Fullerenes from the Geological Environment

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By means of high-resolution transmission electron microscopy, both C_{60} and C_{70} fullerenes have been found in a carbon-rich Precambrian rock from Russia. The fullerenes were confirmed by Fourier transform mass spectrometry with both laser desorption and thermal desorption/electron-capture methods to verify that the fullerenes were indeed present in the geological sample and were not generated by the laser ionization event. The mass spectra were measured under conditions sufficient to resolve the $^{13}C/^{12}C$ isotopic ratios for C_{60} and C_{70} and indicate that these ratios correspond to the normal range of isotopic values.

Fullerenes were discovered as an outgrowth of an investigation of carbon clusters that presumably occur in interstellar atmosphere (*1, 2*). Subsequent studies were made with the goal of locating them in meteorites (*3–6*), but the searches have been unsuccessful. In spite of the intensive research that has occurred since it became possible to make fullerenes in macroscopic quantities (*7, 8*), there are no confirmed occurrences of fullerenes formed in the natural environment. In the laboratory they have been synthesized by laser ablation (*9*), in carbon arcs (*7*), and by burning benzene (*10*). Their apparent absence is perhaps not surprising because they are synthesized at temperatures greater than occur in the natural environment outside of extreme conditions (lightning strikes, stellar interiors). Another constraining factor in natural environments is the effect that oxygen, nitrogen, and other non-inert gases have on inhibiting the growth of fullerenes.

Here we report an occurrence of fullerenes from the geological environment. We found them while examining high-resolution transmission electron microscopy (HRTEM) images of poorly graphitized material by noticing the similarity to images of synthetic fullerenes (*11*). We subsequently confirmed the presence of C_{60} and C_{70} by mass spectrometry. They occur within fracture-filling films in shungite, an usual carbonaceous rock found near the town of Shunga in Karelia, Russia.

Shungite has been the subject of intensive investigation for over a century (*12, 13*). It occurs in a metamorphosed carbon-rich rock within Precambrian sediments; the host formations are, according to Volkova and Bogdanova (*14*), seams of sedimentary origin. The overlying rocks consist of gray dolomitized sandstones and poorly sorted silts and clays, and the underlying rocks are not exposed. The shungite consists of masses containing up to 99% carbon. Diabase is interstratified with shungite-bearing rocks, and the shungite concentration increases with proximity to the diabase. Our sample comes from inclusions in the diabase.

Based on its optical characteristics, Firsova and Yakimenko (*15*) classified shungite into four groups. Our sample belongs to their shungite 1c group. Volkova and Bogdanova grouped it into five types, based largely on carbon content, mineral matter, and luster. Khavari-Khoransani and Murchison (*16*) simply divided it into "bright" and "dull" varieties based on its appearance in hand specimen; the former has a high luster and higher carbon content whereas the "dull" variety has a matte appearance. Our sample is of the bright variety.

The origin of shungite has been a continuing source of controversy ranging from organic (*14*) to volcanic (*17*). Firsova and Yakimenko (*15*) concluded that shungite formation was strongly affected by metamorphic processes. Volkova and Bogdanova (*14*) believe that it represents ancient coal beds within sediments, and that shungite is the most highly metamorphosed coal known; they point out that it has features typical of both humic and sapropelic coals and shows structures that they interpret as woody remains.

Compositionally, shungite represents coals of the meta-anthracite rank, characterized by low ash and sulfur contents, low volatile yields, and high carbon contents. Although Volkova and Bogdanova (*14*) describe organic features that they interpret as evidence of Precambrian life, the last sentence of their paper curiously raises the possibility that the shungite is not Precambrian in age. Schopf (*18*) believes shungite is "coaly" rather than a proper coal and that it is remobilized material formed by devolatilization of organic remains. If a coal, it would probably be best grouped with the anthraxolites, but it seems more likely that it has its origin from bitumens. Clearly, uncertainty exists regarding this unusual rock type.

There are several examples of Precambrian coals, of all which are of interest because of the potential information they provide about early forms of life (*19–22*). Tyler *et al.* (*19*) report the occurrence of thin, highly reflecting graphitic films in their Precambrian coal samples. It is intriguing to speculate that these veins may also contain fullerenes. We obtained a piece of the material collected by Tyler, but we have not observed any of the veinlets.

The samples we studied are pure black, have high reflectivity (resembling jet or obsidian), conchoidal fracture, and contain small, curling fractures that resemble narrow dessication cracks. The fractures are filled with carbonate. In some cases the fracture walls are coated with thin, dark, yellowish-brown films of carbon that have a submetallic luster. TEM study shows the films

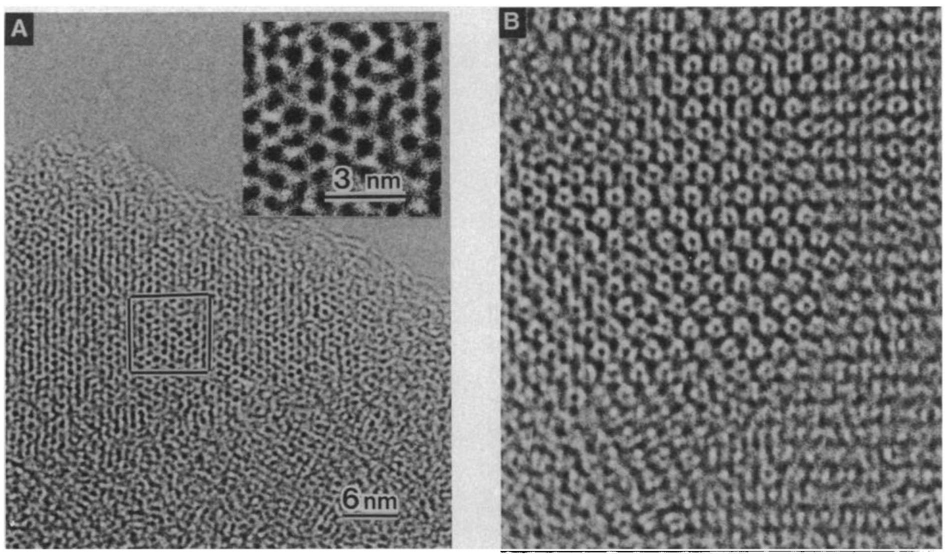

Fig. 1. (**A**) HRTEM image of fullerenes in a shungite sample. The rounded shapes are C_{60} and perhaps C_{70} molecules, arranged in a close-packed array. The inset shows a relatively well-packed array of molecules. (**B**) HRTEM image of synthetic fullerenes at the same magnification as the inset of (A) [S. Wang, Arizona State University]. The contrast between (A) and (B) differs slightly because of small differences in TEM operating conditions.

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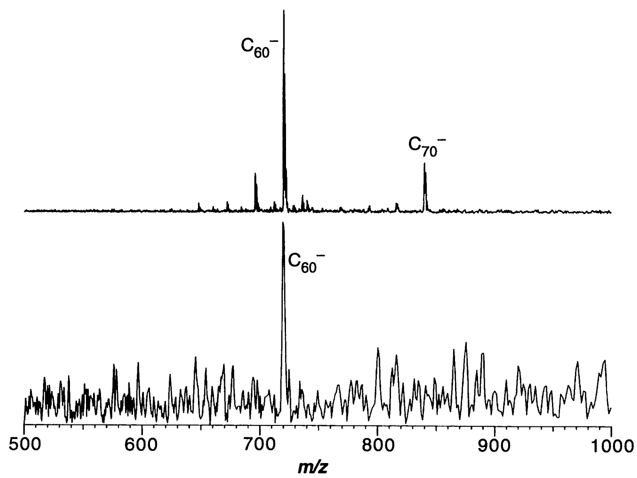

Fig. 2. Negative-ion FTMS spectrum of fullerenes from shungite. The laser-desorption spectrum (top) indicates the presence of C_{60} and C_{70} but no larger fullerenes. Thermal desorption measurements (bottom) verify the presence of significant amounts of indigenous C_{60} in this sample. The vertical scale represents relative abundances and is dimensionless.

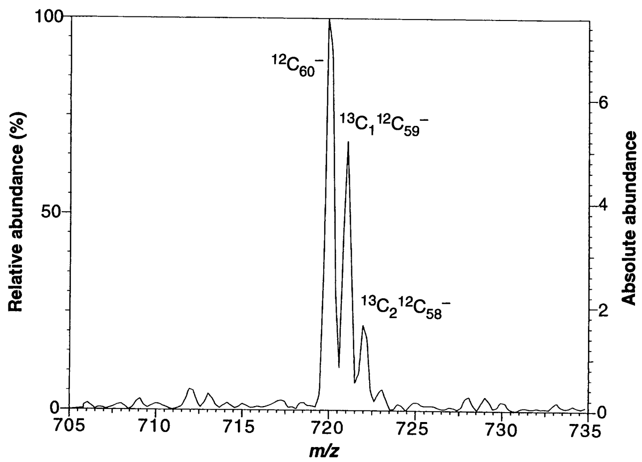

Fig. 3. High-resolution, laser-desorption FTMS spectrum showing the isotopic abundances of C_{60}. The isotope ratios are as follows: for m/z = 720, 100% (calculated) versus 100% (measured); for m/z = 721, 67% (calculated) versus 68% (measured); and for m/z = 722, 24% (calculated) versus 22% (measured).

consist of irregularly shaped particles 0.1 to 1 μm in diameter (*23*). Most are amorphous, but in some HRTEM images we observed features that we interpret as individual molecules and small regions of fullerenes. They are unevenly distributed and minor in abundance. In the images the fullerenes appear round (presumably roughly spherical in three dimensions), with white rims and black centers (Fig. 1A). They look almost identical to images of C_{60} molecules obtained by Wang and Buseck (*11*) (Fig. 1B).

A Fourier transform mass spectrum (FTMS) shows a strong peak at m/z 720, corresponding to C_{60}, and a weaker but still pronounced peak at m/z 840, which was identified to be C_{70}, (Fig. 2). Negative-ion mass spectra were obtained by laser desorption of samples that were ground and then transferred to glass slides (*24*). Laser-desorption FTMS (*25, 26*) is useful for the complete examination of fullerenes of types C_n, where n = 50 to 400. The procedure generates both positive and negative ions, and either can be used to profile the fullerene content of a mixture. A potential problem for these studies is that under certain experimental conditions fullerenes can be generated by the laser process, thereby distorting the results. For example, under laser-ablation conditions (laser power densities greater than 10^8 W/cm^2), fullerenes can be generated from graphitic material (*27, 28*) and from coals (*29–33*). In contrast, low-energy laser desorption (power densities of 10^5 to 10^7 W/cm^2) is often used to profile the constituents of a sample and is less prone to generate species that were not originally present.

To avoid potentially generating fullerenes by high-energy laser ablation of graphitic materials, we used low laser energies and tested these on samples known to contain only graphitic material and no fullerenes. Mass spectra of these blanks were free of C_{60} and other fullerene peaks, verifying that under our experimental conditions we did not generate fullerenes during the laser desorption process. In addition, to check our laser-desorption results, the samples were thermally desorbed up to 350°C into the vacuum chamber, where they were ionized by low-energy electron capture. The ions were trapped, manipulated, and ultimately detected in the FTMS ion cell. While thermal-desorption, electron-capture experiments are dependent on fullerene volatility, it is possible to desorb C_{60} below 350°C (*34*), which was the highest temperature possible with our instrument and is at the threshold of desorbing C_{70}. The importance of the thermal desorption experiments is that there is no evidence that C_{60} can be generated from graphitic material under these experimental conditions. Thus, these experiments definitively established the presence of fullerenes in our samples, increasing our confidence in the initial HRTEM measurements (Fig. 1).

The ions from the shungite samples could be measured with adequate resolution to resolve the ^{13}C isotope contents for both C_{60} and C_{70}. Isotope ratios can be measured with 5% accuracies by carefully controlling the ion population and examining standard materials for isotope calibrations. The results indicate, within these error limits, normal isotopic abundances (Fig. 3).

Fullerenes are known to be highly stable with respect to temperature (*6*) and impact and static high pressure (*35, 36*). Their occurrence in what are probably Precambrian rocks suggests that they are also highly stable with respect to time, although we have no direct evidence that they did not form more recently as secondary products.

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23. Specimens were prepared by hand-picking films from rock chips, first for TEM study and then, some weeks later after the TEM results were obtained, for mass spectrometry. Following initial measurements on a Finnegan MAT312 magnetic sector mass spectrometer at Arizona State University, additional sample fragments were hand-picked and sent to Oak Ridge for Fourier trans-

form mass spectrometry. For the TEM study, we used a JEOL JEM-4000EX transmission electron microscope with a top-entry, double-tilting sample holder (±15° tilt), a structure resolution limit of 1.7 Å, and a spherical aberration coefficient (Cs) of 1.0 mm. A 40-μm objective aperture and a 150-μm condenser aperture were used for high-resolution TEM.
24. Fourier transform mass spectra (FTMS) were obtained using an Extrel FTMS-2000 instrument. Fullerene ions were generated by either laser desorption (using an Nd:YAG pulsed laser) or by thermal desorption/electron capture. For each sample, a few micrograms of solid were loaded onto a stainless steel probe tip for examination. For the laser desorption experiments, the fourth harmonic of the YAG (266 nm) was used at 10^6 to 10^7 W/cm^2 to desorb and simultaneously ionize the fullerenes (37). Single laser shots of the "as is" samples generated ions that were trapped for between 3 and 20 ms at a base pressure of 8×10^{-8} Torr and subsequently detected in the FTMS ion cell. Both positive and negative ions were generated and examined in these studies. For the thermal desorption experiments, the probe tip containing the sample was heated slowly from 30° to 350°C to thermally desorb compounds from the sample. These neutral molecules were then ionized by low-energy electron capture to generate characteristic negative ions that could be monitored and compared to the laser desorption spectra.
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38. We thank S. O. Firsova for providing shungite samples, data, and reprints; R. Dott for loan of a Precambrian anthracite sample from northern Michigan; J. Lehman for help with mass spectrometry at Arizona State University; T. Daly, W. Schopf, S. Wang, and P. Williams for valuable suggestions, N. Bostick for reprints; and T. Daly for helpful reviews. Electron microscopy was performed at the Facility for High Resolution Electron Microscopy at Arizona State University (ASU), which is supported by the National Science Foundation (NSF) and ASU. This study was supported by NSF grant EAR-870529 and private donors.

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Programmed Death of T Cells in HIV-1 Infection

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In human immunodeficiency virus (HIV) infection, functional defects and deletion of antigen-reactive T cells are more frequent than can be explained by direct viral infection. On culturing, both CD4⁺ and CD8⁺ T cells from asymptomatic HIV-infected individuals died as a result of programmed cell death (apoptosis). Apoptosis was enhanced by activation with CD3 antibodies. Programmed cell death, associated with impaired T cell reactivity, may thus be responsible for the deletion of reactive T cells that contributes to HIV-induced immunodeficiency.

Early in HIV infection, abnormalities in the immune system can be demonstrated in clinically stable asymptomatic individuals. HIV infection affects such $CD4^+$ and $CD8^+$ T cell functions as interleukin-2 production and proliferation after stimulation with soluble antigens and CD3 antibodies. These changes occur before $CD4^+$ T cell numbers are decreased (*1–5*) and cannot be attributed to direct HIV infection in vivo because only a relatively small number of T cells are infected (*6, 7*). In addition to deletion of T memory cells, intrinsic nonresponsiveness occurs in both $CD4^+$ and $CD8^+$ cells in long-term infections (*3, 4, 8*). In their nonresponsiveness to antigenic stimulation and lack of interleukin-2 production despite intact cell-signaling pathways (*1–4*), T cells in HIV-infected individuals exhibit the properties of the unresponsive state known as anergy (*9*).

$CD8^+$ T cells from HIV-infected individuals have increased expression of such activation markers as CD38, HLA-DR, and CD57, which suggests that there is continuous immune activation (*10, 11*). $CD8^+$ cells expressing activation markers have severely decreased proliferative responses and clonogenic potential (*12*) and are reported to die in culture (*13*). This suggests hyporesponsiveness in $CD8^+$ cells as a result of hyperactivation.

We considered the possibility that in this apparent anergic or hyperactivated state, T cells could be programmed for death and whether the loss of antigen-reactive cells could occur as a result of apoptosis. Programmed cell death (PCD), also known as apoptosis or activation-induced cell death, is a physiological mechanism of cell deletion that differs morphologically and biochemically from necrosis (*14*). PCD is involved in a wide variety of immunological regulatory processes (*14–16*). The process is characterized by a typical cellular morphology and degradation of the chromatin into discrete fragments that are multiples of about 190 base pairs of DNA (*17*). It has been proposed that in HIV infection interaction of soluble gp120 with CD4, previously shown to lead to impaired lymphocyte function (*18*), would prime $CD4^+$ T cells for PCD (*19*). This hypothesis is supported by results obtained with mature murine lymphocytes that die from PCD after stimulation through the T cell receptor complex when CD4 was previously ligated by CD4 antibodies (*20*).

Peripheral blood mononuclear cells (PBMC) from HIV-infected persons displayed morphology characteristic of PCD after being cultured overnight in the presence of antibodies to CD3 (anti-CD3) (Fig. 1). Cells showed extensive peripheral chromatin condensation, dilation of the endoplasmatic reticulum, and preservation of mitochondrial structures (*21*). To look for DNA fragmentation, we studied PBMC from 29 asymptomatic seropositive (CDC class II or III) homosexual men. They were selected from a prospective cohort study in Amsterdam (*22*) as having normal numbers of circulating $CD4^+$ cells (mean 540 per cubic millimeter, range 320 to 880) and being seropositive for longer than 3.5 years. In all experiments, a healthy male seronegative control was tested for each seropositive individual. All except one of the HIV-infected subjects showed decreased proliferative responses compared to the seronegative controls. Proliferation in response to anti-CD3 ranged from <1 to 80% of control values, as reported (*2–4, 8, 23*).

When low molecular weight DNA fractions were isolated from lysed cells and subjected to gel electrophoresis, the DNA cleavage pattern specific for apoptosis was observed. Fragmentation could be prevented by Zn^{2+}, which inhibits endonuclease activity (*24*) (Fig. 2). DNA fragments corresponding to 1 to 7 nucleosomes were identified by gel electrophoresis, but longer fragments were also detected that formed a smear in the gel near the origin of migration. In unstimulated cultures, DNA fragmentation was observed in

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