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Chromatographic Behavior of Metalloporphyrins by Cellulose Thin-layer Chromatography

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Cellulose thin-layer chromatographic separation of chlorophyll a, b, and c, pheophytin a, b, and c, Zn(II)- and Cu(II)-complexes of protoporphyrin-IX dimethyl ester (PPDE) was studied by using a light petroleum–acetone developing solvent system. The hR_f values of metal–PPDE chelates were found to be virtually linearly related with the electronegativities-to-radius ratio (En/ri) of central-metal ions. The hR_f values of metalloporphyrins become progressively greater with increase in En/ri values of the central metal. This suggests that a dominant factor influencing the hR_f values of metal–porphyrins is the extent of electron transfer from the four nitrogens and/or the π-electron system of porphyrin to central metal.

Metalloporphyrins have been widely investigated because of their biological and chemical importance.[1,2] Recently porphyrins have also been used as a chelating agent for analysis of trace metals since free porphyrins and their metal chelate exhibit intense fluorescence and/or characteristic color.[3] Metal chelates could be further utilized for analysis by applying thin-layer chromatography (TLC). It is being widely used for the separation and purification of metalloporphyrins. In this paper, the chromatographic behavior of cobalt(II) and (III), nickel(II), copper(II), zinc(II), magnesium(II), and iron(III) complexes with PPDE, and magnesium(II) (=chlorophyll a), copper(II), and zinc(II) pheophytin a chelates on cellulose layer is described, considering the dominant factor controlling the mobility.

Experimental

Materials. Chlorophyll a and b: Pigments were extracted from spinach with a methanol–acetone (1:1, v/v) mixed solvent. After partial purification according to the dioxane method,[4] the complexes were separated by chromatography of the crude chlorophyll extract on a glucose column.

Chlorophyll c_1 and c_2: The compounds were isolated from Undaria pinnatifida and Sargassum racemosum, respectively, according to the Jeffrey method.[5]

Pheophytin a, b, c_1, and c_2: The compounds were prepared[6] from their original chlorophylls by shaking an ethereal solution of the chlorophyll with 13% hydrochloric acid (w/w) for 5 min.

Cu and Zn Pheophytin a: These were prepared by heating pheophytin a and five-fold excess of zinc and copper acetate in ethanol at 40 °C, for 10 min.

PPDE and Its Metal Chelates: The carboxyl side chain of protoporphyrin IX (Midori Jūji Co., Ltd.) was esterified[7] with methanol containing 5% (w/v) concentrated sulfuric acid at 0 °C. After esterification, the ester was crystallized from methanol–chloroform solution.

Metal Insertion: Cobalt, nickel, and magnesium PPDE were prepared by the *N,N*-dimethylformamide method as described by Adler *et al.*,[8] using metal chloride. After reaction in *N,N*-dimethylformamide, the crude product was crystallized by addition of water and cooling. Insertion of zinc, copper, and iron to PPDE was carried out by heating the porphyrin with a metal salt [zinc acetate, copper acetate, and iron(II) chloride] in chloroform–ethanol, 40 °C for 30 min. After heating, the metalloporphyrin was crystallized by removing chloroform in a stream of nitrogen.

Purification of Metal Chelates: Purification of Ni, Co, and Zn chelates was carried out on a calcium carbonate column with chloroform eluent. After evaporation of the eluate to a small volume, the metalloporphyrins were crystallized by addition of ethanol. Crude Mg-PPDE was chromatographed on a cellulose column with a light-petroleum: acetone (20:3, v/v) eluent. Crystallization of Mg-chelate from benzene–chloroform solvent was then repeated three times.

Stationary Phase. Thin-layer chromatography was performed with commercial cellulose plates (Merck precoated cellulose plate Art. 5716, 5×20 cm).

Chromatographic Procedure. One μl solution of samples (PPDE and its metal chelates; 0.04 μg/ml in chloroform, chlorophylls, and their derivatives; *ca.* 0.05 μg/ml in diethyl ester) was spotted with a 10 μl micro-syringe at a point 3 cm from the lower edge of TLC. After air-drying, the chromatograms were developed (10 cm/*ca.* 14 min) in thin-layer chambers lined with filter paper, in which the atmosphere had been equilibrated with developing solvent for 15 min before the plates were inserted. Light-petroleum: acetone (20:3, v/v) was chosen as a developing solvent, in view of results obtained for (1) light-petroleum (bp 50—80 °C):acetone (20:3, v/v), (2) light-petroleum:1-propanol (24:1, v/v), and (3) light-petroleum:chloroform (7:3, v/v). After developing, the chromatograms were air dried and examined in daylight and under ultraviolet light (365 nm).

Results

A typical chromatogram of PPDE and its metal chelates on cellulose plate is shown in Fig. 1. The hR_f values obtained for seventeen porphyrins derivatives are given in Table 1. Porphyrins with carboxyl side chain, pheophytin c and Ni-protoporphyrin IX *etc.*, did not move from the origin of chromatogram with a light-petroleum:acetone (20:3, v/v) solvent system. More polar solvent systems such as light-petroleum:acetone (7:3, v/v) and dimethylpyridine:water system[9] should be used in order to separate the above compounds. However, porphyrin derivatives with carboxyl side chain showed extensive tailing and were not separated from each other on the chromatogram in the more polar solvent systems.

Free porphyrins are readily esterified with a alcohol[7] or diazomethane,[10] the esters being hydrolysed without degradation. The esters are more lipophilic than the free porphyrins, as indicated by their solubility in organic solvents. The ester of protoporphyrin IX was

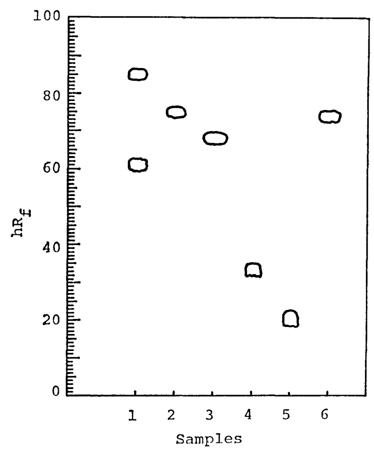

Fig. 1. Thin-layer chromatogram of PPDE and its metal chelates.
Solvent system; light petroleum:acetone=20:3, v/v. TLC; Merck precoated cellulose plate.
1: Co-PPDE, 2: Ni-PPDE, 3: Cu-PPDE, 4: Zn-PPDE, 5: Mg-PPDE, 6: PPDE.

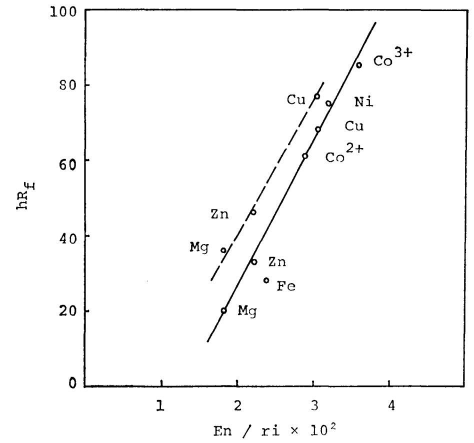

Fig. 2. Relationship between En/ri and TLC hR_f values of metalloporphyrins.
En: Pauling's electronegativity value, ri: Effective ionic radius, ——: Metal-PPDE chelate, ----: Metal-pheophytin-a chelate.

therefore used for the thin-layer chromatographic studies.

Since the hR_f values of the chromatogram were reproducible to about ±2 on repeated runs, porphyrins having hR_f values with a difference of over four were separated perfectly. Under ultraviolet light (365 nm) the separated spots exhibited either a characteristic, very intense red fluorescence (Zn, Mg-PPDE, and PPDE, chlorophylls, and their derivatives, except Cu-pheophytin a) or a dark one against the lighter background of the rest of the plate. The lower limits of detection were about 1 ng for the fluorescent substances, and 10 ng for non-fluorescent porphyrin metal complexes. As shown in Table 1, PPDE and Ni-PPDE were not separated completely, but they could be clearly distinguished by their different behavior under ultraviolet light (365 nm). The former (hR_f: 75) appeared as a black spot, the latter (hR_f: 74) showing red fluorescence.

TABLE 1. hR_f VALUES AND IDENTIFICATION UNDER ULTRAVIOLET LIGHT OF PORPHYRINS AND THEIR CHELATES

Compound	hR_f value[a)]	Identification[b)]
Pheophytin a	86	red-f
Pheophytin b	72	red-f
Pheophytin c	0	red-f
Protoporphyrin IX	0	red-f
Chlorophyll a	36	red-f
Chlorophyll b	21	red-f
Chlorophyll c	0	red-f
Cu-Pheophytin a	77	non-f
Zn-Pheophytin a	46	red-f
Protoporphyrin IX dimethyl ester	74	red-f
Co-Protoporphyrin IX dimethyl ester	85 61	non-f
Ni-Protoporphyrin IX dimethyl ester	75	non-f
Cu-Protoporphyrin IX dimethyl ester	68	non-f
Zn-Protoporphyrin IX dimethyl ester	33	red-f
Mg-Protoporphyrin IX dimethyl ester	20	red-f
Fe-Protoporphyrin IX dimethyl ester	28	non-f

a): Solvent system; light-petroleum: acetone (20 : 3, v/v). b): In ultraviolet light the separated zones exhibited either a red fluorescence or a dark one against the lighter background of the rest of the plate. TLC: Merck precoated cellulose plate (14 min/10 cm).

Co–PPDE has two varieties of hR_f values depending on the preservation time of the chloroform solution of Co–PPDE. The hR_f value of the freshly prepared solution of Co–PPDE was 85, but after being left to stand, it was 61, tailing being observed.

Discussion

The relationship between the individual metals of the porphyrin chelates and the hR_f values on cellulose TLC was studied. As shown in Fig. 2, the hR_f values of the metal chelates were proportional to the En/ri values of the central metals, where En is the Pauling electronegativity values[11)] of the elements, irrespective of their oxidation state, and ri is the effective ionic radii cited from the recent compilation by Shannon and Prewitt.[12,13)] Assuming that the En/ri value of the central atom is a measure of field strength, the extent of electric transfer from four nitrogens of porphyrin to central atom increased with increase in En/ri values. The charge transfer would make the metal atom nearly neutral, and the total positive charge of the central atom might be dispersed over the surrounding π-electron system of porphyrin.[14)] The more delocalized bond between the metal and ligand of the chelates leads to less dipole-dipole interaction between the chelate to cellulose and higher solubility of the chelate in the developing solvent, and accordingly a larger hR_f value.

Co–PPDE shows two kinds of mobility (hR_f value). The hR_f values 61 and 85 could be assigned respectively to low(II) and high(III) oxidation state of cobalt ion for the following reasons. (1) The ionic radius of the cobalt in the two kinds of complexes is estimated to be 65 and 53 pm from the hR_f values 61 and 85, respectively. (2) Cobalt(II)–porphyrins are briefly oxidized to higher oxidation state(III) complex by air.16) (3) The oxidation potentials of metalloporphyrins are Fe: -0.32, Co: $+0.52$, and Ni: $+1.00$.17) Although the data could not be applied directly to this TLC condition, they indicate that the oxidation states of Co, Fe–porphyrins are easily changed by the change in the conditions. It should be expected that cobalt in the crystal of Co(II)–PPDE is air oxydized during storage, but reduced gradually in chloroform.

Fe–PPDE, which have a more sensitive oxidation-potential to the conditions than Co-porphyrin, moved more slowly than expected. The reason for the deviation from hR_f-En/ri relationship is not clear.

Conclusion

The mobility (hR_f values) of metalloporphyrin dimethyl esters on cellulose TLC is closely correlated to the En/ri values of central metal. The En/ri value determines the extent electron transfers from nitrogen to central metal, and it will be a dominant factor to control the mobility of metalloporphyrin. Thus the hR_f values of the metalloporphyrin dimethyl esters can be used for identification and also for metal determination of chelates with unknown central metal. These findings have prompted a study of chromatographic behavior of metalloporphyrin in non-aqueous developing solvent systems.

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