Solid-Phase Labeling

International Edition: DOI: 10.1002/anie.201500331
German Edition: DOI: 10.1002/ange.201500331

Highly Efficient Solid-Phase Labeling of Saccharides within Boronic Acid Functionalized Mesoporous Silica Nanoparticles**

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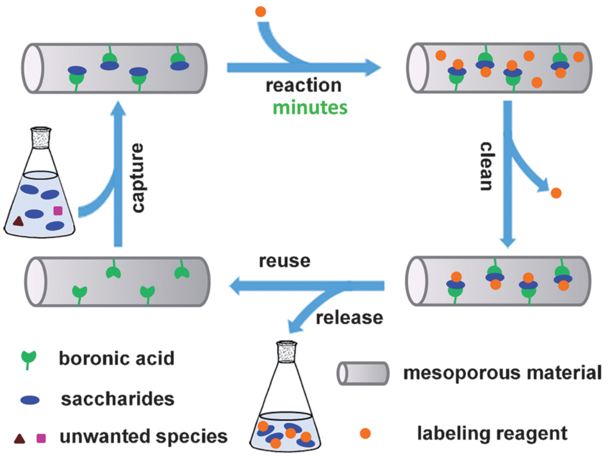
Abstract: *Labeling is critical for the detection, quantitation, and structural identification of saccharides. However, conventional liquid-phase labeling suffers from apparent disadvantages, such as time-consuming, the presence of excessive labeling reagent, and high applicable saccharide concentration. A solid-phase approach is presented for highly efficient labeling of saccharides, using boronic acid functionalized mesoporous silica nanoparticles (MSNs) as a selective extraction sorbent and nanoscale reactor. The solid-phase labeling approach exhibited several significant advantages, including: much faster reaction speed (taking only 2 min), high product purity, and much lower applicable saccharide concentration (four orders of magnitude lower than that of liquid-phase labeling). Thus, this labeling approach opens up new avenues to the facile and efficient labeling of saccharides.*

Saccharides, as essential components of all living systems, are the major compounds of interest in many fields such as biochemistry, biomedicine, glycomics, and glycobiology. Saccharides have no fluorescence, limited UV/Vis absorbance, and a poor mass spectrometry (MS) signal, and therefore labeling with an appropriate signal-producing reagent is thereby a critical step prior to their detection, quantitative analysis, and structural identification.[1] The labeling is usually carried out in the liquid phase.[2] However, it is associated with apparent disadvantages, including time-consuming, the presence of excessive labeling reagent, and higher concentration requirement of the saccharide to be labeled. Therefore, new labeling approaches that can overcome these drawbacks are of significant importance.

Mesoporous materials,[3] which possess highly ordered mesopores and high specific surface areas, have exhibited great potentials for applications in a variety of important areas, such as catalysis,[4] sensing,[5] sorption,[6] separation,[7] and drug delivery.[8] In the last decade, mesoporous materials, and particularly mesoporous silica nanoparticles (MSNs) MCM-41,[9] have been important sorbents for sample pretreatment.[10] They allowed for size-selective extraction and effective enrichment.[10d–f] Besides, enzyme-encapsulated mesoporous materials have been used as nanoscale reactors for enzymatic digestion of proteins and it was found that the digestion speed was significantly enhanced.[11] Moreover, it was revealed that intermolecular interactions within mesoporous materials can be significantly enhanced due to nanoscale confinement effect.[12] However, the merits of mesoporous materials as nanoscale reactors for solid-phase labeling have not yet been well exploited.

In this study, using boronic acid functionalized MSNs as a selective extraction sorbent and nanoscale reactor, we present for the first time a solid-phase labeling approach for the highly efficient derivatization of saccharides. The principle and procedure of this approach are illustrated in Scheme 1. The core for the highly efficient labeling is the pH-controlled capture/release chemistry of boronic acid ligand toward *cis*-diol-containing compounds such as saccharides.[13] The target saccharide in a sample is first covalently captured by boronic acid functionalized MSNs by virtue of boronate affinity interaction at appropriate pH condition (pH higher than the pK_a value of the boronic acid) while non-*cis*-diol containing species are excluded out of the MSNs. Then, the labeling reagent is introduced into the mesopores. Owing to the high abundance and favorable orientation of saccharide molecules within the mesopores, the effective collision



Scheme 1. Representation of the solid-phase labeling within boronic acid functionalized mesoporous silica nanoparticles.

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[**] We acknowledge the financial support of the National Science Fund for Distinguished Young Scholars (No. 21425520) and the general grant (No. 21275073) from the National Natural Science Foundation of China, the Key Grant of 973 Program (No. 2013CB911202) from the Ministry of Science and Technology of China, and the key grant (No. 00551) from the Shanghai Tobacco Group Co. Ltd., China.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201500331.

probability is expected to be dramatically enhanced and thereby the labeling reaction can be completed within only a limited duration. After that, excessive free labeling reagent is moved by washing using a buffer with a pH similar to that for the saccharide capture. Finally, the product molecules are released by elution with an acidic solution (typically pH < 3) while the material can be regenerated for further use. As a comparison, in liquid-phase labeling, the saccharide to be labeled is added with excessive labeling reagent (the reaction usually takes several hours to even tens of hours), and the resultant solution contains the labeled product, excessive free labeling reagent, and unwanted species from the original sample or labeling reagent. We demonstrated that the solid-phase labeling procedure is more advantageous. It exhibited several significant advantages, including: 1) high efficiency (the reaction speed was enhanced by a factor of 210); 2) high purity product, containing no residual labeling reagent and unwanted species from the sample or labeling reagent; and 3) significantly lower applicable saccharide concentration (four orders of magnitude lower than that of liquid-phase labeling). Thus, this approach opened up new avenues to the facile and efficient labeling of saccharides for important applications such as glycomics.

As a proof-of-principle, 4-carboxyphenylboronic acid (CPBA)-functionalized MCM-41-type channel-like MSNs with an average diameter of 77 ± 5 nm and a pore diameter of 2.3 nm (Figure 1) were used as a model mesoporous material. The MSNs exhibited high specific surface area (2.610 $m^2 g^{-1}$). Here we chose the 1-phenyl-3-methyl-5-pyrazolone (PMP) as a representative labeling reagent, because it has been widely adopted for the derivatization of saccharides for UV absorbance detection.[14] Meanwhile, mannose, a typical monosaccharide, was used as saccharide model. The reaction formula is illustrated in the Supporting Information, Scheme S1. A saccharide molecule can be labeled with two molecules of PMP. The reaction condition was slightly modified from the previous method[14c] (see the Supporting Information). It is noteworthy that the solid-phase labeling approach proposed in this study should be applicable to other boronic acid functionalized meseporous materials, other labeling reagents, and other saccharides with matching configuration.

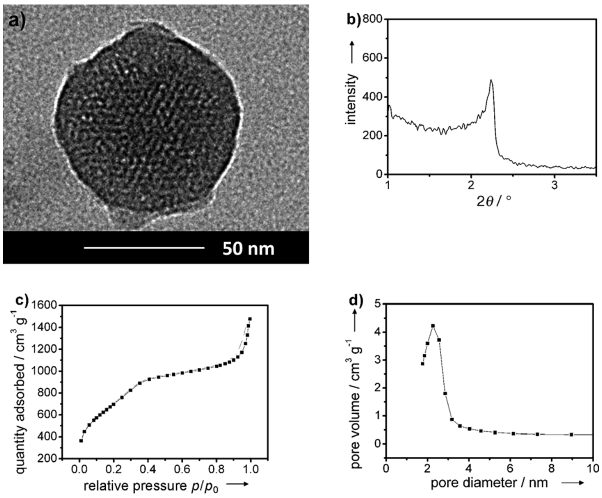

Figure 1. a) Transmission electron microscopic image, b) X-ray diffraction pattern, c) N_2 adsorption–desorption isotherms (△ adsorption, ■ desorption), and d) pore size distribution of the CPBA-functionalized MSNs.

The affinity of the boronic acid functionalized MSNs was first confirmed using adenosine as a test compound while deoxyadenosine as an interferrant. Adenosine contains a pair of *cis*-diol groups and it has UV absorbance at about 260 nm. As a comparison, deoxyadenosine contains no *cis*-diol moiety. As shown in the Supporting Information, Figure S2, the boronic acid functionalized MSNs exhibited excellent selectivity toward adenosine. Meanwhile, it was observed that the affinity of the MSNs toward PMP was limited (Supporting Information, Figure S3), which ensured that free PMP molecules can be effectively removed.

The temperature for saccharide capture was found to play a critical role in the solid-phase reaction between saccharides and PMP. Mannose exists preferentially as β anomer at low temperature but as α anomer at high temperature.[15] As shown in the Supporting Information, Scheme S2, both α and β forms of mannose can be captured by a boronic acid ligand. However, the hydroxy group at carbon 1 of β anomer, which is reactive toward PMP, is occupied by the boronic acid ligand. As a result, the labeling reaction is prohibited. We investigated the effect of saccharide capture temperature on the reaction. It was observed that when the capture temperature was as high as 60 °C, almost all mannose molecules have been labeled (Supporting Information, Figure S4). Thus, for further labeling the saccharide capture was carried out at 60 °C.

The product for the labeling of mannose with PMP was analyzed by capillary electrophoresis (CE) and matrix-assisted laser desorption ionization time-of-flight MS (MALDI-TOF MS). As shown in Figure 2a, when the product of liquid-phase labeling was analyzed by CE, three peaks were observed, which were assigned as the labeling product, free PMP, and impurity from the labeling reagent. Because PMP was present in a high concentration, its presence generated a much broad peak, which greatly degraded the resolution of the CE separation. As a comparison, when the product of the solid-phase labeling was analyzed, only one sharp peak was observed, which exhibited

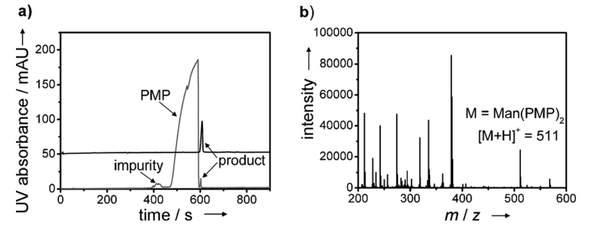

Figure 2. a) Electropherograms of the products of PMP-labeled mannose by liquid-phase (gray trace) and solid-phase labeling (black trace); and b) MALDI-TOF MS spectrum of PMP-derivatized mannose by solid-phase labeling.

much high signal as compared with the peak at the same location for liquid-phase labeling. This peak was assigned as the labeling product, as confirmed by MALDI-TOF MS (Figure 2 b). The peaks within the range of 200–500 m/z in the MS spectrum was due to the fragmentation of the MALDI matrix (Supporting Information, Figure S5). Clearly, the solid-phase labeling approach was advantageous over liquid-phase labeling, providing pure product.

In the solid-phase labeling approach, the reaction occurs at the surface of the mesopores and the high surface area of the MSNs is highly favorable for reaction speed; meanwhile, and the effective collision probability between PMP and mannose is significantly increased owing to the fixed orientation of mannose molecules on the wall of the mesopores. Thereby, we expect that the solid-phase labeling can provide much faster reaction speed as compared with liquid-phase labeling. This was confirmed by the reaction equilibrium test, as shown in Figure 3. In liquid-phase labeling, it took 7 h to reach reaction equilibrium. In the solid-phase labeling, the reaction was completed within only 2 min, indicating that the reaction speed was facilitated by a factor of 210. Although mesoporous materials are widely employed as catalysts, it should be noted that no catalytic function was involved in this study and the improvement in reaction speed was due to only the abundance and fixed orientation of the saccharide molecules within the mesopores.

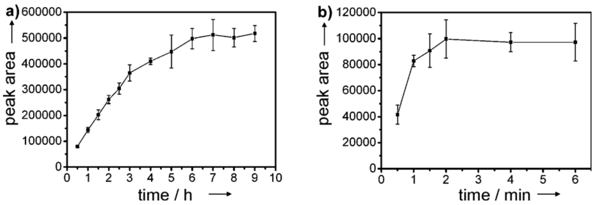

Figure 3. Dependence of the UV absorbance of the labeling products on the reaction time for a) liquid-phase and b) solid-phase labeling.

For labeling reactions, there is a requirement of minimum concentration for the target to be labeled. If the concentration is too low, there will be no enough product. Therefore, a labeling reaction with high efficiency is favorable for the labeling of target of low concentration. The lowest applicable concentration of mannose for the reaction with PMP was investigated for the solid-phase labeling and compared with that for conventional liquid-phase labeling. As shown in Figure 4, the minimum applicable mannose concentration was 10^{-5} M for liquid-phase labeling, while it was 10^{-9} M for the solid-phase labeling, with a peak area slightly higher than that for the former. This means that the minimum applicable target concentration was reduced by four orders of magnitude by the solid-phase labeling using boronic acid functionalized MSNs as nanoreactor. Such a beneficial feature was due to the enrichment capability of the mesoporous material as well as the improved reaction efficiency.

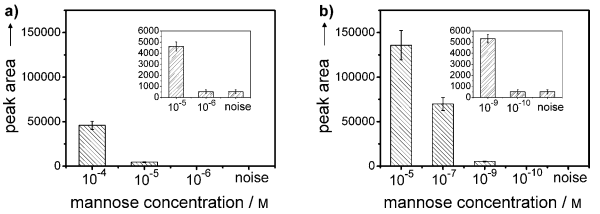

Figure 4. The lowest applicable mannose concentration in a) liquid-phase labeling and b) solid-phase labeling.

To reveal the importance of mesopores for the performance of solid-phase labeling, CPBA-functionalized non-porous silica nanoparticles (NSNs) with an average diameter of 100 ± 4 nm were synthesized as an alternative sorbent and nanoreactor for solid-phase labeling. Although solid-phase labeling on NSNs could also provide high product purity (Supporting Information, Figure S6), it needed 10 min to reach reaction equilibrium (Supporting Information, Figure S7) and the lowest applicable concentration of mannose was just 10^{-6} M (Supporting Information, Figure S8). These results clearly suggested that MSNs functioned as a much more efficient nanoreactor for solid-phase labeling.

The feasibility of the solid-phase labeling approach for the applications to complicated samples was demonstrated with the selective labeling of monosaccharides from tobacco. Tobacco leaves contain a variety of saccharides and their composition is critical for the quality of tobacco. As shown in Figure 5, when the extracts of tobacco leaves by methanol–water were labeled with PMP in liquid-phase, a very complex profile was observed due to the interference of PMP as well as other unwanted species from the sample. As a comparison, when the extracts were first selectively captured and then labeled within boronic acid functionalized MSNs, a much clearer profile was observed. The cleaned profile is favorable for further analysis of the sample.

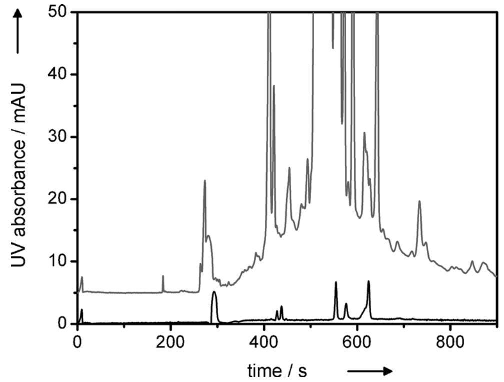

Figure 5. Electropherograms of the liquid-phase (gray trace) and solid-phase labeling (black trace) products of tobacco leaf extracts.

In summary, we have proposed a solid-phase approach for the labeling of saccharides by using boronic acid functionalized MSNs as nanoreactors. The solid-phase labeling was demonstrated to be much superior to conventional liquid-phase labeling. It exhibited several significant advantages, including faster reaction speed (only 2 min), high product purity, and much lower applicable saccharide concentration (10^{-9} M). These features are highly favorable for the selective labeling of trace saccacharides in complex sample matrix. The approach can be applicable to other labeling reagents and other mesoporous materials. Meanwhile, the saccacharide capture method can be extended to other means such as hydrophilic interaction chromatography (HILIC). Therefore, this approach opened up new avenues to the facile and efficient labeling of saccharides and can be a promising approach for important applications such as glycomics. Besides, the application of mesoporous materials as nanoreactors can be expanded to other solid-phase reactions.

Keywords: boronic acid · carbohydrates · mesoporous materials · nanomaterials · solid-phase labeling

How to cite: *Angew. Chem. Int. Ed.* **2015**, *54*, 6173–6176
Angew. Chem. **2015**, *127*, 6271–6274

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Received: January 14, 2015
Revised: February 19, 2015