nor the end products of its reaction have been defined.

Why does *Thermoplasma* need TRI in addition to the proteasome? Like the proteasome, TRI appears to cooperate with other factors (albeit in a different manner) to achieve its full proteolytic activity. These include two low molecular weight protein factors, F1 and F2 (*2*). Surprisingly F1, a 33-kD polypeptide (*5*), is itself a protease with homology to certain proline iminopeptidases and is capable of hydrolyzing a wide variety of substrate peptides. By means of a physical interaction with TRI, F1 may act on the proteolytic products generated by TRI. Thus, the picture of a new type of modular proteolytic system is emerging with TRI as its core; upon interacting with low molecular weight factors, intrinsic proteolytic activities of TRI may be enhanced and new activities generated (*2*, *5*).

The proteasome cooperates with an adenosine triphosphate (ATP)–dependent protein machine that is thought to unfold polypeptide substrates in preparation for subsequent cleavage (*3*). The proteolytic chamber of the proteasome, located in the center of the barrel, is flanked by two antechambers and, in the crystal structure (*3*), is accessible through narrow channels of no more than 1.3-nm width that can only be traversed by unfolded polypeptides (on the right in part B of the figure is a vertical cut through the proteasome cylinder). So far, there is no evidence for an ATP requirement for TRI. The access to the large central chamber in the TRI complex appears much wider, and less extensive unfolding of a substrate may be required for cleavage. TRI may cooperate with the proteasome by cutting substrates, such as thermally denatured polypeptides, into smaller pieces that unfold spontaneously to become competent for final degradation by the proteasome. No doubt future studies will provide answers to many of these questions and will clarify the significance of TRI as a novel proteolytic concept. Who knows what other secrets TRI keeps under its hat.

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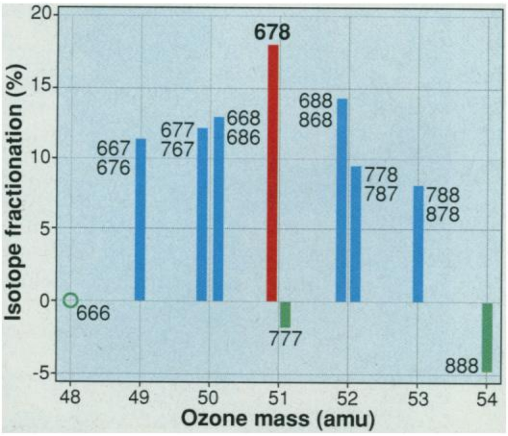
ATMOSPHERIC CHEMISTRY

Heavy Ozone—A Difficult Puzzle to Solve

Dieter Krankowsky and Konrad Mauersberger

Ozone makes up a small percentage of the atmosphere but is one of its most important constituents. It has a unique molecular composition consisting of three atoms of the same element that form an equilateral open triangle. Ozone is produced in a three-body reaction $O + O_2 + M = O_3 + M$, with M being a stabilizing molecule. Because there are three oxygen isotopes—^{16}O, ^{17}O, and ^{18}O—a variety of ozone isotopomers, usually called ozone isotopes, can be formed, ranging in mass from 48 to 54 atomic mass units (amu). Because ^{17}O and ^{18}O are minor species in atmospheric oxygen, only three ozone isotopes are present in measurable quantities in our atmosphere: the dominant molecule $^{48}O_3$ and, considerably lower in abundance, $^{49}O_3$ and $^{50}O_3$. Numerous laboratory studies and atmospheric measurements have shown that the heavy isotopes are enriched by 10% or more over values one would expect from a statistical distribution of oxygen in ozone. This imbalance is an unusually large isotope effect for such molecules. Theoretical analysis of the ozone formation process in terms of the standard kinetic isotope effect actually predicts a small mass-dependent depletion. Past attempts to find the physical processes leading to the large enrichments have not been successful. A report by Gellene (*1*) on page 1344 of this issue of *Science* provides a quantitative prediction of the symmetry-controlled ozone isotope fractionation.



Measured enrichment or depletion of all possible ozone isotopes [after Mauersberger *et al.* (*7*)]. The labels 6, 7, and 8 stand for ^{16}O, ^{17}O, and ^{18}O, respectively. Ozone was produced in two isotopically enriched oxygen mixtures of well-known composition at 70 torr and room temperature. The statistical abundance of each isotope was calculated and used to derive from the measured values the isotopic fractionation. The values in the graph are normalized so that $^{48}O_3$ has zero enrichment.

The first indication of an unusual isotope effect was found in stratospheric ozone, initially by mass spectrometric measurements on board balloons (*2*) and later through analysis of cryogenically collected ozone samples (*3*). Infrared emission and absorption spectroscopy from the ground and airborne vehicles (*4*) have confirmed the enrichments in heavy ozone. Tropospheric ozone repeatedly collected and analyzed has also shown enrichments of about 9 and 7% in $^{50}O_3$ and $^{49}O_3$, respectively (*5*).

Inspired by atmospheric measurements, investigators have studied in the laboratory the ozone formation process in great detail. Ozone has been produced both in oxygen of atmospheric composition and in isotopically enriched samples, extending the number of ozone isotopes to be studied. Results show that the isotope effect is large, typically 10 to 20%, and its magnitude depends on the pressure and temperature of the gas in which ozone is formed. This isotope effect cannot be explained by known physical processes such as diffusion or by kinetic effects, in which most isotope fractionations have their origin. The effect is mass independent, occurs in the gas-phase formation process, and exhibits a strong dependence on molecular symmetry, as first recognized by Heidenreich and Thiemens (*6*).

The symmetry dependence was clearly demonstrated when isotopically enriched oxygen mixtures were used to produce all possible isotopomers of ozone from 48 through 54 amu (*7*) (see figure). The asymmetrical molecule $^{16}O^{17}O^{18}O$ of mass 51 amu shows the largest enrichment, whereas six others composed of symmetric and asymmetric molecules are less enhanced independent of their mass. The two completely symmetrical molecules $^{17}O^{17}O^{17}O$ and $^{18}O^{18}O^{18}O$, however, are reduced in abundance in a mass-dependent

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manner. It is indeed surprising that ozone molecules of mass 51 amu either can carry a large enrichment of nearly 20% or can be depleted by a few percent depending on the oxygen atoms and molecules from which they were formed. Kaye (*8*) used conventional recombination theory based on approximate unimolecular rate theory and statistical thermodynamics to derive the ozone isotope distribution. His results show a mass-dependent depletion in all heavy isotopes, which is only observed in the two completely symmetrical molecules (see figure).

Despite the fact that much experimental information is at hand, the isotopic anomaly has lacked plausible explanations. A fair number of attempts have been made (*6*, *9*), but so far they have been unable to explain all of the experimental results. Most recently, Anderson *et al.* (*10*) proposed that metastable states in the ozone molecule may be responsible for the symmetry-controlled isotope effect that has been observed in other molecules. Metastable electronic states of ozone have been found, but the connection to the isotope anomaly has not been made. The lifetime of the metastable molecule will be of critical importance.

The work of Gellene (*1*) now permits a quantitative prediction of the isotope fractionation pattern in the ozone formation process. The theory is able to reproduce the general features of the isotope distribution shown in the figure. The new permutation inversion symmetry scheme developed by Gellene and co-workers, taking into account nuclear symmetrization in reactive collisions, is particularly well suited for determining the effects of isotopic substitution in gas-phase collision processes. In contrast to the mass-dependent kinetic isotope effects well known in reaction dynamics, the unusually large isotope effects recently found by Gellene in his laboratory experiments [references in (*1*)] have been explainable in terms of the new symmetry correlation scheme, lending credibility to the new approach.

The jury is still out on which explanation will best describe the ozone isotope enrichments. In particular, the subtle effects seen in the symmetrical and asymmetrical variety of isotopomers wait for a solution. Compared to the laboratory and tropospheric results, the stratospheric data show often very high abundances of heavy ozone. Gellene's theory, in principle, has the potential to explain such large and variable isotope enrichments. Other processes such as symmetry selection through metastable states (*10*) or reactions involving highly vibrationally excited O_2 (*11*) are still competing to be the correct explanation of the ozone isotope effect; future experiments will have the task of proving one or the other to be the right explanation.

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NOTA BENE: PHOTOPHYSICS

The 110% Solution

Supersaturated solutions contain more dissolved substance than the solvent can normally carry. As a result, they are on the verge of crystallizing; all that is needed is a seed or trigger to provide a nucleus for crystal formation. A familiar example is rock candy, which crystallizes onto a string dipped in sugar water. In the 1860s, John Tyndall studied the condensation of supersaturated vapors triggered by light (*1*). A dramatic form of light-induced precipitation was discovered in the 1970s by researchers at Princeton. When they passed a laser beam through sodium vapor, they observed laser "snow" (*2*) as the sodium atoms clustered and precipitated. In this and other cases of laser-induced nucleation of supersaturated solutions, the light produced a chemical change causing crystallization or precipitation. However, as recently reported in *Physical Review Letters*, Garetz *et al.* have now observed the photonucleation of supersaturated urea solution—but without a photochemical change (*3*).

Urea is well known to laser physicists because it has important nonlinear optical properties. Garetz *et al.* were investigating whether the nonlinear process of second harmonic generation, whereby laser light can be converted to light at twice the optical frequency, could be observed in aqueous solutions of urea. The solutions had concentration ratios c/c_{sat} between 1.10 and 1.29, where c_{sat} is the solubility of urea, and so were highly supersaturated. The incident laser light was at a wavelength of 1.06 μm, with a pulse duration of 20 ns and a pulse power intensity of 250 MW/cm^2. At this intensity, the laser pulses caused nucleation of the urea solution as a single small crystallite, followed by crystallization of the entire sample.

In contrast to previous examples of photonucleation, the authors believe that the crystallization occurred by an entirely nonphotochemical process. It is conceivable that the laser could have photolyzed some impurity, but the samples were carefully prepared to be pure and free of dust. Urea itself has no absorption bands at the infrared laser wavelength, so a direct light-induced chemical reaction is unlikely, as is some process involving the absorption of multiple photons. The authors also checked for chemical production of nucleation centers by redissolving the crystallized urea and saw that they could re-create the supersaturation. Thermal effects were discounted because heating would make the solution more, not less, stable.

One key characteristic implicated the electric field produced by the laser as the cause of the crystallization: The urea crystals that formed by laser action were oriented along the polarization direction of the light. This orientation suggests a kind of electric field–induced alignment of the molecules known in other contexts as the optical Kerr effect. Kerr effect optical shutters use electric fields to change the optical polarizing properties of liquids and are used in high-speed movie cameras to record projectile motion or the time evolution of explosions. Garetz *et al.* speculate that the Kerr effect assists the formation of nucleation sites by aligning existing prenucleating clusters that normally form and break up continually in solution.

Although more work will be needed to confirm this picture, the light-induced crystallization of solutions may offer ways to study the fundamental physics of nucleation and might provide a method for cleanly and controllably inducing the crystal formation of technologically useful substances.

—**David Voss**

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