Cleavable ester linked magnetic nanoparticles for labeling of solvent exposed primary amine groups of peptides/proteins

Ujwal S. Patil, Laura Osorno, Angela Ellender, Casey Grimme, Matthew A. Tarr

Department of Chemistry, University of New Orleans, 2000 Lakeshore Drive, New Orleans, LA 70148, USA
Southern Regional Research Center, 1100 Robert E. Lee Blvd., New Orleans, LA 70124, USA

Abstract

Covalent labeling of solvent exposed amino acid residues using chemical reagents/crosslinkers followed by mass spectrometric analysis can be used to determine the solvent accessible amino acids of a protein. A variety of chemical reagents containing cleavable bonds were developed to label abundantly found lysine residues on the surface of protein. To achieve efficient separation of labeled peptides prior to mass spectrometric analysis, magnetic nanoparticles can be decorated with amino acid reactive functional groups and utilized for quick recovery of labeled peptides. In this work, iron oxide magnetic nanoparticles (Fe3O4 MNPs) were synthesized by thermal decomposition method and coated with silica (SiO2@Fe3O4 MNPs) by reverse micro emulsion approach. The Fe3O4 MNPs and SiO2@Fe3O4 MNPs were characterized by TEM and XRD. The SiO2@Fe3O4 MNPs were further coated with amine groups and conjugated to N-hydroxysuccinimidyl (NHS) ester groups via a cleavable ester bond. Fluorescence based qualitative analysis of ester linked NHS ester modified SiO2@Fe3O4 MNPs was performed to confirm the presence of NHS ester group. The active NHS ester sites on the surface of SiO2@Fe3O4 MNPs were determined by depletion approach and found to be 694 active sites per 1 mg of SiO2@Fe3O4 MNPs. Free amine groups of a small peptide, ACTH (4–11) were labeled by ester linked, NHS ester modified SiO2@Fe3O4 MNPs under physiological conditions.

DOI of original article: http://dx.doi.org/10.1016/j.ab.2015.05.006
* Corresponding author.
E-mail address: mtarr@uno.edu (M. Tarr).
Superparamagnetic nature of SiO₂@Fe₃O₄ MNPs allowed quick and efficient magnetic separation of labeled peptides from the solution. The ester bond was further cleaved to separate labeled peptides followed by mass spectrometric analysis. The ester linked, NHS ester modified SiO₂@Fe₃O₄ MNPs introduced a mass shift of 115.09 Da on amine groups of ACTH (4–11), which was confirmed by mass spectrometry.

© 2015 Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Specifications table

| Subject area       | Biochemistry, Materials chemistry |
|--------------------|-----------------------------------|
| More specific subject area | Surface Proteomics               |
| Type of data       | Text file, figure                 |
| How data was acquired | X-ray diffraction, mass spectrometry, and fluorescence spectroscopy |
| Data format        | Analyzed                          |
| Experimental factors | The SiO₂@Fe₃O₄ MNPs were modified with NHS ester groups via a cleavable ester bond. Quantitative flurometric characterization of ester linked NHS ester modified SiO₂@Fe₃O₄ MNPs was performed to determine active NHS ester sites on the surface of SiO₂@Fe₃O₄ MNPs. Solvent exposed free amine residues of peptides were labeled using cleavable ester linked NHS ester linked silica coated iron oxide magnetic nanoparticles. The labeling reaction was performed under physiological conditions to preserve the native structure of proteins. The ester bond was subsequently cleaved followed by magnetic separation of nanoparticles |
| Experimental features | The label generated on the solvent exposed free amine groups of peptides and proteins were identified by mass spectrometric analysis. |
| Data source location | New Orleans, Louisiana, USA       |
| Data accessibility | Data is included in this article   |

2. Value of the data

- The surface exposed amine groups of peptides can be determined by labeling with ester linked NHS ester modified SiO₂@Fe₃O₄ MNPs under physiological conditions.
- Cleavable ester linked NHS ester modified SiO₂@Fe₃O₄ MNPs provide an effective approach to magnetically separate the labeled peptides from the solution without adding extra step of purification.
- The flurometric quantification of active NHS ester sites on the surface of SiO₂@Fe₃O₄ MNPs can allow quantitative control over the labeling reaction.

3. Data, experimental design and methods

The data shown here is divided into four major steps: a) synthesis and characterization of SiO₂@Fe₃O₄ MNPs, b) synthesis of ester linked NHS ester modified SiO₂@Fe₃O₄ MNPs, c) fluorometric quantification of active NHS ester sites on the surface of SiO₂@Fe₃O₄ MNPs and, d) labeling and identification of primary amine groups of ACTH (4–11) using ester linked NHS ester modified SiO₂@Fe₃O₄ MNPs.
4. Materials and methods

Iron (III) oxyhydroxide (FeO(OH)), oleic acid, dimethyl suloxide (DMSO, anhydrous), (3-aminopropyl)triethoxysilane (APTES, 95%), tetraethylorthosilicate (TEOS, 99%), Igepal CO-520, cyclohexane, dansylcadaverine, ≥ 97%, hydroxylamine hydrochloride, bovine serum albumin (BSA) and β-lactoglobulin were purchased from Sigma-Aldrich (St. Louis, MO). 1-Octadecene was purchased from Alfa Aesar (Ward Hill, MA). Ethylene glycolbis(succinimidylsuccinate) (EGS, +99%) was purchased from ProteoChem (Loves Park, IL). Phosphate buffered saline (PBS) was purchased from Calbiochem (Billerica, MA). Dithiothreitol and iodoacetamide were purchased from Piercenet, Thermo Scientific (Rockford, IL). ACTH (4–11) was purchased from American Peptide Company (Sunnyvale, CA). Ethanol was purchased from Pharmco-AAPER (Brookefield, CT). Nanopure, deionized and distilled water (18.2 MΩ) was used for all experiments. Fluorescence measurements were performed by using Agilent Cary Eclipse fluorescence spectrophotometer (Santa Clara, CA).

5. Experimental design and data

5.1. Synthesis of iron oxide nanoparticles

Iron oxide nanoparticles were synthesized as described [2] and characterized by XRD (Fig. 1).

5.2. Silica coating of Fe₃O₄ MNPs by reverse micro-emulsion approach (scheme 1)

Silica coating was performed as reported earlier. [3] The Fe₃O₄ MNPs (400 μL of 10 mg/mL) were dissolved in cyclohexane (4 mL) and Igepal-CO-520 (0.247 g) then sonicated for 15 min. The suspension was further mixed with tetraethylorthosilicate (25 μL) and sonicated again for 10 min. In the last step, ammonium hydroxide (50 μL) was added and sonicated for 15 min. The suspension was stirred using a magnetic stirrer at room temperature for 24 h. The SiO₂@Fe₃O₄ MNPs were magnetically recovered, washed using ethanol several times, and dried at room temperature.

Scheme 1: synthesis of cleavable ester linked, NHS ester modified SiO₂@Fe₃O₄ MNPs

5.3. Functionalization of SiO₂@Fe₃O₄ MNPs with amine groups (scheme 1)

Amine groups were introduced on the surface of SiO₂@Fe₃O₄ MNPs using amine containing silane coupling reagent [4]. The SiO₂@Fe₃O₄ MNPs (10 mg) were resuspended in ethanol (10 mL) followed by 20 min of sonication. In the next step, APTES (95%, 100 μL) was added dropwise and the mixture was

Fig. 1. XRD data of Fe₃O₄ MNPs.
mechanically stirred at room temperature for 24 h. Amine modified Fe$_3$O$_4$ SiO$_2$@Fe$_3$O$_4$ MNPs were magnetically separated, washed several times with ethanol, and air dried at room temperature.

5.4. Synthesis of cleavable linked, NHS ester modified SiO$_2$@Fe$_3$O$_4$ MNPs (scheme 1)

Amine modified SiO$_2$@Fe$_3$O$_4$ MNPs (1 mg) were mixed with ethanol (100 µL), followed by sonication for 10 min. EGS (12 mg in µL DMSO, 100 final conc. 0.13 M) was added dropwise to the solution of amine modified SiO$_2$@Fe$_3$O$_4$ MNPs and allowed to react for 20 min at room temp. EGS modified SiO$_2$@Fe$_3$O$_4$ MNPs were recovered by magnetic separation, washed with ethanol, and dried under vacuum.

Presence of NHS ester was determined by conjugating dansylcadaverine and measuring the fluorescence of dansylcadaverine conjugated SiO$_2$@Fe$_3$O$_4$ MNPs.

5.5. Labeling amine groups of peptides/proteins using cleavable ester linked, NHS ester modified SiO$_2$@Fe$_3$O$_4$ MNPs

Labeling of ACTH (4–11), BSA and β-lactoglobulin was performed by following a protocol as reported earlier [5] with minor modifications. Protein sample (BSA or β-lactoglobulin, 10 µL, 10 mg/mL) solution was mixed with ester cleavable, NHS ester modified SiO$_2$@Fe$_3$O$_4$ MNPs followed by addition of PBS, pH = 7.4 (190 µL). The mixture was allowed to stir at room temperature for 40 min. Protein conjugated SiO$_2$@Fe$_3$O$_4$ MNPs were magnetically separated and washed with water (6X). The unreacted NHS ester groups on the surface of SiO$_2$@Fe$_3$O$_4$ MNPs were quenched by reacting with Tris–HCl (100 µL of 50 mM) for 15 min followed by washing with water (3X). Protein conjugated SiO$_2$@Fe$_3$O$_4$ MNPs were incubated with urea (8 M, aq. 80 µL) and DTT (5 µL of 200 mM) at 45°C for 1 h. Free thiol groups were alkylated with iodoacetamide (10 µL of 200 mM) for 1 h in the dark. Trypsin was added (6 µg) with ammonium bicarbonate (1 mL of 50 mM) followed by digestion for 15 h at 37°C. The tryptic peptide conjugated SiO$_2$@Fe$_3$O$_4$ MNPs were magnetically separated and washed with water (3X), and water:ACN (30:70, 6X). Labeled tryptic peptides were isolated from SiO$_2$@Fe$_3$O$_4$ MNPs by cleaving the ester bond using hydroxylamine (200 µL of 2 M, pH = 8.5) for 4 h at 37°C. The SiO$_2$@Fe$_3$O$_4$ MNPs were magnetically separated, and the supernatant was saved for further analysis.

5.6. Mass spectrometric analysis of labeled ACTH (4–11), BSA and β-lactoglobulin

Chromatographic separation was performed by using a chip consisting of a 160 nL enrichment column and a 150 mm analytical column packed with C18, 5 m beads with 300 Å pores. The sample (2 µL) was transferred to the enrichment column via the capillary pump. Capillary pump was operated...
at a flow rate of 4 μL/min. The flow rate of nano pump was set to 600 nL/min. The MS source was operated at 300 °C with 5 L/min N2 flow and a fragmentor voltage of 175 V. Quad and TOF were operated in the positive ion mode. The calibration standards contained reference compounds of 322.048121 and 1221.990637 Da, which were continually released into the source for mass calibration. LC chromatograms and mass spectra were analyzed using Mass-Hunter software (Version B.0301; Agilent Technologies).

5.7. Quantification of active NHS ester groups on the surface of SiO2@Fe3O4 MNPs using ‘depletion’ approach

Cleavable ester linked, NHS ester modified SiO2@Fe3O4 MNPs were prepared as shown in Scheme 1. Dansylcadaverine (700 μL of 20 μM) was mixed with cleavable ester linked, NHS ester modified SiO2@Fe3O4 MNPs (1 mg) for 40 min. The dansylcadaverine conjugated SiO2@Fe3O4 MNPs were magnetically separated, and the supernatants were collected for quantitative fluorescence
measurements (Fig. 4). The quantity of conjugated dansylcadaverine was determined by subtracting the quantity of remaining dansylcadaverine after conjugation to NHS ester modified SiO₂@Fe₃O₄ MNPs from the initial quantity of dansylcadaverine.

Acknowledgments

L. Osorno was supported by a National Science Foundation Research Experiences for Undergraduates site (DMR-1004869 and DMR-1262904). We would like to thank Dr. Leonard Spinu for his help with magnetization measurements of magnetic nanoparticles.

References

[1] U.S. Patil, H. Qu, D. Caruntu, C.J. O’Connor, A. Sharma, Y. Cai, M.A. Tarr, Labeling primary amine groups in peptides and proteins with N-hydroxysuccinimidyl ester modified Fe₃O₄@SiO₂ nanoparticles containing cleavable disulfide-bond linkers, Bioconjug. Chem. 24 (2013) 1562–1569, http://dx.doi.org/10.1021/bc400165r.
[2] U.S. Patil, L. Osorno, A. Ellender, C. Grimm, M.A. Tarr, Cleavable ester linked magnetic nanoparticles for labeling of solvent exposed primary amine groups of peptides/proteins, Anal. Biochem. (2015) In press.
[3] S. Santra, R. Tapec, N. Theodoropoulou, J. Dobson, A. Hebard, W. Tan, Synthesis and characterization of silica-coated iron oxide nanoparticles in microemulsion: the effect of nonionic surfactants, Langmuir 17 (2001) 2900–2906, http://dx.doi.org/10.1021/la0008636.
[4] R.A. Bini, R. Fernando, C. Marques, F.J. Santos, J.A. Chaker, M. Jafelicci Jr., Synthesis and functionalization of magnetite nanoparticles with different amino-functional alkoxysilanes, J. Magn. Magn. Mater. 324 (4) (2012) 534–539.
[5] J. Qian, R.B. Cole, Y. Cai, Synthesis and characterization of a ‘fluorous’ (fluorinated alkyl) affinity reagent that labels primary amine groups in proteins/peptides, J. Mass Spectrom. 46 (2011) 1–11, http://dx.doi.org/10.1002/jms.1854.