PLASMALOGENS IN THE GILL LIPIDS OF AQUATIC ANIMALS

J. C. NEVENZEL, A. GIBBS and A. A. BENSON
Scripps Institution of Oceanography, A-002, La Jolla, CA 92039, USA

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Abstract—1. Lipids constituted 0.6-2.2% wet wt of the gills of 11 species of aquatic animals (4 bivalves, a crustacean and 6 fishes).
2. Phospholipids, largely phosphatidylcholine (PC) and phosphatidylethanolamine (PE), are major components of all species.
3. The plasmalogen contents of these lipids were 47-291 μmol/g, with the highest values found for bivalve gill total lipids and the catfish phospholipid fraction.

INTRODUCTION

Preliminary to a study of the effect of lowered pH on the gills of aquatic animals we analyzed the gill lipids of 11 species for their content of plasmalogens, 1-(1'-alkenyl)-2-acyl-phospho-glycerides [and any 1-(1'-alkenyl)-2,3-diacyl-glycerols].

The plasmalogen content of gills has previously been determined quantitatively only for an Octopus sp. (Dembitskii, 1981) and the trout, Salmo trutta (Bolis et al., 1984). Dumont (1958) found a "high" plasmalogen level in the posterior gill of Eriocheir sinensis and Rapport (1961) reported that the highest plasmalogen levels in both an unspecified bivalve and a Loligo sp. were in gill tissues. Total lipid contents, phospholipid fraction, the phospholipid classes present and their fatty acid compositions in gills of aquatic animals have been investigated in several laboratories; specific papers are cited below in Results and Discussion.

Vaskovsky, Dembitskii and colleagues in the USSR have published extensive data on the plasmalogens of the total body lipids of over 60 marine species from sponges to tunicates (Dembitskii and Vaskovsky, 1976; Dembitskii et al., 1977; Dembitskii, 1979, 1980, 1981; Kostetsky and Gerasimenko, 1984). Other investigations published since the reviews in Snyder's (1972) book Either Lipids report the plasmalogens in coral (Parker et al., 1984); molluscs, including abalone (de Koning, 1966; Joh and Hata, 1979), a top shell (Joh and Hata, 1979) and bivalves (Samugna et al., 1972; Joh and Hata, 1979; Chelom and Zhukova, 1981); and in a tunicate (Hayashi et al., 1979). Plasmalogen analyses of fish tissue other than gills are: brain (Selivonchick and Roots, 1976; Driedzic et al., 1976; Kruglova, 1979), muscle mitochondria (Wodtke, 1981), optic nerve (Matheson et al., 1981) and erythrocyte membranes (Nelson, 1972; 1979; Warren et al., 1979).

MATERIALS AND METHODS

The catfish was purchased from Aquatic Systems Incorporated, La Jolla, CA; the crayfish and freshwater mussels from College Biological Supply Co., Escondido, CA; the trout and beef heart (as a reference standard) from Mayfair Markets, La Jolla, CA. The rock scallops were cultured in the Bivalve Mariculture Laboratory, S10; the oysters were purchased on the Atlantic Coast of Florida and maintained in running local seawater. The mussels, leopard shark and kelp bass were collected off Scripps; the tuna was caught on hook and line off San Diego; and the sturgeon netted in the Sacramento River Delta, California.

The freshwater mussels and crayfish were maintained under the irregular light regimen of the working laboratory in aerated tap water, changed every second day. The catfish were fed canned fish or a pelleted pet food twice a week, and the clams received yeast once a week. The gills were dissected out and extracted with chloroform–methanol by the Bligh and Dyer (1959) technique, using either the whole gills (invertebrates and tuna) or the soft tissue (muscosa) scraped from the filaments (remaining fish species). The solvent was evaporated under nitrogen with slight warming to obtain the total lipids. For some species this was further fractionated into neutral, glyco- and phospholipid by column chromatography on silicic acid (Bio-Rad A, Bio-Rad Laboratories, Richmond, CA), eluting the neutral lipids with 4 column volumes (=4 vol.) of chloroform, "glycolipids" with 3 vol. of acetone and the phospholipids with 3 vol. of methanol (Patton and Thomas, 1971).

Total 1'-alkenyl (vinyl) ethers were determined by the iodine-uptake method of Williams et al. (1962), simplified. Aliquots of 50-800 μg of lipid were dissolved in 0.9 ml of methanol with shaking and warming as necessary for complete solution. Buffer (3.2 ml of 0.094 M citrate of pH 5.5), 0.85 ml of 3 M KI, and 50 μl of 5 Mm L-1-in-3-M-KI were added. After 40 min at room temperature the absorbance at 363 nm was measured in a Beckman DU Spectrophotometer (Beckman Instruments, South Pasadena, CA) fitted with a Gilford Photometric Accessory (Gilford Instrument Laboratories, Inc., Oberlin, OH). Two blanks were run: (a) omitting lipids and (b) with 0.9 ml 3 M KI but no iodine. Our simplification consisted in measuring the absorbance of the reaction mixture directly, rather than that of an N-butyl acetate extract. The net iodine uptake was calculated assuming for KI 3 at 363 nm a molar extinction (1 cm) of 2.12 x 10^4 (Williams et al., 1962). The weight of plasmalogen was estimated assuming (a) a ratio of PE-plasmalogen to PC-plasmalogen of 2, (b) an 18:1 alkenyl ether chain (cf. Bell et al., 1983) and (c) an acyl moiety of the average molecular weight found for the polysaturated acids of the three lipid phospholipids we analyzed (Table 1).
analyses of tuna gill and beef heart total lipids using the original 66 mM reagent gave generally higher values than obtained by I2-uptake. When the reagent was diluted 100-fold the background measured was very high, probably because of carbonyl compounds present, or that on the chromarods the glyco- and phospholipids gravimetric value of 25% (Table 2). The latroscan Crayfish values are in good agreement if we assume that on the chromatarods the glyco- and phospholipids appear as a single zone. Teleost gill phospholipids are ca 54% in Anguilla anguilla (Zwingelstein et al., 1980) or 60–76% in nine marine species (Thomas and Paton, 1972), all considerably higher than our 36 ± 2% for two marine species.

Dumont (1958) found a high content of plasmalogens in the posterior gill of Eriocheir sinensis, while Rapport (1961) reported that the highest plasmalogen contents in both a bivalve and a squid were in their gill tissues, being over 400 μmol/g in the bivalve; compare our 264 ± 17 μmol/g for four species of bivalves—the highest values in Table 2 except for the catfish gill phospholipids. The Octopus sp. gills investigated by Dembicki (1981) contained 13.7% of the total lipid phosphorous as PE and phosphatidylserine (PS) plasmalogens, while the PE and PC plasmalogens in trout gills totaled 30.7% (Bolis et al., 1984). In our hands the iodine-uptake method when applied to beef heart gave results (plasmalogens 25.7 wt% of total phospholipids) in agreement with Dawson et al.’s (1962) maximum plasmalogen value of 28.5 mol % of total lipid phosphorous. The 1-alkenyl ethers are expected to be major components only of the phospholipids, as in our catfish analyses (Table 2), so the kelp bass data are apparently anomalous in having a higher vinyl ether content in neutral lipids than in phospholipids (115 vs 48 μmol/g), although ‘neutral plasmalogen[s]’ (i.e. 1-(1’-alkenyl)-2,3-diacylglycerols) are well known in aquatic animals (cf. Snyder, 1970, 1972).
Table 2. Lipid and 1'-alkenyl glyceryl ether contents of gills

<table>
<thead>
<tr>
<th>Species</th>
<th>% Lipid wet wt</th>
<th>NL %</th>
<th>GL %</th>
<th>PL %</th>
<th>Lipid class</th>
<th>Lipid class</th>
<th>I$_7$ uptake (μmol/g) (wt %)</th>
<th>Vinyl ethers (μmol/g) (wt %)</th>
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<tr>
<td>Mussel, <em>Mytilus californianus</em></td>
<td>0.57</td>
<td>291 ± 12</td>
<td>22.4 ± 0.9</td>
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<td>Unk. sp., family Unionidae†</td>
<td>0.71 ± 0.03</td>
<td>265 ± 22</td>
<td>20.4 ± 1.7</td>
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<td>Oyster, <em>Crassostrea virginica</em></td>
<td>0.78 ± 0.05</td>
<td>249 ± 30</td>
<td>19.2 ± 2.3</td>
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<tr>
<td>Rock scallop, <em>Haliotis multirugosus</em></td>
<td>(15.5)*</td>
<td>249 ± 30</td>
<td>19.2 ± 1.7</td>
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<td>Crayfish, <em>Procambarus clarkii†</em></td>
<td>0.38 ± 0.04</td>
<td>130 ± 13</td>
<td>10.0 ± 1.0</td>
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<tr>
<td>Mussel, <em>Mytilus californianus</em></td>
<td>0.64 ± 0.03</td>
<td>95 ± 15</td>
<td>7.4 ± 1.2</td>
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<td>Leopard shark, <em>Triakis semifasciata</em></td>
<td>&gt;0.07</td>
<td>150 ± 50</td>
<td>11.6 ± 3.8</td>
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<td>Sturgeon, <em>Acipenser transmontanus</em></td>
<td>1.03</td>
<td>85 ± 18</td>
<td>6.5 ± 1.4</td>
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<td>Catfish, <em>Ictalurus punctatus†</em></td>
<td>1.62</td>
<td>104 ± 17</td>
<td>8.0 ± 1.3</td>
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<tr>
<td>Phospholipids</td>
<td></td>
<td>286 ± 53</td>
<td>22.0 ± 4.1</td>
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<td>Neutral lipids</td>
<td></td>
<td>19 ± 10</td>
<td>1.5 ± 0.8</td>
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<td>Kelp bass, <em>Paralabrax clathratus</em></td>
<td>0.98</td>
<td>107 ± 26</td>
<td>8.2 ± 2.0</td>
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<tr>
<td>Phospholipids</td>
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<td>48 ± 15</td>
<td>3.7 ± 1.2</td>
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<tr>
<td>Neutral lipids</td>
<td></td>
<td>115 ± 49</td>
<td>8.9 ± 3.8</td>
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<td>Trout, <em>Salmo gairdneri†</em></td>
<td>2.19</td>
<td>47 ± 23</td>
<td>3.6 ± 1.8</td>
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<td>Tuna, <em>Thunnus alalunga</em></td>
<td>1.23</td>
<td>68 ± 19</td>
<td>5.2 ± 1.5</td>
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<td>Beef heart, total lipid</td>
<td>1.8 ± 0.4</td>
<td>334 ± 57</td>
<td>25.7 ± 4.4</td>
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NL = neutral lipid, GL = glycolipid, PL = phospholipid, nd = no data.
*Dry wt basis.
†Freshwater species.
§The glycolipid fraction was lost before weighing.
||Assuming a molecular weight of 770 for the plasmalogen; see text.
[Includes glycolipid; iatroscan analysis.]
Cursory examination by TLC showed PC and PE to be the main components of the phospholipids of catfish, tuna and kelp bass; Thomas and Patton (1972) found 62.3% PC and 17.6% PE in kelp bass gills. Semiquantitative analyses using the Iatroscan (Ackman, 1980) confirmed these observations for crayfish gills (PC 64 ± 4%, of total phospholipids, PE 23 ± 3%) and oyster gills (PC 61%, PE 39%). These values are high since minor components were not quantified, but the ratios of PC/PE found, 1.56–3.35, are commensurate with the literature range of 1.80–2.50 for total gill lipids in three species of marine crustaceans (Chapelle et al., 1976, 1982b; Chapelle, 1977); in the gill mitochondria this ratio tends to be lower: 1.27–2.20 (Chapelle et al., 1981, 1982a). The gill of Dembitski’s (1981) Octopus sp. contained less PC than PE for a ratio of 0.733. In teleost gills the published PC/PE ratios are: fresh water species, trout 2.06 (Bolis et al., 1984), goldfish gill mitochondria 1.55–2.04 (Anderson, 1970; Caldwell and Vernberg, 1970); nine species of marine fish 2.77 ± 0.59, range 1.93–3.61 (Thomas and Patton, 1972; Zwingelstein et al., 1973, 1975). A mesopelagic marine species with a low PE content had PC/PE of 12.95 (Thomas and Patton, 1972). Environmental temperature (Anderson, 1970; Caldwell and Vernberg, 1970; Chapelle et al., 1981; Hazel, 1984), acidity (Bolis et al., 1984), or salinity (Chapelle et al., 1976, 1982a) affect this ratio.

The fatty acid analyses of Table 1 are representative of other gill data, with a high content of polyunsaturated C20 and C22 components (27–46%) and a comparatively high level of 18:0 (15–32%) in phospholipids. The tuna gill neutral lipids still have high polyunsaturated fatty acids (33%), the highest 16:0 content (19%) but lower 18:0 (7%).

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