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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF CHLOROPHYLLS AND SOME OF THEIR DERIVATIVES

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It has been developed a micro-scale high-performance liquid chromatographic method for qualitative analysis of chlorophylls and some of their derivatives. The method combines simplicity, sensitivity and reproducibility without degradation of chlorophylls and their derivatives.

It has been reviewed[1] a number of publications on the analytical and preparative separation of plant pigments by column, paper, countercurrent distribution, and thin-layer chromatography. None of these, however, is sufficient method for both analysis and preparative studies of chlorophyll. Recently, Eskins et al[2] proposed a liquid chromatographic method for separation of photosynthetic pigments extracted from the brown sea diatom, *Nitzschia closterium*, to add a suitable method for both analysis and preparation of the pigments. However, it has been still needed to develop a micro-scale method for both qualitative and quantitative analysis of plant pigments and their derivatives during a course of our chlorophyll studies.[3)-9)] In this letter, we report on a high-performance liquid chromatographic method for separation of chlorophylls and some of their derivatives as a preliminary study by aiming to add a suitable method for the micro-scale analysis.

All the experiments were performed at room temperature in total darkness or under green dim light. Chlorophylls a and b were prepared according to the method of Iriyama et al.[3),4)] Chlorophylls a' and b' were obtained by re-fractionation of chlorophylls a and b according to the method of Sievers and Hynninen.[10)] Pheophytin a was prepared according to the method of Hynninen.[11)] High-performance liquid chromatography was run on a FAMILIC-100 instrument (Japan Spectroscopic Co., LTD, Tokyo, Japan) with 0.5x65 mm teflon tube packed with silica gel powder SS-05 (ϕ0.5 μm). Elution patterns were monitored at 380 nm with UVIDEC-100 (Japan Spectroscopic Co., Tokyo, Japan). Runs were made at room temperature with a solvent flow rate of 16 μl/min. All solvents used were analytical reagent grade without further purification.

Pigments were characterized by comparison of their visible spectra with literature values. In addition, the pigments were thin-layer chromatographed according to the method of Shiraki et al.[9]

Fig. 1 shows typical chromatogram for the mixed pigments (chlorophylls a, a', b, b', and pheophytin a). The good resolution of the pigments in the test material was obtained with hexane containing acetone by a solvent program of 8, 10, and 12 % acetone. The total elution time was 80 min. This method enables the micro-scale qualitative analysis of chlorophyll a to the order of 10^{-10}g.

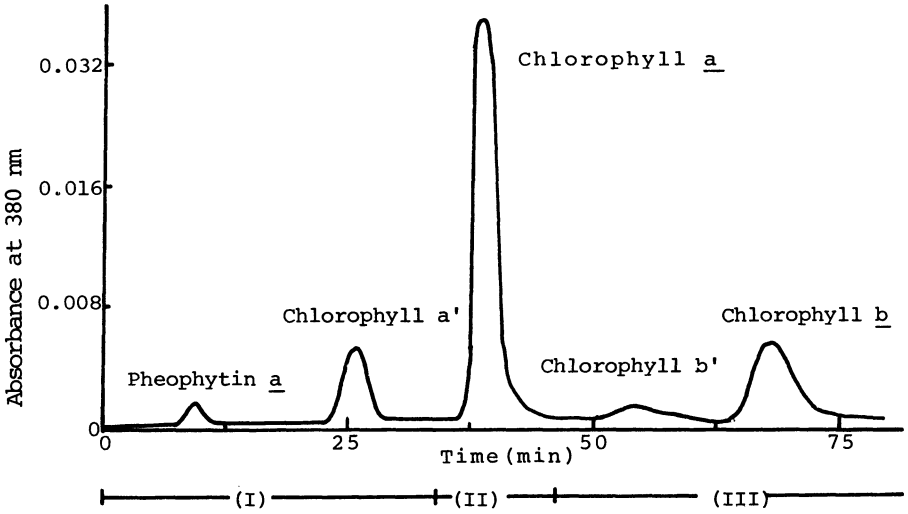

Fig. 1. Chromatogram of mixed pigments (chlorophyll a, a', b, b', and pheophytin a) run on FAMILIC-100 with 0.5x65 mm teflon tube packed with silica gel powder SS-05 at a flow rate of 16 µl/min. 1 µl of sample solution(8% acetone in hexane) containing the mixed pigments was injected. Solvent systems for (I), (II), and (III) are 8, 10, and 12% acetone in hexane respectively.

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