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Non-methylene-Interrupted Fatty Acids in Phospholipids of the Membranes of the Mussel *Crenomytilus grayanus*

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Abstract—The distribution of non-methylene-interrupted fatty acids (NMIFAs) in the subcellular fractions of the gill and hepatopancreas of the mussel *Crenomytilis grayanus* is investigated. A greater percentage of NMIFAs in the subcellular fractions of both organs, notably the gills, was found in aminophospholipids (phosphatidylethanolamine and phosphatidylserine) compared to that of phosphatidylcholine. The greatest percentage of NMIFAs occurred in phosphatidylserine of the mitochondrial fraction of both organs. Hence, functionally different organs exhibited similar patterns of NMIFA distribution by phospholipid classes. NMIFAs in the membranes of marine bivalves may act as inhibitors of lipid peroxidation processes.

A variety of unique components and structural features that have been found in the lipids of marine organisms seem to be important factors in the adaptation of bottom organisms to their specific habitats. Hence, this class of compounds has attracted intense interest.

Non-methylene-interrupted fatty acids (NMIFAs) were identified some time ago [22]. However, the largest body of information on these compounds was obtained in the 1970 and 1980s from studies of the lipids of marine invertebrates [6, 15–17, 20]. A number of interesting properties and peculiarities in the distribution of NMIFAs in the organs of marine invertebrates have been reported. Thus, in marine invertebrates, NMIFAs are synthesized *de novo* [23]; irregularly distributed among the organs and tissues [2, 4, 14]; and localized in aminophospholipids, mainly in the plasmalogenic form [4]. The most interesting properties of these acids are their lower melting point [3] and higher oxidation resistance compared to corresponding methylene-interrupted homologues [21].

Despite the large number of publications on NMIFAs, their role in the functioning of membranes is still unclear. One unknown aspect is the distribution of NMIFAs in the inner cell membranes. The study of this question may give us an understanding of the role of NMIFAs in biological membranes. There are grounds to believe that the appearance of NMIFAs in lipids of the membrane matrix exerts a significant effect on the structure and functioning of the membranes. This fact provides an example of the variety of forms of biochemical adaptation.

The aim of this study is to investigate the distribution of NMIFAs in the enriched subcellular fractions of the marine mollusk *Crenomytilus grayanus*.

MATERIALS AND METHODS

The subcellular fractions were obtained from the gills and hepatopancreas of the marine mollusk *Crenomytilus grayanus*. The mollusks were collected in May from Amursky Bay at a water temperature of 5–8°C. The tissues were homogenized in a Teflon homogenizer in a medium containing 0.25 M sucrose, 0.05 M Tris-HCl (pH 7.5), and 0.5 M NaCl. The subcellular fractions were separated by differential centrifugation [11]. The microsomal fraction was precipitated by the Kamath method [12].

Lipids from each subcellular fraction were extracted according to Bligh and Dyer [7]. Phospholipid classes were separated by two-dimensional microscale TLC on silica gel plates [19] in a series of solvents proposed by Rouser *et al.* [18]. After the plates were sprayed with water, silica gel zones containing phospholipids were transferred onto the filter and phospholipids were eluted with a chloroform–methanol mixture (1 : 1) and methylated by the method of Carreau and Dubacq [9]. The resulting fatty acid methyl esters (FAMEs) were purified by microscale TLC in benzene. Purified FAMEs were analyzed by GLC on a Shimadzu GC9A chromatograph equipped with a flame ionization detector on a stationary quartz capillary column (30 m × 0.25 mm) Supelcowax-10. The carrier gas was He; the injector pressure was 1.5 atm; the split ratio was 1/60; the column temperature, 210°C; the injector temperature, 240°C. Fatty acids were identified from the calculation of equivalent chain-lengths.

RESULTS AND DISCUSSION

Non-methylene-interrupted fatty acids are structural isomers of unsaturated fatty acids that have long been

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Table 1. Relative content of diunsaturated, nonmethylene-interrupted, and polyunsaturated fatty acids (% of total acids) in total lipids and phospholipids of the hepatopancreas and gills of the mussel *Crenomytilus grayanus*

Group of fatty acids	Hepatopancreas				Gills			
	Total lipids	PC	PE	PS	total lipids	PC	PE	PS
DUFAs	2.5	4.3	2.0	5.1	1.1	–	5.0	6.8
NMIFAs	4.0	3.5	8.9	5.9	10.4	5.6	9.1	30.0
PUFAs	33.6	30.5	23.5	17.5	44.4	30.6	33.8	4.6

Table 2. Relative content of diunsaturated, nonmethylene-interrupted, and polyunsaturated fatty acids (% of total acids) in membrane phospholipids of the cellular fractions of the hepatopancreas of the mussel *Crenomytilus grayanus*

Group of fatty acids	Nucleus			Mitochondrion			Microsome		
	PC	PE	PS	PC	PE	PS	PC	PE	PS
DUFAs	2.3	1.6	2.2	2.4	1.6	2.3	3.2	2.6	2.6
NMIFAs	6.0	5.6	6.2	2.5	4.5	12.7	2.7	3.2	4.2
PUFAs	28.0	23.6	19.8	37.2	12.3	18.9	25.3	30.6	21.0

identified in marine invertebrate tissues [14, 16, 17]. Screening studies have revealed NMIFAs in 30 species of 11 phyla of marine invertebrates [3]. Marine bivalves stand out among invertebrates because of their quantitative content of these acids [2, 4, 10]. For example, the plasmalogenic form of phosphatidylserine (PS), identified in the lipids of the mussel *Crenomytilus grayanus*, contains up to 73% of NMIFAs [4]. It is known that such isomers as 20:2Δ5,13, 20:2Δ5,11, and 22:2Δ7,15, 22:2Δ7,13 are the major acids in marine bivalves [17, 24]. As a rule, these acids are contained in phospholipids, among which amine-containing phospholipids, phosphatidylethanolamine (PE), and phosphatidylserine (PS) stand out because of their content of the acids [4]. The irregular distribution of NMIFAs in bivalve tissues is also worth mentioning [2, 14]. The largest amounts of these acids are found in the lipids of erythrocytes and gill cells, and small amounts occur in the hepatopancreas [4]. The proportion of NMIFAs in the total lipids of the organs of marine mollusks is inversely related to the content of ω3 PUFAs—eicosapentaenoic 20:5ω3 (EPA) and docosahexaenoic 22:6ω3 acids (DHA). The content of NMIFAs shows seasonal variability; its content decreases from summer to winter [4] following the decrease in the quantity of plasmalogen forms of phospholipids [1], since NMIFAs are largely contained in the plasmalogen form of PE [4]. Localization of NMIFAs in phospholipids indicates that they are needed for the membranes, and the inverse relationship of NMIFA content to the sum of EPA and DHA in the phospholipids suggests that the presence of these fatty acids in the membrane is in contrast with the need for PUFAs. In studying NMIFA synthesis, Zhukova [23] showed that marine mollusks are able to synthesize them *de novo*. It is known that ω3 PUFAs are essential fatty acids for marine mollusks. This likely indicates that mollusks can maintain the membrane parameters at the expense of NMIFAs and, in this case, they do not depend on outside sources of NMIFAs.

Based on the above data, we used the cell membrane structures of the gill and hepatopancreas of a typical representative of marine bivalves, the mussel *Crenomytilus grayanus* in our study.

More than 35 acids, including NMIFAs, were identified among fatty acids of the major phospholipid classes (PC, PE, and PS) of the investigated tissues and membrane structures of mussels. For better interpretation of a large volume of numerical data, several classes of fatty acids were distinguished: diunsaturated (DUFAs), excluding NMIFAs; diunsaturated nonmethylene-interrupted (NMIFAs); and polyunsaturated fatty acids (PUFAs).

Attention is drawn to the fact that the lipids of the gills and hepatopancreas of mussels differ substantially in the composition of fatty acids, primarily NMIFAs. The content of NMIFAs in the total lipids and individual phospholipids of the gills is several times that of the hepatopancreas (Table 1). In individual phospholipids, the highest content of these acids in the gills occurred in PS (up to 30%), while in the hepatopancreas, the highest percentage of NMIFAs was found in PE (8.9%). Mitochondrial phospholipids contain a notable amount of NMIFA (Tables 2, 3). Moreover, in the membranes of these organelles in both organs, the highest content of these acids occurred in PS.

The ratio of dienoic acids with regularly and irregularly positioned double bonds (Table 4) reflects, to some extent, the preference for specific constituent NMIFAs in a given membrane organelle. In PC of the membranes (particularly the gill cells), the preferred acids are dienoic acids with normal structures; the only exception is PC of the nuclear fraction of hepatopancreas cells. At the same time, aminophospholipids (PE

Table 3. Relative content of diunsaturated, nonmethylene-interrupted, and polyunsaturated fatty acids (% of total acids) in membrane phospholipids of cellular fractions of the gills of the mussel *Crenomytilus grayanus*

Groups of fatty acids	Nucleus			Mitochondrion			Microsome		
	PC	PE	PS	PC	PE	PS	PC	PE	PS
DUFAs	4.1	2.0	5.3	8.8	4.9	1.7	4.9	2.7	4.5
NMIFAs	1.2	8.7	1.2	2.8	17.5	21.3	2.1	13.3	12.4
PUFAs	11.0	9.3	55.9	21.7	11.4	0.84	10.6	11.0	8.1

Table 4. The proportion of NMIFAs (%) of the total diunsaturated (with regular and irregular position of double bonds) fatty acids in phospholipids of the subcellular fractions of the hepatopancreas and gills of the mussel *Crenomytilus grayanus*

Phospholipids	Hepatopancreas			Gills		
	nucleus	mitochondrion	microsome	nucleus	mitochondrion	microsome
Phosphatidylcholine	72.3	51.0	45.8	22.6	24.1	30.0
Phosphatidylethanolamine	77.8	73.8	55.2	81.3	78.1	83.1
Phosphatidylserine	73.8	84.7	61.8	18.5	92.6	72.9

and PS) with a high proportion of NMIFAs are of major importance for the construction of the lipid matrix of cell membranes of the hepatopancreas and gills. Mitochondrial PS of both tissues, except for nuclear PS of the gills, also stand out by this index.

Based on the concept of the asymmetry of the membrane lipid matrix, primarily of choline- and amine-containing phospholipids [8], and the results of our study, we could infer a pronounced transbilayer asymmetry of NMIFAs in the molluskan tissue membranes.

The non-methylene-interrupted position of the double bonds in NMIFAs cause some of their physicochemical properties to be different from those of the regular homologues. The rate of autooxidation is reduced [21]; and the melting point shifts to a lower region (T_m° of the 20:2 NMIFA from *Bugula neretina* is −13.5°C, which is about 10°C lower than in the corresponding homologue 20:2ω6 [3]). On the basis of these properties, it can be assumed that NMIFAs in phospholipids can substantially influence the mesomorphic structure of the lipid matrix and act as inhibitors of peroxidation processes in the membrane, enhancing the antioxidative activity of lipids by their presence. This assumption may be supported by the fact that the erythrocyte membranes of the bivalve *Scapharca broughtoni* contain large amounts of NMIFAs (up to 26%) [2]. This may account for the low oxidizability of membrane lipids of *S. broughtoni* [5] and the high resistance of erythrocytes to some hemolytic agents [13] and ions of certain heavy metals [5]. Moreover, the NMIFA content in phospholipids of mollusks varies with the season [4], also indicating the importance of these acids in the structure of the membrane lipid matrix.

NMIFAs, being structural isomers, and their homologues with regularly positioned double bonds (diunsaturated fatty acids) are products of the concordant functioning of a number of biosynthetic enzymes [23]. We are far from understanding the biochemical mechanism of the "switch" of metabolic pathways leading to NMIFA synthesis. From the principle of structural and functional correlation, it can be inferred that the appearance of phospholipids containing NMIFAs in the hydrophobic matrix of the membrane structure results from peculiarities of the functioning of membranes in marine mollusks.

This study of the distribution of non-methylene-interrupted fatty acids in the subcellular fractions of the gills and hepatopancreas of the mussel *Crenomytilus grayanus* has shown that the NMIFA content in the subcellar fractions of both organs is markedly higher in aminophospholipids (PE and PS) than in PC. This is particularly pronounced in the gills. Among PE and PS of the subcellular fractions, the highest percentage of NMIFAs occurs in PS of the mitochondrial fraction of both tissues. Hence, functionally different tissues exhibit the same pattern of NMIFA distribution by phospholipid classes in the subcellular fractions.

The results of this study suggest that NMIFAs in the membranes of marine bivalves act as a peculiar "structural antioxidant," and their presence reduces the oxidizability of the lipid matrix.

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