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Nanoparticles Secreted from Ivy Rootlets for Surface Climbing

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ABSTRACT

Using atomic force microscopy, we observed ivy secretes nanoparticles through adhering disks of the ivy aerial rootlets which allow the plant to affix to a surface. We analyzed the organic composition of the secretions using high-performance liquid chromatography/mass spectrometry and were able to determine the formula of 19 compounds. This study suggests that the nanoparticles play a direct and important role for ivy surface "climbing". Weak adhesion and hydrogen bonding seem to be the forces for the climbing mechanism. This ivy secretion mechanism may inspire new methods for synthesizing nanoparticles biologically or new approaches to adhesion mechanisms for engineering applications.

Ivy, a root-climber, is an evergreen plant having palmately lobed leaves. It belongs to the genus *Hedera*. Ivy consists of five species, including English ivy (*H. helix*), Boston ivy (*Parthenocissus, Ampelopsis, tricuspidata*), and those plants referred to as "poison" ivy. Boston ivy is a clinging woody vine of the Vitaceae family commonly applied in domestic and commercial landscaping and known for its coloration in autumn when the leaves turn bright scarlet. English ivy has dark-green leaves with three to five lobes, and tends to droop horizontally from the stem. Ivy can affix itself to, and extend its growth upward on, rocks, fences, trees, and many other surfaces. In this "climbing" process, ivy uses adhering disks of the aerial rootlets developed from the stem to affix to the surface. Removal of climbing ivy from a surface can be difficult, even after plant death at the root.

Charles Darwin reported in 1876 that ivy rootlet secretes yellowish matter while climbing a surface.[1] But the questions are still open for what is contained in the secreted material and how the matter is related to the ivy climbing mechanism. The first and the only study for micro-/nanostructure properties of the ivy in the open literature is imaging the ultrastructure of an isolated ivy leaf cuticles using atomic force microscopy (AFM).[2] Nevertheless, it does not discuss anything related to the secreted materials. It is particularly interesting for scientific communities to know how the adhering force is generated and why ivy can adhere to and climb various surfaces. We have first applied optical microscopy and AFM to study morphology of the secreted materials on a substrate surface and then treated these materials to solvent extraction and to use high-performance liquid chromatography/mass spectrometry (HPLC/MS) for analyzing the nanoparticle chemical composition. Here we report the results.

Optical Microscopic Images of the Ivy Rootlet. As shown in Figure 1A, large numbers of adhering disks from the rootlets are seen. The adhering disks themselves usually consist of four to seven tendrils or "fingers". These fingers make direct contact and affix to the surface. Figure 1B is the optical image of the adhering disk left on a silicon wafer. The image was obtained by pressing an ivy stem against the wafer surface. The length of these fingers is about 250–350 μm. As a reference, the left-hand side of the Figure 1B shows the length of an AFM tip of 125 μm.

AFM Image of the Secreted Materials. To study details of the fingers and secreted materials, we placed a 3 in. silicon wafer and a piece of mica at the end of an ivy branch. After the ivy climbs onto the silicon wafer and mica for a week, the branches were removed and the traces left on the surfaces were imaged using AFM. To ensure no external matter interfered with the process, we varied the experimental conditions, including (1) ivy grown on a silicon wafer in a place open to the clean air, (2) ivy grown on a silicon wafer in a sealed paper envelop, (3) ivy grown on a silicon wafer covered by a plastic film, and (4) ivy grown on mica in a sealed paper envelop.

As shown in Figure 2, we observed that the ivy secreted large numbers of nanoparticles from tendrils of the adhering disk for all the above experimental conditions. Figure 2A is an AFM image of the traces with a scan size of 14 × 14

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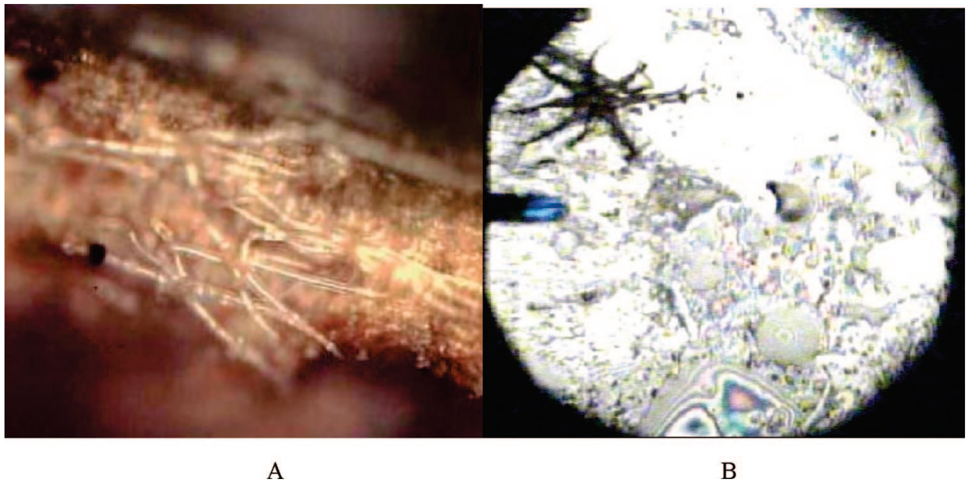

Figure 1. (A) An optical microscopic image of the ivy adhering disks from a rootlet. Each adhering disk consists of four to seven fingers. (B) An optical microscopic image of the leftover adhering disks on a silicon surface. Each finger is about 250–350 μm.

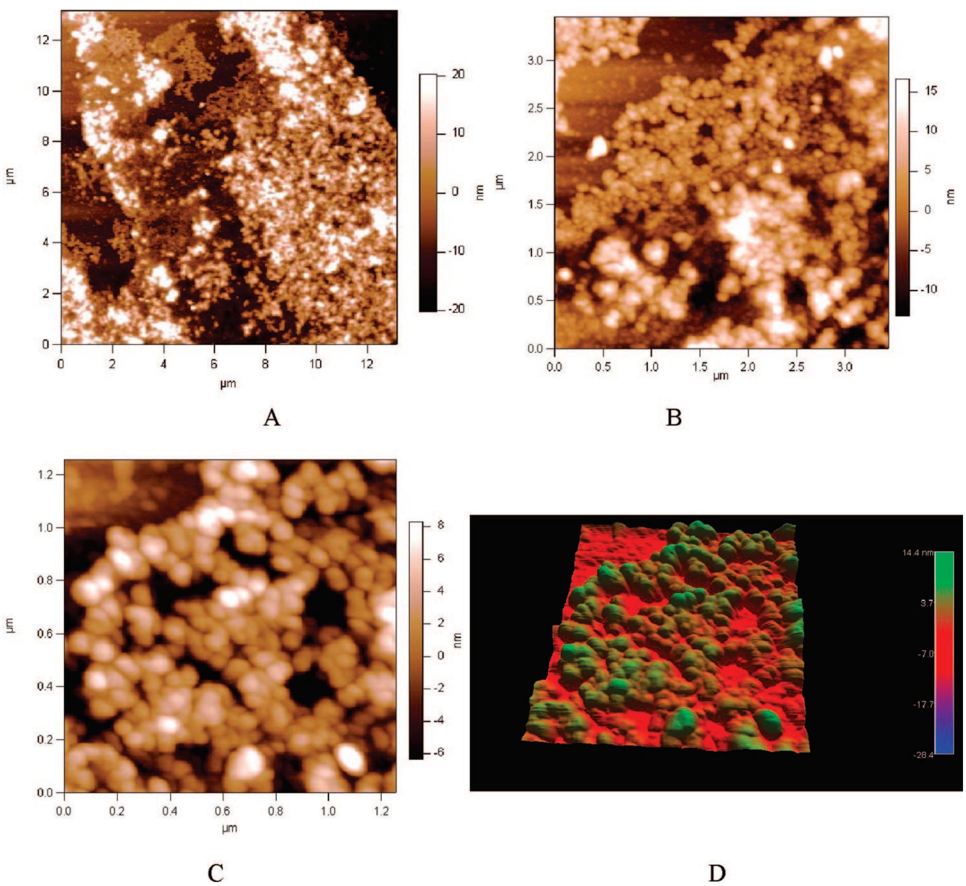

Figure 2. Nanoparticles secreted from fingers of the adhering disks of an ivy rootlet. (A−C) AFM topographic images of scan size of 14 × 14 μm, 3.5 × 3.5 μm, and 1.25 × 1.25 μm, respectively. (D) A 3-D view of (C).

μm. Panels B and C of Figure 2 show higher magnifications of the image and Figure 2D is a three-dimensional (3-D) image. The most salient feature revealed in the images is the high degree of uniformity of the nanoparticles. The particles are about 70 nm in diameter measured from cursor profiles of the AFM image. The apparent average height is about 20–30 nm, and the differences between the vertical and horizontal aspects are due to deformation caused by the AFM tip.

Figure 3 shows an image of the nanoparticles from traces of the ivy growing on two different surfaces: (A) a silicon wafer and (B) a mica wafer. Both show similar features for nanoparticles.

Every AFM image has consistently indicated that a large number of nanoparticles is secreted from the ivy rootlets. The nanoparticles are delivered from the ivy rootlets of the stem, to the adhering disk, and finally to its fingers. Observations support the hypothesis that the nanoparticles

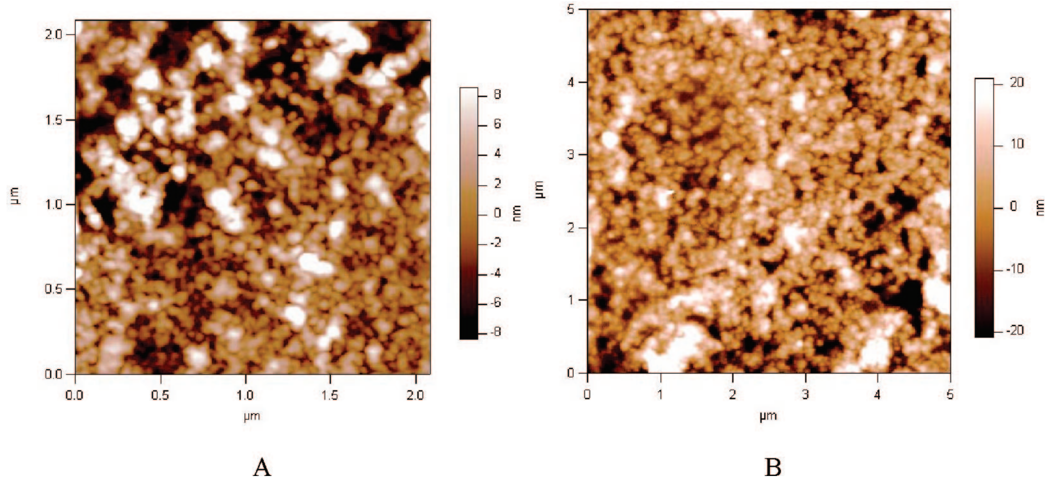

Figure 3. Nanoparticles secreted from fingers of the adhering disks along a silicon wafer surface (A) and mica surface (B).

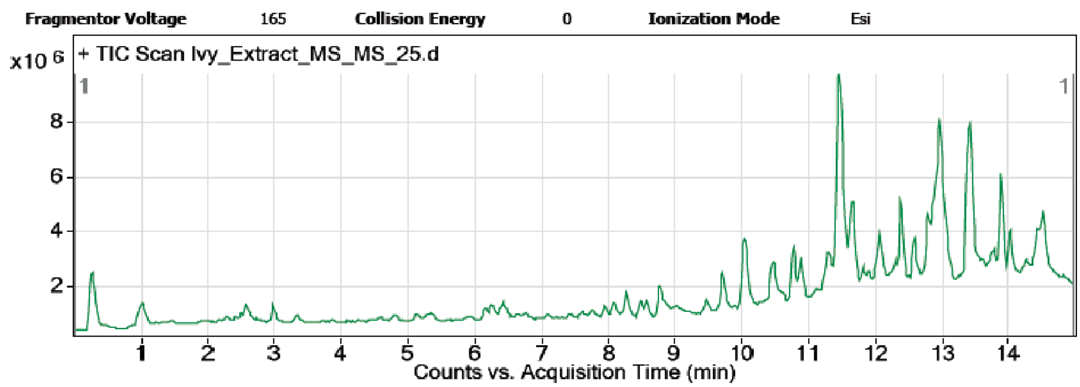

Figure 4. Reconstructed total ion chromatogram from HPLC/Q-TOF MS/MS.

play a direct and important role for ivy surface climbing and are directly related to the ivy affixing capability. After this physical analysis, the nanoparticles were submitted to a chemical composition analysis through extraction of the nanoparticles and extract analysis by HPLC/MS.

HPLC/MS Composition Analysis. Sample Preparation. A few milligrams of nanoparticles secreted from the ivy rootlets as shown in Figure 1A were extracted in chloroform/methanol (1:2) at room temperature for 2 days. The sample was evaporated to dryness (by SPEEDVAC), brought up in methanol, and analyzed by HPLC with Q-TOF detection (Agilent Technologies, Inc., Santa Clara, CA).

Experimental Condition. A rapid resolution, 1200 series HPLC system with a binary SL pump, well plate autosampler with thermostat and heated column compartment was used. Detection was performed using an Agilent 6520 Q-TOF with dual electrospray ion source (ESI). A reverse phase Zorbax Sb-Aq, 2.1 × 50 mm, 1.8 μm particle size column was used to separate the ivy extract. Solvent A was water + 0.1% formic acid. Solvent B was acetonitrile + 0.1% formic acid. Flow: 0.6 mL/min. A gradient of 2% B at time zero to 95% B at 13 min with a stop time of 15 min was used. The column temperature was 65 °C. Data were acquired in positive ion mode with auto MS/MS set at 100 ms for MS only mode acquisition and a maximum of three ions per cycle were subjected to MS/MS. The collision energy was set to either 25 or 35 eV.

Results and Analysis. Figure 4 shows a reconstructed total ion chromatogram of the nanoparticle extraction obtained by HPLC/Q-TOF. Clearly, there are many components and the data indicate that most peaks in the chromatogram contain multiple closely eluting compounds. A comprehensive composition analysis is very difficult so instead preliminary empirical formulas of the most intense 19 compounds that could have empirical formulas assigned using MS and MS/MS data in the extract were considered. The chemical formulas of composition are listed in Table 1. The empirical formulas calculated are based on the observed assumed M + H ion in the MS data and the MS/MS fragment ion data. For an empirical formula to be accepted as being likely to be correct, the MS/MS spectra must include fragments ions that are consistent with the proposed empirical formula from the MS data. This is a higher criterion to pass than if only MS data were required.

Although the (relative) response factors are unknown, the relative trend in the chromatogram toward more peaks and more intensity later in the TIC chromatogram suggests many lower polarity compounds. This is consistent with the idea of a more waxy leaf composition which is widely known. However the formulas indicate that most of these compounds contain oxygen, nitrogen, and sulfur. These compounds are widely known for their ability to generate polar materials and, most importantly, hydrogen bonding. It is likely that the typical hydrophobic, saturated hydrocarbon "tail" with

Table 1. Empirical Formulas for the Top 19 Components As Determined by High-Resolution Q-TOF Analysis Organized by Decreasing Abundance

index (ordered decreasing by abundances)	retention time (min)	compound formula
1	11.436	$C_{26}H_{42}N_8O_{10}$
2	13.369	$C_{18}H_8ClNO_{11}S_5$
3	11.506	$C_{21}H_{46}N_{20}O_3$
4	13.385	$C_{38}H_{76}N_2O_3$
5	12.773	$C_{14}H_6Cl_3NO_{12}S_3$
6	12.068	$C_{20}H_7N_5O_{11}S_5$
7	11.641	$C_{38}H_{74}N_2O_4$
8	12.513	$C_{28}H_{46}N_6$
9	10.461	$C_{38}H_{76}N_2O_5$
10	13.47	$C_{38}H_{76}N_2O_3$
11	11.66	$C_{23}H_{26}N_{12}O_{10}$
12	11.643	$C_{32}H_{33}N_7OS_3$
13	12.359	$C_{29}H_{28}N_{12}O_{10}$
14	13.368	$C_{38}H_{76}N_2O_3$
15	10.854	$C_{37}H_{63}N_5O_3$
16	10.752	$C_{21}H_{24}N_{12}O_5S$
17	13.489	$C_{38}H_{76}N_2O_2$
18	10.016	$C_{21}H_{45}N$
19	8.476	$C_{18}H_{23}N_5O_2S_2$

a polar "head" molecular structure is expressed. Considering the surfaces that the nanoparticles normally or typically attach to are substrates like rocks, bricks, etc., which are inorganic or at least polar in nature, the composition suggests that the nanoparticles rely on hydrogen bonding to affix to different surfaces.

Observing the evolution of the nanoparticle during the climbing process, we noticed that a yellowish material was gradually secreted which, in the earliest stage, is in the gel state and later becomes dry. Once the secretion and drying are completed, the materials are attached firmly to the surface. Apparently water is evaporated during the process.

Millions of adhering disks can generate remarkable adhesion for the ivy to affix to a surface. The adhering disk along the secreted nanoparticles can fit into various surfaces due to their small size and flexibility. This feature allows ivy to generate enough force for surface climbing. The affixing mechanism formed by the nanoparticles and the adhering disk is unique. The ivy climbing mechanism by secreting nanoparticles has many advantages for surface climbing. First, joints are not needed and complex mechanics are avoided, yet the mechanism is flexible enough to adapt to various environments.

The above finding may also inspire mechanisms for fabricating nanoparticles through plants. Considerable efforts have been made to generate nanoparticles biologically.[3–5] Alfalfa plants have been demonstrated to formulate and grow metallic gold nanoparticles.[3] Extracts from the lemongrass plant, when reacted with aqueous chloroaurate ions, have been shown to yield a high percentage of thin, flat, single-crystalline gold nanoparticles.[4] Aloe vera plant was used to synthesize both gold and silver nanoparticles.[5] However, no research in the open literature reports that plants can actually secrete nanoparticles naturally for climbing purpose. This study demonstrates that ivy can secrete nanoparticles for surface climbing.

In summary, we discovered ivy secrete nanoparticles for surface climbing. HPLC/MS analysis suggests empirical formulas for the 19 prevalent compounds of organic composition from the secreted nanoparticles. The study suggests that the weak adhesion and hydrogen bonds are the likely forces for ivy surface climbing. The finding may inspire new method to synthesize nanoparticles biologically, or new climbing mechanisms for engineering applications.

References

(1) Darwin, C. *Movements and Habits of Climbing Plants*; D. Appleton and Company: New York, 1876; pp 183–206.

(2) Canet, D.; Rohr, R.; Chamel, A.; Guillain, F. *New Phytol.* **1996**, *134*, 571–577.

(3) Gardea-Torresdey, J. L.; Parsons, J. G.; Gomez, E.; Peralta-Videa, J.; Troiani, H. E.; Santiago, P.; Yacaman, M. J. *Nano Lett.* **2002**, *2*, 397–401.

(4) Shankar, S. S.; Raiv, A.; Ankamwar, B.; Singh, A.; Ahmad, A.; Sastry, M. *Nat. Mater.* **2004**, *3*, 482–488.

(5) Chandran, S. P.; Chaudhary, M.; Pasricha, R.; Ahmad, A.; Sastry, M. *Biotechnol. Prog.* **2006**, *22*, 577–583.

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