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Probing Solid-phase Reactions by Monitoring the IR Bands of Compounds on a Single "Flattened" Resin Bead

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Abstract: The resolution of the IR spectra recorded on a single resin bead is greatly improved by flattening the bead. This technique allows the differentiation of carbonyl groups of resin-bound compounds with minor structural differences. Three solid-phase reactions on Rink AM, Rink Amide and Wang resins were directly monitored by observing the disappearance and the appearance of the resolved carbonyl bands. It was possible to quantify the yields of two of the solid phase reactions directly on bead.

INTRODUCTION

Solid-phase synthesis is the principal tool for constructing combinatorial libraries [1-6]. It is now painfully apparent that there is a serious lack of quick and sensitive analytical tools for monitoring solid phase reactions directly on the polymer supports. The approaches used for solution chemistry, such as TLC, NMR, MS and FTIR, are not convenient for characterizing resin-bound compounds. One is forced to rely on indirect techniques, such as the "cleave and analyze" method, to study chemical reactions on polymer supports.

We have recently developed[7] a single bead FTIR microspectroscopy method for real-time monitoring of solid phase reactions. The method enabled us to identify synthesis products, analyze the time course of solid-phase reactions and, in some cases, quantitate reaction yields directly on beads. The method is potentially useful for monitoring the synthesis of combinatorial libraries.

The method is very fast and highly sensitive. An IR spectrum recorded this way contains essentially the same information as that obtained from a KBr pellet made of multi-milligram quantities of beads. However, the quality of the spectrum recorded on a single bead is not always satisfactory. IR bands thus detected tend to be broad and flat-topped. Interference caused by moisture and carbon dioxide also appeared to be significant. Results from the current study demonstrated that the spectral quality of single bead FTIR microspectroscopy can be substantially improved by using a flattened resin bead. The improved spectral resolution allowed us to resolve the IR bands of carbonyl groups coming from slightly different chemical environments and monitor organic reactions directly on resin bead.

RESULTS AND DISCUSSION

The IR spectrum recorded on a single globular Merrifield resin bead (Fig.1A) is representative of the single bead spectra in general. Peak broadening blurs the resolution of adjacent bands. The major bands are flat-topped. The spectrum was distorted in such a way that many bands, especially those at lower wavenumbers, were out of proportion compared with the spectrum of the polystyrene standard. Interference from moisture and carbon dioxide is significant. One cause for this is probably the long and irregular pathlength the incident beam has to pass through a globular bead. A straightforward solution to this is to flatten the bead to produce a sample with short and uniform pathlength. The spectrum in Fig.1B is the result of such a treatment. The comparison shows that the spectrum from a flattened bead features higher resolution, sharper peaks and less interference from moisture and carbon dioxide. The spectral alteration observed in the fingerprint region of the bead spectrum is not observed in the spectrum of the flattened bead. The 1000 - 1800 cm^{-1} region is the region containing rich structural information. The higher spectral resolution achieved by flattening the resin bead makes single bead FTIR microspectroscopy a more powerful tool for the direct structural identification of solid-phase reaction products.

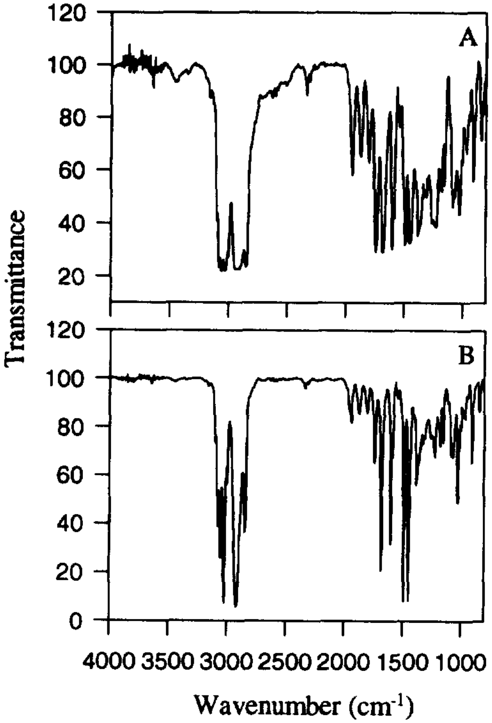
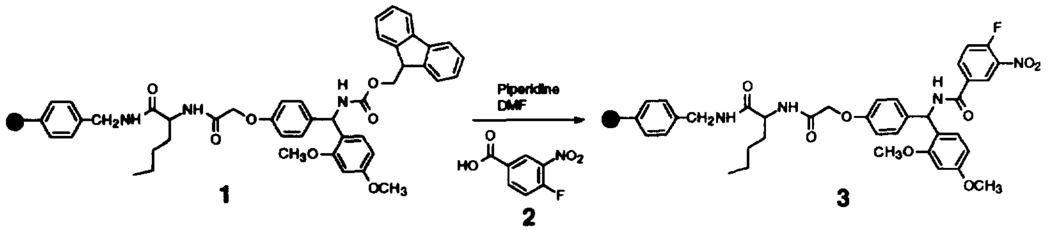


Figure 1. IR spectra of a single Merrifield resin bead (A) and a flattened bead (B).

Reaction I



Bead flattening also improved the spectral quality of Wang, Rink Amide and Rink AM resins in addition to Merrifield resin. Fig.2 shows the IR spectra of the starting material (Fig.2A) and the product (Fig.2B) of Reaction I carried out on Rink AM resin. The Fmoc carbonyl exhibits only minor differences compared to the amide carbonyls which already exist in the starting material. However, the IR spectra showed the complete shift of the Fmoc carbonyl frequency at 1720 cm^{-1} to the amide carbonyl frequency at 1670 cm^{-1}. An independent NMR analysis of the cleaved product showed the existence of the clean product. No starting material was detected. This indicated that the increase in intensity at 1670 cm^{-1} represented the conversion of a compound containing two amide bonds to a compound containing three amide bonds. The formation of the desired product was also indicated by a new shoulder at 1540 cm^{-1} which is the vibration frequency of the nitro group. Furthermore, this conversion was nearly complete as seen from the disappearance of the Fmoc carbonyl band and independent NMR analysis. Fmoc and Boc are popular protecting groups. Removal or addition of these groups is frequently involved in solid-phase synthesis. The direct quantitation of these reactions on beads will speed up the analysis of multi-step synthesis.

Reaction II was carried out on Rink Amide resin (Fig.3A). The success of the first step which involves the formation of an amide linkage was observed by the formation of a band at 1665 cm^{-1} (Fig.3B). Additional evidence is the appearance of the nitro frequency at 1540 cm^{-1}. The next step is a nucleophilic replacement of a fluorine by a group containing a methyl ester. The success of this conversion is shown by the formation of an ester carbonyl frequency at 1738 cm^{-1} (Fig.3C). Even though the yields of these reaction steps cannot be directly estimated from the IR information as we did for Reaction I, the reaction time course can be obtained by taking a single bead IR spectrum at a certain time intervals since the newly formed peak is so distinct (Fig.3). Decisions such as when to stop the reaction and move on to the next step can be made based on the information.

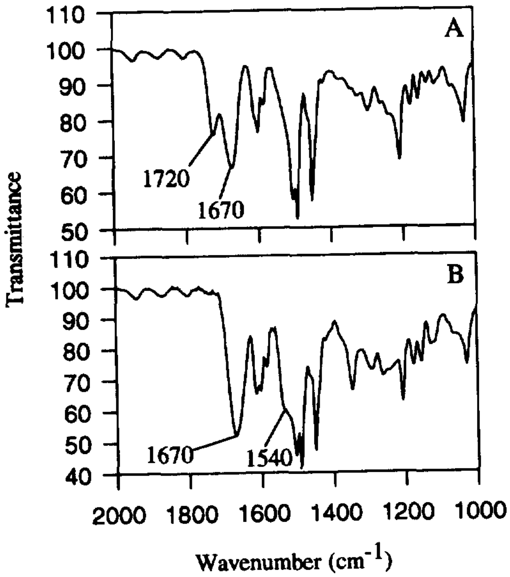
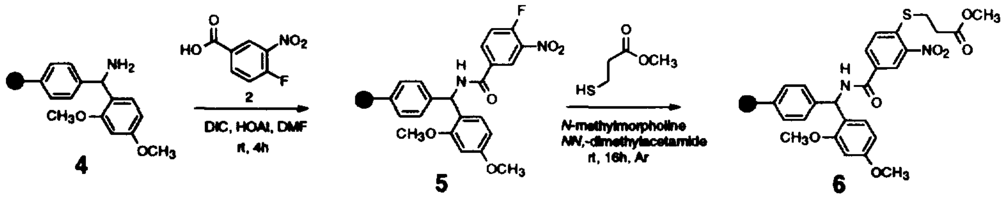


Figure 2. FTIR spectra of 1 (A) and 3 (B).

Reaction II



The proper resin selection can benefit solid-phase reaction monitoring. The first step of Reaction II was also carried out on Wang resin (Reaction III see Fig.4). In this case, the product formation was confirmed by the appearance of the 1725 cm^{-1} band (Fig.4B). The conversion was nearly complete as seen by the disappearance of the hydroxyl band at 3407 cm^{-1} (Fig.4A). The selection of Wang resin allows the quantitation of the reaction yield in this case in addition to determining the time course of the reaction[7].

In summary, we have improved the spectral quality of single bead IR spectra of Merrifield, Wang, Rink Amide and Rink AM resins by recording the spectra on flattened resin beads. By this technique, we successfully monitored several synthetic reactions involving compounds having slightly different carbonyl frequencies. In some cases, the reaction yields can be directly determined by analyzing the IR spectra alone. The ability to optimize solid-phase reaction monitoring by proper resin selection is also demonstrated.

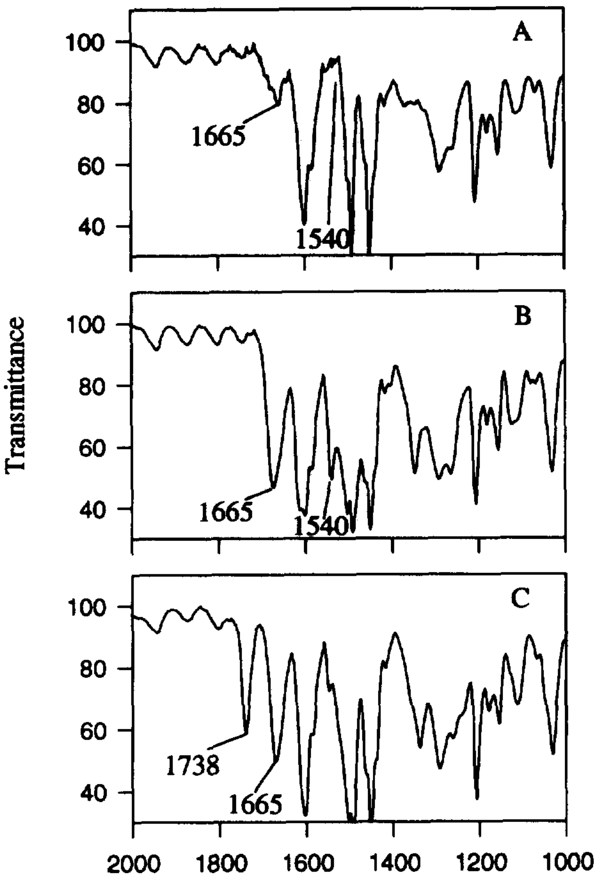
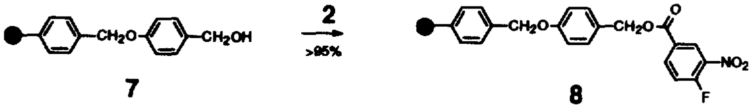


Figure 3. Single bead FTIR spectra of **4** (A), **5** (B) and **6** (C).

Reaction III



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EXPERIMENTAL

Materials and Synthetic Reactions. In Reaction I, Rink Amide AM resin from Peptide International (2g, 0.44 mmol/g) was washed with DMF. Fmoc groups were cleaved by treatment with 50:50 DMF:piperidine for 10 min and the resin was washed with DMF (3 times), methanol (3 times) and DMF (3 times). The resin was suspended in DMF (15 ml) and argon was bubbled through the resin suspension. 4-Fluoro-3-nitrobenzoic acid was added to 10 ml of DMF. Diisopropylcarbodiimide (688 µl, 4.4mmol) was added after compound 1 and AcOH was added after 1 min. After the mixture was stirred for 5 min, diisopropylcarbodiimide (344 µl, 2.2mmol) was added and the mixture was stirred for 5 min and then was added to the resin. Argon was bubbled through the resin suspension for 24 hrs. One drop of resin suspension was washed with DMF (3 times) and methanol (3 times), and then dried under vacuum (0.5 mmHg) for 30min.

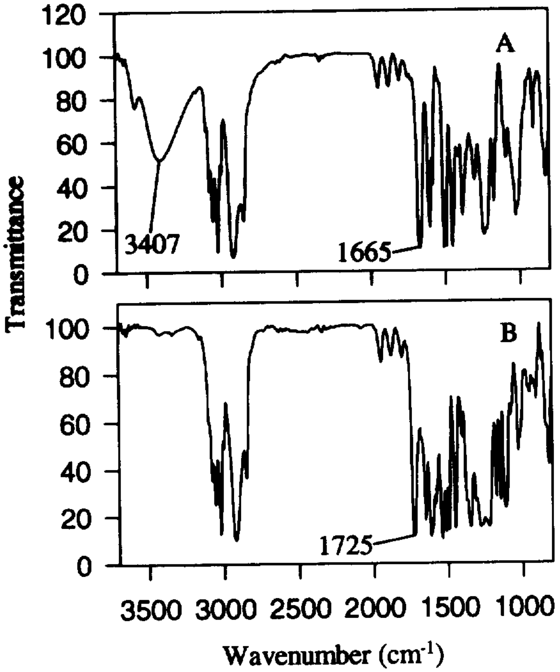

Figure 4. Single bead IR spectra of 7 (A) and 8 (B).

For Reaction II, Rink Amide resin from DuPont (0.1mmol/g) was washed with DMF three times, treated with 50:50 DMF:piperidine for 10 min and washed with DMF (3 times), methanol (3 times) and DMF (3 times). The resin was suspended in DMF (10 ml) and argon was bubbled through the resin suspension. 4-Fluoro-3-nitrobenzoic acid (92.5 mg, 0.5 mmol) was activated as described above and added to the resin suspension. Argon was bubbled through resin suspension for 4 hrs. The product was washed with DMF (3 times), methanol (3 times) and DMF (3 times). Methyl-3-mercaptopropionate (60 mg, 56 µl, 0.5 mmol) was added to the resin suspension followed by N-methylmorpholine (0.5 ml) in DMF. Argon was bubbled through the resin suspension for 16 hrs. A drop of resin suspension was treated as described above for IR study.

Wang resin was purchased from Midwest Bio-tech and Merrifield resin from Aldrich. The reaction procedure on Wang resin has been previously described^{7}.

FTIR Microspectroscopy. All spectra were collected on a BIO-RAD, FTS-40 Spectrophotometer, using an SPC-3200 data station. The optical bench is coupled with a UMA-300 IR microscope. The microscope is equipped with a 36X Cassegrain objective and liquid nitrogen cooled Mercury-Cadmium-Telluride (MCT) detector. In the view mode, the total visual magnifications was 360X, which facilitated the locating of a single bead. Using the view mode and the X-Y platform of the microscope, the incident radiation was focused on a single resin bead (for globular bead measurement). Data collection is feasible in both the transmission and reflectance modes. In this study, the transmission mode was employed.

A few resin beads were transfered to the surface of a NaCl window (6 mm diameter). The window was put into a manual pellet maker with a diameter of 9 mm. The pressure was adjusted and kept constant to reduce the thickness of the flattened bead to 10-15 μm (calculation based on the diameter of the globular and flattened bead measured under the microscope). The area of the NaCl window adjacent to the flattened bead was used to collect the background spectrum. Data were collected with 4 wavenumber resolution. Sixty-four scans were averaged.

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