Journal of Chemical Ecology, Vol. 11, No. 11, 1985

USE OF DYE-LABELED PROTEIN AS SPECTROPHOTOMETRIC ASSAY FOR PROTEIN PRECIPITANTS SUCH AS TANNIN

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(Received January 24, 1985; accepted March 14, 1985)

Abstract—Bovine serum albumin has been covalently labeled with Remazol brilliant blue R to provide a substrate for a convenient spectrophotometric assay for protein precipitants. The blue protein is especially useful for measuring protein precipitation by vegetable tannins because its absorption maximum is at a wavelength where plant pigments exhibit minimum absorption. Blue BSA has been used to determine, by competition experiments, the relative affinity of various proteins for tannins. A procedure for purifying condensed tannin from commercially available quebracho extract is described.

Key Words—Tannin assay, protein precipitation, sorghum tannin, quebracho tannin, condensed tannins.

INTRODUCTION

The biological effects of the complex polyphenols known as tannins are considered to be the result of their binding and precipitating proteins (McManus et al., 1981). Assays of protein binding and precipitation are therefore of considerable importance in characterizing tannins.

Unfortunately, the assays presently available for measuring protein binding/precipitation are not satisfactory in all respects. The use of hemoglobin as a spectrophotometric marker for protein precipitation (Bate-Smith, 1973; Schultz et al., 1981) is absolutely dependent on freshly prepared hemoglobin (I. Baldwin, personal communication); commercial (lyophilized) preparations are unsatisfactory. Pigments such as anthocyanidins, often present in plant extracts containing tannins, absorb at similar wavelengths as hemoglobin, interfering with the precipitation assay and causing high blank values. The hemoglobin

0098-0331/85/1100-1535$04.50/0

precipitation assay has given unsatisfactory results on high-tannin sorghum (Bullard et al., 1981).

Martin and Martin (1983) devised an indirect assay using the Bradford protein test (Bradford, 1976) to measure unprecipitated protein. This technique is versatile with respect to assay conditions and has recently been adapted to the analysis of multiple samples (Wilson, 1984). It is inherently less accurate because it does not measure the precipitated protein directly. Moreover, controls to eliminate the effect of interfering materials are laborious.

There have been several attempts to estimate tannin concentration by its inhibition of various enzymes (Davis and Hoseney, 1979; Becker and Martin, 1982), but the correlation between tannin concentration and degree of inhibition is unsatisfactory (Daiber, 1975; Gupta and Haslam, 1980; Earp et al., 1981; Bullard et al., 1981). This may be due to retention of variable activity in enzyme–tannin complexes (Armstrong, 1983; Butler et al., 1984).

In this laboratory we have directly measured protein binding and precipitation by utilizing standard proteins labeled with radioisotopes in order to facilitate their detection (Hagerman and Butler, 1980a; Asquith et al., 1983). This method is sensitive and reliable, but it depends upon the availability of radioisotope equipment, and preparation of labeled protein may be difficult.

We report here a direct spectrophotometric assay which obviates most of the difficulties mentioned above. The assay utilizes a standard soluble protein, bovine serum albumin (BSA), covalently labeled with a blue dye. The assay can be adapted to measurement of materials which do not precipitate proteins but which compete with precipitants for binding them.

METHODS AND MATERIALS

All chemicals were reagent grade and used without further purification. Bovine serum albumin (fraction V, fatty acid free), chicken egg ovalbumin, and fetuin were purchased from Sigma Chemical Corp. (St. Louis, Missouri). Calf skin gelatin was from Eastman Organics (Rochester, New York). Rat submaxillary gland glycoprotein GP_{66}-SMX (Mehansho and Carlson, 1983) was generously provided by Dr. Haile Mehansho. Remazol brilliant blue R was purchased from Aldrich Chemical Co. (Milwaukee, Wisconsin). Cyanidin was purchased from K & K Laboratories Inc. (Plainview, New York). Condensed tannin (Gupta and Haslam, 1980) was purified from *Sorghum bicolor* Moench, DeKalb BR 64, as described by Hagerman and Butler (1980b). Crude quebracho condensed tannin (Roux, 1957) was obtained from Trask Chem. Corp. (Marietta, Georgia).

Preparation of Labeled BSA. The protein-labeling procedure was adapted from Rinderknecht et al. (1968). To 2 g of BSA dissolved in 40 ml of 1% (w/v) $NaHCO_3$, pH 8.2, was added 150 mg of Remazol brilliant blue R, and