REMOVAL OF PHENOLICS FROM PLANT EXTRACTS BY GRINDING WITH ANION EXCHANGE RESIN

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SUMMARY

A new method is described for removing phenolics during the preparation of crude plant extracts. Tissue was ground in Tris-glycine buffer with insoluble polyvinylpyrolidone (PVP) or Dowex 1-X8 anion exchange resin. Assays for peroxidase, o-diphenol oxidase, total protein and total phenolics showed that the resin removed interfering phenolics far more effectively than did PVP.

INTRODUCTION

When cell membranes are broken by grinding, the phenolics in the vacuolar sap are exposed to the action of oxidases with the formation of quinones and ultimately of brown polymers (1), thus inhibiting enzyme activity or even causing protein precipitation. The formation of protein-quinone complexes can be prevented, either by inhibiting the oxidation of phenolics with reducing agents or by the addition of an adsorbant during grinding. Both unoxidized phenolics and reducing agents affect enzyme assays in crude extracts by interfering with the determination of the substrate or by inhibiting enzyme activity.

Insoluble polyvinylpyrolidone (PVP) has been employed as an insoluble adsorbant for clearing protein extracts

by Loomis and Battaile (2) and is now widely used for this purpose. It has, however, certain disadvantages. It probably binds only those phenolics of molecular weight greater than chlorogenic acid (MW 354) (3). Moreover, it inhibits some enzymes (4,5) and its capacity for adsorbing phenolics is very low, necessitating its use in large amounts so that it is sometimes difficult to grind the tissue in a mortar.

METHODS

Flax (Linum usitatissimum L.) was chosen as experimental material because of its high phenolic content. Flax cotyledons were ground in a mortar with either (a) Tris-glycine buffer (0.05M) at pH 8.3, or (b) buffer plus 2 gm dry PVP per gm fresh weight of tissue or (c) a 10% (w/v) suspension of Dowex 1-X8 chloride (200-400 mesh) in buffer. The resin was washed repeatedly with deionized water and equilibrated with buffer overnight. The supernatant was decanted and fresh buffer added to give approximately a 10% (w/v) suspension. The percentage of Dowex used and the pH of the buffer can be varied depending upon the phenolic content of the tissue to be extracted and the requirements of the experimenter.

Peroxidase, o-diphenol oxidase, total protein and total phenolics were determined after removal of PVP or resin by centrifugation. Peroxidase was assayed with guaiacol (6) and o-diphenol oxidase with chlorogenic acid (7) as substrate. Protein was precipitated with TCA, washed with acetone and measured according to Lowry et al. (8). Phenolics were estimated according to Nair and Vining (9) using chlorogenic acid as a standard.

RESULTS AND DISCUSSION

The results in Table I show that the Dowex resin is

TABLE I. A comparison of the different methods for protein extraction.

Extraction	PEROXIDASE ACTIVITY ΔOD470/0.2ml Extract/min*	ΔOD470/mg. protein/min.	O-DIPHENOL OXIDASE ACTIVITY ΔOD330/0.2 ml Extract/min*	ΔOD330/mg. protein/min.	PROTEIN CONTENT (mg/ml)	PHENOLIC CONTENT (μg/ml)
(a) Buffer	0.600	1.875	0.075	0.234	1.700	500
(b) Buffer + PVP	0.390	1.814	0.065	0.302	1.075	164
(c) Buffer + Dowex	0.750	3.846	0.103	0.528	0.975	0

*Crude extracts were prepared from 2 gm fresh wt. of flax cotyledons and made up to 12 ml with buffer. Activity per mg protein depends on protein estimations which are affected by the degree to which phenolics are complexed and retained with the protein in (a) and (b).

an efficient adsorbant for phenolics under these conditions. Protein content was apparently the lowest in (c), but it must be emphasized that the higher readings in (a) and (b) were at least partly due to interference caused by phenolics bound to the protein. This view is supported by the observation that the protein prepared from (b) and especially that from (a) had a yellowish colour. It cannot therefore be concluded that (c) had the lowest protein content (8) and it is clear that removal of the phenolics was largely responsible for increasing the apparent activity per mg protein for both enzymes. In addition, the activities of both peroxidase and o-diphenol

oxidase expressed per unit volume of extract were distinctly higher in (c) than in (a) or (b). This may reflect the more complete removal of inhibitory phenolics in (c) and possibly also an inhibitory effect of PVP.

Since most phenolic compounds are small molecules and are more negatively charged than proteins, they can readily be removed from extracts with anion exchange resin. The method described offers distinct advantages over the use of PVP; it cannot, however, be recommended for the extraction of enzymes having low isoelectric points, since highly negatively charged proteins may also be adsorbed by the exchange resin.

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