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Fabrication of nanoscale multimolecular structures of lanthanum stearate using Langmuir monolayers for laser desorption/ionization mass spectrometry

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Matrix-assisted laser desorption/ionization (MALDI) from the surface of nanosized multimolecular structures based on lanthanum stearate monolayers (FLa) has been studied. The presence of FLa on the surface of MALDI target was confirmed experimentally by laser desorption of lanthanum-containing organic ions. MALDI target functionalization with FLa is shown to significantly increase the yield of target peptide ions, with the optimum being achieved at a film thickness of about 6 monolayers. An approach is proposed in a „lab-on-a-plate" format, which allows specific extraction of peptides modified with chlorine-containing compounds and includes the following steps: functionalization of the target surface, metal affinity extraction, matrix deposition and MALDI mass spectrometric analysis.

Keywords: mass spectrometry, matrix assisted laser desorption/ionization (MALDI), surface, Langmuir monolayers, metal affinity chromatography.

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Mass spectrometry combined with matrix-activated laser desorption/ionization (MALDI) is widely used in molecular biophysics and biochemistry for the protein molecules analysis. Functionalization of the MALDI target surface with self-assembled monolayers makes it possible to provide new unique properties to the surface, which contribute to a significant efficiency increasing of mass spectrometric analysis [1]. We have previously demonstrated that monolayers of metal stearates (FMe), obtained by self-assembly of stearic acid (HSt) molecules on the surface of an aqueous subphase containing metal ions, exhibit the properties of a metal-affinity sorbent [2–4]. Besides, we recently proposed an approach that makes it possible to form such a regular two-dimensional structure directly on a solid substrate, which was a MALDI target (MTP 384 target plate polished steel BC, Bruker Daltonics) [5]. It was shown that the application of a solution of stearic acid in hexane onto the arranged on solid substrate of droplet of aqueous subphase containing lanthanum ions leads to the formation of a collapsed monolayer of lanthanum stearate (FLa). The resulting structure was characterized by a developed surface formed from well-defined metal stearate aggregates with an average diameter of 300−400 nm, a high level of adhesion to the surface of the polished MALDI target, and the presence of properties corresponding to metal-affinity sorbents. Note that such surface functionalization is intended for carrying out metal-affinity chromatography directly within the target cell (format „Laboratory on the target") [1], which allows not only to significantly reduce the cost of reagents, to reduce time of the experiment, but also significantly increase the yield of ions of the target compounds and, as a result, the sensitivity of the analysis. The effectiveness of the sorbent largely depends on the surface available for interaction with the analyte. Accordingly, it seemed interesting to investigate the formation of FLa multimolecular structures within the MALDI target cell, to study the morphology and composition of the obtained sorbent, and to explore the possibility of carrying out metal affinity chromatography in the format „Laboratory on the target" using the example of human globin adducts with oxidation products (OP) of the chlorine-containing drug amodiaquine (AQ) [6].

For this, on the target cells (inner diameter 3.1 mm) by the organic phase deposition on the aqueous subphase droplet (see additional materials, Fig. S1) one, two, four, six, and eight FLa layers were formed, in the last three cases the water droplet was removed and reapplied after the formation of every two layers. In all experiments a solution of $LaCl_3$ ($C = 10^{-2}$ mol/l, pH = 3, $V = 0.7$ μl) was used as water subphase, and as an organic subphase HSt solution in n-hexane ($V = 0.7\,\mu$l) saturated at 20°C was used. Then the formed FLa were washed twice with water and dried at 25°C. Light microscopy using scattered light was used to study the morphology of the resulting structures directly on

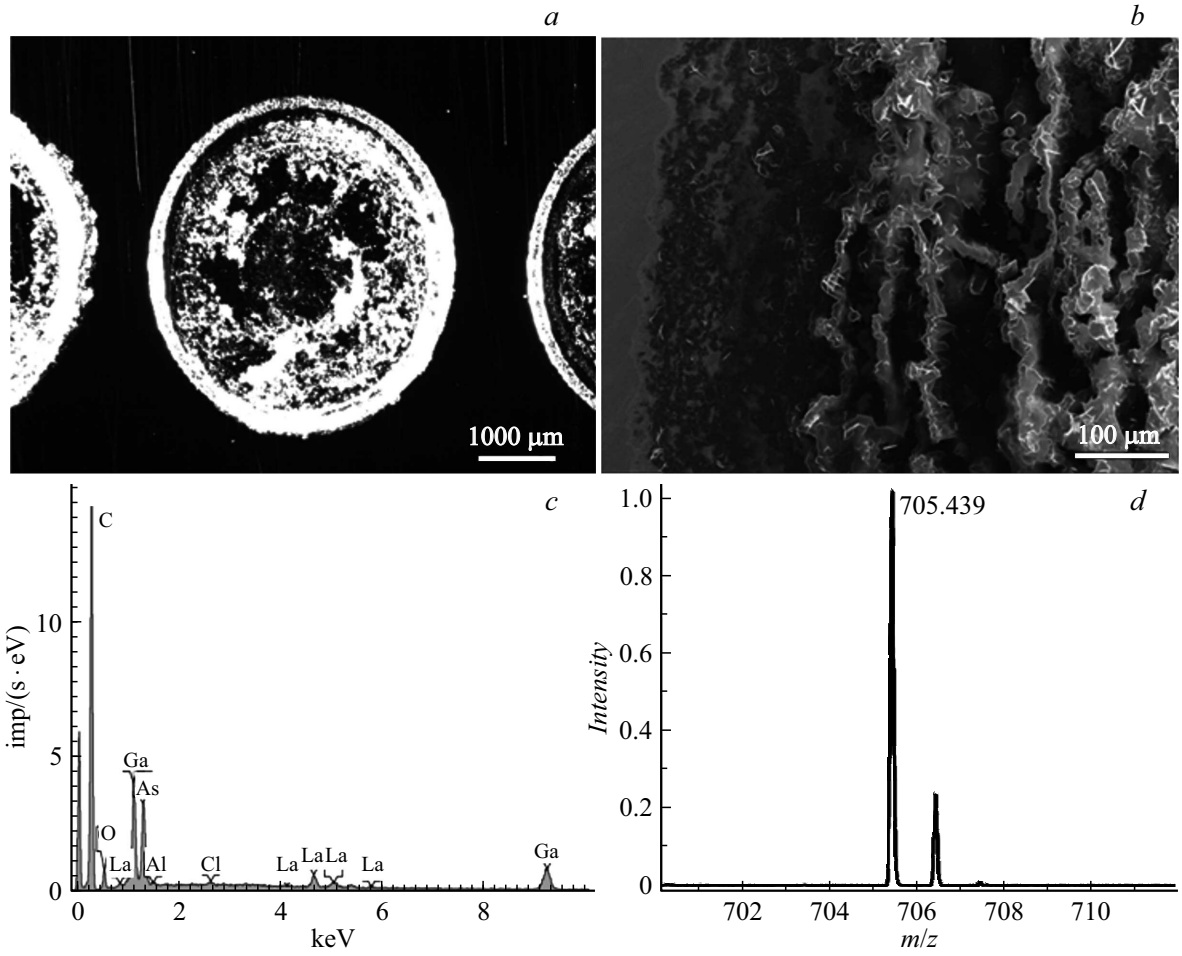

Figure 1. *a* — image of six FLa layers formed on a MALDI target (SMZ 1500 stereo microscope with DS-2MBWc digital camera (Nikon, Japan)), *b* —image of six FLa layers formed on MALDI target (S-3400N scanning electron microscope (Hitachi, Japan)), *c* — EDX spectrum of the substrate with six FLa layers formed Hitachi S-3400N scanning election microscope with EDX analytical attachment), *d* — fragment of the MALDI mass spectrum of FLa.

the substrate surface. According to the intensity of the glow it was found that with the number of layers increasing, the clusters of heteromorphic particles are formed, while the largest amount of material remains after washing when six layers of FLa are formed (Fig. 1, *a*).

Apparently, when applying a larger number of layers, the interactions between the layers are violated, and part of the formed material is not retained on the substrate. The sorbent obtained directly on the MALDI target has a characteristic structure. Multiple overlay of layer upon layer generates a specific three-dimensional structure with surface fractures. As flowing down from the droplet near the edges of the cell, n-hexane rapidly evaporates, which leads to multiple creeping of new parts of the layer onto the previous ones, their deformation and destruction. As a result, a structure with periodic thickenings and cracks is formed on the target. The results obtained using scanning electron microscopy indicate the formation on the target of the material with a fairly developed surface (Fig. 1, *b*), which, accordingly, made it possible to expect a good sorption capacity from the formed FLa. To confirm the composition the energy dispersive X-ray (EDX) spectra were obtained with six formed FLa layers, which confirmed the presence of lanthanum atoms in the composition of the structure (Fig. 1, *c*, see also additional materials, Fig. S2). The structural unit of the sorbent was determined by MALDI mass spectrometry in the reflectron mode when registering positive ions using UltrafleXtreme TOF/TOF (Bruker Daltonics, Germany) at the resource center of the Science Park of St. Petersburg State University „Development of molecular and cellular technologies“.

The analysis was carried out after applying the matrix solution (2,5-dihydroxybenzoic acid in 70

Previously, we demonstrated for the first time the possibility of extracting chlorine-containing organic compounds from samples by metal-affinity chromatography [7]. Accordingly, it seemed interesting to evaluate the specific and selective properties of the obtained FLa multimolecular structures using the example of human globin adducts with chlorine-containing compounds. We obtained amodiaquine

Table 1. The sequence of preparation of the sorbent and sample for MALDI mass spectrometric analysis in the format „Laboratory on the target"

Number of stage	Stage description	Number of stage	Stage description
1	**Application of aqueous subphase** Apply 0.7 μl of aqueous solution of lanthanum (III) nitrate with a concentration of 10^{-2} mol/l (subphase)	7	**Sorbent incubation** to the cell For sorption apply 7 μl of buffer onto the sorbent, hold for 5 min
2	**Applying a solution of stearic acid in hexane** Apply 0.7 μl of a saturated solution (20°C) of stearic acid in hexane three times on the surface of the subphase	8	**Sample sorption** Add 1 μl of the sample solution to a droplet of buffer, incubate for 20 min, place the target under the cover for the time of sorption
3	**Removal of subphase residues** Remove residual water solution	9	**Removal of unbound fraction** Collect the unbound fraction from the surface of the sorbent and, if analysis is necessary, transfer it to a free cell, add a matrix solution, i.e. α-cyano-4-hydroxycinnamic acid (5 μg/ml in 70
4	**Sorbent drying** Keep the target at room temperature for 2 min	10	**Sorbent washing** For sorption apply 7 μl of buffer onto the sorbent, hold for 1 min, delete the droplet. For sorption apply 7 μl of buffer onto the sorbent, hold for 1 min, delete the droplet
5	**Sorbent washing** On surface of layers apply 7 μl of distilled water, hold for 1 min, delete the droplet. Repeat procedure one more time	11	**Preparation for analysis** Apply 3 μl of 30trifluoroacetic acid and 2 μl of matrix solution — α-cyano-4-hydroxycinnamic acid (5 mg/ml in 70
6	**Sorbent drying** Keep the target at room temperature for 20 min	12	**MALDI mass spectrometric analysis**

Note. Stages 1–6 — blocks related to the sorbent formation on the MALDI target cell, stages 7–11 — procedure of metal affinity extraction, stage 12 — MALDI mass-spectrometric analysis.

oxidation products (see additional materials, Table S1) and modified human globin according to the method presented in the paper [8]. The object of study was the peptide LLGNVLVC$_{112}$VLAHHFGK, which is formed as a result of tryptic hydrolysis of the β-subunit of human globin. The sorbent formation and the sample preparation for analysis were carried out according to the sequence presented in Table 1. The results of MALDI mass spectrometric analysis are shown in Fig. 2. As shown in Fig. 2, in the m/z range corresponding to the peptide LLGNVLVC$_{112}$VLAHHFGK ($m/z = 1719.898$) modified by AQ OP, there are a number of intense signals with $m/z = 1999.971$, 2015.943, 2037.977, two of which, according to the results of fragment analysis, were identified as adducts of LLGNVLVC$_{112}$VLAHHFGK with OP1 and OP4 (Table 2, see also additional materials, Fig. S4, S5). By the type of isotopic distribution we also determined as adduct the signal with $m/z = 2037.977$ (OP7), but we could not attribute the difference in weights $\Delta = 318$ Da to any of the previously described AQ OPs. Nevertheless, this compound was identified by the Mascot program with a sufficient level of reliability as the peptide LLGNVLVCVLAHHFGK containing the βCys112 modification (Table 2; see also additional materials, Fig. S6). $\Delta = 318$ Da can correspond to structure with the hypothetical gross-formula $C_{16}H_{15}ClN_2O_3$. Note that in the mass spectrum of the same sample before metal-affinity extraction the signal with $m/z = 2037.977$ is absent, and the signals corresponding to adducts with OP1 and OP4 slightly exceed the noise level and are masked by signals from other peptides (see additional materials, Fig. S7). The off-target peptide signals disappearance (or a significant intensity decreasing) from the mass spectrum indicates a high level of selective and specific properties of multimolecular FLa structures formed on the MALDI target for metal-affinity extraction in the format „Laboratory on the target".

Thus, the possibility of functionalizing the MALDI target surface by multimolecular structures based on self-assembling monolayers of lanthanum stearate was shown. It was shown that the structures formed directly on the target surface have a developed surface and can be used for metal-affinity extraction of protein adducts with chlorine-containing compounds when conducting experiment in the format „Laboratory on the target", since they exhibit a high level of specific and selective properties and provide high yield of target adduct ions.

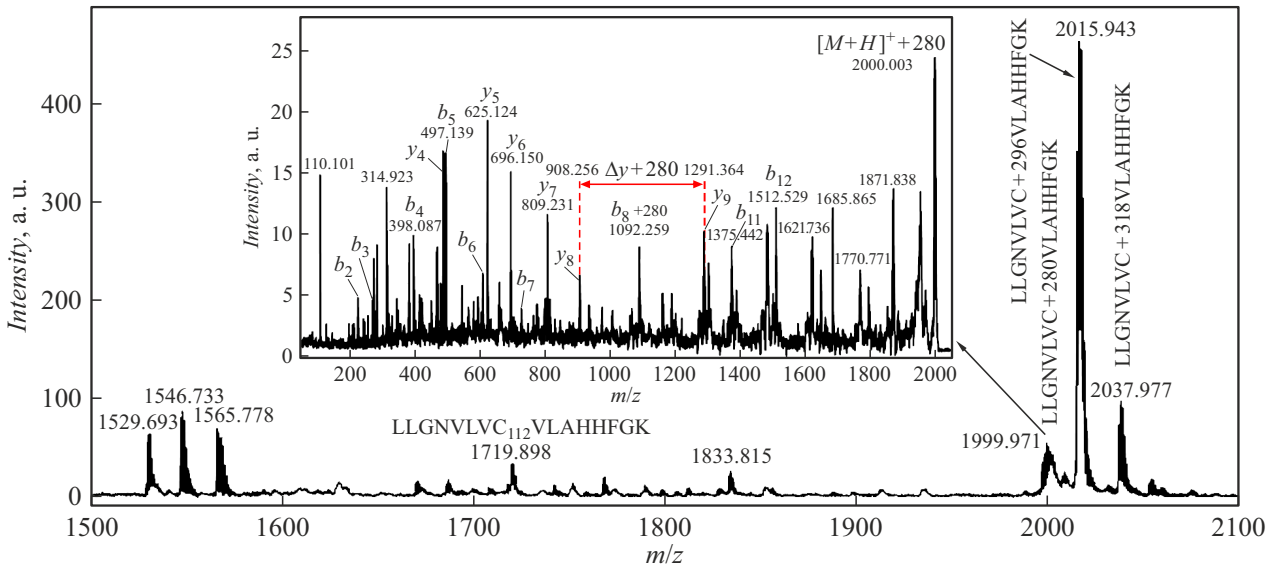

Figure 2. MALDI mass spectrum of tryptic digest of human globin modified by amodiaquine oxidation products (the LLGNVLVCVLAHHFGK β globin subunit peptide modified with AQ OP is shown: OP1, OP4 and OP7). On the insert there is a fragment of the mass spectrum of the LLGNVLVCVLAHHFGK peptide modified with PO1 over βCys112 (m/z = 1999.971).

Table 2. Identified AQ OPs forming adducts with human globin

Designation	Gross-formula $[M+H]^+$	m/z		Difference of weights, Da	Mascot score
		OP $[M+H]^+$	Adduct $[M+H]^+$		
AQ	$C_{20}H_{23}ClN_3O$	356.15242	–	–	–
OP1	$C_{20}H_{21}ClN_3O$	354.13678	1999.971	280*	86
OP4	$C_{16}H_{12}ClN_2O_2$	299.05832	2015.943	296	66
OP7	–	–	2037.977	318	47

*Taking into account the neutral loss of diethylamine 73 Da.

This paper does not contain any studies with participation of people as the object of studies.

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Conflict of interest

The authors declare that they have no conflict of interest.

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