Time-extended experiments were carried out, to assure that the substrate concentration was not limiting the reaction. To remove non-tannin phenolics, the tannins were purified by chromatography on LH20 columns (Pharmacia). The equilibrium of the binding reaction of (trypsin-tannin) was reached between 3 and 5 min, depending on the room temperature. The inhibitory effect exerted by tannins toward trypsin was not competitive.

Results: BSA was used in order to determine whether or not proteins are capable of binding tannins at pH 8.2, without being precipitated. The results showed, that BSA binds unspecifically and only little amounts of tannin. At a concentration of 50 µg/ml BSA, 10 µg/ml of tannic acid reduced already significantly the trypsin activity, indicating that a vast amount of available tannin did bind rather to trypsin than to BSA. Increasing tannic acid concentrations increased the inhibitory effect. The result supports the assumption, that scenarios involving tannin binding trypsin without inhibiting it or simutaneoulsly binding tracer enzyme and test protein are not very

likely. Additionaly, the high A-405-value without tannic acid showed, that BSA and trypsin did not interfere with each other in any inhibitory way. To further evaluate the properties of BSA regarding its binding to tannin, we assayed 50 µg/ml of tannin with increasing amounts of BSA. 70 µg/ml BSA was needed to completely filter the tannins and to reach a level of tryptic activity equal to the value reached without tannin. These data support previously described results (Hagerman and Klucher, 1986). Further experiments were carried out with lysozyme, human crude saliva, gelatin and PVP. Surprising was the relative binding of the tested compounds at pH 8.2. Not only did all tested compounds reverse the tannin caused inhibition of trypsin, in most cases their relative binding to tannin had inversely changed compared to the findings at pH 4.8. According to the binding assay, the tannin-affinity ranking order was as follows: PVP > lysozyme > human crude saliva > BSA > gelatin. We also examined acid phosphatase and B-galactosidase (two metabolic proteins which exert their enzymatic activity under acidic conditions) regarding their binding to tannic acid and quebracho. Both proteins did not display any binding capability to either tannic acid or quebracho. These results will be discussed extensively in the poster.

References

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