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TANNIN-BINDING PROTEINS IN SALIVA OF DEER AND THEIR ABSENCE IN SALIVA OF SHEEP AND CATTLE

PAUL J. AUSTIN,[1] LISA A. SUCHAR,[1] CHARLES T. ROBBINS,[2] and ANN E. HAGERMAN[1]

[1]*Department of Chemistry*
Miami University
Oxford, Ohio 45056

[2]*Program in Wildlife Biology*
Washington State University
Pullman, Washington 99164

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Abstract—A method has been developed for detecting tannin-binding proteins in the saliva of herbivores. The method is simple and requires only small quantities of crude saliva. The saliva of deer, a browsing ruminant, has been compared to that of domestic sheep and cow, which are grazing ruminants. The browser, which normally ingests dietary tannin, produces tannin-binding proteins, while the grazers do not produce such proteins. The tannin-binding protein from deer saliva is a small glycoprotein containing large amounts of proline, glycine, and glutamate/glutamine. The protein is not closely related to the proline-rich salivary proteins found in rats and other nonruminant mammals.

Key Words—Tannin, saliva, ruminant, proline-rich protein, electrophoresis, deer, sheep, cattle, grazers, browsers.

INTRODUCTION

Dietary tannins may affect herbivores by reducing protein digestibility (Rhoades and Cates, 1976; Robbins et al., 1987a) or by systemic toxicity (Dollahite et al., 1966; Martin et al., 1987). Successful herbivores have developed mechanisms for overcoming these adverse effects. For example, some insects have modified digestive tracts in which tannin does not complex protein (Martin et al., 1987). In tree locusts, absorbed phenolics are not toxic but are utilized in

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synthesis of the cuticle (Bernays and Woodhead, 1982). Rats synthesize salivary proline-rich proteins (PRPs) in response to tannin-containing diets (Mehansho et al., 1983). PRPs apparently protect rats by binding to the tannin and preventing its interaction with other proteins. Salivary tannin-binding proteins could also minimize absorption of tannins and reduce their toxicity.

We are currently seeking to evaluate differences in the abilities of various ruminants along the grazer-to-browser continuum to consume tannin-containing diets. Grazers consume virtually tannin-free diets and have a minimal ability to tolerate soluble phenolics, while browsers normally consume a variety of phenolic-containing plants and are well prepared to tolerate phenolics (Robbins et al., 1987b). The saliva of mule deer (*Odocoileus hemionus hemionus*), a browser, contains two to three times more nitrogen than the saliva of grazers such as cows or domestic sheep (Robbins et al., 1987b). Deer saliva is more proline rich than cow or sheep saliva and has a greater tannin-binding capacity than the saliva of the grazers (Robbins et al., 1987b). These findings suggest that the tolerance of browsers for dietary tannins may be due, in part, to the production of salivary PRPs. We have developed new methodology that facilitates the detection of tannin-binding proteins in saliva samples and have applied those methods to a detailed characterization of the salivary proteins of deer and sheep.

METHODS AND MATERIALS

Electrophoresis. Nondenaturing, discontinuous, pH 8.3 polyacrylamide gels (Davis, 1964) were used for identification of tannin-binding proteins. The gels were poured in a 1-mm slab gel apparatus using Laemmli's (1970) modification of the method, omitting the sodium dodecyl sulfate (SDS). The acrylamide concentration varied linearly from 7.5% at the top of the gel to 12% at the bottom of the gel, and the bisacrylamide was 0.2% throughout the gel. The stacking gel contained 3.0% acrylamide and 0.08% bisacrylamide. The gels were run at a constant voltage of 300 V for 10–12 hr at 4°C with 0.05% bromophenol blue as the tracking dye.

SDS gels were run to establish whether the composition of saliva changed when animals were fed tannin. The gels were prepared as described by Laemmli (1970). The resolving gel contained 12% acrylamide, 0.3% bisacrylamide, and 0.1% SDS. The stacking gel contained 3.0% acrylamide, 0.08% bisacrylamide, and 0.1% SDS. Samples were prepared by mixing the sample with β-mercaptoethanol, glycerol, SDS, and bromophenol blue and heating the mixture in a boiling water bath for 4 min to denature the proteins. The gels were run at a constant voltage of 90 V for 20 hr at room temperature.