THE REPLACEMENT OF PHOSPHATIDYLETHANOLAMINE AND ACIDIC PHOSPHOLIPIDS BY AN ORNITHINE-AMIDE LIPID AND A MINOR PHOSPHORUS-FREE LIPID IN *PSEUDOMONAS FLUORESCENS* NCMB 129

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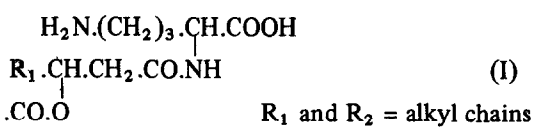
1. Introduction

Zwitterionic acylated ornithine amide lipids having the general structure (I) have been



(I)

R_1 and R_2 = alkyl chains

characterised as constituents of the lipids of several Gram negative bacteria [1–4]. It has been suggested [1] that such lipids might be interchangeable in function with the Zwitterionic phospholipid phosphatidylethanolamine (PE) which usually co-occurs with the ornithine-containing lipids. The studies reported here show that the major lipids of certain magnesium-limited chemostat cultures of *Pseudomonas fluorescens* NCMB 129 are PE, phosphatidylglycerol (PG) and diphosphatidylglycerol (DPG); in phosphate-limited cultures, however, no detectable amount of phospholipids are present and an ornithine-containing lipid is almost the sole polar lipid component.

2. Materials and methods

Batch cultures (1 litre) of *Ps. fluorescens* NCMB 129 were prepared in a Nutrient Broth medium used previously [5] for the cultivation of bacilli; cells were harvested after 24 hr incubation on a gyrotatory shaker at 25°C. The basic medium used for continuous cultures of this organism was that employed previously [6] for the cultivation of bacilli with the addition of L-glutamic acid, sodium salt (1.84 g/l), L-leucine (0.80 g/l) L-valine (0.30 g/l), L-threonine (0.17 g/l) and L-methionine (0.07 g/l); this medium was devised originally for another purpose and no attempt was made to determine the exact growth requirements of this strain of *Ps. fluorescens*. Continuous cultures (25°C, pH 7.0 ± 0.1) were prepared aerobically in a 600 ml chemostat; magnesium-limited cultures (dilution rate 0.2 hr^{-1}) contained 8 mg/l of $MgSO_4$ and phosphate-limited cultures (dilution rates 0.1 and 0.2 hr^{-1}) had 15 mg/l of KH_2PO_4.

Lipids were extracted from cell pellets by modified Bligh and Dyer procedures [7,8] and examined by thin-layer chromatography as described previously [9] (see fig. 1); the relative proportions of the individual polar lipids were estimated by densitometry after charring [10]. The identity of the individual phospholipids was established by analysis of their deacylation products by paper chromatography in the system propan-1-ol–ammonia (0.880)–water (6:3:1) [11]. The same paper chromatographic system, in conjunction with a specific vanillin reagent [12], was used to detect the presence of ornithine (R_{lysine} 0.98) in acid hydrolysates of a phosphorus-free nitrogeneous lipid isolated by preparative thin-layer chromatography.

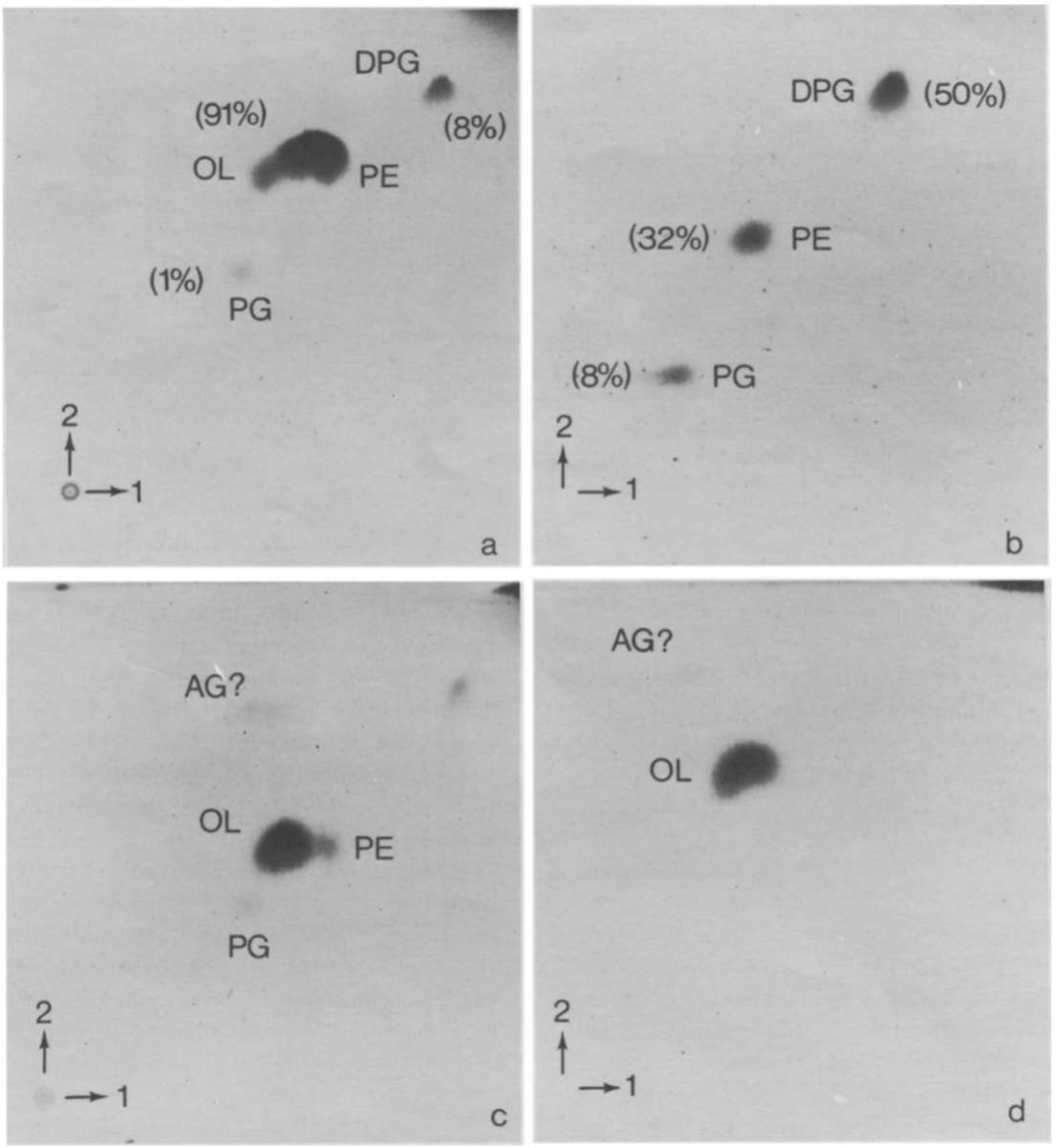


Fig. 1. Two-dimensional thin-layer chromatograms of polar lipids of *Ps. fluorescens:* a) 24 hr batch culture; b) magnesium-limited chemostat culture (0.2 hr^{-1}); c) and d) phosphate-limited chemostat cultures (0.1 and 0.2 hr^{-1}, respectively). Chloroform–methanol–water (65:25:4) was used in the first direction and chloroform–acetic acid–methanol–water (80:18:12:5) in the second direction. Spots on developed chromatograms were revealed by charring after spraying with dichromate–sulphuric acid reagent and the proportions of lipids were estimated by densitometry [10]. Abbreviations: AG? – possible acidic glycolipid; other abbreviations are described in the text.

3. Results and discussion

The polar lipid composition of batches of *Ps. fluorescens* (fig. 1a) was similar to that reported previously for this organism [13]. The phospholipids were identified as PE, PG and DPG; acid hydrolysates of a phosphorus-free lipid were shown to contain ornithine but the structure of this lipid was not studied in more detail. The polar lipids of cells from magnesium-limited chemostat cultures (fig. 1b) contained all three phospholipids but the ornithine-contain-

ing lipid (OL) was absent. The relatively enhanced proportions of the acidic lipids PG and DPG under these conditions support the suggestion that these lipids are important in the uptake of metalions [6]. In cells from phosphate-limited cultures prepared at the lower dilution rate (0.1 hr^{-1}) OL was the major lipid but small proportions of the three phospholipids were present (fig. 1c); the polar lipids of cells prepared in the same medium at the higher dilution rate (0.2 hr^{-1}) consisted essentially of a single component (OL) no phospholipids being detected (fig. 1d). By changing back and forth from phosphate to magnesium limitation (dilution rate 0.2 hr^{-1}) it was demonstrated that the alterations in lipid composition (fig. 1b and d) were reversible. Cells from both phosphate-limited cultures contained traces of another phosphate-free lipid which co-chromatographed with and had staining properties similar to a glucuronosyl diacylglycerol isolated from *Pseudomonas diminuta* [14]; no further structural studies were performed on this lipid. It was noted previously [15] that another strain of *Ps. fluorescens* (NCTC 10038) produced a hexuronosyl diacylglycerol when cultivated on solid media. The lipids represented in fig. 1 were extracted by a minor modification [17] of the Bligh and Dyer procedure; recently, however, it has been shown [8,16] that DPG is not quantitatively removed from some bacterial cells by the usual extraction solvents. Consequently a modified Bligh and Dyer procedure [8], shown to extract DPG efficiently, was also used on lyophilised cells from the chemostat cultures; the lipid patterns obtained on analysis of these extracts were closely similar to those shown in fig. 1b, c and d.

Several important conclusions may be drawn from the above results. It is apparent that the polar lipid composition of a microorganism can be completely changed by controlled variation of growth environment. The results also support the hypothesis of the interchangeability of certain polar lipid types in bacterial membranes [5,6,10,17]. In this case it appears that the acidic phospholipids (PG and DPG) are replaceable by a very small amount of a component presumed to be an acidic glycolipid and PE is interchangeable with an ornithine-containing lipid presumed to have a Zwitterionic structure similar to (I). The polar lipid composition of one phosphate-limited culture (fig. 1d) of *Ps. fluorescens* is also quite unique and must represent perhaps the simplest polar lipid composition yet observed for a living cell. *Ps. fluorescens* NCMB 129 cultivated in a chemostat under phosphate limitation is, to our knowledge, the first living organism capable of sustained growth without substantial proportions of phospholipids in its membranes. In previous studies [17] it was found that certain phosphate-limited cultures of *Pseudomonas diminuta* had almost vanishing proportions (approximately 0.3%) of PG, acidic and neutral glycolipids forming the bulk of the membrane polar lipids. Evidence has also been produced [5,6,16] that PE and diglucosyl diacylglycerols may be possibly interchangeable in the membranes of certain bacilli.

These interrelations between the various polar lipid types are in accord with the principle of different substitution groups for polar lipids [18,19]. One major substitution group contains acidic polar lipids while the other consists of the neutral or Zwitterionic polar lipids and sulphatides. The results presented here are, however, not in total agreement with the substitution group concept which suggests that the sum of the proportions of the lipids in a particular substitution group should be a constant fraction of the total polar lipid for a given tissue [18,19]. Inspection of the lipid patterns given in fig. 1 shows that the total proportion of the acidic lipids in one substitution group (PG, DPG and acidic glycolipid) may vary with respect to the sum of the proportions of the lipids (PE and OL) in the other major substitution group. In certain bacteria, therefore, changes in environment as well as bringing about changes in proportions of lipids within their respective substitution classes may also alter the relative distribution of polar lipids between the substitution groups. Finally it should be noted that *Ps. fluorescens* NCMB 129 is a marine bacterium which has possibly adapted to a low-phosphate environment by developing the capacity to reduce the proportions of the phosphorus-containing components of its membrane lipids to an absolute minium.

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