sponsible for transfer of the electron to the primary acceptor. If this is the case, from the kinetics derived from Fig. 3, a and c, we can deduce a rise time of approximately 150 psec for the oxidation of the reaction center bacteriochlorophyll. However, to confirm this, it will be necessary to measure the appearance of the 1250-nm band, since this is generally accepted as being associated with the oxidized reaction center bacteriochlorophyll.

If the proposed P^f state were a pure excited singlet, then the previous 10-psec singlet lifetime estimate made from fluorescent yield experiments (*14*) would have to be more than an order of magnitude in error. A lifetime of several hundred picoseconds would be necessary to explain the relatively small three- to fivefold increase in fluorescence yield on reduction of the primary acceptor. In contrast, Parson *et al.* (*16*) estimated the P^f lifetime to be a few nanoseconds when the primary acceptor was reduced. This estimate should be regarded as an upper limit because activation was performed with a 20-nsec flash. A pure singlet lifetime of several hundred picoseconds corresponds to a quantum yield for oxidation of reaction center bacteriochlorophyll in the 0.6 and 0.8 range. This is less than the value determined by Wraight and Clayton (*17*). At the present, there is no evidence to confirm or rule out with any certainty the various other possible assignments to this state, including the possibility of a triplet, as has been suggested by several workers, or perhaps a charge transfer dimer (*10, 18*) in which the bacteriopheophytin chromophore may actually play a role or undergo an electrochromic shift. We expect that fluorescence decay measurements on photosynthetic bacteria will promote spectroscopic identification of this state.

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A Tunneling Spectroscopy Study of Molecular Degradation due to Electron Irradiation

Abstract. *Electron tunneling spectroscopy has been used to characterize the degradation of β-D-fructose after electron bombardment in a scanning electron microscope. The decrease in intensity of various vibrational bands is correlated with structural changes in the molecule, thereby providing a detailed picture of the degradation process.*

In high-resolution electron microscopy the action of fast electrons on condensed molecular films has been of great interest, since it is now realized that it is specimen degradation and not the resolving power of the instrument that has limited the atomic imaging of biological molecules (*1*). Energy loss spectroscopy (*2*) and mass loss spectroscopy (*3*) have provided some information about changes in molecular specimens under the electron beam. However, changes in the physical structure of the molecules are difficult, if not impossible, to ascertain from such studies. The application of tunneling spectroscopy to molecular degradation induced by electron beams has been suggested as a possibly useful way of examining such changes (*4*); we present here preliminary results that not only identify the changes in the molecular structure but also indicate for a particular electron irradiation what fraction of the molecules has undergone a specific change.

Inelastic tunneling spectroscopy (*5*) reveals the vibrational modes of organic compounds included in the insulating layer of a metal insulator–metal tunneling junction. A vibrational mode of energy $\hbar\omega$ ($\hbar$ is Planck's constant, and ω is the frequency) is observed as a small change (<1 percent) in the electrical resistance of the junction at a voltage $V = \hbar\omega/e$ (e is the electronic charge). Both infrared and Raman active vibrational modes can be observed with a resolution of 16 cm^{-1} at a junction temperature of 4.2 K (4 cm^{-1} at 1 K) over a spectral range of 300 to 4000 cm^{-1}. The effect of sandwiching the molecules between the metal electrodes apparently does not deform the physical structure of the molecules, since the vibrational frequencies from tunneling spectroscopy correspond quite well to those from neat infrared and Raman spectra (*6*).

Crossed film tunnel junctions (Al-Al_2O_3-Pb) were fabricated in a clean, oil-free, high-vacuum evaporator and liquid-doped with β-D-fructose by general procedures described in detail elsewhere (*6*). In outline, a thermally oxidized Al strip (0.2 mm wide) on a glass slide was uniformly doped with a solution of β-D-fructose in water (0.5 mg/ml). After any excess solution had been spun off, the slide was returned to a high-vacuum evaporator where four Pb strips (0.2 mm wide, 2000 Å thick, and 1.5 mm apart) were evaporated across the doped, oxidized Al strip. Thus we obtained four closely spaced junctions, on the same Al strip, with nearly the same characteristics.

Electron irradiations of the junctions (and thus of the sandwiched molecule) were carried out in a scanning electron microscope (ETEC Autoscan). The exposed area was slightly larger than the junction area, thereby ensuring the even exposure of the entire junction. In most cases three of the four junctions on each substrate were given different electron fluences (*7*) and the fourth was left unexposed. Typical irradiation parameters were as follows: electron beam voltage, 30 kev; beam current, 0.1 to

30 na; exposure time, 2 to 10 minutes; and pressure, 10^{-5} to 10^{-6} torr.

After irradiation the junctions were removed from the microscope and immersed in liquid He. We measured the tunneling spectra, which are plots of d^2V/dI^2 (I is the tunneling current) as a function of V, using a second harmonic detection technique (*5, 6*) that employed an a-c modulation at 1120 hertz and a second harmonic voltage detection with a lock-in amplifier. One measures d^2V/dI^2 since small changes in the resistance dV/dI due to inelastic tunneling give rise to peaks in the second derivative.

Some results from our experiments with β-D-fructose are shown in Fig. 1. Other experiments (*8*) have shown that all our spectra depend on the total electron fluence and not on the fluence rate; thus varying the electron beam current i over the range from 3.7 to 19 na and varying the exposure duration τ over the range from 2 to 10 minutes while keeping the total electron fluence ($\phi = i \cdot \tau$) constant produces the same spectrum.

The COH functional groups in the fructose molecule can be expected to have three strong vibrational bands at positions close to those found (*9*) for this functional group in primary and secondary alcohols. These are a CO stretching vibration in the range 1050 through 1100 cm^{-1}, an OH bending vibration in the range 1260 through 1300 cm^{-1}, and an OH stretching vibration above 3600 cm^{-1}. Figure 1 shows that there are three strong bands at these wave numbers. These are labeled ▲ at 1100 cm^{-1}, ▼ at 1261 cm^{-1}, and ● at 3580 cm^{-1}. The effect of the electron irradiation is to produce comparable large decreases in the intensities of these bands; thus we deduce that one of the primary effects of electron irradiation is to disrupt the COH functional group in fructose. The large broad peak at 2900 cm^{-1} (labeled △) can be unambiguously associated with CH_2 and CH stretching modes. The peak at 1461 cm^{-1} (labeled ▽) can be tentatively associated with CH_2 deformation [usual frequency range (*9*), 1465 ± 15 cm^{-1}] although other modes may contribute in this frequency range. Both of these modes decrease in intensity with irradiation at comparable rates (for example, both have decreased by approximately 40 percent at a fluence of 30 mcoulomb/cm^2), a result that supports their identification with the same functional groups. The growth of a peak at 1638 cm^{-1} (labeled ○), a position characteristic of C=C stretching vibrations, suggests that, as the OH group and the peripheral protons are removed by electron action, double bonds are formed between C

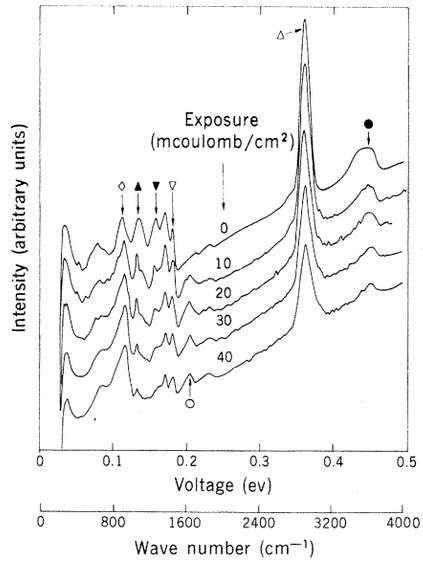

Fig. 1. Inelastic electron tunneling spectra of β-D-fructose irradiated with different fluences of the electron beam in a scanning electron microscope. The curves are labeled by exposure, or equivalently electron fluence (1 mcoulomb/cm^2 = 0.625 electron/Å^2), and are offset for clarity. See the text for the association of the symbols with identified peaks. It should be noted that some peaks decrease more rapidly than others; moreover, a peak (labeled ○) grows at 1638 cm^{-1} as a result of the formation of C=C bonds upon irradiation. Each curve was traced in approximately 100 minutes with a lock-in sensitivity of 2 μv (full scale) and a time constant of 3 seconds.

atoms. Finally, the symmetrical peak at 900 cm^{-1} (labeled ◊) in the unirradiated junction becomes upon electron irradiation a broad asymmetrical peak (at 940 cm^{-1}) with a shoulder (at 880 cm^{-1}). Infrared study (*10*) of 2-keto sugars has made it possible to assign a mode at 925 cm^{-1} to a ring-stretching vibration; other modes dependent on molecular configuration are observed at 872, 822, and 781 cm^{-1}. In this region, a tunneling spectrum of a junction with Al_2O_3 insulator has a prominent broad mode at 920 cm^{-1} (*5, 6*). Tentatively, without the explicit subtraction of this background peak, we associate the shoulder at 880 cm^{-1} with the contribution of the ring-stretching mode. The persistence of this shoulder, even at the highest fluence values, leads us to speculate that the deformation of the ring occurs with a much smaller probability than the disruption of the COH group and of the CH bonds.

In summary, we have obtained by inelastic electron tunneling spectroscopy a detailed picture of the degradation of β-D-fructose in the electron microscope. Although our results are at present qualitative, it is already possible to ascertain that: (i) significant disruptions in the COH functional groups and the CH bonds occur at an electron fluence as low as 10 mcoulomb/cm^2; (ii) new double bonds between C atoms are formed; and (iii) there is some evidence that the ring structure of β-D-fructose persists even at high fluences; however, this needs detailed verification.

Although our work has exposed the potential of this technique, we feel that much work remains to be done. For example, an ultrahigh-vacuum electron microscope (or an electron probe), equipped with a stage cooled to liquid He temperatures and the necessary electronics, would make possible the measurement of successive tunneling spectra of the same junction, continuously and during irradiation. Moreover, the influence of the metal films on the sandwiched organic molecules has to be considered before individual molecular fragmentation cross sections can be deduced. Studies in an electron irradiation instrument that has provisions for in situ evaporation of metal would yield information on the effect of the overlayer of metal as well as on the evaporation of fragments. Thus, we believe that in situ studies involving the use of inelastic tunneling spectroscopy will yield detailed quantitative data on molecular degradation in the electron microscope.

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