all require TonB to transport their ligands across the outer membrane, although active transport has not been demonstrated in all cases. The best example of active transport is the BtuB transporter, which can establish a vitamin B_{12} concentration in the periplasmic space that is 1000 times that in the external milieu (*2*). From the periplasmic space, the substrates follow the usual path of ATP-dependent transport across the inner membrane.

By itself, the active transport of a nutrient across the outer membrane seems unremarkable, except that the outer membrane has no direct access to energy-producing pathways. Instead, this active transport step depends on activation of TonB by the proton electrochemical potential of the inner membrane. Activated TonB can then bind to the outer membrane iron transporters and transduce energy to them. In the absence of TonB or the proton gradient, ligands still bind with high affinity to their transporters but are unable to cross the outer membrane. These TonB-dependent outer membrane transporters were erroneously classed as ligand-gated porins before the crystal structure of FhuA (*3*) revealed that ligand binding did not open a pore within the transporter.

Like every protein in the outer membrane, the active transporters FhuA, FepA (*4*), and FecA (*1*) have a β-barrel skeleton; however, that structure merely reflects the requirement for their localization in the outer membrane. Outer membrane proteins cannot contain the 20–amino acid stretches of hydrophobic residues characteristic of inner membrane proteins because these sequences would prevent newly synthesized outer membrane proteins from reaching their destination. Outer membrane proteins that allow passive transport of ligands are all pores of some sort. In contrast FepA, FhuA, and now FecA have an internal globular domain (the gate) that completely occludes the β barrel. The crystal structures for both FhuA and FecA (bound and unbound to ligand) have been solved. In both cases, binding of ligand to the external face of the transporter causes a large conformational shift in the region of the protein at the periplasmic face. Intriguingly, this conformational change does not alter the location of the internal globular domain or create a passageway through which ligand can exit. Clearly, we have been confused—these outer membrane transporters do not form pores and are not gated open by their ligands, which suggests that henceforth they should be called TonB-gated transporters to reflect the way they work.

In the new study, Ferguson *et al.* (*1*) reveal that FecA is even more dynamic than other TonB-gated transporters. It has a second gate, primarily composed of external loops 7 and 8 of the β barrel. The ligand, in this case ferric citrate, binds to transporter and closes the newly discovered external gate behind it, preventing access to the external milieu. It will be interesting to learn whether this is a general feature of all TonB-gated transporters. Ferguson *et al.* propose a four-step model for iron transport by FecA: (i) The iron-siderophore complex is adsorbed by low-affinity sites on external loops of the β barrel of FecA, (ii) the complex is transferred to high-affinity sites within the external loops of FecA's internal globular domain, (iii) the external loops reposition themselves to close the external pocket of the β barrel, and (iv) a TonB-dependent conformational change in the globular domain opens the gate to allow release and transport of the ligand into the periplasmic space (see the figure).

Like any important scientific advance, this one raises interesting questions. Does the newly discovered external gate stay closed once ligand has been bound, or does it "flutter" between open and closed positions? The on-off binding rates for iron-siderophore complexes have not been measured and could be very rapid. Work on vitamin B_{12} transport has shown that once in the periplasmic space, vitamin B_{12} can return to the external medium (*2*). Is this reverse transport mediated by the TonB-gated transporter BtuB? Bacterial toxins (colicins) that attack *E. coli* and *E. coli*–specific bacteriophages both gain access to their bacterial hosts through TonB-gated transporters. But how? While it does not answer that question, the FecA crystal structure does explain how iron-siderophore complexes decrease bacterial susceptibility to colicins (*5*). Presumably, when the external gate closes behind the iron-siderophore complex, this completely sequesters the colicin binding site. The colicins are enormous (~60 kD) relative to iron-siderophore complexes (~1 kD), and bacteriophages are even larger. Is closing of the transporter loops a requirement for iron-siderophore complex and vitamin B_{12} transport? The size of colicins and bacteriophage ϕ80 would almost certainly preclude closing of the external gate.

The crystal structure of FecA, the third member of the TonB-gated transporter family, has provided us with a new way of thinking about ligand transport. It also confirms the structural aspects of iron transport revealed by the structure of other bacterial iron transporters. Thus, crystal structures of homologous proteins continue to reveal insights that could not have been predicted from the amino acid sequence.

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PERSPECTIVES: LASER CHEMISTRY AND PHYSICS

The Next Frontier

Kaoru Yamanouchi

How do atoms and molecules interact with light? One might think that sophisticated modern laser spectroscopy has already answered this fundamental question and that nothing remains but to apply our wealth of knowledge. However, the development of ultrashort, superintense pulsed lasers has led to the realization that much remains to be learned about light-matter interactions.

It is well known that ultrashort pulsed lasers allow probing of molecular processes in real time on the femtosecond time scale. The latest advances originate not, however, from the ultrashort temporal width of the laser light but from its extremely high intensity. The advent of chirped pulse amplification (CPA) has greatly increased the output energy of ultrashort pulsed lasers (*1–3*). Even in university laboratories, laser light fields as high as 10^{15} W/cm^2 (1 PW/cm^2) can now be generated routinely with a table-top, high-power CPA laser system. This intensity is comparable in magnitude to the Coulomb field generated by an atomic nucleus. In large-scale facilities such as JAERI in Japan and LOA in France, a laser field intensity of 10^{20} W/cm^2 (100 EW/cm^2) could be achieved.

At intensities well below ~10^{12} W/cm^2 (the perturbative regime), atoms and molecules absorb one or multiple photons through a weak interaction with the light

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field. At intensities of ~10^{12} to ~10^{17} W/cm^2 (the Coulombic regime, see the first figure), a fundamentally different behavior is observed. Most studies have focused on the characteristic dynamical behavior of molecules at these intensities (*4*).

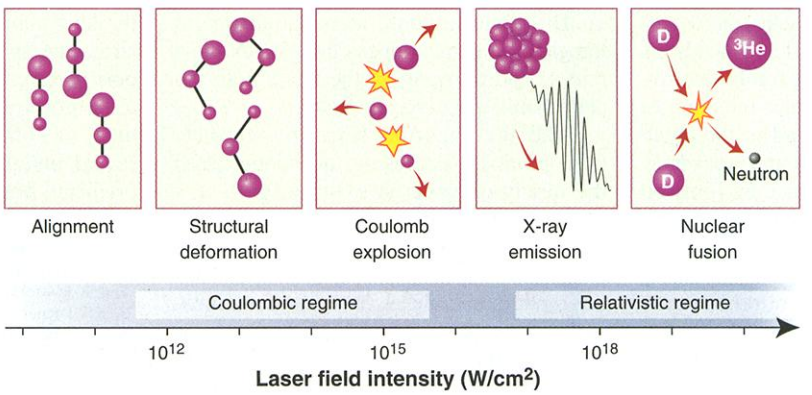
When laser field intensities reach ~10^{12} W/cm^2 (1 TW/cm^2), molecules are aligned along the laser polarization direction by the torque generated by the interaction between nonresonant light fields and the induced dipole moment of the molecule. Such an alignment process can be realized by focusing an intense laser field with relatively long pulse duration (~10 ns). Recently, the alignment of gaseous molecules along the polarization direction of a TW laser field was observed directly by pulsed-gas electron diffraction measurements (*5*, *6*).

When the magnitude of the laser field is increased to between 0.1 and 1 PW/cm^2, strong mixing of electronic states of molecules occurs. This means that the potential energy surface (PES) of a molecule, whose shape is determined by its electronic configuration, could be deformed in intense laser fields. If the shape of the PES can be varied in a desired way in intense laser fields, the fate of a molecule could be actively controlled by light.

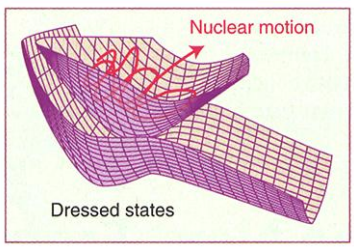
The idea of changing the shape of a pair of PESs by a light field is shown schematically in the second figure. The newly formed PESs are called light-dressed PESs, that is, PESs wearing a dress of light. By controlling the direction of nuclear motion on the potential energy surface, whose shape is dependent on the variation of the laser field intensity, the nuclear motion leads to breaking of a specific chemical bond. Selective bond breaking of a polyatomic molecule using the ideal shape of intense laser pulses was recently achieved using a genetic algorism (*7*). This process might be interpreted in terms of the formation of light-dressed PESs.

At intensities of ~1 PW/cm^2, the geometrical structure of molecules becomes deformed within as little as 100 fs. Linear molecules such as CO_2 and CS_2 become bent while bent molecules such as H_2O and NO_2 become stretched. These phenomena have been attributed to dressed state formation (*8*).

During such structural deformation, tunneling ionization occurs, in which an electron escapes through a barrier of the deformed Coulombic well and singly, doubly, and multiply charged molecular ions are formed. After the formation of multiply charged molecules, abrupt chemical bond fission (Coulomb explosion) occurs, triggered by the strong Coulombic repulsive force, and atomic and molecular fragment ions with large kinetic energies (10 to 100 eV) are ejected. The structural deformation of small polyatomic molecules has been characterized by detecting such fragment ions and measuring their momentum vector distributions with new detection schemes such as mass-resolved momentum imaging (*8*) and coincidence momentum imaging (*9*).



Exotic behavior of molecules and clusters in intense laser fields.



A pair of light-dressed potential energy surfaces.

When the laser intensity reaches 1 EW/cm^2, plasmas are produced from atomic and molecular substances in the focal region of the laser, and the effect of the magnetic field component of the light on electron motion becomes as large as that of the electric field component. In such a superintense laser field, the electron velocity increases so much that relativistic mass correction becomes necessary. Also, the electrons move in figure-eight patterns due to the contribution from the Lorentz force, rather than along the direction parallel to the laser polarization direction.

In the relativistic regime (above 1 EW/cm^2), pulsed laser light propagating through the plasma causes a wake field, which accelerates electrons in the plasma to GeV. This laser wake field acceleration is regarded as a novel technique in accelerator physics (*1*, *2*). Researchers have also discovered that atomic clusters are very efficiently ionized in superintense laser fields to form plasmas containing highly charged atomic ions. Bright x-rays in the 0.1- to 10-keV range have been generated from rare gas clusters with high conversion rate (up to 10 %) from the incident laser energy. Ions ejected from the clusters have a high kinetic energy: For Xe_n ($n > 1000$) clusters, Xe ions with energies as high as 1 MeV have been detected (*3*).

Another important phenomenon occurs in superstrong laser fields: nuclear fusion. When D_2 clusters were irradiated with a short pulsed superintense laser field, near monoenergetic neutrons with energy of 2.45 MeV were generated, indicative of the fusion reaction D + D → n + He^3 (*10*). This observation was attributed to collisions between energetic deuterons ejected from different D_2 clusters in the laser-heated volume at the focal region. The short pulsed (less than 1 ns) neutrons generated by laser-assisted fusion could be an efficient neutron source, for example, for the structural analysis of solid materials by neutron diffraction.

These developments illustrate the variety of characteristic phenomena related to atoms, molecules, clusters, plasmas, nuclei, and elementary particles that have been discovered through the use of intense laser fields. A new interdisciplinary research field is emerging through cooperative efforts among chemists, physicists, and laser engineers. Efforts are underway to increase the laser field intensities even further to 10^{28} W/cm^2 where electron-positron pair creation from vacuum could be realized (*11*).

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