Preparation of Chlorophyll-a and Chlorophyll-b by Means of Column Chromatography with Sephasorb HP Ultrafine

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Summary Chlorophyll-a and chlorophyll-b were prepared from spinach leaves by means of column chromatography with Sephasorb HP Ultrafine.

THE need for highly purified chlorophyll preparations is increasing. Recently, the use of Sephadex LH-20 has been extended to the application of gel filtration to the separation of chloroplast pigments, although the separation of chlorophyll-a (Chl-a) and chlorophyll-b (Chl-b) has not yet been attained.[1-3] We report here an improved method for the preparation of Chl-a and Chl-b by means of column chromatography with Sephasorb HP Ultrafine (Pharmacia, Uppsala, Sweden), which is similar in structure to Sephadex LH-20.

All the procedures for chlorophyll preparation were carried out in total darkness or under dim green light at 15 °C. Chlorophyll extracted from spinach leaves with acetone was selectively precipitated with dioxan and water[4] and was then washed with 80% (v/v) aqueous methanol.[5] T.l.c. analysis, according to the method of Shiraki *et al.*,[6] revealed that the crude chlorophyll preparation (C.C.P.) thus obtained did not contain any yellow leaf pigments which would disturb the separation of Chl-a and Chl-b by column chromatography. 13·8 mg of the C.C.P. was dissolved in diethyl ether–hexane (10 ml; 1 : 9 v/v) and the solution was added to the top of a Sephasorb HP Ultrafine column (25ϕ × 260 mm). The column was washed with diethyl ether–hexane until all the pigments

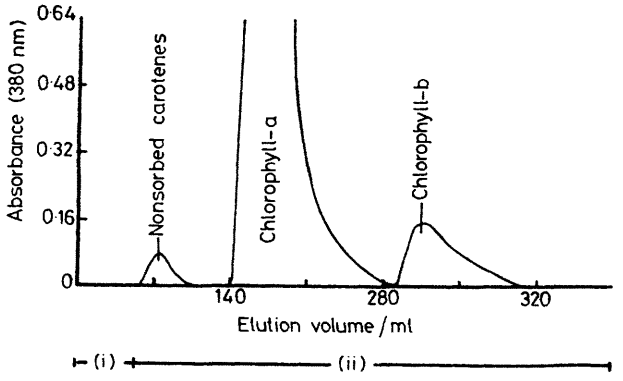

FIGURE. Chromatogram for the crude chlorophyll preparation: (i) diethyl ether–hexane (1 : 9, v/v); and (ii) 0·5% (v/v) acetone in the mixture (i), were used as eluants at a flow rate of 110 ml h^{-1}.

had been adsorbed on the column and subsequently was washed with 5% (v/v) acetone in diethyl ether–hexane to separate Chl-a from Chl-b. The flow rate of the eluants was 110 ml h^{-1} and the chromatographic elution pattern of the pigments was monitored at 380 nm. The elution pattern is shown in the Figure. Chl-a and Chl-b emerged as separate peaks in the chromatogram. T.l.c. revealed that the Chl-a and Chl-b preparations each showed a single spot on commercial silica gel sheets (Tokyo Kasei, Japan). 8·1 mg of Chl-a and 2·8 mg of Chl-b were obtained from 13·8 mg of the C.C.P. in this case. Absorption spectra of the Chl-a and Chl-b preparations dissolved in anhydrous diethyl ether (AR grade) at 20 °C did not show any significant differences (within experimental error) when compared with the literature values.†

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† Spectral details for Chl-a and Chl-b were as follows: red peak (nm) 660·0 ± 0·5 (Chl-a) and 642·3 ± 0·3 (Chl-b); blue peak (nm) 427·0 ± 1·0 (Chl-a) and 452·8 ± (Chl-b); absorbance ratio (blue peak/red peak) 1·30 ± 0·01 (Chl-a) and 2·83 ± 0·01 (Chl-b).

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