Separation of Plant Membrane Proteins by Ion Exchange Chromatography[1]

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Abstract

A fraction enriched in plasma membranes from onion stem was fractionated on a DEAE-cellulose column using a linear gradient of KCl from 0.0 to 0.4 molar in 1 per cent Triton X-100. Qualitative resolution by gel electrophoresis showed that a major protein component was separated from the other membrane proteins. The procedure provides a purified fraction in sufficient quantity to permit biochemical characterization.

Progress toward an understanding of the nature and function of membrane proteins has been hampered by the lack of methods for preparing a single species of membrane protein in sufficient quantity for biochemical analysis. This note describes a technique suitable for separating plant membrane proteins.

Membrane fractions were isolated from stems of green onions as previously described (2, 3, 6). A fraction rich in "heavy" plasma

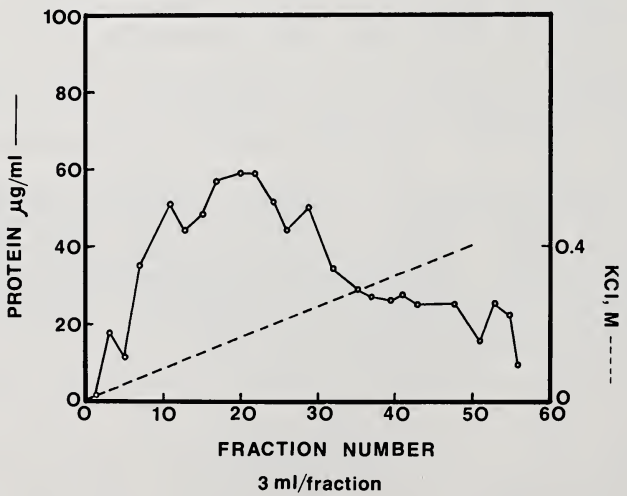

FIGURE 1. *Elution profile on DEAE-cellulose showing the distribution of protein as measured by the Lowry procedure. Solid line represents µg protein per ml and the dashed line represents the linear elution gradient of KCl from 0.0 to 0.4* M.

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membrane (8) was chromatographed on DEAE-cellulose (diethylaminoethyl cellulose) (1). Membranes (10 mg protein) solubilized in Triton X-100 (1%) were placed on the column and the proteins were eluted from the column using a linear gradient of KCl from 0.0 to 0.4 M (containing 1% Triton X-100). Fractions were collected and protein was precipitated in 5% trichloroacetic acid and 50% acetone (final concentration). The insoluble material was analyzed qualitatively using polyacrylamide gel electrophoresis (5, 7) and quantitively by measuring total protein (4).

The protein elution profile from the DEAE-cellulose column as determined by the Lowry method (Fig. 1) showed high amounts of protein in Fractions 9 through 30. When resolved by electrophoresis on polyacrylamide gels, a sample from pooled Fractions 28-29 showed a single major protein having an electrophoretic mobility corresponding to protein band A of the total membrane fraction (Fig. 2). In contrast, a sample from pooled Fractions 17-22 gave a mixture of proteins with band B being predominant. Other fractions from the column contained varying amounts of these major bands and smaller amounts of minor bands. The results show that a specific protein fraction (band A) from plant membranes can be prepared using DEAE-cellulose chromatography. By expanding the procedure, sufficient quantities of a protein fraction can be obtained to permit a biochemical characterization.

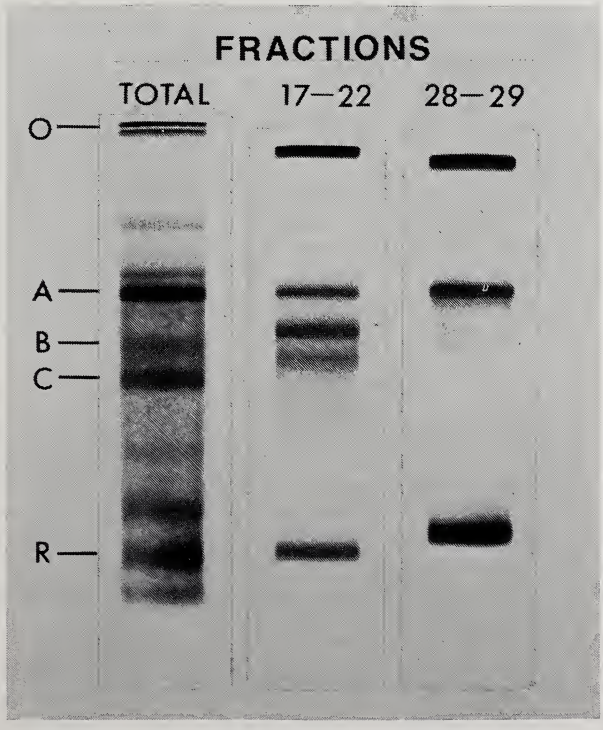

FIGURE 2. *Polyacrylamide gel electrophoresis of membrane proteins. Total = total membrane. R = ribonuclease. O = origin.*

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