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ABSORPTION OF POLYPHENOLS BY POLYVINYLPYRROLIDONE AND POLYSTYRENE RESINS

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Abstract—Absorption of polyphenols by insoluble polyvinylpyrrolidone (PVP) and by polystyrene resins has been examined. Dowex-1 was the most efficient absorbent of polyphenols extracted from leaves of spinach, bean or tobacco. Dowex-50 and Amberlite XAD-2 were more effective than PVP for the removal of leaf polyphenols from solution. With purified polyphenols, Dowex-1 efficiently absorbed chlorogenic acid, flavonol glycosides and catechins but did not absorb condensed proanthocyanidins. PVP absorbed all classes of polyphenols examined but showed a low affinity for chlorogenic acid.

INTRODUCTION

The removal of phenolic compounds during the preparation of tissue extracts is often essential for the successful isolation of plant proteins and enzymes in a native state. Generally the removal of polyphenols is achieved by the inclusion of absorbents in the homogenisation medium. The most widely used absorbent is insoluble polyvinylpyrrolidone (PVP) which removes polyphenols from solution by the formation of strong hydrogen-bonds with the phenolic OH groups [1–3]. However, Lam and Shaw [4] showed that Dowex-1, a polystyrene resin containing strongly basic anion-exchange substituents, was much more effective than PVP for the removal of polyphenols from extracts of flax. Dowex-1 has subsequently been used for the removal of polyphenols from several plant tissue extracts [5–7]. Loomis [3] has suggested the use of the uncharged polystyrene Amberlite XAD resins as absorbents of phenolic compounds, but there has been no comparison with Dowex-1 with regard to the efficiency of polyphenol absorption.

The aim of this investigation was to obtain a direct comparison of the ability of polystyrene resins and PVP to remove various polyphenols from solution in order to produce a rational approach to the problem of the removal of phenolic compounds from plant tissue extracts.

RESULTS AND DISCUSSION

The absorption of leaf polyphenols by PVP and polystyrene resins was examined in experiments designed to simulate the absorption of polyphenols during the homogenisation of plant tissue. A solution of polyphenols (1 ml), previously extracted from fresh leaves and equivalent to the polyphenols of 1 g fr. wt of leaves, was mixed on a vortex mixer for 30 sec with 3 ml buffer containing a weighed amount of absorbent. After centrifugation to remove the absorbent the concentration of polyphenols remaining in solution was determined. The results obtained with polyphenols extracted from spinach leaves are shown in Fig. 1; essentially similar results were obtained with polyphenols extracted from the leaves of French bean and tobacco. Dowex-1 was the most efficient absorbent of leaf polyphenols from these 3 sources, being about an order of magnitude better than PVP (the dry wt of absorbent required to remove 50% of the spinach polyphenols was 10 mg Dowex-1 or 125 mg PVP). Lam and Shaw [4] suggested that the superiority of Dowex-1 over PVP was due to its ability to bind ionically the negatively charged polyphenols. However, ionic binding cannot be the sole explanation of the efficiency of Dowex-1, because Dowex-50, a cation-exchange resin, and Amberlite XAD-2, an uncharged polystyrene resin, are also effective absorbents of spinach polyphenols (Fig. 1).

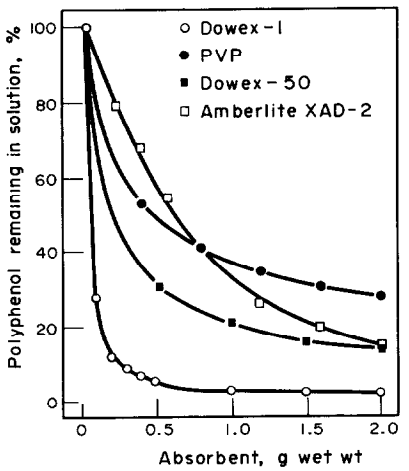

Fig. 1. Absorption of spinach polyphenols by insoluble PVP and polystyrene resins. Spinach polyphenol extract contained 500 μg polyphenol/ml determined by Folin–Ciocalteu assay using gallic acid as standard. Concentrations of polyphenols remaining in solution were determined by Folin–Ciocalteu assay. Moisture contents of absorbents were. PVP, 73.6%; Amberlite XAD-2 (20–50 mesh), 43.3%; Dowex-1 × 2 (200–400 mesh), 75.4%; Dowex-50 × 4 (100–200 mesh), 65.4%.

This suggests that the absorption of polyphenols by polystyrene resins is predominantly due to hydrophobic interactions with the aromatic rings of the polyphenols. This is in contrast to the absorption of polyphenols by PVP which is due to hydrogen bonding with the phenolic OH groups [1].

Differences in the mechanisms by which polystyrene resins and PVP absorb polyphenols were indicated by the different affinities of the absorbents for various classes of polyphenols. A comparison of the absorption of purified polyphenols by Dowex-1 and by PVP is shown in Fig. 2. The affinities of the absorbents for the various polyphenols were markedly different. For example, Dowex-1 showed the greatest affinity for chlorogenic acid whereas this polyphenol was only poorly absorbed by PVP. In contrast, Dowex-1 did not absorb grape proanthocyanidin whereas PVP was able to remove quantitatively this polyphenol from solution. The inability of polystyrene resins to absorb condensed proanthocyanidins was confirmed by repeating the experiment with a highly polymerised condensed proanthocyanidin from bracken; Dowex-1, Dowex-50 and Amberlite XAD-2 were unable to bind this polyphenol.

These results indicate that with a knowledge of the polyphenol composition of a tissue it should be possible to suggest a rational approach for the removal of polyphenols. With herbaceous tissue, containing hydroxycinnamic acid derivatives and flavonol glycosides, but with little or no proanthocyanidins, it should be possible to remove polyphenols by absorption with Dowex-1 alone, as has been shown for polyphenols extracted from spinach leaves. With bean and tobacco leaves, Dowex-1 was able to remove sufficient polyphenols to prevent the covalent modification of leaf proteins by polyphenols [5, 6]. In woody tissues, or tissues containing high concentrations of proanthocyanidins, a combination of Dowex-1 and PVP will probably be the most effective means of polyphenol removal. Indeed, Gross *et al.* [7] have reported that PVP and Dowex-1 were necessary to achieve optimal extraction of enzyme activity from yew leaves, and Loomis [3] has reported that a combination of PVP and Amberlite XAD-4 gave clear protein-containing solutions from potato tubers and walnut hulls.

EXPERIMENTAL

Extraction of polyphenols. Leaves (50 g) were homogenised in 10 vol. 50% aq. MeOH for 30 sec in a Braun blender at 4°. The homogenate was centrifuged at 12000 *g* for 10 min and the clear supernatant was reduced to 50 ml under vacuum. An equal vol. of MeOH was added, any ppt. which formed was removed by centrifugation and the vol. was again reduced to 50 ml. Extracts were prepared from leaves of spinach (*Spinacia oleracea* L.), French bean (*Phaseolus vulgaris* L cv Canadian wonder) and tobacco (*Nicotiana tabacum* L. cv Turkish samsun). Condensed proanthocyanidin from bracken (*Pteridium aquilinum*) was prepared by blending 50 g pinnae in 200 ml 0.05 M Tris–HCl pH 7.4, 10 mM 2-mercaptoethanol for 60 sec at top speed in a Waring blendor at 4°. The homogenate was strained through cheesecloth and centrifuged at 77000 *g* for 1 hr. The clear supernatant was heated at 100° for 10 min to remove protein and the proanthocyanidin was purified by passage through DEAE-Sephadex, CM-Sephadex and Sepharose 6B. The proanthocyanidin conc was determined by boiling with 2 M HCl for 30 min, extraction of the red colour into an equal vol. of *iso*amyl alcohol and measurement of the *A* at 510 nm [8]. Spectral and chromatographic analyses indicated that the predominant anthocyanidin formed was cyanidin, as shown previously in ref. [9]. Grape proanthocyanidin was a gift from Dr. E. C Bate-Smith to Dr. D. S. Bendall. All other polyphenols were obtained commercially.

Absorption of polyphenols. Polyphenol soln (1 ml containing either polyphenols extracted from 1 g leaves or 2 mg purified polyphenols) was mixed for 30 sec by vortex mixer (Whirlimixer, Fisons Scientific Apparatus) with 3 ml 0.05 M NaPi buffer pH 7.5 containing weighed amounts of the absorbents. After centrifugation at 1500 *g* for 5 min to remove the absor-

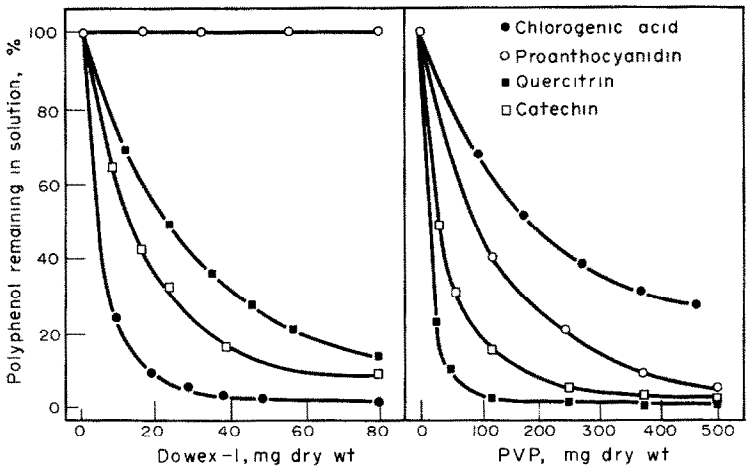

Fig. 2. Absorption of purified polyphenols by Dowex-1 and insoluble PVP. Incubations contained 2 mg polyphenol and were carried out as described in the Experimental. The concentration of polyphenols remaining in solution was determined from the *A* at 280 nm.

bents, the supernatants were assayed for polyphenol content by Folin–Ciocalteu reactivity [10] or by their A at 260 nm or 280 nm. Insoluble PVP (GAF Corp. Polyclar AT) was washed as described in ref. [3], collected by filtration and stored as a damp powder. Amberlite XAD-2 (BDH Ltd.) was washed with H_2O until free of chloride. Dowex resins were equilibrated with 0.05 M NaPi buffer pH 7.5, collected by filtration and stored as the moist resin. Dry wts of absorbents were determined to standardise the amounts of absorbents added.

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