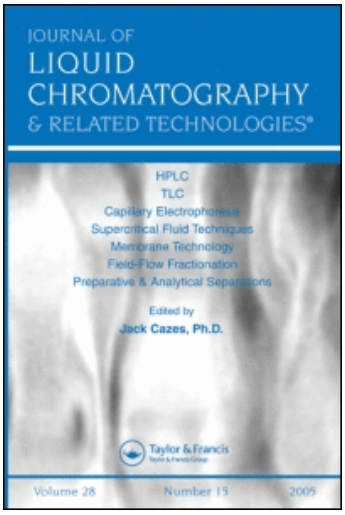

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An Improved Method for the Preparation of Chlorophyll by Means of Column Chromatography with Sepharose CL-6B

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AN IMPROVED METHOD FOR THE PREPARATION OF CHLOROPHYLL BY MEANS OF COLUMN CHROMATOGRAPHY WITH SEPHAROSE CL-6B

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ABSTRACT

An improved method for the preparation of chlorophyll "a" and chlorophyll "b" from fresh spinach leaves by means of the column chromatography with Sepharose CL-6B has been developed. A good separation of the green pigments was attained on the column (ϕ25 x 163 mm) with a mixed solvent program of 2, 3, 10 and 20 % 2-propanol in hexane.

INTRODUCTION

Recent progress in synthetic adsorbents has made possible the separation and isolation of chlorophyll(Chl) with good reproducibility as well as with high yield by means of column chromatography with synthetic adsorbents such as Sephadex LH-20 (1), Sephasorb HP Ultrafine (2, 3), DEAE-Sepharose CL-6B (4, 5) and Sepharose CL-6B (4, 5). After the conventional comparative examinations, it has been emphasized (5) that Sepharose CL-6B may be one of the best adsorbents for preparative separation and isolation of chlorophyll "a" and chlorophyll "b".

Omata and Murata (4) have been able to obtain pure chlorophyll "a" and chlorophyll "b" from Ppt I (6) prepared according to the dioxane method (6, 7) as a starting material by combining column chromatography with DEAE-Sepharose CL-6B with that using Sepharose CL-6B. Subsequently, we have developed an improved method for the preparation of chlorophyll "a" and chlorophyll "b" from fresh spinach leaves by means of column chromatography with Sepharose CL-6B. In this paper, we report an improved method for the preparation of Chl.

MATERIALS AND METHODS

Precleaning of Chl extracted from fresh spinach leaves was achieved according to the procedures described earlier (6, 8). Ppt III, thus obtained after the precleaning of the acetone extract (6), contained chlorophyll "a" and chlorophyll "b" and non-sorbed carotenes on the Sepharose CL-6B column developed in the solvent program used in this study. As will be described later, it has been found that Ppt III is extremely useful as a starting material to separate photosynthetic pigments from each other.

The chemical stability of Chl molecules was examined during the course of the Chl preparation by a thin-layer chromatographic method (9). In addition, the purity of chlorophyll "a" and chlorophyll "b" was determined by a high-performance liquid chromatographic method (10) as well as by a spectrophotometric method described elsewhere (6).

The Sepharose CL-6B column (ϕ25 x 163 mm) was prepared according to the procedures of Omata and Murata (4). The column was connected, through the UV monitor (JASCO UVIDEC-100-II W), to a fraction collector. Percolation of the pigment solution and the developing

solvents was accelerated by slight pressure from a rotor pump (SJ-1210, Mitsumi; flow speed, 126-138 ml/hr) connected to the top of the chromatographic tube by means of a teflon tube. For example, 80 mg of Ppt III, freshly prepared, was dissolved in about 30 ml of the mixed solvent (2-propanol/hexane = 2 : 98, v/v). The resultant green solution was added to the top of the column and then the column was washed using solvent program of 2, 3, 10 and 20 % 2-propanol in hexane. The column temperature was maintained at 16°C, although it has been already confirmed (4) that the separation should be carried out at lower temperatures, for example, at 4°C. Because the equipment used in the column chromatographic separation was not practical to operate below 15°C. The thin-layer chromatographic examinations of each collected fraction of 10 ml were performed in order to avoid contamination of chlorophyll "a" and chlorophyll "b" fractions with any other photosynthetic pigments or degradation products of Chl molecules.

RESULTS

Fig. 1 shows the elution pattern. The complete separation between the pigment peaks was achieved. The thin-layer chromatographic analyses of the column chromatographic fractions (170-240 ml) yielded only one spot, corresponding to chlorophyll "a". The chlorophyll "a" solution thus collected was evaporated to obtain chlorophyll "a" preparation in a solid state in a vacuum desiccator (3 mmHg) which was kept in a thermostated water bath at 10°C. Chlorophyll "a", 49.3 mg, on a dry weight basis, was obtained. From the fractions (260-340 ml), 16.8 mg of chlorophyll "b" on a dry weight basis was obtained according to the same procedures used in the above preparation. Thin-layer chromatographic analysis

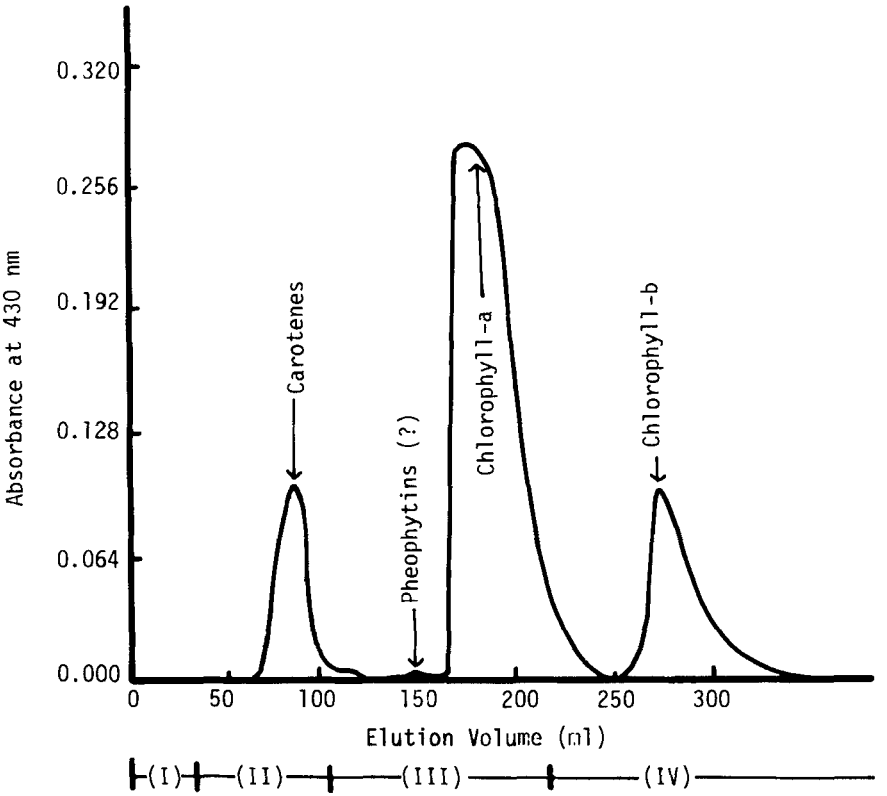

FIGURE 1

Chromatogram of chlorophyll in a Sepharose CL-6B column monitored at 430 nm with flow speeds of 126-138 ml/hr. We have not been able to flow the developing solvents at a constant rate, as it was observed that the bed volume of the adsorbent in each developing solvent system was different, and thus the swelling of the adsorbent prevent us from achieving constant flow. Fractions of 10 ml were collected.

Eluent: (I) 2, (II) 3, (III) 10, and (IV) 20 % (v/v) 2-propanol in hexane.

of the chlorophyll "b" preparation revealed that it did not contain any other coloured materials. In addition, 21.4 mg of non-sorbed carotenes was obtained.

The visible absorption spectral characteristics of the chlorophyll "a" and chlorophyll "b" preparations were in good agreement with the literature values (3) with experimental error.

DISCUSSION

Recently, Höxtermann (11) emphasized, after comparative investigations, that application of the dioxane method (7) is effective for extraction and precleaning of Chl obtained from plant materials. Subsequently, we have confirmed (5) that the combination of the dioxane method and the column chromatographic method developed by Omata and Murata (4) may be practically useful for the preparation of chlorophyll "a" and chlorophyll "b". In the column chromatographic procedures (4), the DEAE-Sepharose CL-6B column was employed to separate the photosynthetic yellow pigments from the green pigments. By contrast, we have developed a method for the elimination of most carotenoids from Ppt II by washing with 80 % (v/v) aqueous methanol prior to the powdered-sugar column chromatographic separation (8), and then Ppt III was obtained. By the use of Ppt III as a starting material for the column chromatographic isolation of chlorophyll "a" and chlorophyll "b", we have been able to obtain pure chlorophyll preparations very rapidly and very easily. For example, we have developed a method for the preparation of Chl by means of column chromatography with powdered-sugar (6). However, we have confirmed (3) that special care is required to prepare a powdered-sugar column of good quality with good reproducibility, since powdered sugar has a strong

tendency to adsorb moisture from air, and the presence of any trace water affects the separation of the pigments. Conversely, for example, columns of Sepharose CL-6B of good quality can be very easily obtained with good reproducibility. In addition, it has been found (5) that Sepharose CL-6B has great binding capacity for Chl. Hence, we have developed the method for the preparation of Chl as has been described in this report.

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