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DISTRIBUTION OF AN AMINO ACID-CONTAINING PHOSPHOLIPID IN BROWN ALGAE

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Abstract—The phospholipid composition of 30 species of brown algae belonging to eight different orders has been analysed by TLC. A new amino acid-containing phospholipid was detected in members of the orders, Ectocarpales, Chordariales, Dictyotales, Desmarestiales, Scytosiphonales, Laminariales and Fucales. The occurrence of this new amino acid-containing phospholipid has chemotaxonomic value for brown algae.

INTRODUCTION

The phospholipid composition of marine brown algae has been examined by many researchers [1–8]. The lipids of these plants are represented by the usual phospholipids but, in addition, there are some unknown components [2, 4, 5, 8]. One of these was detected about 10 years ago in algae from the Sea of Japan; it contained phosphorus and gave an intensive pink stain with ninhydrin, demonstrating the presence of an amino acid–containing polar head group [9, 10]. Recently, Schmid *et al.* [11] have described a new phospholipid from brown algae with the same colour characteristics and similar chromatographic properties. Based on IR, NMR, mass spectroscopic and chemical data, they suggested the most likely structure of the new phospholipid as:

phosphatidyl–CH_2–CH_2–NH–$CH(NH_2)$–CH_2–CH_2–CO_2H

We have studied the phospholipid composition of brown algae and special attention was paid to the presence or absence of amino acid-containing phospholipids (amino-PL) in order to obtain a representative picture for the distribution of this unusual phospholipid and to examine its importance in algae of the Phaeophyceae. It has been shown that polar lipids have taxonomic value for marine macrophytic algae and each division of seaweeds has distinctive peculiarities in their phospholipid composition [4, 5]. The significance of our study is further emphasized by the fact that many species of brown algae are used as human food [12]. Thus, their lipid constituents are of interest also from the viewpoint of nutrition.

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RESULTS AND DISCUSSION

Phospholipid composition of 30 species representing eight orders of brown algae were determined. Phospholipids were separated by two-dimensional TLC (Fig. 1) and detected by spraying the plates with specific spray reagents. Phospholipids were also identified by comparison of their R_f values with those of standards.

A new amino-PL was found in 29 of the algal species examined: Ectocarpales—*Analipus japonicus*; Chordariales—*Leathesia difformis, Chordaria flagelliformis, Sphaerotrichia divaricata*; Dictyotales—*Dictyopteris divaricata*; Desmarestiales—*Desmarestia ligulata*; Dictyosiphonales—*Dictyosiphon chordaria, Punctaria plantaginea*; Scytosiphonales—*Colpomenia sinuosa, Hydroclathrus clathratus, Scytosiphon lomentaria, Petalonia fascia*; Laminariales—*Chorda filum, Agarum cribrosum, Arthrothamnus kurilensis, Costaria costata, Laminaria japonica, L. cichorioides, L. bongardii, Alaria angusta, A. fistulosa, Undaria pinnatifida*; Fucales—*Fucus evanescense, Pelvetia wrightii, Sargassum pallidum, S. miybei, S. thunbergii, Coccophora langsdorfii, Cystoseira crassipes*. The taxonomic arrangement is based on the system of Wynne [13].

The amino-PL was present in appreciable amounts and its content varied from 5.1% of the total phospholipids in *Scytosiphon lomentaria* to 15.7% in *Pelvetia wrightii* [9]. However, this lipid was not detected in *Dictyota dichotoma*. This algal species is distinctly different from all other brown algae, because phospholipids constitute only 1.5% [4] or 1.9% of the total complex lipids [6] present, much lower than in *Scytosiphon lomentaria*, for example, where phospholipids comprised

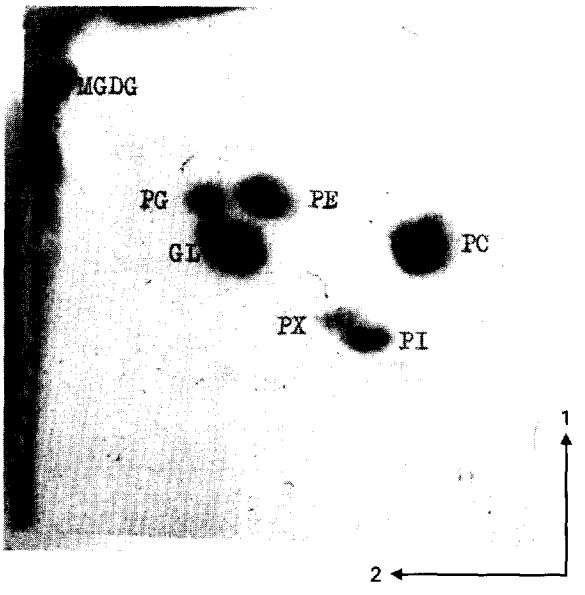

Fig. 1. Two-dimensional TLC of lipids from *Analipus japonicus*. Lipid detection with 10% H_2SO_4 in methanol and charring. Solvent systems: first direction, $CHCl_3$–MeOH–benzene–28% NH_4OH (65:30:10:6); second direction, $CHCl_3$–MeOH–benzene–Me_2CO–HOAc–water (70:30:10:5:4:1). Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PX, new amino-phospholipid; GL, glycolipids; MGDG, monogalactosyldiacylglycerol.

24% of total lipids [9]. Therefore, only the main lipid components, glycolipids, betaine lipid and phosphatidylglycerol, were detected under high loading of lipid extracts onto TLC.

There is some suggestion that variations between reports on the presence or absence of some phospholipids, for example phosphatidylcholine, in members of the brown algae may be due to environmental conditions or other factors [8]. In order to examine the influence of environmental conditions on the detection of the new amino-PL in algae, several species of brown algae were collected in different regions of the World Ocean. *Undaria pinnatifida*, *Laminaria japonica*, *Sargassum pallidum* and *S. miybei* were collected from the Sea of Japan and also from the Yellow Sea. The amino-PL was found in all species examined.

The new amino-PL has been found previously only in a few members of three orders. It was observed for the first time in *Ectocarpus siliculosus* and 'later' was detected in brown algae belonging to Fucales (*Fucus serratus*, *F. vesiculosus*, *Ascophyllum nodosum*, *Cystoseira* sp.), Sphacelariales (*Sphacelaria rigidula*) [11] and Ectocarpales (genera *Ectocarpus*, *Feldmannia*, *Hincksia*) [14].

The amino-PL resembles phosphatidylserine (PS) in its colour reactions with specific spray reagents. But the two phospholipids can be distinguished by chromatographic mobility; for example, the amino-PL migrates faster than phosphatidylinositol (PI), but PS migrates slower than PI in TLC solvent systems for separation of phospholipids [4, 15]. Hence, the very similar properties of the new phospholipid and PS may lead to incorrect identification of phospholipids. Some researchers have found PS in 12 species of brown algae belonging to the Chordariales, Scytosiphonales, Laminariales, Dictyotales and Fucales [6]. We did not detect PS in any of the algal species studied in this work, in agreement with other researchers [2, 3, 5, 7]. It is possible that Araki *et al.* [6] found the new amino-PL in brown algae, but not PS.

Based on the results of our work and literature data, we suggested that amino-PL is characteristic of all brown algae and, therefore, has taxonomic value because it was not found in red algae [4, 16, 17], green algae [4, 18] nor in seagrasses [19]. These results call for a continuation of the study of the distribution of amino acid–containing phospholipids in brown algae and an extension of the algal species list. It should be noted that the analysis of phospholipids in seaweeds requires great caution in obtaining reliable data, because marine brown algae contain several unknown phospholipids [4, 5].

EXPERIMENTAL

Most of the algal species were collected in the Sea of Japan in the southern region of the Russian Far East in May–September. *Laminaria bongardii*, *Alaria angusta*, *A. fistulosa*, *Fucus evanescence*, *Arthrothamnus kurilensis* were sampled on the coast of the Kurile Islands (Sea of Okhotsk). *Laminaria japonica*, *Undaria pinnatifida*, *Colpomenia sinuosa*, *Sargassum pallidum*, *S. miyabei* were collected in the Yellow Sea (China) and also in the Sea of Japan (Russia). *Sargassum thunbergii* was collected from the Yellow Sea and *Hydroclathrus clathratus* from the South China Sea (South Vietnam).

Fresh algae were thoroughly cleaned to remove epiphytes, small invertebrates and sand particles. Algae were then heated for 2 min in boiling H_2O to inactivate enzymes, dried and ground up with a small amount of acid washed sand using a mortar and pestle. Lipids were extracted with $CHCl_3$–MeOH (1:2) by a modified method of ref. [20], as described in ref. [4]. TLC was carried out using silica gel G plates. Polar lipids were separated by 2D TLC using solvent systems for complex mixtures of phospholipids [15] and those of ref. [21]. Nonspecific visualization was achieved by plate-charring after spraying with 10% H_2SO_4 in MeOH. Lipids were identified by comparison with authentic standards and using spray specific reagents, Molybdenum Blue for phospholipids [22], Malachite Green for phosphorus-containing substances [23], 0.5% ninhydrin in Me_2CO for aminophospholipids and Dragendorff's reagent for lipids with quarternary ammonium groups [24].

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