In Situ and Timed Extraction of Cellular Peptides from Live HeLa Cells by Photo-Switchable Mesoporous Silica Nanocarriers

Yi Zhang,‡ Jiaxi Peng,†‡ Zheyi Liu,†‡ Hanfa Zou,†§ and Ren’an Wu*†‡

†CAS Key Lab of Separation Sciences for Analytical Chemistry, National Chromatographic R&A Center, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, Liaoning 116023, China
‡University of Chinese Academy of Sciences, Beijing, 100049, China

Supporting Information

ABSTRACT: In situ and timed extraction of cellular peptides is a great challenge for dynamic and global proteomic investigation of live cells. In this work, a mesoporous silica nanocarrier with photoswitchable off/on coumarin gates (MSNcg) was developed for capturing peptides from the cytosol of living HeLa cells. The MSNcg was constructed from mesoporous silica nanoparticle (MSN) and its subsequent modifications with TAT peptides and coumarin, to endow the features of the size-exclusion effect of the mesoporous silica and the localization of nanocarrier at cytosol by TAT peptide and to control the closing and opening of the coumarin gates by reversible photodimerization and photocleavage. With the pre-endocytosing of MSNcg, 126 cytosol peptides were harvested and identified from living HeLa cells. Moreover, 3 peptides were captured containing dynamic and changeable information. The extraction strategy of using MSNcg exhibited promising potentials in the in situ and dynamic extraction of endogenous peptides and/or proteins from living systems.

Pepidome, the low-molecular-weight (LMW) subset of proteome, could be highly dynamic as proteome in localization, abundance, and interaction for living systems.1,2 Trials on the in situ and dynamic profiling of peptidome/proteome have been tested at subcellular range by performing the prefraction of subcellular components in vitro with the isotope labeling at different time points and the subsequent quantitative approaches.3–5 Other than the in vitro collection and labeling strategies, the strategies to collect peptides and proteins from physiological circumstances by keeping the highly temporal and spatial resolution information such as the in situ extraction with live cells without the chemical derivatizations are greatly preferred for proteomic investigation.6

Nanoprobes, owning high biocompatibility and abilities for subcellular targeting and controllable intracellular transporta-
tion, have promising potential to extract cellular components from live cells in situ. In earlier attempts, heparin sulfate proteoglycans (HSPGs) antibody functionalized magnetic particles were employed to collect targeted endocytic vesicles for proteomic analysis of HSPGs induced endocytosis;7 methotrexate (MTX) linked dendrimers were applied in proteomic discovery of drug substrates from live cells,8 and magnetic carbon nanotubes were used to harvest nucleic acid associated proteins in situ coupled with proteomic exploration.9 However, cellular proteins/peptides would be adsorbed on the nanocarriers as soon as the nanocarriers inside the cells were exposed, which makes the dynamic extraction of proteins/peptides at certain loci and time periods not possible as the timed trigger action in situ for conventional nanoprobes is lacking.

To achieve the in situ and timed harvest of intracellular proteins and/or peptides from a living system, the extraction probes should meet the following essential requirements: (1) be biocompatible with live cells; (2) be suitable for both general or selective extraction of intracellular proteins/peptides; be capable to harvest compounds at (3) designated intracellular loci and at (4) an appointed time period. So far, the conventional proteome/peptidome sampling methods including microextraction and electroporation could hardly satisfy all of the above requirements.10–12

In this paper, a photoswitchable mesoporous silica nanocarrier (MSN with coumarin gates, MSNcg) was constructed as a general nanoplatform to collect cytosol peptides from live HeLa cells for the in situ and timed proteomic investigation. Mesoporous silica nanoparticles (MSNs) with high specific area, size exclusion effect, excellent biocompatibility, and capability in peptide sampling/drug delivery13–17 were selected as the base material. Coumarin gates functionalized on MSN (MSNcg) were the photocontrollable switches, which were robust, controllable, and reversible under the control of UV irradiation with different wavelengths (>310 nm to photo-
dimerize as cyclobutane coumarin dimers in head-to-head configuration for closing; 256 nm to photomonomononimize for opening. (Figure 1). Meanwhile, ingestion of MSN-based nanomaterials in cells goes through endocytosis, hinting that MSN would mostly be localized in endocytotic vesicles, beyond the contact with cytosol polypeptides. TAT peptides, which come from HIV-tat proteins and could assist nanoprobes in escaping from the endocytotic vesicles, were functionalized on the MSN to assist the cytosol localization of MSNcg. In this case, MSNcg was assigned to be ingested by live HeLa cells and localized in cytosol for in situ contact with cytosol peptides; the opening/closing of the coumarin switches of MSNcg could be optimized for the initiation/termination of the in situ extraction (Scheme 1).

MSN was synthesized by the hydrolysis of TEOS with CTAB as template, with particle size under 100 nm (TEM in Figure S-1a), specific area of 887.7 m²/g, and pore size of ca. 3.0 nm (Figure S-1b). The maximum adsorption of ca. 270 mg/g on MSN for peptides could be reached within 15 min (Figure S-1c). The main mechanism for peptide adsorption in the MSN might be the hydrophobic interaction, since the elution of peptides was more efficient using higher concentrations of CH₃OH solution (Figure S-1d). The MSN functionalized with coumarin gates was characterized by FT-IR to illustrate the existence of lactone from coumarin at 1710 cm⁻¹ (Figure S-1e). After amination of MSNcg, TAT peptides were linked on MSNcg by the EDC/sulfo-NHS approach.

At first, the optimal operation of the coumarin gates was monitored by UV spectra during UV irradiation. The dimerization of coumarin (closing) could be observed according to the decrease of the adsorption band at 324 nm under a 10 min UV irradiation at 310 nm (1.67 W/cm²), which could be monomerized (opening) with the band increase at 324 nm by UV irradiation at 256 nm (0.50 W/cm²) for 2 min. The opening/closing of the coumarin gates on MSN could be repeated for more than 3 times, showing good reversibility of the molecular switches in MSNcg (Figure 1). Then, the retention of peptides in the opened/closed MSNcg (with the coumarin gates opened or closed) was performed to evaluate the feasibility for timed extraction of peptides in vitro. (1) To test the preservation of collected peptides by coumarin gates, opened MSNcg (4 mg) was utilized to collect peptides (heavy isotope labeled BSA digested peptides, H pep, +32 Da), after which the MSNcg was closed by UV treatment (>310 nm) and washed completely with an elution buffer (15% methanol, 85% H₂O, 0.1% TFA) until no peptides could be detected in the washing solution. After the coumarin gates reopened under UV treatment (256 nm), peptides elution, and MALDI-TOF MS analysis, abundant H pep could be detected from those closed MSNcg; in contrast, almost no peptides (light isotope labeled BSA digested peptides, L pep, +28 Da) were left in pristine MSN during the washing procedure (Figure 2a). Moreover, MSNcg containing peptides were treated with the elution buffer for 2 h, while no peptide signals could be detected in the supernate by MALDI-TOF MS analysis, hinting that the coumarin gates could preserve the extracted peptides inside the MSNcg from the washing step. (2) The capability of MSNcg to resist interferences during extraction was evaluated by mixing interference peptides (L pep, 4 mg) with the closed MSNcg (1 mg) containing extracts (H pep, 4 mg). After washing and analysis, only precollected H pep was detected (Figure 2b), hinting that the closed pores could effectively prevent the diffusion of irrelevant components into pores. (3) The repeatability of the timed and in situ extraction with MSNcg (4 mg) was investigated by comparing the relative abundance of extracted peptides (1 mg of L pep in TEST 1 and 1 mg of H pep in TEST 2) in different tests by LC-MS/MS (3 group of tests in 3 days). The ratios of intensities for the identified H pep against the L pep were all around 1, and 80% (32/40) of peptides were identified in more than 4 tests during 2 days, exhibiting good repeatability during the whole sampling and analysis procedures (Figure S-2). (4) Since intracellular peptides are dynamic in physiological environments, the controllable and timed extraction by MSNcg should be able to obtain the changing details of the peptides. Thus, peptide mixtures (with ratios of H pep/L pep of 1/2 and H pep/L pep of 2/1, respectively) were assigned as samples at different time points. The MSNcg could successfully obtain the artificially changeable peptides with different ratios of isotope labels (Figure 2c).

Scheme 1. Schematic Illustration of the Photo-Controllable and in Situ Extraction of Cytosol Peptides from Live HeLa Cells by MSNcg
The cytotoxicity of MSNcg and UV irradiation was evaluated before further application in living cells. MSNcg with concentrations of 50, 100, 150, 200, and 250 mg/mL was incubated with HeLa cells for 3, 6, 12, 24, and 48 h, with cell viabilities kept above 90% according to the Cell Counting Kit-8 (CCK-8) assay (Figure S-3), exhibiting excellent biocompatibility of the MSNcg. The operation of coumarin gates employed UV irradiation, the influence of which for cells was also investigated (Figure S-4), and no obvious apoptosis could be observed during the gate opening procedure. When closing the coumarin gates inside cells, changes inside cells could hardly disturb the peptides that already adsorbed in the channel of MSNcg. Thus, the light operating procedure would be of little influence for the in situ extraction of peptides in living cells. The localization of MSNcg in HeLa cells was observed with TEM imaging, with most of the MSNcg found in the cytosol (Figure 3). Without TAT modification, pristine MSN could only be found in intracellular vesicles. Therefore, the MSNcg with the TAT modification could be utilized for the in situ extraction of polypeptides from the cytosol of live cells.

MSNcg could be ingested by living HeLa cells and localized at the cytosol, suggesting the direct and in situ contact with cytosol peptides. According to the in vitro investigation, the coumarin gates of MSNcg could preserve and maintain the states of the extracted peptides, so the MSNcg was able to initiate and terminate the timed extraction by opening and closing the coumarin gates with photons at different UV wavelengths. The feasibility of the coumarin gates in living cells was also examined with MSNcg containing doxorubicin (DOX) as the fluorescent dye, and more DOX could be observed after the coumarin gates in MSNcg were opened (Figure 4). Later, the MSNcg was utilized in the extraction of cytosol peptides in situ of living HeLa cells. By LC-MS/MS analysis, 126 cytosol polypeptides could be extracted during the opening and closing of the MSNcg; in contrast, only 4 peptides could be obtained with the pristine MSN due to the washing treatments for eliminating the nonspecifically adsorbed compounds outside the nanocarriers (Table S-1a,b). Furthermore, less than 10% of peptides obtained with MSNcg were over 2000 Da, while there...
were more than 60% of peptides collected using ultrafiltration (MWCO 10 000) over 2000 Da, implying the feasibility of the size exclusion effect of MSNcg in living HeLa cells (Figure S-5). As a further attempt for the dynamic and in situ extraction of changing peptides from live cells, the HeLa cells were treated with RPMI 1640 culture medium containing isotope labeled lysine (stable isotope labeling by amino acids in cell culture, SILAC22), from light lysine (K0, +0 Da) to heavy lysine-D4 (K4, +4 Da) for 0, 12, and 24 h (Figure S). At first, cytosol polypeptides in HeLa cells were light peptides containing K0. As the replacement with culture medium containing K4, newly synthesized peptides would become heavy peptides with K4. Thus, the ratio of heavy peptides and light peptides would keep changing during the SILAC treatments. MSNcg was utilized to harvest the cytosol peptides after SILAC treatments for 0, 12, and 24 h, and with LC-MS/MS analysis, 3 peptides (VIDNGSGMCK, CTGESGAGKTENT, and SSQTQGGG-88) were synthesized with K4. Namely, the method which could only be synthesized with K4. As the replacement with culture medium containing K4, newly synthesized peptides would become heavy peptides with K4. Therefore, the abundance of heavy lysine (K4, +4 Da) labeled peptides (red) increased and light lysine (K0, +0 Da) labeled peptides (blue) decreased.

In summary, a photoswitchable nanocarrier of the MSNcg was constructed for the in situ and timed sampling of cytosol peptides during in situ extraction (PDF) from live HeLa cells. During the isotope labeling treatments, abundance of heavy lysine (K4, +4 Da) labeled peptides (red) increased and light lysine (K0, +0 Da) labeled peptides (blue) decreased. Moreover, MSNcg could accomplish the timed extraction of peptides by opening and closing of the coumarin gates, and TAT peptides guided the localization of MSNcg in cytosol for in situ capture. Molecular characterization of materials, repeatability of the controllable extraction with MSNcg, viability of cells under treatments with MSNcg and UV irradiation, molecular weight of cellular peptides obtained with MSNcg and ultrafiltration, and detailed information on extracted peptides during in situ extraction (PDF).

**REFERENCES**

(10) Spengler, B. Anal. Chem. 2015, 87, 64–82.

**ACKNOWLEDGMENTS**

Financial support from the Creative Research Group Project of NSFC (21321064), the National Natural Science Foundation of China (Nos. 21235006, 21175134, 21375125, and 21675156), the Instrument Developing Project of the Chinese Academy of Sciences (YZ201503), and the Translational Medicine Research Program of Dalian Institute of Chemical Physics is gratefully acknowledged.

**AUTHOR INFORMATION**

**Corresponding Author**

E-mail: wurenan@dicp.ac.cn.

**Notes**

The authors declare no competing financial interest.


**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.6b02447.

Detailed information for the experimental procedure, characterization of materials, repeatability of the controllable extraction with MSNcg, viability of cells under treatments with MSNcg and UV irradiation, molecular weight of cellular peptides obtained with MSNcg and ultrafiltration, and detailed information on extracted peptides during in situ extraction (PDF).