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The importance of glyceraldehyde radiolysis in chemical evolution

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Abstract Studies in chemical evolution are intended to demonstrate how compounds of biological importance are generated from substances that could have been found in abiotic conditions on the primitive Earth or in extraterrestrial environments. In this context, the aim of the present work was to examine the behavior of DL-glyceraldehyde in both aqueous solution and solid samples under gamma irradiation. We irradiated DL-glyceraldehyde at different doses and temperatures with a gamma source; even at low doses and temperature (77 K), free radicals were detected. Among the products formed were ethylene glycol and glycolaldehyde. Some sugar-like compounds were also detected.

Keywords Gamma irradiation · Glyceraldehyde · Chemical evolution · Primitive Earth

Introduction

The field of chemical evolution encompasses the formation of biologically relevant compounds under primitive-earth conditions; in this context, life arose from inorganic molecules, which became organic molecules and eventually biological ones. In addition to the nucleic acids and proteins that provide the foundations of life, sugar plays a critical role, as it is part of nucleic acid components (e.g., RNA) and is linked to almost every life process in biological systems [1].

The synthesis of organic matter in a simulated primitive environment (terrestrial or extraterrestrial) has been widely studied [1]. An important aspect of chemical evolution is the stability of the organic molecules that have biological significance under primitive conditions, especially in the presence of constant energy sources. Some protective mechanisms may be enacted to ensure that important compounds endure these primitive conditions.

One set of biologically relevant organic compounds comprises the sugars and their precursors, such as glyceraldehyde, the simplest triose. The presence of these compounds in prebiotic environments is critical in the formation of more complex systems. These compounds, which might have been carried to Earth by extraterrestrial bodies such as comets or meteorites, must be sufficiently stable to persist in hostile environments whether they are in an aqueous solution or a dry state. According to Weber and Pizzarello [2], glyceraldehyde was probably present on the prebiotic Earth, as it has been synthesized under prebiotic conditions [2 and the references therein].

Sources of energy that existed on the early Earth and that were useful in the abiotic synthesis of organic matter are essential parts of chemical evolution because energy is responsible for initiating, promoting, and directing all physicochemical processes. Energy in the form of ionizing radiation was probably of great importance in the chemical reactions that occurred on the early Earth and in extraterrestrial environments [3–6] due to its high efficiency in inducing the synthesis of organic compounds, its

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penetration into matter, and its relative abundance. Two sources contributed to this type of energy: ionizing radiation from radionuclides in the Earth's crust and radiation from extraterrestrial sources such as cosmic rays or the solar wind [3, 4].

Extraterrestrial bodies are exposed to various types of high-energy radiation, mainly in the form of cosmic rays (high-speed particles), ultraviolet (UV) rays, and gamma photons [3]. Cataldo et al. [7] described calculations related to the energy deposited by the decay of radionuclides in comets, asteroids, meteorites and larger bodies of the solar system on the time scale of the age of the solar system (i.e., 4.69×10^9 years about 14 MGy) [7]. In these bodies, the presence of minerals and other solid surfaces may influence the behavior of organic molecules by acting as catalysts, concentrators or protecting agents [5]. On the primitive Earth and on small bodies, the most relevant minerals were carbonates, sulfides and, in particular, clays and silicates [5].

The aim of this work is to study the stability of prebiotic organic molecules such as glyceraldehyde under ionizing radiation (gamma rays of ^{60}Co). The study was carried out on aqueous solutions and solid samples at 298, 198 and 77 K, thus simulating the environments of prebiotic Earth and a comet's core, which has an icy phase consisting of water mixed with glyceraldehyde.

Experimental

Reagents and glassware

High-purity DL-glyceraldehyde (Merck Co., USA) was employed in all of the experiments. Sodium-montmorillonite, hectorite, and attapulgite (from the Clay Mineral Society's Source of Clay Minerals Repository) were used to anchor the aldehyde. The glassware was treated with a warm mixture of HNO_3 and H_2SO_4 for 60 min, rinsed with bi-distilled water, and heated in a 350 °C oven overnight. Triple-distilled water was used for all aqueous solutions, according to the standards of radiation chemistry [8].

Preparation of samples

Three types of samples were prepared: aqueous solutions of glyceraldehyde, aqueous solutions of glyceraldehyde in the presence of a clay mineral and solid samples with only powdered glyceraldehyde. Aqueous solutions of glyceraldehyde from 1×10^{-1} to 2×10^{-4} mol/L were saturated with argon and prepared with triple-distilled water in sealed glass tubes. The pH of this solution was 6.9.

For the solid samples, the DL-glyceraldehyde powder was placed inside of a glass tube and evacuated for 20 min.

Sorption experiments

Three clays were used for adsorption experiments: Sodium-montmorillonite, attapulgite and hectorite. To prepare the clay-glyceraldehyde system, 0.1 g of clay was mixed with 3 mL of the glyceraldehyde standard solution (1×10^{-2} mol/L). The pH was adjusted with formic acid and ammonium hydroxide, and left on a plate undergoing continuous agitation at 150 rpm for 30 min. After this time, a Beckman Allegra XL-90 centrifuge was used (for 30 min at 25,000 rpm and 20 °C) to separate the fine-particle solids from the liquid phases. High-performance liquid chromatography (HPLC) mass spectroscopy was employed to determine the percentage of glyceraldehyde that adsorbed onto the clay, relative to the amount available in the standard solution.

A desorption test was carried out to determine how much glyceraldehyde could be recovered from the mineral; this was done by changing the pH of the solid previously separated by centrifugation from 2 to 11. Glyceraldehyde was recovered after three cycles of treatment with KOH (0.1 mol/L). The clay was dried at 80 °C overnight and then ground in an agate mortar for X-ray diffraction analysis. The solution was analyzed using HPLC mass spectrometry.

Radiolysis experiments

The irradiation was carried out at Institute de Ciencias Nucleares-UNAM using a high-intensity radiation source, ^{60}Co (Gammabeam 651PT). The samples were irradiated at room temperature (298 K). The dose rate was determined to be 221 ± 3 Gy/min using the ferrous ammonium sulfate-cupric sulfate dosimeter [8]. The samples were bubbled with argon for 20 min in a warm container to eliminate dissolved oxygen.

Aqueous solution

The aqueous samples without clay were irradiated from 0 to 25 kGy and pH 6.9.

Solid samples

These samples were irradiated for 0.5–10 h in liquid nitrogen (77 K), in dry ice (195 K), and at room temperature (298 K) using the same position at the source. These samples were analyzed using electronic paramagnetic resonance (EPR). Another set of solid samples was irradiated from 0 to 308 kGy and analyzed using polarography.

Analysis

The compounds were analyzed using HPLC coupled with mass spectrometry detector. Other analyses were performed using UV spectroscopy, polarography, and EPR spectroscopy. The samples were analyzed immediately after irradiation. The clays were analyzed using X-ray diffraction spectroscopy.

UV–Vis spectroscopy

After irradiation, the aqueous samples were analyzed using UV spectroscopy with a Carry 100 spectrometer in the range of 200–350 nm. The samples with clay were separated beforehand by centrifugation.

Polarography

All polarography measurements for the irradiated samples were performed at room temperature (25 °C) with an initial 1×10^{-2} mol/L DL-glyceraldehyde aqueous solution. Polarographic curves were registered on a Metrohm polarography Model 797 VA Computrace, The working conditions included a start potential of 0.0 V, a final potential of -1.7 V, a scan rate of 0.005 V/s, a pulse width of 0.05 V, a pulse time of 0.04 s, and support electrolytes of ammonium hydroxide and ammonium chloride (pH 8.24). The working electrode was dropping mercury with a drop time of 1 s; the reference electrode used Ag and AgCl, and the counter electrode used platinum. The program (797 VA Computrace Version 1.2) controlled the potential of the mercury cathode.

HPLC–MS analysis

Direct analysis of liquid samples was performed using electrospray-ionization negative-mode (ESI-) mass spectrometry with a Single Quadrupole Mass Detector (SQ-2, manufactured by Waters Corp., USA). The working conditions included a capillary of 2.1 kV, a cone of 10 V, a source temperature of 150 °C, a desolvation temperature of 350 °C, a desolvation gas flow of 650 L/h, and a sample rate of 100 μL/min. The samples were analyzed at 18 °C. In addition, the samples were introduced to the mass detector via HPLC using a HPLC Column XBridge C18 measuring 3.5 μm 3.0 × 100 mm (WT186003027) with a liquid phase of methanol and water (50:50 ratio) at 0.4 mL/min. Some samples were analyzed by HPLC coupled to an ELSD detector. The column used was a Chirobiotic T, and the liquid phase was methanol and water (70:30 ratio) at 1.3 mL/min.

EPR analysis

EPR measurements were performed at Instituto de Química-UNAM, using 30 ± 0.1 mg of the sample in a quartz tube at room temperature and a JEOL JES-TE300 spectrometer, which was operating on the X-band at a 100 kHz modulation frequency and which had a cylindrical cavity in the TE_{011} mode. The external calibration of the magnetic field was made using a precision JEOL ES-FC5 gauss meter. The spectrometer was set to measure all spectra with a center field of 335.0 mT, a microwave power of 1 mW, a microwave frequency of 9.43 GHz, a modulation width of 0.079 mT, a time constant of 0.1 s, an amplitude of 1×100, and a sweep time of 120 s; two scans were completed. The readings were made at the vertical peak-to-peak height of the line. Spectral acquisitions and manipulations were performed using the ES-IPRITS/TE program. The EPR spectra were recorded as a first derivative.

X-ray diffraction spectroscopy

The adsorption of glyceraldehyde by montmorillonite was followed by X-ray diffraction spectroscopy using a Bruker 08 Advance diffractometer, with filtered CuKa radiation ($A = 1.5406$ A) Ni filter; 40 kV, 40 mA, at 2θ angles from 2 a 90° during 30 min. Specimens were prepared by pressing the organic adsorbed montmorillonite on the diffractometer's plastic holder (polymethylmethacrylate). The spectrometer was calibrated using Si, quartz, and kaolinite reference materials.

Results and discussion

Aqueous and solid samples without clay minerals

The glyceraldehyde molecule contains both an aldehyde group and two hydroxyl groups. It is highly reactive and occurs in the biosphere [2, 9, 10]. Preliminary results show that glyceraldehyde is unstable under irradiation in aqueous solutions; even at low doses, it starts to decompose rapidly. It forms a compound that has a much higher absorption coefficient in the UV spectra, accompanied by a hypsochromic shift in the maximum absorption from 274 to 246 nm, which can indicate the formation of a product with a new chromophore or functional group (Fig. 1). The adsorption increases with the dose, and the maximum absorption changes with the pH at the same dose.

This shift probably corresponds to the formation of malonaldehyde (also known as malondialdehyde, MDA), which has a UV absorption spectrum pH-dependent. It presents a maximum at 245 nm (molar absorptivity, $\in = 1.34 \times 10^4$) below pH 3, due to it's configuration as

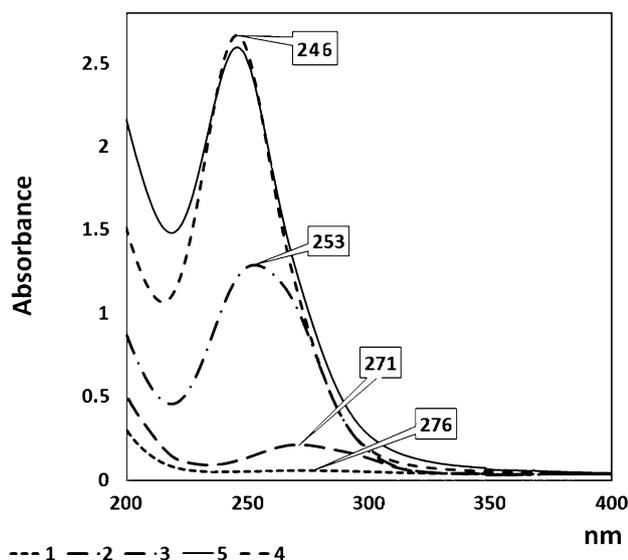**Fig. 1** UV spectra for 1×10^{-2} mol/L aqueous solution samples of DL-glyceraldehyde at various irradiation doses and pH of 6.9: *1* 0 kGy, *2* 0.22 kGy, *3* 2.2 kGy, *4* 11 kGy and *5* 20 kGy

Fig. 2 Decomposition of DL-glyceraldehyde as a function of irradiation dose: **a** aqueous solution, **b** solid-state samples

s-cis planar structure with an intramolecular H bond; and another one above pH 7 at 267 nm (molar absorptivity, $\in = 3.18 \times 10^{4}$) due to it's enolate anion structure completely dissociated [11]. Irradiated solution change the maximum in the spectrum when the solution is measured at different pH, in a similar way that MDA. Even with a very small irradiation dose (61 Gy), the change in the spectrum is apparent. The resulting product has a very high molar extinction coefficient, and its spectral changes associated with pH may indicate that malonaldehyde is responsible for this new absorption band in irradiated carbohydrates [9, 10, 11, 12]. This change in the absorption spectrum is also observed with an aqueous solution of the irradiated solid samples. Other identified products are ethylene glycol and glycolaldehyde.

Figure 2 shows the decomposition of glyceraldehyde in an aqueous solution without clay and in a solid state as a function of an irradiation dose followed by polarography.

For irradiated solid samples an aqueous solution was prepared and the identification of products was made by HPLC–MS, based in their retention times and molecular weight (Fig. 3). Even when MDA is present in small amounts, as shown by the mass spectrum, it has a very large extinction coefficient.

Solid samples irradiated at different temperatures

Although solid DL-glyceraldehyde is relatively stable, it produces free-radical species even at low irradiation doses
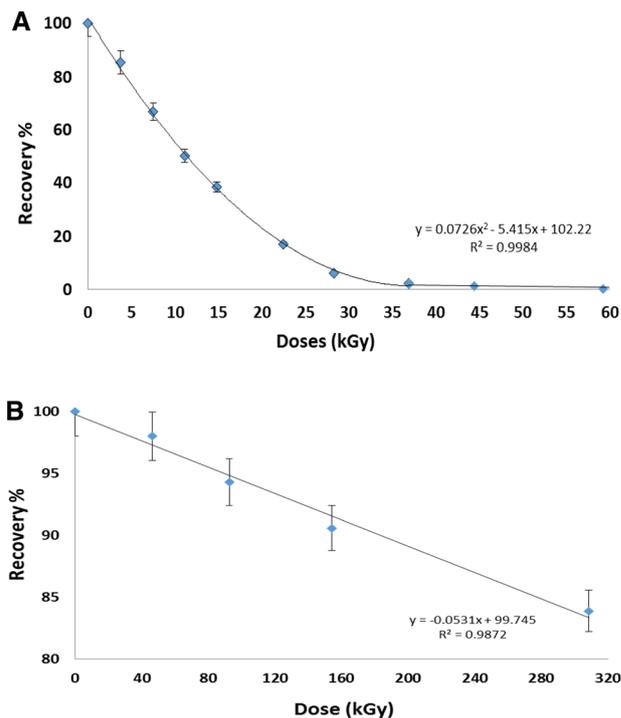
(Fig. 4). The irradiations at low temperature and at room temperature showed the same spectral patterns (Fig. 5).

Glyceraldehyde showed a composite EPR spectrum. Upon irradiation, different paramagnetic radicals formed, creating broad and overlapping lines. Polyalcohols' primary radicals are usually very unstable and are observed at low temperatures. Figure 5 shows the EPR spectra for samples irradiated at room temperature at different irradiation doses, and Fig. 6 shows the spectra for samples irradiated at room temperature and at the temperatures of dry ice and liquid nitrogen for a constant time. The same pattern of signals is present at the different temperatures and doses. The signal at $g = 2.0090$ is the most important line in the spectrum. Steenken and Schulte-Frohlinde [12] detected two secondary radicals by EPR from the photolysis of glyceraldehyde, which was derived from the radical at C-1: $CH_2OH^{\bullet-}CHCOOH$ produced by 1, 2 elimination of water and $^{\bullet}CHOHCH_2OH$ produced by the 1, 1 elimination of water and by decarbonylation. Those substances are stable for several weeks and are formed at low doses and temperatures.

Information about the radiation chemistry of ketones and aldehydes, whether in aqueous solution or in a solid state, is scarce [9]. In particular, the radiation-induced transformation of aldehydes is a complex problem. The entire mechanism may be elucidated by conducting a detailed analysis of the transient intermediates, identifying

Fig. 3 HPLC-MS for dissolved solid samples irradiated at 265 kGy. Legend: *1* formic acid (reagent), *2* glycolaldehyde + ethylene glycol, *3* malonaldehyde, *4* 2-hydroxypropanal, *5* glyceraldehyde, *7* ethylene glycol dimer (tetritol), *10* pentitrol, and *11–12* sugar-like compounds, *6*, *8* and *9* are unknown
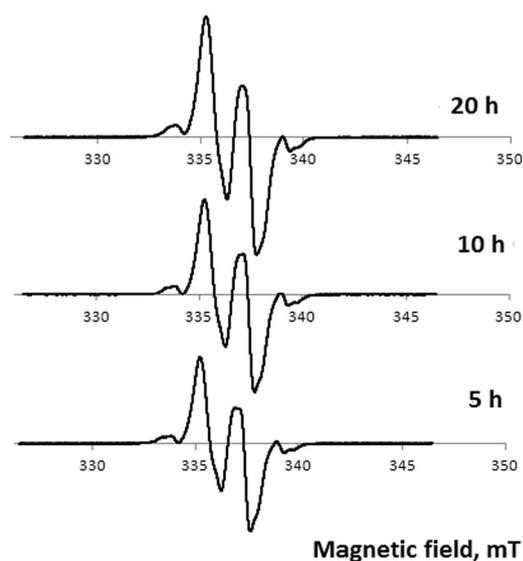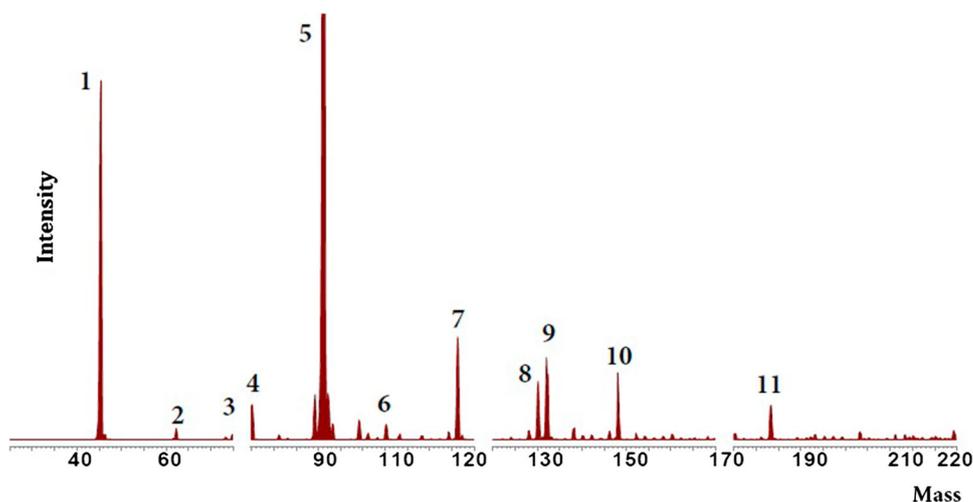
Fig. 4 EPR spectra of glyceraldehyde irradiated at 298 K and at different radiation doses

the primary stable-radiolysis products, and taking into account the kinetics and yields of their formation [9].

Non-irradiated aqueous samples in the presence of a clay mineral

According to chemical evolution studies, minerals (like clays) may have played an important role, such as by serving as sites for the concentration and catalysis of different reactions or possibly as a protector against external sources of energy for the molecules' adsorbed, for which dilution acts against the local conditions needed for effective prebiotic synthesis. For this reason, as a first step, the adsorption of glyceraldehyde was studied in different clay minerals and at various pH levels. The experiments with clays showed that the adsorption percentage varied for the three types of clay
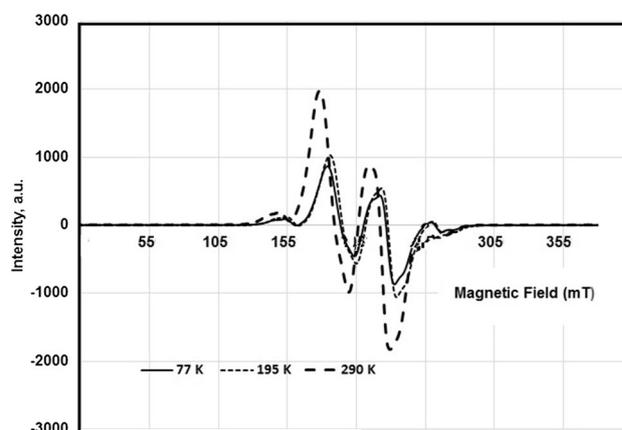
Fig. 5 EPR spectra of glyceraldehyde irradiated at three temperatures for the same irradiation time
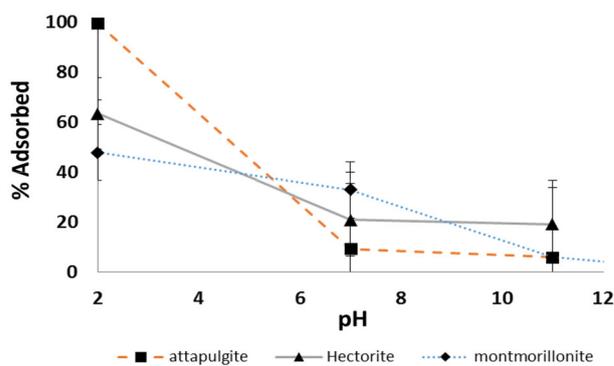
Fig. 6 Effect of pH on the adsorption of glyceraldehyde (1×10^{-2} mol/L) in sodium-montmorillonite, attapulgite and hectorite

used. In all cases, the maximum adsorption was at an acid pH (especially so for attapulgite). At alkaline pH, the adsorption was very low. For sodium-montmorillonite, the maximum adsorption was about 48 % at pH 1.5; the adsorption decreased as pH increased (Fig. 6). Organic compounds bind

Fig. 7 Scheme for the possible mechanism of radiolysis from water radiolysis products
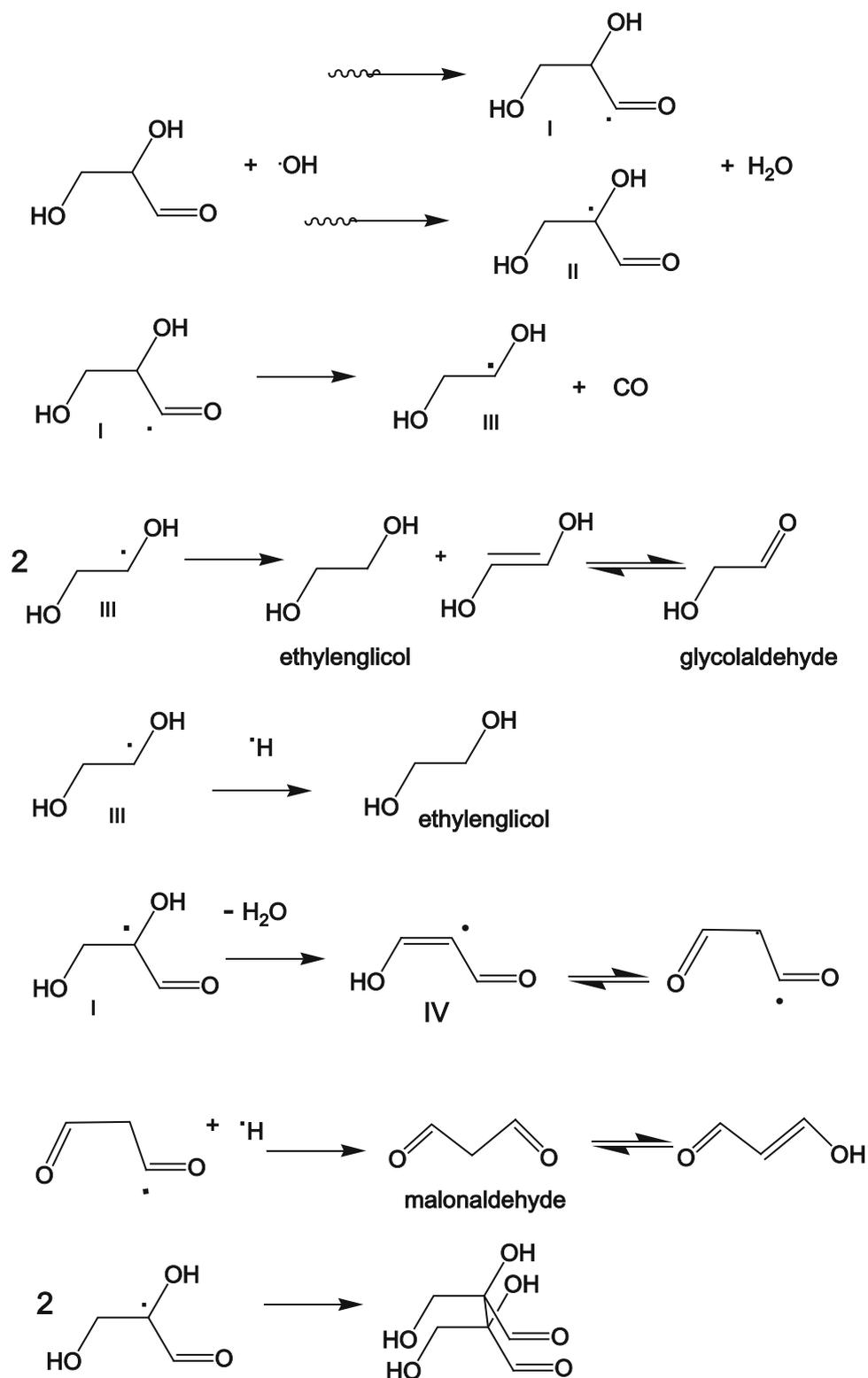
to sodium-montmorillonite in two places: the interlamellar channel and the edges of the crystal [13]. The X-ray diffraction spectrum shows that the binding of glyceraldehyde occurs in the interlamellar channel that expanded from 11.86 to 14.59 Å. The presence of the clay enhances the shift in the maximum absorption of the UV spectrum, which is accompanied by a hypsochromic shift in the peak absorption to 245 nm. This change is probably due to the decomposition

of glyceraldehyde, which is catalyzed by acid sites in the clay, forming malonaldehyde in a way that is similar to what happens with irradiation.

Glycolaldehyde, one of the products detected in this radiolysis, has been identified in interstellar space [14], where it could have been exposed to cosmic radiation and thus generated sugar-like products. Glycolaldehyde, by condensing with formaldehyde via the formose reaction, yields sugars [9, 15, 16]. Glyceraldehyde and other sugars has been detected from the ultraviolet irradiation of interstellar ice analogs [15].

The identification of the products after radiolysis reveals that oxidation is one of the predominant transformations. This process yields oxidized products with unchanged or shorter carbon chains. In the aqueous samples, the water-radiolytic products, mainly $^\bullet$H and $^\bullet$OH, are responsible for forming the observed products via a secondary attack. The $^\bullet$OH radical is a powerful oxidizing agent that is very reactive with aldehydes [17, 18].

$$H_2O \rightsquigarrow \; ^\bullet H \; + \; ^\bullet OH \; + \; e_{aq}^- \; + \; H_2 \; + \; H_2O_2$$

The patterns of hydroxyl radicals' reactions with a solute depends on their chemical structures. EPR and other methods have shown that OH's most typical reaction with polyhydroxy compounds is the abstraction of hydrogen atoms bound to carbon [9, 12, 16, 17]. Some possible pathways for the decomposition of DL-glyceraldehyde [9, 12, 18] via water elimination reactions and CO are shown in Fig. 7.

Our results suggest that radiation-induced reactions of glyceraldehyde may yield compounds of biological importance in both terrestrial and extraterrestrial environments.

Conclusions

The synthesis and preservation of aldoses in prebiotic conditions is fundamental to their role in energetic functions and to the abiotic formation of nucleotides and nucleic acid components (e.g., RNA). On the primitive Earth, clay minerals may have contributed to the concentration, catalysis, or protection of organic compounds such as glyceraldehyde, which is readily absorbed in different clays, especially attapulgite at acid pH.

Glyceraldehyde is an important molecule in chemical evolution studies. The glyceraldehyde molecule also may be formed in the ice of star-forming clouds—and thus could be a source of sugars carried to Earth on extraterrestrial bodies (such as comets). Glyceraldehyde is unstable under irradiation in aqueous solutions, even at low doses. Radiolytic decomposition forms compounds, including some sugar-like products, so decomposition may be a pathway for the formation of sugars and other compounds that are related to

bioorganic compounds under primitive Earth conditions or in icy bodies. In particular, the formation of glycolaldehyde is important because this compound has been identified in interstellar space; it could thus be an intermediate step along the path to forming more complex molecules.

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