Analytical Methods

DOI: 10.1002/anie.200605190

Direct and Label-Free Detection of Solid-Phase-Bound Compounds by Using Surface-Enhanced Raman Scattering Microspectroscopy**

Carsten Schmuck, Peter Wich, Bernd Küstner, Wolfgang Kiefer, and Sebastian Schlücker**

Solid-phase-bound compounds are nowadays widely used in chemistry not only for chemical synthesis itself (as for DNA, peptides, and carbohydrates) but also for further studies and direct applications of such bead-bound compounds in supramolecular[1] or medicinal chemistry.[2] In general, the solid support, in most cases a modified polystyrene resin, has a loading of typically about 100 pmol per bead. Owing to this low loading, it is rather difficult to analyze the solid-phase-bound compound on the bead directly. The properties of the compound on the bead (for example, binding to a specific substrate) are normally probed in screening experiments by using fluorophore- or chromophore-labeled substrates.[3] However, in such experiments it is not possible to determine any structural information of the complex formed, for example, or even to establish the identity of the actual library member on a specific bead.[4] For libraries of chemically diverse substances, direct MS analysis of the solid-phase-bound compounds is sometimes possible.[5] However, mass spectrometry (MS) requires a cleavage of the compound from the bead prior to analysis and can therefore not be performed directly in a screening assay. An alternative, chemical tagging, significantly increases the complexity of the solid-phase synthesis as, in addition to the actual compound, a separate coding strand, which makes it possible to later identify the library member, also has to be synthesized. Additionally, the tags should not interfere with the synthesis of the compound and need to be inert under the subsequent screening conditions,[4] which often limits the scope and diversity of solid-phase-bound compound libraries.[6]

Hence, a direct and label-free spectroscopic detection of solid-phase-bound compounds would have significant advantages. However, any spectroscopic attempt to directly detect a molecule bound to a single bead has to cope with the low loading and the presence of a significant excess of the solid support itself. This prevents, at least for standard resins with picomolar capacity, the use of techniques such as solid-state NMR spectroscopy (which is not sensitive enough) and even highly sensitive techniques, such as UV absorption or fluorescence spectroscopy. Generally, vibrational spectroscopic techniques such as Raman[7] and IR spectroscopy are ideally suited for this task[8] as they offer very detailed chemical information about both the molecular composition and structure when compared with electronic absorption (UV/Vis) and emission (e.g. fluorescence) spectroscopy.[9] However, Raman and IR spectroscopy do not normally allow one to distinguish between the actual compound and the matrix background of the bead itself. In this context, we report herein to the best of our knowledge the first time that surface-enhanced Raman scattering (SERS) has been used for direct, label-free, and surface-selective detection of compounds bound to a single polystyrene bead within a few seconds.[10] SERS combines the advantages of Raman spectroscopy with surface selectivity and ultrasensitive detection of substances located close to the surface of noble metal nanostructures.[11]

Noble-metal nanoparticles tremendously enhance Raman signals by up to a factor of 10^{14}, but only of those molecules that are located close to their surface as the SERS effect falls off at approximately r^{-10}. By using this distance dependence, a discrimination of a solid-phase-bound compound from the matrix of the bead itself should become possible (Figure 1). As a proof of concept for the use of SERS as a direct detection method for solid-phase-bound compounds, we first investigated the artificial peptide receptor CBS-Lys-Lys-Phe-NHR **1** (CBS = guanidiniocarbonyl pyrrole cation) synthesized on a standard TentaGel-NH_2 resin.[12]

Silver nanoparticles were prepared by reduction of a silver nitrate solution with sodium citrate. The colloidal solution of silver nanoparticles (400 μL) was mixed with the swollen polystyrene resin (previously prepared by soaking for at least 3 h in 0.1M KCl) onto which compound **1** had been attached by using a standard solid-phase Fmoc-protection-group peptide synthesis (Fmoc = 9-fluorenylmethoxycarbonyl) as described previously.[12b] Aggregation was induced by adding 60 μL of 0.1M KCl. After aggregation of the nanoparticles, the solution was adjusted to pH 2.5 with 30 μL 0.1M HCl. SERS spectra were then recorded by using a Raman microspectrometer with excitation at 633 nm. As the size of the silver nanoparticles used in this case is around 10000 times smaller than the size of the TentaGel beads (Figure 2), the silver particles can only interact with small areas on the surface of the solid support. Hence, the nanoparticles only "see" compound **1**, which is attached through long polyethylene glycol chains ($M_r \approx 2000\ g\,mol^{-1}$, $n = 45$) to the polystyrene

[*] Prof. Dr. C. Schmuck, Dipl.-Chem. P. Wich
Institut für Organische Chemie
Universität Würzburg
Am Hubland, 97074 Würzburg (Germany)
Fax: (+49) 931-888-4625
E-mail: schmuck@chemie.uni-wuerzburg.de
Dipl.-Chem. B. Küstner, Prof. Dr. W. Kiefer, Priv.-Doz. Dr. S. Schlücker
Institut für Physikalische Chemie
Universität Würzburg
Am Hubland, 97074 Würzburg (Germany)
Fax: (+49) 931-888-6332
E-mail: sebastian.schluecker@uni-wuerzburg.de

[**] We thank the DFG (SFB 630, TP A3 and C1) and the Fonds der Chemischen Industrie for ongoing financial support of our work.

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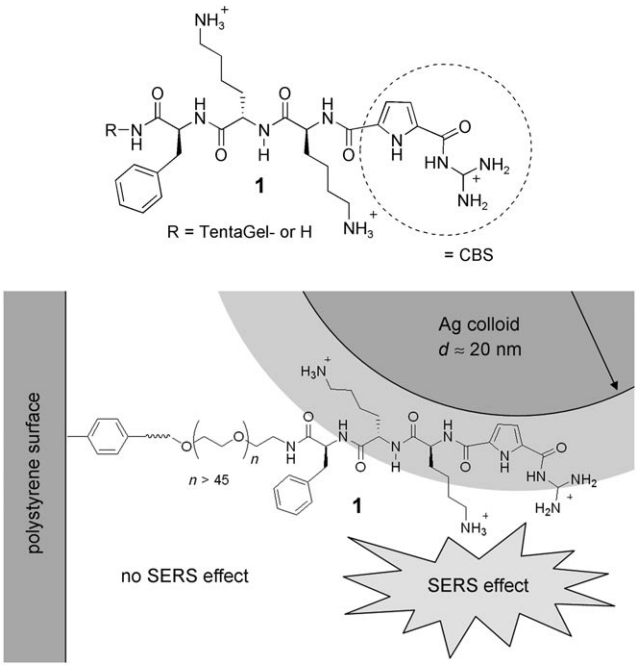

Figure 1. Silver colloidal particles as the SERS substrate lead to a tremendous enhancement in the Raman signal but only for substances that are located in close proximity to the nanoparticle surface. Hence, the signals of **1** are enhanced but not that of the polystyrene matrix.

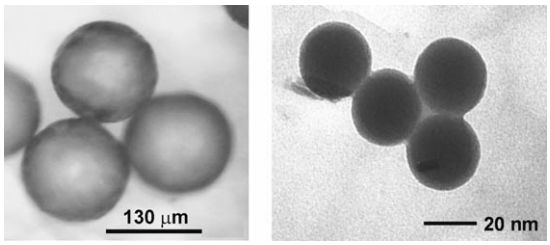

Figure 2. Microscopic image of the polystyrene beads (TentaGel) onto which compound **1** is attached (left) and a TEM image of the silver nanoparticles used for the SERS effect (right). The nanoparticles are around four orders of magnitude smaller than the polystyrene beads.

matrix. The actual polystyrene matrix is too far away to experience any significant SERS enhancement. Under these conditions, we were able to directly record a Raman spectrum with about 50 femtomole of compound **1** still attached to the TentaGel bead within a few seconds (Figure 3, upper spectrum). A qualitative comparison with the conventional Raman spectrum of **1** (R = H) in a 20 mM aqueous solution, which takes around 30 minutes to record, indicates that indeed no contributions from the polystyrene resin itself are observed (Figure 3, lower spectrum). This method of on-bead detection by using SERS is about 10^6–10^7 times more sensitive than a conventional Raman spectrum recorded in solution and takes only a few seconds compared with minutes.[13] Therefore, a direct and label-free detection of molecules bound on a solid phase is possible even with the standard resins and the rather low loadings that are conventionally used in solid-phase synthesis.

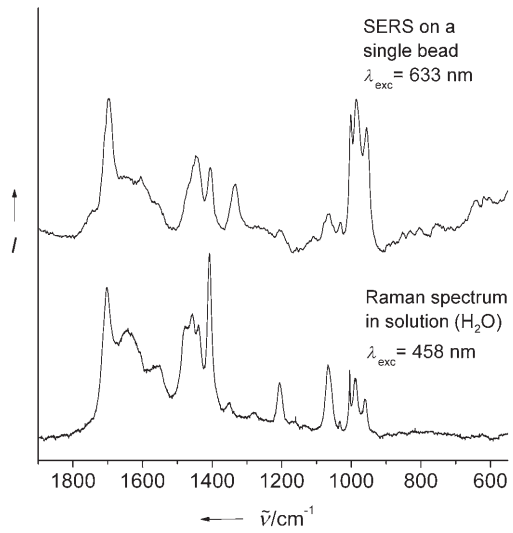

Figure 3. SERS spectrum of **1** on a single polystyrene bead (upper spectrum). The lower reference spectrum is a conventional Raman spectrum recorded in aqueous solution.

Similarly, the Fourier transform (FT) Raman spectrum of **1** in the solid state was recorded and compared with the theoretical Raman spectrum (Figure 4, middle and bottom, respectively). Based on the similarity of these two spectra, individual bands can be identified and assigned in the SERS spectrum of **1**. For example, the marker band at approximately 1700 cm^{-1} in the Raman spectrum (indicated by an asterisk in Figure 4) arises mainly from the CBS moiety. This was also demonstrated by comparison with an experimental FT-Raman reference spectrum for a guanidiniocarbonyl pyrrole diester **2** (CBS diester, Figure 4, top). Compound **2** lacks the tripeptide part of **1** but still shows the same characteristic band around 1700 cm^{-1} in the spectrum.

The reproducibility of this new vibrational microspectroscopic approach for the detection of solid-phase-bound compounds was probed by recording spectra from different beads as well as from different surface spots of one bead. In all cases, essentially the same SERS spectrum was obtained for **1** (see the Supporting Information).[14] Even the intensity of the bands varies only slightly. This is indicated by the consistent baseline-corrected intensity for the marker band at 1700 cm^{-1}, which is taken from different positions of the bead (Figure 5). This method is indeed capable of distinguishing between different compounds attached to a solid support. This was tested by recording the SERS spectrum of a structurally related compound, CBS-Ala-Ile-Val-NHR, with a different amino acid sequence in the tripeptide part. Again, the SERS spectrum of this compound, which was recorded from a single TentaGel bead within a few seconds, is the same as the corresponding conventional Raman spectrum that was recorded in the solid state. However, the spectrum exhibits characteristic differences when compared with the spectrum of **1**. Our method therefore allows us to distinguish between different compounds based on the differences in their Raman spectra. This opens the possibility for structure determination and identification of unknown compounds or, by means of spectral changes, detection of complex formation with

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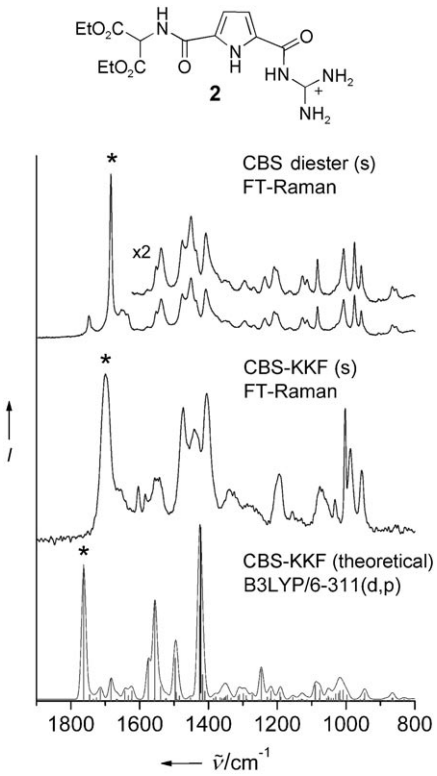

Figure 4. Experimental solid-state FT-Raman spectra of the CBS diester **2** (top) and of CBS-Lys-Lys-Phe-NH_2 **1** (middle) obtained with an excitation wavelength at 1064 nm. The theoretical Raman spectrum of **1** (bottom) was calculated at the B3LYP/6-311G(d,p) level. The marker band indicated by an asterisk arises mainly from the CBS and was used for the bead mapping in Figure 5.

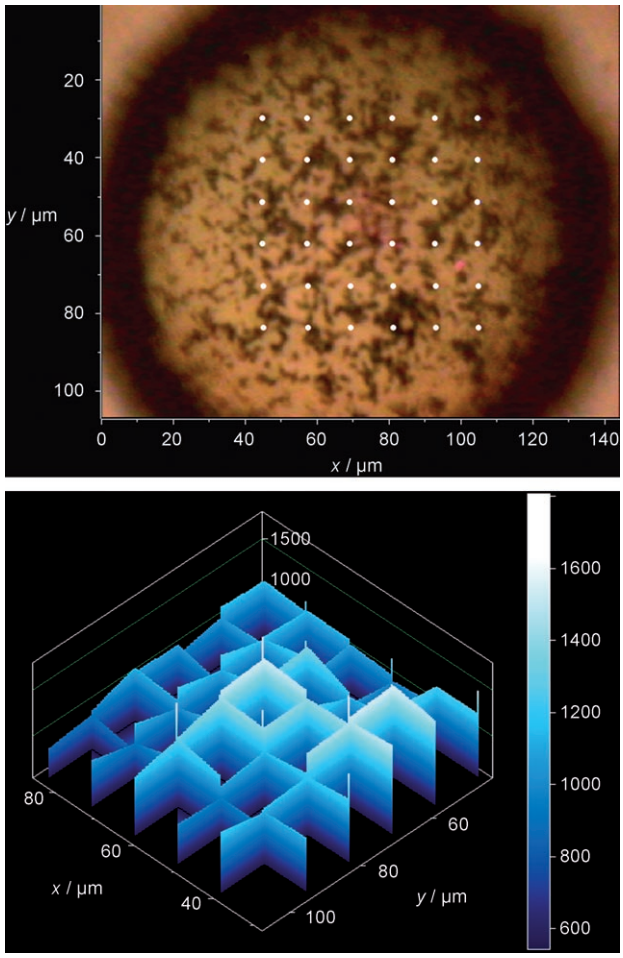

Figure 5. Microscopic image of a single TentaGel bead with aggregated silver nanoparticles on its surface (top). At each white dot, a SERS spectrum of **1** was recorded for 10 s. The reproducibility of these spectra is indicated by the 3D plot showing the baseline-corrected intensity of the Raman band at 1700 cm^{-1} (see SERS spectrum in Figure 3).

another molecule in an on-bead screening experiment. The method is furthermore not limited to TentaGel resin; other resins such as phenylacetamidomethyl (PAM) resin can also be used even though the spectrum quality is not as good. We attribute the more-intense spectral background from the polystyrene matrix to the lack of spacer groups that are present in TentaGel (SERS distance dependence).

To the best of our knowledge, this is the first time that direct detection of a compound attached to a single polystyrene bead by using SERS was achieved. SERS microspectroscopic mapping, that is, the combination of Raman microscopy with a spatially resolved detection, should allow the screening of entire combinatorial libraries.[15] Future studies employing SERS for combinatorial chemistry will also explore how substrate complexation can be directly monitored on bead.

Experimental Section

The preparation of silver nanoparticles is based on the Lee and Meisel method.[16] Freshly degassed (with argon) and deionized water (18 MΩ) was used in the experiments. All glassware was cleaned with aqua regia before use and thoroughly rinsed with water. The reaction was performed under an argon atmosphere in a 500-mL round-bottom flask equipped with a condenser. Sodium citrate solution (1%, 5 mL) was added to a boiling solution of $AgNO_3$ (45 mg) in H_2O (250 mL) with vigorous stirring. The reaction mixture was left to boil gently for 90 min.

SERS spectra were recorded with a Raman microspectrometer (Horiba-Jobin-Yvon, model LabRam with a holographic grating having 1800 grooves mm^{-1}) by using the 632.8-nm line from a HeNe laser. The spectra were collected in a backscattering geometry with a 50× microscope objective (Olympus, model LMPlanFL). The laser power on the sample used in our measurements was approximately 10 mW. The spectrally dispersed Raman signal was detected with a Peltier-cooled CCD camera. Autofluorescence of the receptor molecules on bead, as observed in conventional Raman microspectroscopic experiments, was efficiently quenched in our on-bead SERS methodology.

Received: December 22, 2006
Published online: May 11, 2007

Keywords: analytical methods · Raman spectroscopy · SERS (surface-enhanced Raman scattering) · supramolecular chemistry

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[14] A statistical analysis of 36 spectra showed that after baseline correction and normalization the mean relative standard deviation (RSD) was only 10% (for more details see the Supporting Information).

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