Investigation of chemical transformations of thiophenylglycoside of muramyl dipeptide on the fumed silica surface using TPD-MS, FTIR spectroscopy and ES IT MS

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Abstract

In this study, chemical transformations of benzyl ester of O-(phenyl-2-acetamido-2,3-dideoxy-1-thio-β-D-glucopyranoside-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine (SPhMDPOBn) on the fumed silica surface were examined, and the surface complex structure was characterized by temperature-programmed desorption mass spectrometry (TPD-MS), infrared spectroscopy (FTIR) and electrospray ion trap mass spectrometry (ES IT MS). Stages of pyrolysis of SPhMDPOBn in pristine state and on the silica surface have been determined. Probably, hydrogen-bonded complex forms between silanol surface groups and the C = O group of the acetamide moiety NH-(CH3)-C = O…H-O-Si≡. The thermal transformations of such hydrogen-bonded complex result in pyrolysis of SPhMDPOBn immobilized on the silica surface under TPD-MS conditions. The shifts $\Delta \nu$ of amide I band (measured from 1,626 to 1,639 cm$^{-1}$ for SPhMDPOBn in pristine state) of 33 and 35 cm$^{-1}$ when SPhMDPOBn was immobilized on the silica surface may be caused by a weakening of the intramolecular hydrogen bonding of the SPhMDPOBn because the interaction with the silica surface as hydrogen bond with silanol groups is weaker than that in associates.

Keywords: Muramyl dipeptide; Temperature-programmed desorption mass spectrometry (TPD-MS); Pyrolysis; Thioglycosides; Electrospray ion trap mass spectrometry (ESI IT MS); Fourier transform infrared spectroscopy (FTIR)

Background

It has long been known that non-specific stimulation of the immune system can be brought about by exposure to bacteria or components extracted from bacterial cells [1]. The minimum effective structure responsible for the immunoadjuvant activities of the bacterial cell wall was identified as a sugar-containing peptide of the peptidoglycan component [2,3]. The smallest effective synthetic molecule was found to be an N-acetylmuramyl-L-alanyl-D-isoglutamine (MDP) [2,3]. MDP was found to exert numerous immunomodulatory activities. However, the administration of MDP into different hosts was always associated with serious toxicity that hampered its use in man [4]. Therefore, in an effort to generate MDP analogues with reduced toxicity and enhanced biological activities, several hundred derivatives were synthesized by chemical modification of the parent molecule [5-8].

Sulfur-containing compounds play an important role in living organisms in energy metabolism (energy production), blood clotting, and synthesis of collagen (the main protein of connective tissue in animals which is the major constituent of bones, fibrous tissues of the skin, hair, and nails) and also participate in enzyme formation. Thioglycosides are less investigated in contrast to O-glycosides. It is known that O-glycosidase is able to split O-glycosides, including of O-arylglucosides, in biological systems. Enzymes capable of cleaving the thioglycosidic bond are less common in nature and occur mainly in plants [9,10]. While O-glycosidases are ubiquitous, plant myrosinase is the
only known S-glycosidase [11]. Thioglycosides possess significantly lower susceptibility to enzymatic hydrolysis than the corresponding oxygen glycosides [12]. Also, thioglycosides have gained widespread use in carbohydrate chemistry as inhibitors of O-glycosidase and O-glycosyltransferase inhibitors [13]. Nevertheless, unlike intensively investigated O-glycosides of MDP, S-glycosides have received relatively little attention. Currently, only three S-alkyl glycosides of MDP, namely, methyl and butyl β-glycosides and hexadecyl S-glycoside, have been obtained [8], although 1-thiomuramyl dipeptide itself was found to possess the adjuvant effect close to the action of muramyl dipeptide [8]. For this reason, we synthesized the thioglycosides of MDP.

Fumed silica with controlled particle size, morphology and surface area, along with its chemical, thermal and easy functionalization properties, is suitable for application in adsorption, catalysis, chemical separation, drug delivery and biosensors [14-20]. Silica nanoparticle-MDP thioglycoside complexes’ synthesis is a way of structure modification that results in enhanced bioavailability of MDP thioglycosides and prolonged action and simplifies delivery in biological systems [21]. Partenyuk et al. [21] have demonstrated the possibility of the application of silica nanoparticles for topical delivery of the immunomodulatory drug glucosaminylmuramyl dipeptide (GMDP; the chemically synthesized natural equivalent of peptidoglycan) to the peritoneal macrophages of women with endometriosis. Researchers have shown that the immunomodulatory effect of GMDP can be increased by its immobilization on silica nanoparticles.

The aim of this study was to examine chemical transformations of thiophenylglycoside of MDP with silica surface and to characterize the structure of the adsorbed films on silica by temperature-programmed desorption mass spectrometry (TPD-MS) and Fourier transform infrared spectroscopy (FTIR).

**Methods**

**Materials**

Powdery fumed silica (pilot plant at the Institute of the Surface Chemistry, Kalush, Ukraine; with a specific surface area of 270 m²/g) was used in this work. Fumed silica was previously heated on air for 2 h at 400°C to remove adsorbed organic substances.

Benzyl ester of O-(phenyl-2-acetamido-2,3-dideoxy-1-thio-β-D-glucopyranoside-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine (SPhMDPOBn; Figure 1) was synthesized at the Department of Biological and Organic Chemistry of Taurida National V.I. Vernadsky University: SPhMDPOBn ¹H-NMR (DMSO-d₆) SAr: 7.11 to 7.24 (m, CH₃); GlcNac: 4.75 (d, J = 10 Hz), 1.79 (s, NAc), 7.98 (d, NHAc), 5.58 (d, C₄-CH₂), 4.69 (bt, C₅-CH₂); Ala: 1.25 (d, CH₃), 7.11 to 7.24 (m, NH); Glu: 12.48 (bs, CO₂R), 2.10 (t, γ-CH₂), 1.74, 1.95 (m, β-CH₂), 6.79, 7.24 (s, CONH₂), 8.28 (d, NH) [22].

The details of the synthesis procedure of SPhMDPOBn have been previously reported [22].

**Loading of MDP arylthioglycosides on the fumed silica surface**

The sample of SPhMDPOBn with a concentration of 0.6 mmol/g on the silica surface was obtained by impregnation. It is known that the concentration of free silanol groups (isolated Si-OH groups), the main active sites, on the silica surface is equal to 0.6 mmol/g of silica [23]. The weight of the MDP thioglycoside batch was such as to ensure a ratio of the concentration of modifier to that of silica surface silanol groups of 1:1. A 0.0121 g of SPhMDPOBn dissolved in 0.8 mL of 96% ethanol was added to 0.03 g of fumed silica in a Petri dish. The components were mixed and left on air at approximately 20°C till the solvent is evaporated (approximately 12 h). In the experiment, the air-dried sample was under investigation.

**Figure 1** Structure of O-(phenyl-2-acetamido-2,3-dideoxy-1-thio-β-D-glucopyranoside-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine (SPhMDPOBn).

**Figure 2** The positive-mode ESI IT mass spectrum of O-(phenyl-2-acetamido-2,3-dideoxy-1-thio-β-D-glucopyranoside-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine (SPhMDPOBn).
Instrument and procedures

**Electrospray ionization ion trap mass spectrometry analysis**

Mass spectra were obtained with the ion trap mass spectrometer Bruker HCT Plus (Bruker Daltonics, Bremen, Germany) equipped with an electrospray ionization source. Ionization was performed under electrospray conditions (flow rate 1.0 μL/min, spray voltage 4.8 kV, sheath gas 40 arb). All spectra were acquired at a capillary temperature of 25°C, and all ion guide voltages were tuned to maximize the abundance of the total ion current.

The analyte solutions (250 pmol/μL) were prepared in methanol. Methanol was of HPLC grade (Sigma, St. Louis, MO, USA).

**Fourier transform infrared spectroscopy**

FTIR spectra were recorded using a FT IR NEXUS spectrometer (Thermo Fisher Scientific Inc., Madison, WI, USA) at room temperature in the frequency range of 4,000 to 400 cm⁻¹ in diffuse reflection mode at a resolution of 4 cm⁻¹, a scan rate of 0.5 cm/s and number of scans of 150. In diffuse reflectance mode, the powdered samples were mixed with freshly calcined and milled KBr (1:100).

**Method of temperature-programmed desorption mass spectrometry**

TPD-MS experiments were performed in a MKh-7304A monopole mass spectrometer (Electron, Sumy, Ukraine) with electron impact ionization, adapted for thermodesorption measurements. A typical test comprised placing a 20-mg sample on the bottom of a molybdenum-quartz ampoule, evacuating to approximately 5 × 10⁻⁵ Pa at approximately 20°C and then heating at 0.15°C/s from room temperature to approximately 750°C. For all the samples, the sample vials were filled approximately 1/16 full, which helped limit interparticle diffusion effects [24-28]. Limiting the sample volume along with the high vacuum should further limit readsorption and diffusion resistance as described elsewhere [24-33]. The volatile pyrolysis products was passed through a high-vacuum valve (5.4 mm in diameter, a length of 20 cm and a volume of 12 mL) into the ionization chamber of the mass spectrometer where they were ionized and fragmented by electron impact. After mass separation in the mass analyzer, the ion current due to desorption and pyrolysis was amplified with a VEU-6 secondary-electron multiplier ("Gran" Federal State Unitary Enterprise, Vladikavkaz, Russia). The mass spectra and the P-T curves (where P is the pressure of volatile pyrolysis products, and T is the temperature of the samples) were recorded and analyzed using a computer-based data acquisition and processing setup. The mass spectra were recorded within 1 to 210 amu. During each TPD-MS experiment, approximately 240 mass spectra were recorded and averaged.
During the thermodesorption experiment, the samples were heated slowly while keeping a high rate of evacuation of the volatile pyrolysis products. The diffusion effects can thus be neglected, and the intensity of the ion current can be considered proportional to the desorption rate.

Results and discussion

Electrospray ionization ion trap mass spectrometry analysis of O-(phenyl-2-acetamido-2,3-dideoxy-1-thio-β-D-glucopyranoside-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine

The electrospray ionization ion trap mass spectrum (ESI IT MS) of SPhMDPOBn (Figure 2) shows a peak at m/z 697 which corresponds to the sodium adduct molecular ion \([\text{M + Na}]^+\). The product ion at m/z 469 is most probably derived from m/z 402 fragment ion of SPhMDPOBn: \([\text{M-C}_10\text{H}_11\text{O}_2\text{-C}_6\text{H}_5\text{S} + 3\text{Na}^+-2\text{H}^+]^+\). The ion at m/z 247 was identified as [M + 3Na]^{3+}/3.

TPD-MS analysis of O-(phenyl-2-acetamido-2,3-dideoxy-1-thio-β-D-glucopyranoside-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine

As can be seen from the P-T curve (Figure 3), pyrolytic degradation of thiophenylglycoside of MDP in the pristine state proceeds in a relatively narrow temperature range from 150°C to 250°C in two main stages (Figure 4). The same two main stages are observed on the TPD-curves (Figure 5). Probably, these stages of pyrolysis result from the existence of SPhMDPOBn in α- and β-anomeric forms. Figure 4 illustrates a possible pyrolytic pattern and products.

At the first and the second stages (Figure 5), the elimination of the benzyl ester-protected carboxylic group of isoglutamine fragment takes place, which gives rise to a peak of the molecular ion of benzyl alcohol at m/z 108 (Figure 4). Fragmentation of benzyl alcohol via loss of the -OH group at m/z 17 leads to a common fragment seen for alkyl benzenes at m/z 91. Loss of CH₂OH at m/z 31 from the molecular ion gives m/z 77 corresponding to the phenyl cation (Figure 4). Loss of aglycone and carbohydrate moiety occurs during the first and the second stages of pyrolysis. But it is observed that there are different ratios of peak intensities on the TPD-curve for molecular and fragment ions of corresponding products. Thus, the first stage proceeds via preferential removal of benzyl alcohol, while the second stage-by elimination of thiophenol. Aglycon is easily removed in the form of thiophenol under the pyrolysis of SPhMDPOBn. The intensity of a thiophenol molecular ion peak is high as the thiophenol molecular ion is stable. The thiophenol molecular ion is stabilized by the presence of π-electron systems, which are capable of accommodating a loss of one electron more easily. The fragmentation of thiophenol molecular ion under electron impact is shown in Figure 6. Isomerization of the molecular ion of thiophenol to thioketone can take place, as the fragmentation pattern used here provides a reasonable explanation of the observed release of -CS and fragment ions from the molecular ion (Figure 6).

Moreover, the characteristic peak at m/z 125 common to amino sugars is observed in the mass spectrum [34]. Pyrolysis of SPhMDPOBn on the silica surface is more complex. As can be seen from the P-T curve (Figure 7), pyrolysis begins at a lower temperature and proceeds in a wider temperature range. At the same time, there are products such as thiophenol, benzyl alcohol and carbohydrate fragment with m/z 125, which were observed during the pyrolysis of SPhMDPOBn in the pristine state. However, the sequence of their stages...
and temperature range are changing. Thermal decomposition of SPhMDPOBn on the silica surface (Figures 7 and 8) also proceeds via the elimination of aglycon and carbohydrate moieties. The set of peaks in mass spectra of SPhMDPOBn adsorbed on the silica surface (Figure 8) is the same as that for the pyrolysis of pristine SPhMDPOBn (Figure 5).

Probably, a hydrogen-bonded complex forms between the silanol surface groups and the C = O group of the acetamide moiety: NH-(CH₃)-C = O...(H-O-Si≡. The thermal transformations of such hydrogen-bonded complex results in the pyrolysis of SPhMDPOBn immobilized on the silica surface under TPD-MS conditions.

**FTIR spectroscopy**

The IR spectra of the silica sample are depicted in Figure 9. The band at 3,745 cm⁻¹ is assigned to the stretching vibration of isolated silanol groups (≡Si-OH). The wide band in the 3,700- to 3,000-cm⁻¹ interval corresponds to the overlapping of the O-H-stretching modes of adsorbed water and Si-OH stretchings [35,36]. A small peak at approximately 1,628 cm⁻¹ can be attributed to the proton-containing components σOH (silanol groups and the deformation vibrations of the O-H groups in physically adsorbed molecular water at the silica surface) [37-39]. Bands centered at 1,980 and 1,867 cm⁻¹ represent overtones and combinations of intense Si-O fundamental modes (two component bands of Si-O-Si stretching modes) (Table 1).

The Si-O-Si and Si-O vibration bands appeared, respectively, at 1,083 and 809 cm⁻¹ for the silica sample. The symmetric vibrations of the silicon atoms in a siloxane bond occur at approximately 809 cm⁻¹ (νas-Si-O-Si). The largest peak observed in the silica spectrum is present at approximately 1,197 cm⁻¹ and is dominated by
antisymmetric motion of silicon atoms in siloxane bonds ($\nu_{\text{as}}\text{-Si-O-Si}$).

The infrared spectra of SPhMDPOBn can be divided into several spectral regions. The IR spectra of SPhMDPOBn in the range 4,000 to 3,100 cm$^{-1}$ are dominated by absorption arising from the symmetric and asymmetric N-H stretching modes. The IR spectrum of SPhMDPOBn adsorbed on the silica surface in the range 4,000 to 3,100 cm$^{-1}$ shows a widened band near 3,313 cm$^{-1}$ representing the N-H stretching mode, which is partially overlapped by the bands of the silica matrix (Figure 9). The maximum at 3,313 cm$^{-1}$ is assigned to the N-H groups which were involved in hydrogen bonding interactions with the surface hydroxyl groups.

The bands in the IR spectra of SPhMDPOBn in the pristine state and adsorbed on the silica surface in the region 3,100 to 2,800 cm$^{-1}$ are assigned as the symmetric and antisymmetric stretching vibrations of the C-H bonds in a methylene group (in pristine state: $\nu_s = 2,850$ cm$^{-1}$ and $\nu_{\text{as}} = 2,925$ cm$^{-1}$; on the silica surface: $\nu_s = 2,850$ cm$^{-1}$ and $\nu_{\text{as}} = 2,931$ cm$^{-1}$).

The 1,800- to 1,700-cm$^{-1}$ region involves bands due to the C=O stretching modes of benzyl ester-protected carboxylic group of isoglutamine fragment. The bands at 1,724 cm$^{-1}$ in the spectrum of SPhMDPOBn in pristine state and at 1,728 cm$^{-1}$ on the silica surface referred to the ester C=O stretch mode.

Table 1 Assignments of the main silica bands in the 700- to 4,000 cm$^{-1}$ region

| Band maximum (KBr powder, cm$^{-1}$) | Assignment | Reference |
|----------------------------------|------------|-----------|
| 3,745                            | $\nu$ (isolated silanol groups) Si-OH | [38,40] |
| 3,700 to 3,000                   | $\nu$ hydrogen-bonded silanols (overlapping of the stretching modes in hydrogen-bonded hydroxyl bands produced by O-H bonds in adsorbed water and Si-OH) | [38,40] |
| 1,867 and 1,980                  | Si-O-Si stretching modes | [38,40] |
| Approximately 1,628 to 1,630     | Proton-containing components $\sigma_{\text{OH}}$ (silanol groups and the deformation vibrations of the O-H groups in physically adsorbed molecular water at the silica surface) | [37-39] |
| Approximately 1,083               | Si-O-Si stretching | [38,40] |
| 1,000 to 1,300                    | $\nu_{\text{as}}$, anti-symmetric stretching of Si-O-Si bonds | [38] |
| 932 to 939                       | Si-OH stretching | [38,40] |
| Approximately 809                | Bending vibration of Si-O-Si bonds | [38,40] |
| Approximately 790                | Bending modes in Si-OH bonds | [38,40] |

$\nu_{\text{as}}$, asymmetric/symmetric stretching mode.
Table 3 Assignments of amide bands to the secondary structure of peptides and proteins (literature data)

| Assignment     | Amide I (ν (cm⁻¹)) | Amide II (ν (cm⁻¹)) | Reference |
|----------------|--------------------|---------------------|-----------|
| α-helix        | 1,649; 1,653 to 1,657; 1,655 | 1,545 | [45] |
|                | 1,648 to 1,660 | - | [46] |
|                | 1,650 to 1,652 | 1,540 to 1,546; 1,516 | [47] |
| β-sheet        | 1,621 to 1,623; 1,630; 1,634 to 1,639; 1,647 to 1,648 | 1,530 | [45] |
|                | 1,620 to 1,640; 1,670 to 1,695 | - | [46] |
|                | 1,633 | 1,530 | [47] |
| β-turn         | 1,661; 1,667; 1,673; 1,677 | 1,528; 1,577 | [45] |
|                | 1,620 to 1,640; 1,650 to 1,695 | - | [46] |
|                | 1,663; 1,670; 1,683; 1,688; 1,694 | - | [47] |
| Random coil    | 1,648; 1,654; 1,642 to 1,657 | - | [45] |
|                | 1,640 to 1,657; 1,660 to 1,670 | - | [46] |

This is indicated on the hydrogen bonding of the SPhMDPOBn molecule with silanol groups. The amide I band at 1,626 and 1,639 cm⁻¹ was shifted to 1,659 and 1,674 cm⁻¹, respectively, for adsorbed-on-silica SPhMDPOBn molecules. That is, the amide I band is shifted to higher wavenumbers (Figure 9, Table 2). The shift of the amide I band of the adsorbed SPhMDPOBn by 33 and 35 cm⁻¹, respectively, to higher wavenumbers may be caused by a weakening of the intramolecular hydrogen bonding of the SPhMDPOBn because of the interaction with the silica surface [41,42]. This testifies that the binding to the silica surface occurs due to peptide fragment resulting in the change of its conformation under adsorption. The amide II band represents mainly N-H bending with the C-N stretching vibrations and is conformationally sensitive. The amide II of SPhMDPOBn in pristine state absorbs at 1,535 and 1,568 cm⁻¹. The amide II of SPhMDPOBn on the silica surface has a complex structure and centered at 1,547 cm⁻¹ (Figure 9, Table 2).

Earlier using 1H-NMR and nuclear Overhauser effect spectroscopy, it was shown that MDP consists of two type II adjacent β-turns forming an S-shaped structure [43,44]. The first β-turn is formed by a C10 hydrogen bonding between the Ala NH and the acetamido C = O of N-acetylmuramic acid. The existence of the second β-turn is assumed by the presence of a free carboxamide group of isoglutamine [43,44]. Correlation between amide frequency and protein secondary structure found in the literature is listed in Table 2. We can assume from the comparison correlation between amide frequency in FTIR spectra of SphMDPOBn (Table 2) and protein secondary structure found in the literature (Table 3) that, probably, SphMDPOBn in the pristine state adopt β-sheet conformation and in the adsorb state, combination of β-sheet and β-turn structures.

The spectral region 1,400 to 1,200 cm⁻¹ is characterized by overlapping deformation vibrations of the C-H bond in...
methyl and methylene groups of peptide fragment, stretching vibrations of the C-O bond in carbonyl group and amide III vibrations (stretching vibrations of C-N bond and N-H bend in plane) and the Si-O-Si, Si-O and O-Si-O vibration bands of the silica matrix.

Conclusions

The stages of pyrolysis of aglycone, peptide fragment and carbohydrate residue of thiophenylglycoside of muramyl dipeptide in the pristine state and adsorbed on the silica surface have been determined. Decomposition of thiophenylglycoside of muramyl dipeptide in pristine state occurs within the narrow temperature range from 150°C to 250°C. The decomposition of thiophenylglycoside of muramyl dipeptide adsorbed on the silica surface undergoes certain reactions to produce pyrolys products such as thiophenol, benzyl alcohol and carbohydrate fragment with m/z 125 in the temperature range from 50°C to 450°C. Probably, the hydrogen-bonded complex forms between silanol surface groups and the C = O group of the acetamide moiety NH-(CH₃)-C = O...H-O-Si≡. The thermal transformations of such hydrogen-bonded complex result in the pyrolysis of SPhMDPOBn immobilized on the silica surface under TPD-MS conditions.

The intensity of the infrared band at 3,745 cm⁻¹ assigned to the OH stretching vibrations of isolated silanol groups on silica decreased after the immobilization of SPhMDPOBn. This indicated the hydrogen-bonding of SPhMDPOBn molecule with silanol groups.

The shifts Δν of the amide I band (measured from 1,626 to 1,639 cm⁻¹ for SPhMDPOBn in the pristine state) of 33 and 35 cm⁻¹ which occurred when SPhMDPOBn was immobilized on the silica surface may be caused by a weakening of the intramolecular hydrogen bonding of the SPhMDPOBn because the interaction with the silica surface as hydrogen bond with silanol groups is weaker than that in the associates.

Abbreviations
ESI-IT MS: electrospray ion trap mass spectrometry; FTIR: Fourier transform infrared spectroscopy; MDP: muramyl dipeptide; NMR: nuclear magnetic resonance; SPhMDPOBn: O-(phenyl-2-acetamido-2,3-dideoxy-1-thio-β-D-glucopyranoside-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine; ESI IT MS: electrospray ion trap mass spectrometry; FTIR: Fourier transform infrared spectroscopy; MDP: muramyl dipeptide; NMR: nuclear magnetic resonance; SPhMDPOBn: O-(phenyl-2-acetamido-2,3-dideoxy-1-thio-β-D-glucopyranoside-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine; TPD-MS: temperature-programmed desorption mass spectrometry.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
LRA obtained the silica-supported SPhMDPOBn sample, carried out the electrospray ionization trap mass spectrometric investigation and FT-IR spectroscopic investigation, and drafted the manuscript. LRA together with TVK conceived of the study and participated in its design and interpretation of TPD-MS and FT-IR investigation results. BBP obtained the TPD-MS spectra of SPhMDPOBn in the pristine state and on the silica surface. VNT together with LRA carried out the synthesis of SPhMDPOBn. AEZ together with VYC participated in the design and coordination of the synthesis of SPhMDPOBn. All authors read and approved the final manuscript. We declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

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