Oxidative degradation of oligo(ethylene glycol)-terminated monolayers†

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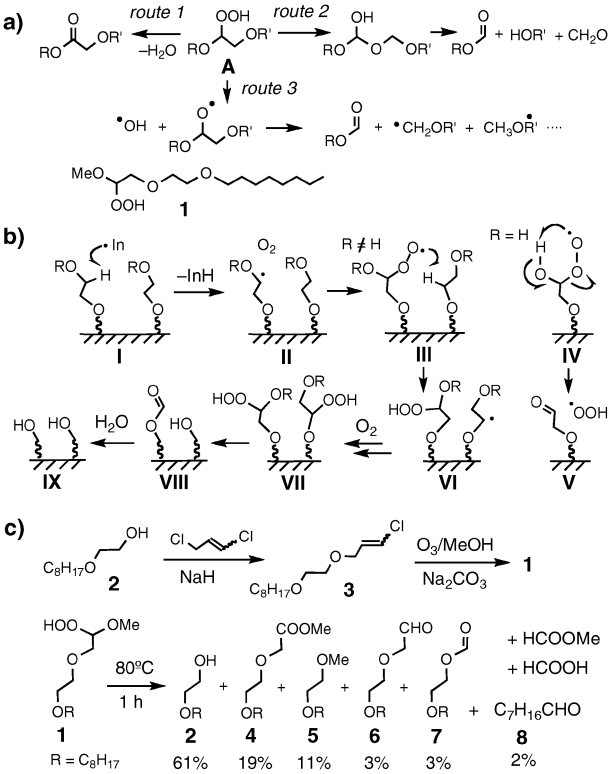
The observation that oligo(ethylene glycol) (OEG)-terminated monolayers remained highly protein-resistant for a month at 37 °C in PBS buffer while they degraded faster in air can be rationalized by a proposed mechanism formulated from a model study using the first internal hydroperoxide of OEG.

Monolayers presenting poly- or oligo(ethylene glycol) (PEG/OEG) have attracted extensive research activities.[1] However, PEG/OEG derivatives are susceptible to degradation *via* autoxidation.[2–6] Although the long-term stability of PEG/OEG films has been investigated,[7] their oxidative degradation and the composition of the degraded surfaces are not well understood. Herein, we present a model study using the first internal hydroperoxide of an OEG derivative to represent the primary products of autoxidation of PEG/OEG. The availability of this model compound allowed for verification of the decomposition products for the first time. A mechanism differing from the general autoxidation process for oxidative degradation of OEG films is proposed. It suggests that the degraded surfaces are dominated by alcohols that can terminate the autoxidation process, in agreement with the experimental observations.

Autoxidation of OEG/PEG has been described to proceed *via* internal hydroperoxides **A** (Scheme 1a).[2–6] They were assumed to decompose *via* three routes: (1) dehydration to an ester, (2) rearrangement to a hemiformate that decomposes to a formate, an alcohol and formaldehyde, and (3) homolytic cleavage followed by chain scissoring leading to a formate and reactive radicals.[3–5] Route 2 was proposed to be the major pathway, accompanied by route 1.[5] Both routes do not involve radical intermediates. Methyl ethers were assumed to form *via* route 3, which also generates radicals and the products of route 2.[3–5] In these studies, PEG/OEG derivatives were exposed to air for days at 150 °C or for months and even years at room temperature. Regardless of the conditions, the hydroperoxides **A** were detected in the mixtures,[3,5] but have never been obtained in a pure form. Hence, their proposed degradation products have not been validated. In this work we synthesized **1** as the first internal hydroperoxide of OEG, which closely represents the hydroperoxides initially formed on methoxy-terminated OEG films.

The proposed mechanism for autoxidation of OEG films starts with hydride abstraction by a radical initiator ($In^{\bullet}$, Scheme 1b) on the film surface **I** to generate a carbon radical in **II**. For the methyl-terminated films (R = Me), hydride abstraction at the accessible –CH_2O– group is energetically more favorable than at the H_3CO– group.[8] Rapid trapping of the radicals by O_2 generates the peroxy radicals in **III**. For the OH-terminated OEG films (R = H), the peroxy radical **IV** should rapidly fragment to an aldehyde **V** and a hydroperoxy radical,[3,6] the calculated activation energy for similar reactions being ~9 kcal mol^{-1}.[6] On the other hand, the peroxy radical in **III** should abstract a hydride from an adjacent molecule, leading to **VI**, rather than intramolecular hydride abstraction which has a higher energy barrier (~25 *vs.* 20 kcal mol^{-1}).[6] Repeating the process accumulates hydroperoxides as the primary oxidation products on the film surface **VII**. We studied their decomposition using the model compound **1**.

The hydroperoxide **1** was synthesized from the alcohol **2** *via* ozonization of the chlorovinyl derivative **3** followed by selective cleavage of the primary ozonide to form the desired carbonyl oxide[9] that is quenched with methanol (Scheme 1c). Thermo gravimetric analysis and differential thermal analysis (TGA-DTA) of **1** showed that its exothermic decomposition occurred rapidly at 80 °C (see ESI†). The decomposition of **1**



Scheme 1

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† Electronic supplementary information (ESI) available: Synthesis, TGA-DTA and decomposition of **1**, NMR, GC-MS of the decomposition mixture, and proposed mechanism of oxidative degradation of OEG films. See DOI: 10.1039/b911155g

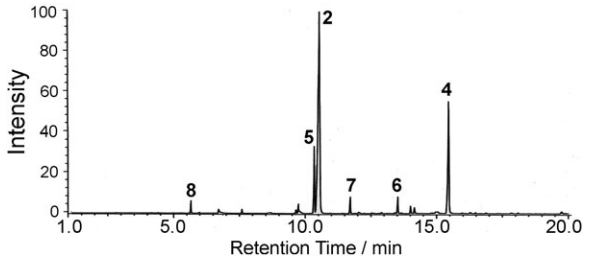

Fig. 1 GC-MS diagram after heating of **1** at 80 °C in air for 1 h.

was then performed at 80 °C in air for 1 h. The reaction mixtures were analyzed by GC-MS (Fig. 1), showing the presence of the products **2** and **4–8**. The yields listed in Scheme 1c were obtained by GC, indicating that **1** was decomposed mainly through route 2 (Scheme 1a) to the alcohol **2**. ^{1}H- and ^{13}C-NMR spectroscopy of the mixture showed that methyl formate and formic acid were also formed (ESI†), the latter probably by oxidation of formaldehyde in air.[5] To a lesser extent, the ester **4** was formed in 19% yield *via* route 1. The aldehyde **6** (3%) and formate **7** (3%) as minor products might be formed by reactions of **1** with formaldehyde, and **2** with formic acid, respectively. Note that the methyl ether **5** was formed in 11% yield. Only trace amounts of other byproducts, mainly the aldehyde **8** (2%), were present. This result indicated that **5** was not generated from route 3, which would also produce equal amounts of highly reactive HO$^{\bullet}$ and carbon radicals leading to substantial amounts of other products (see ESI† for a speculative, non-radical route).

The above model study establishes that the internal hydroperoxides on the OEG film **VII** (Scheme 1b) decompose mostly to alcohols and formates as in **VIII**. The latter are hydrolyzed to alcohols as in **IX**. Note that rather than undergoing hydride abstraction to propagate the radical chain reaction, *i.e.* **III** to **VI** (Scheme 1b), the α-peroxy radicals of alcohols rapidly decompose to aldehydes and HOO$^{\bullet}$, *i.e.* **IV** to **V**.[3,6] If the HOO$^{\bullet}$ radicals can be removed from the film, *e.g.* by forming hydrogen bonds with water[11] and diffusion into the bulk solution (see below), the radical chain process is then terminated. Otherwise, HOO$^{\bullet}$ radicals will generate the carbon radicals **II** (Scheme 1b), and the process is repeated from **II** to **IX** leading to mostly alcohols and aldehydes (*via* **IV** to **V**) on the surface.

We found that films prepared by hydrosilylation of MeO-EG$_7$(CH$_2$)$_8$CH=CH$_2$ on hydrogen-terminated silicon (111) surfaces[10] remained protein resistant in phosphate buffered saline (PBS) for weeks, while they were easier to degrade in air. Preparation of the OEG monolayers on silicon substrates was described in ref. 10. The stability of the monolayers for protein-resistance were evaluated by incubation of the samples in PBS buffer (pH 7.4, Sigma) at 37 °C for 28 days, or placed in semiconductor wafer containers in air at room temperature for 24 days. The samples were then immersed in a solution of fibrinogen (1 mg mL^{-1}) in PBS buffer for 1 h, washed under a gentle flow of deionized water (Millipore) for about 15 s, and dried with a flow of Ar. The samples were immediately measured by X-ray photoelectron spectroscopy (XPS, Fig. 2). The XPS N1s signal intensity is proportional to the amount of adsorbed protein. A hydrogen-terminated

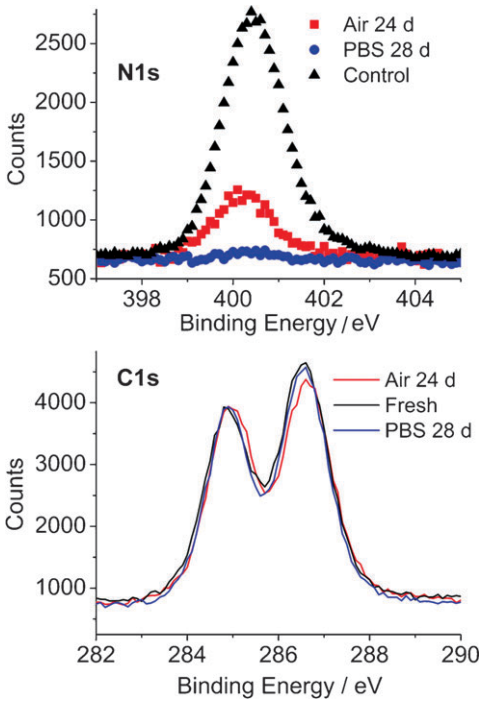

Fig. 2 Top: XPS N1s narrow scans of MeO-EG$_7$-terminated monolayers on Si substrates after storage in air for 24 days, or in PBS buffer at 37 °C for 28 days, followed by incubation in 0.1% fibrinogen for 1 h. A freshly prepared hydrogen-terminated silicon substrate with an adsorbed monolayer of fibrinogen is used as a reference. Bottom: XPS C1s narrow scans of MeO-EG$_7$-terminated monolayers before and after storage in air for 24 days, or in PBS buffer for 28 days followed by treatment with fibrinogen.

silicon (111) substrate was subjected to an identical fibrinogen adsorption experiment, forming a monolayer of fibrinogen. This sample was used as a reference for the measurement of the % monolayer adsorption of fibrinogen onto the OEG films. Thus, after 4 weeks in PBS buffer at 37 °C, the OEG films adsorbed only a ~1.1% monolayer of fibrinogen, while they adsorbed a 23% monolayer of fibrinogen after 24 days in air, as derived from the N1s data (Fig. 2). The higher stability in PBS buffer might be due to the decomposition of the OEG hydroperoxides to alcohols that stop the propagation of peroxy radicals, leading to HOO$^{\bullet}$ radicals that hydrogen-bond to water[11] and diffuse into the solution. The short singlet state lifetime of O_2 in water[12] may also suppress the oxidation. The alcohols accumulated on the monolayer surface can terminate the propagation of OEG peroxy radicals on the surfaces if the resultant HOO$^{\bullet}$ radicals can be removed. This advantage for OEG/PEG monolayers is not shared by OEG/PEG in the bulk. For the films maintained in air, HOO$^{\bullet}$ radicals will remain in the film, and may initiate the degradation process as discussed above. Indeed, after storage of the OEG films in PBS buffer at 37 °C for 28 days, the number of EG units remained the same, as indicated by the same intensity of the etheric carbon peaks at 286.6 eV (Fig. 2). In comparison, as calculated from the decrease of the etheric C1s peak, one out of seven EG units was lost for films stored in air for 24 days. However, EG$_6$-terminated monolayers on Si are expected to adsorb only 3% instead of 23% monolayer of fibrinogen.[10] The substantial

increase in protein binding is attributed to the formation of imines with the aldehydes on the film surface,[13] although the C1s XPS signal for aldehydes at 288 eV was at the noise level (Fig. 2) due to the low density and the presence of 22 other carbon atoms in the molecule. In fact, the C1s signal (blue curve in Fig. 2) was obtained on the sample that absorbed 1.1% fibrinogen. Yet, the carbonyl signals at 288 eV and 289 eV were also at the noise level. In a relevant study, Leggett and co-workers performed nanopatterning with photochemical oxidation of thiolate monolayers terminated with a short OEG (EG_3) on gold substrates. A high density of aldehydes and OC=O species were observed by XPS, likely as a result of exposure of the films to UV light in air which generates a high concentration of oxy radicals.[13]

In conclusion, we demonstrated for the first time that internal OEG hydroperoxides are decomposed mainly through non-radical pathways to alcohols and esters. When stored in an aqueous solution (PBS buffer), OEG monolayers remained highly protein-resistant for at least a month at 37 °C. They were easier to degrade when stored in air. This observation can be rationalized by a proposed mechanism suggesting that oxidation on the surface of OEG monolayers by reactive oxy radicals generates mostly alcohols and aldehydes. The mechanism may be applied to other processes involving oxy radicals, such as photooxidation,[13] electro-chemical oxidation,[14] and interactions with polymorpho-nuclear leukocytes that secrete reactive oxy radicals.[7a]

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