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P.S. DMITRENOK

Metabolomic approaches in the studies of holothurian and starfish glycosides

Some results of the using of metabolomic approaches in the studies of holothurian and starfish glycosides are discussed. LC-ESI MS and LC-ESI MS/MS approach are quite applicable for the profiling of holothurian triterpene glycosides and starfish polar steroid compounds in such complex mixtures as holothurian and starfish extracts and useful for searching of new structures, comparing metabolomic profiles of different these marine animal species and populations for ecological, dietary and biosynthesis studies.

Key words: metabolomics, sea cucumber, starfish, glycosides, mass spectrometry

Metabolomics is a rapidly developing field of research based on the study of low molecular weight metabolic profiles of various objects - tissues, fluids, organs etc. including whole organisms. Since both genetic factors and environmental factors affect the level of metabolites, the metabolic approach provides an assessment of the physiological state of the organism and helps identify potential chemical markers that can be used as indicators of physiological and pathological biological processes or their responses to changes in various factors. At the moment, metabolomics is successfully used in biology, medicine, toxicology, ecology, as well as in studies of the influence of environmental factors [1, 2, 6].

Metabolic fingerprinting and metabolic profiling are the main methods of metabolomic approaches [3]. The first is the analysis of biological objects on the basis of the common types of chromatograms formed by the qualitative and quantitative ratio of the including metabolites. This approach is aimed at a comparative analysis of the type of chromatograms that reflect changes in the disease or external effects on the body and covers the widest possible range of metabolites. Another type of metabolomic analysis - metabolic profiling – is an analysis in which a group of compounds of a certain class is studied. Metabolic profiling is used in the analysis of metabolites, belong to the same chemical class of substances or a particular biochemical pathway, it is carried out in order to establish or refine the biosynthetic pathways search biomarkers diseases, research target groups of metabolites, diagnosis or targets for drug research effects. Metabolic profiling is widely used to study the influence of environmental factors on organisms, for example, to study the change in the composition of secondary metabolites of plants [5].

Mass spectrometry has been playing an important role in the structural analysis of complex mixtures of natural products mainly due to its high sensitivity, rapid analysis time and selectivity. Liquid chromatography sequentially combined with mass spectrometry, more often using LS-ESI-MS methods, have been extensively applied to the analysis of complex mixtures of many natural compounds, and proved to be the appropriate analytical tool to obtain the necessary structural information for a great diversity of natural products. Metabolic profiling, including

* DMITRENOK Pavel Sergeevich – PhD, Acting Director (G.B. Elyakov Pacific Institute of Bioorganic Chemistry, FEB RAS, Vladivostok, Russia). *E-mail: paveldmt@piboc.dvo.ru

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the analysis of low-molecular secondary metabolites in biological objects by modern HPLC-MS methods, has been widely used in recent years to study the metabolites of terrestrial plants and animals, for example, glycoside conjugates of phenolic substances, plant saponins, lipids and other compounds. However, the number of papers devoted to metabolic research of marine invertebrates is very limited.

We have applied this method for investigation of metabolite profiles of triterpene glycosides and polar steroid constituents of some Far Eastern holothurian and starfish, respectively. Marine glycosides and related highly polar compounds are a large group of secondary metabolites found in various marine organisms, in particular, in marine invertebrates. Of particular interest are triterpene glycosides of holothurians and steroidal glycosides and related polar steroids of starfish. These substances have an original chemical structure, significantly differing from similar metabolites of terrestrial origin, and exhibit a variety of physiological activities, including antitumor, antiviral, anti-inflammatory, analgesic, hemolytic, immunomodulating and other properties. Usually these substances are studied by isolation of individual compounds, and only in recent years there have been works devoted to the study of mixtures of triterpene glycosides of holothurians and steroid glycosides and related starfish compounds without the isolation of their individual components.

We have studied detailed mass spectrometric information about a wide range of individual previously known and new triterpene glycosides of holothurians and steroidal glycosides and related polar steroids of starfish with the aim of compiling databases of their MS/MS spectra. In particular, the features of mass spectrometric fragmentation of series of asterosaponins previously isolated from the starfishes *Lethasterias fusca* and *Aphelasterias japonica*, and 38 triterpene glycosides isolated earlier from the sea cucumber *Eupentacta fraudatrix* were obtained. We have also been studied mass spectrometry characteristics of five representatives of a rare type of steroid oligoglycosides starfish cyclic steroid glycosides that have trisaccharide chain cyclized between C-3 and C-6 of the aglycone isolated early from the starfish *Echinaster luzonicus*.

Mass spectra, including high resolution (HR) and tandem (MS/MS) ESI mass spectra, of several new sea cucumbers triterpene glycosides and starfish asterosaponins were also obtained. Thus, the new asterosaponin of aphelasteroside F isolated from the Far Eastern starfish *Aphelasterias japonica*, which has a new type of carbohydrate chain not previously encountered in starfish asterosaponins, 11 asterosaponins from the starfish of *Pentaceraster regulus*, including seven new pentaregulosides A-G, two of which are representatives of the furostanic steroid glycosides rare for starfish, and two new triterpene glycosides of fallaxosides B1 and D3 isolated from the Far Eastern holothurian *Cucumaria fallax*, having disulfated and trisulfated carbohydrate chains and unique aglycons, were investigated. The data obtained expand the mass spectrometric database that we are creating for carbohydrate-containing metabolites of echinoderms.

Metabolic profiling of triterpene glycosides of the sea cucumber *Eupentacta fraudatrix* widely distributed near the coast of Primorsky Krai was made. Metabolomic approach using LC-ESI-MS technique made it possible to identify known compounds and establish elemental composition and suggested the structures of new triterpene glycosides. LC-MS approach allowed a multitude of new as well as previously isolated triterpene glycosides to be characterized. Analyzed profile revealed at least 54 compounds, including 26 sulfated, 18 non-sulfated and 10 disulfated glycosides (Figure 1).

In accordance with the proposed structures of oligosaccharide chains, all the detected triterpene glycosides of *E. fraudatrix* were subdivided into eleven groups (I-XI). Five of the eleven types of oligosaccharide chains were not found previously in *E. fraudatrix*. In addition, we discovered new glycosides having methylated glucose as the terminal monosaccharide residue.

Also the content of the detected triterpene glycosides in separate organs of the sea cucumber *E. fraudatrix* was studied by metabolic approach. The walls of the body contained the largest amounts of most of the detected triterpene glycosides, which confirms the supposed protective

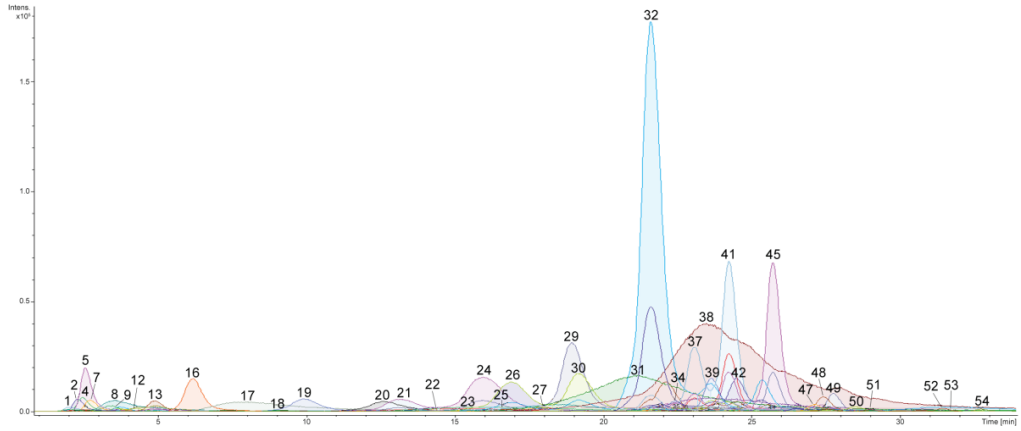

Figure 1. LC-ESI MS total compounds chromatogram of detected triterpene glycosides in negative ion mode (sulfated, disulfated and non-sulfated glycosides were detected as $[M - Na]^-$, $[M - 2Na]^{2-}$ and $[M - H]^-$ ions) in ethanol extract of sea cucumber *Eupentacta fraudatrix*

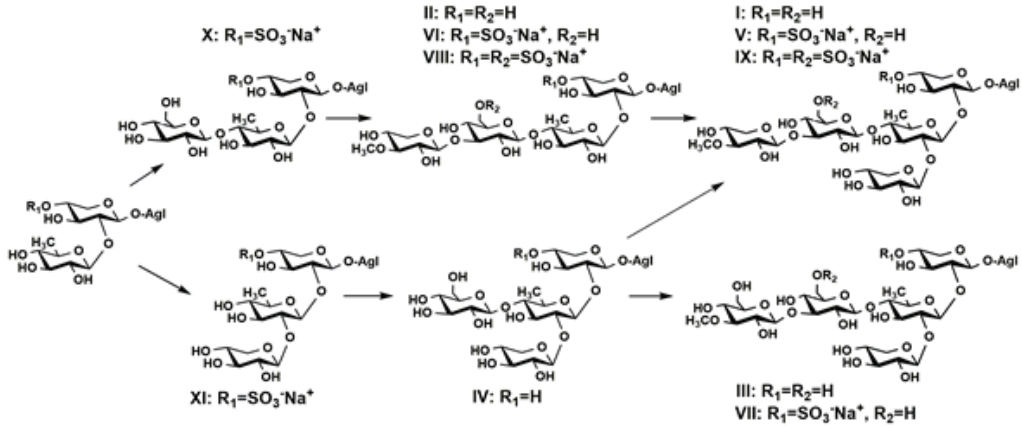

Figure 2. Hypothetic scheme of biosynthesis of oligosaccharide chains in *E. fraudatrix*

role of these substances. Differences in relative amounts of some compounds in different organs of this holothurian indicate additional biological functions of triterpene glycosides in the producing organism that need to be investigated.

Obtained data allowed us to propose a biosynthetic pathway for oligosaccharide chains in *E. fraudatrix* (Figure 2). These data correspond to theoretical biosynthetic pathway of oligosaccharide chains proposed early [4].

Using a metabolic approach, the total fraction of steroid compounds from the starfish *Lethasterias fusca* was studied and the study of fraction of triterpene glycosides of the sea cucumber of the genus *Psolus* was started.

Our investigation demonstrated that LC-ESI MS and LC-ESI MS/MS approach are quite applicable for the profiling of holothurian triterpene glycosides and starfish polar steroid compounds in such complex mixtures as holothurian and starfish extracts and useful for searching of new structures, comparing metabolomic profiles of different these marine animal species and populations for ecological, dietary and biosynthesis studies.

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