Clonal propagation technology

The National Symposium on 'Commercialization of Clonal Propagation Technology in Plant Improvement' was organized by G.M. Reddy Research Foundation, Hyderabad during 24–26 March 2000. The theme of the symposium was 'Integration of frontier technologies with traditional farmers' wisdom and scientific temper in upgrading economic status of common man, farmer and rural poor'. The topics ranging from commercialization of transgenics, conservation, propagation of medicinal plants, forest trees spices, horticultural crop like banana, biopesticides in control of crop disease besides intellectual property rights were discussed.

V. L. Chopra (National Biotech Centre for Indian Agricultural Research Centre, New Delhi) in his inaugural address, reviewed the present scenario of commercialization of clonal propagation. He pointed out that in the last decade very large investments have been made in India both by public and private sectors for creating an infrastructure for tissue culture multiplication of plants. It is estimated that the capitalized cost of investment in the private sector is over rupees 350 crores. The capacity for producing tissue culture plants is of the order of 170 million plants per year even though only a fraction of the capacity is currently utilized. There were close to 70 units about a decade ago, when tissue culture started as fashionable activity, of which 40 have already wound up. Many of the remaining units are financially in a bad state because of defaulting in the payment schedule of loan instalments. In the public sector, almost every university and research institute has made large investments in creating plant tissue culture facilities.

It was emphasized that there was no dearth of competent technical persons or market demand. For successful operation, a change of mindset is needed so that synergies are exploited between technical know-how, entrepreneurial capacities and management skills to turn around the situation and transform loss-making investments into profitable enterprises.

The session on transgenics and functional genomics dealt with various aspects of commercialization by A. R. Reddy, N. Seetharama, K. V. Krishnamurthy, P. B. Kirti and S. Y. Anwar. Emphasis was laid on commercialization of genetically modified plants including oil quality which is the need of the hour. P. S. Rao emphasized the need to cultivate medicinal plants and C. M. Rao dealt with a new technology in clonal propagation of high-yielding eucalyptus. Low cost media were developed for efficient micropropagation of banana by G.M. Reddy Research Foundation. As a result, the cost of production has come down to almost one-third of the regular price. A session on biopesticides identified neem-based products in the control of crop diseases by Srimannarayana and Shiva Prasad. In the final session, E. A. Siddiq dealt with in great detail the intellectual property rights. The symposium concluded with the following recommendations.

The tissue culture protocols developed for micropropagation of horticultural, forest and medicinal plants may be listed and registered. Conservation of endangered species may be propagated through tissue culture technology for prosperity and also for other purposes. Need-based basic and applied research may be identified in different areas of clonal propagation in collaboration with research institutes, industrial organizations and forest and horticultural departments to promote joint ventures in product development for commercialization.

G. M. Reddy, Prof. G.M. Reddy Research Foundation, 4-123/E, Swaroop Nagar, Uppal Kalan Municipality, Hyderabad 500 039, India.

RESEARCH NEWS

Chemical bond formation at atomic and molecular scale by STM

Ujjal Kumar Sur

Since the invention of the scanning tunnelling microscope (STM) in the early eighties by Binnig and Rohrer, it has been a powerful tool in imaging surfaces of metals and semiconductors. There have been reports on the positioning of single atoms and molecules[1–3] and dissociation of single molecules on metal and semiconductor surfaces[4,5] using STM. Eigler and Schweizer have first used the STM for positioning atoms and fabricating patterns on metal surface[1]. Using a polycrystalline tungsten STM tip at 4 K they positioned individual xenon atoms on a single crystal nickel surface (110) and fabricated various patterns. The process they described was also applicable to molecules. Utilization of the atomic resolution of the STM gives the prospect of atomic-scale logic circuits and nanotechnology. Over the past few years much has been learnt from experimental and theoretical studies regarding the mechanism of atomic and molecular manipulation by STM. Stipe *et al.*[4] have used the STM to dissociate single O_2 molecules on Pt (111) surface in the temperature range of 40 to 150 K. From their experimental results, they have found a relationship between the dissociation rate and tunnelling current and voltage. Their results opened the concept of 'angstrochemistry' with atomic resolution in the initiation of reactions. Using the concept of 'angstrochemistry', scientists have tried to perform chemical bond formation

at atomic and molecular levels which is the reversal of bond dissociation. Recently, a breakthrough has been made in the field of single atom chemistry, where a chemical reaction has been controlled at both atomic and molecular levels. Lee and Ho have used a low temperature STM in an ultrahigh vacuum environment to form a single bond between a diatomic molecule CO and an Fe atom[6]. They have characterized the reactants and products by the STM topographical imaging and Inelastic Electron Tunnelling Spectroscopy (IETS).

In their method, a clean Ag (110) single crystal surface was taken and Fe atoms were evaporated and coadsorbed along with CO molecules at 13 K with the formation of Fe(CO) and $Fe(CO)_2$ molecules on Ag surface using a STM tip. The entire experimental set up was cooled to 13 K for reducing the contamination rate of the sample Ag surface during the coadsorption of Fe atoms and CO molecules in the gas phase. Fe atoms and CO molecules formed a monolayer of coverage around 0.001 on the Ag surface. Such a low coverage will prevent the possible interactions among the Fe atoms and the CO molecules on the Ag surface, which makes the formation of Fe(CO) and $Fe(CO)_2$ virtually impossible at the cryogenic temperature.

The formation of a single bond between an Fe atom and CO molecule involved a series of steps. The topographical image of the surface with coadsorbed Fe atoms and CO molecules was taken at 70 mV sample bias voltage and 0.1 nA tunnelling current (Figure 1). In the first step, the polycrystalline tungsten tip was positioned over a single CO molecule adsorbed on the top of a Ag atom and the CO molecule was transferred to the tip by increasing the bias voltage to 250 mV and ramping the tunnelling current from 0.1 to 10 nA. A 180° rotation of the CO molecule has been observed during its transfer from the surface to the STM tip. The tip with the attached CO molecule was translated and positioned over an Fe atom. Finally by reversing the bias voltage (– 70 mV) and the flow of electrons in the form of a tunnelling current, the CO molecule was transferred from the tip to the Fe atom on the Ag surface. Thus Fe–CO single bond was formed. An additional CO molecule was bonded to Fe(CO) molecule in a similar way to form $Fe(CO)_2$. In the STM images, Fe atoms and CO molecules appeared as protrusions and depressions, respectively (Figure 1 *a*). The STM topographical image of the Fe(CO) molecule indicates that the CO ligand is not bonded perpendicular to the Fe atom, but has an inclined configuration. The inclined configuration of the CO ligand on the Fe atom is probably due to the localized electronic properties of the Fe atom. The symmetric location of two CO ligands on the Fe atom in $Fe(CO)_2$ was also observed in the STM topographical image (Figure 1 *d*).

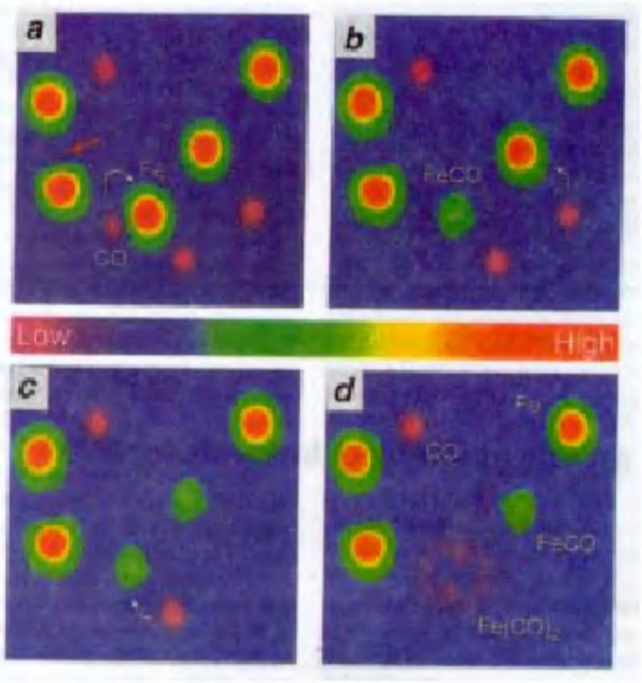

Figure 1. STM topographical images showing chemical bond formation between an Fe atom and a CO molecule. Image size, 63 Å × 63 Å; Bias voltage, 70 mV; tunnelling current, 0.1 nA; Temperature, 13 K. ***a***, Adsorbed Fe atoms and CO molecules on Ag (110) surface. Red arrow indicates close presence of a CO molecule to an Fe atom and white arrow indicates the CO molecule and Fe atom involved in bond formation; ***b***, A CO molecule is bonded to an Fe atom to form Fe(CO) molecule; ***c***, Formation of another Fe(CO) molecule; ***d***, Formation of $Fe(CO)_2$. Reprinted with permission from Lee, H. J. and Ho, W., *Science*, 1999, **286**, 1719. © 1999 American Association for the Advancement of Science.

The results obtained from IETS are strong proofs for the identification of reaction products. IETS is a powerful technique for the study of vibrational modes of molecules adsorbed on the surface of oxide layers in a metal–insulator–metal tunnel junction[7]. Elastic tunnelling involves the tunnelling of electrons from one metal to another without loss of any energy. However if molecules are adsorbed on the insulating tunnelling junction between two metals, there is loss of energy involved in the vibrational transition of molecules. Beyond a certain threshold value of bias voltage, inelastic electron tunnelling occurs and the plot of d^2I/dV^2 vs bias voltage gives sharp peaks similar to those in infrared (IR) absorption spectroscopy. The peaks in the second derivative curves correspond to various vibrational levels of the molecules adsorbed on the insulating tunnel junction between two metals. The positions of the peaks give information about the structure and bonding of adsorbed molecules. The major advantage of IETS over IR absorption spectroscopy is its better sensitivity and resolution. Extremely sharp peaks are observed and there is no broadening effect of peaks at the cryogenic temperature. $^{12}C^{16}O$ and $^{13}C^{18}O$ cannot be distinguished from the STM topographical images. Each isotope in the products $Fe(^{12}C^{16}O)$, $Fe(^{13}C^{18}O)$, $Fe(^{12}C^{16}O)_2$, $Fe(^{13}C^{18}O)_2$ and $Fe(^{12}C^{16}O)$

($^{13}C^{18}O$) can be differentiated from the peak positions of IETS. The Ag(110) spectrum was considered as a reference to all other vibrational peaks. The dc sample bias voltage was ramped from 180 to 280 mV in each scan. Sharp peaks were observed at 236 and 224 meV, respectively for Fe($^{12}C^{16}O$) and Fe($^{13}C^{18}O$) which correspond to C–O stretching. The observed isotope shift between Fe($^{12}C^{16}O$) and Fe($^{13}C^{18}O$) is 12 meV, which matches closely with other calculated and experimental values. For Fe($^{12}C^{16}O)_2$ and Fe($^{13}C^{18}O)_2$ peaks were observed at 234 and 220 meV, respectively. The product of Fe($^{12}C^{16}O$) ($^{13}C^{18}O$) consists of mixed isotopes. The IETS was recorded separately over $^{12}C^{16}O$ and $^{13}C^{18}O$ and peaks were observed at 235 and 223 meV. The observed isotope shift is 12 meV, which is the same as in the previous case.

Comparison of the corresponding activation energy of desorption values indicates that CO can form stronger bonds with an Fe atom compared to the Ag surface or tip. It was also observed that due to the very low temperature of the study there was no bond formation between an Fe atom and a CO molecule in spite of their close presence as shown by the STM topographical images (Figure 1 *a*). The STM tip plays a very important role in the bond formation between an Fe atom and a CO molecule even at very low temperatures.

In addition to the manipulation of atoms and molecules by STM, atomic manipulation can be done by other scanning probe microscopes. A modified Scanning Force Microscope (SFM) has been used for manipulation of various atoms and molecules on various substrates (mica, silicon, graphite, etc.)[8,9] in a controlled manner. This instrument is called a Nano Manipulator. A hand-held force stylus is interfaced with the scanning tip of the microscope. When the microscope is switched to the manipulating mode, the scanning tip moves according to the motion of the hand-held stylus, thus enabling controlled manipulation of the sample. The manipulation force, speed and manipulation direction can be controlled by the user. After manipulation, the microscope can be switched back to the imaging mode to view the manipulation. This cycle can be repeated as desired. The hand-held stylus is connected to the scanning tip by an integrated force feedback loop. During manipulation, several parameters (modifying force, lateral force, topography) are recorded simultaneously. This Nano Manipulator has been used in the manipulation of tobacco mosaic virus[8] and carbon nanotubes[9] and other biological and nonbiological materials.

The STM junction behaves as a reactor of atomic dimension in which tunnelling electrons and the electric field are probably responsible in overcoming the energetic barriers between the stable bonding sites of CO on the surface, tip and the Fe atom. Lee and Ho have done a remarkable job by performing one of the simplest chemical transformations of binding a diatomic molecule to an atom using the STM. The scientific community, especially chemists hope that using the STM more complicated chemical reactions can be performed at the atomic and molecular levels in the future.

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Ujjal Kumar Sur is in the Raman Research Institute, Bangalore 560 080, India (e-mail: uks@rri.ernet.in)

Random selections

High pressure research

'Hydrogen at high pressure'
E. G. Maksimov and Yu. I. Shilov
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Nearly 80 years ago, Wigner and Huntington predicted on theoretical basis that hydrogen that solidifies into a molecular insulating phase would transform into a monatomic metallic phase at around 25 GPa (1 bar = 10^5 Pa). This transition pressure got upgraded by further calculations by others to a wide range up to 1500 GPa.

Experiments in the late eighties and nineties showed that the molecular crystalline phase exhibits a rich variety of unusual properties as a result of formation of several anisotropic crystalline transforms (see review by H. K. Mao and R. J. Hemley, *Rev. Mod. Phys.*, 1994, **66**, 671). The phase diagrams of isotopically pure hydrogen and deuterium (H_2 and D_2) have been established on the basis of diffraction data and spectroscopy of optical vibrations of their solids.

Theoretical studies in the seventies led to the prediction of Hi-T_c superconducting behaviour of hydrogen in the metallic phase with a T_c around 200 K. The insulator–metal transition reportedly observed by experimental groups is shrouded with debate and controversy. The discussions cover a wide variety of opinions – from (i) reliability of experimental facts themselves to (ii) possibility of metallic behaviour in the molecular phase itself instead of need to go through a molecular to atomic phase of hydrogen. The story of Hi-T_c in the metallic phase is more complex and at the same time interesting.

The article cited here provides a comprehensive review of this fascinating subject.