The Possible Role of Surface Oxygen Species in Quartz Pathogenicity

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The inhalation of particulate quartz or of some other silica polymorphs causes silicosis and, as recently suspected, lung cancer [1, 2]. In spite of the massive amount of work performed on this subject [3–6], the primary cause of quartz toxicity has not yet been clarified at the molecular level. It is widely accepted that, as a consequence of a failed phagocytosis of the particle by alveolar macrophages, an abnormal production of a growth factor (MFF, a low-weight protein) occurs, which stimulates fibroblasts to produce fibrotic tissue [7–9]. Some immunological reactions have also been found in the tissue response to silica, involving the release of interleukin 1 [10, 11].

Alongside these processes, silica exerts its well-known cytotoxicity by attacking the phagolysosome membrane, with consequent release of lytic enzymes into the cytoplasm, eventual death of the cell and release of the free silica particle [3–6].

The question arises as to what is the role played by the surface chemistry of SiO_2 in all the above processes. We note that the SiO_2 particle may act at different stages with various surface functionalities, causing on the one hand the rupture of the phagolysosome membrane (cytotoxicity) and, on the other hand, triggering a series of reactions yielding the formation of the growth factor (fibrogenicity) and related stimulation of the immune system.

Much emphasis has been given so far to the membranolytic action of silica, and different mechanisms have been proposed, mainly invoking the acid–base properties of surface hydroxyls (silanols) [3, 6, 12]. Cytotoxicity, wich is an essential step in quartz pathogenicity, is not sufficient, however, to explain fribrogenicity and, moreover, cancerogenicity. Beside hydroxyls, which are always present at the surface of any silica, when the surface is created by grinding (as in most quartz dusts) several radical species are present, originating from the interaction of the atmospheric components with the cleaved Si–O–Si bonds. These species have been considered so far by few authors [13, 14] only as an alternative cause of membranolysis via lipid peroxidation. We propose here that the oxygen surface radicals created in this way are one of the possible initiators of the fibrogenic process. The existence and stability of these radicals in air has already been reported [15–17]. We have now evidence that a peroxy radical ($SiO_2^{\bullet}$) and a superoxide anion ($O_2^{-\bullet}$) are always present on quartz ground in air.

The EPR spectrum taken at 77 K *in vacuo* of a pure quartz crystal freshly ground in air is reported in Fig. 1. Most powdered quartzes used as standards for medical work, e.g. DQ-12 [18], Min-U-sil [1, 6], exhibit similar spectra [19]. In the Figure among the various lines originating from several radical species and bulk defects are indicated the components of the g tensor arising from the peroxy radical and the superoxide anion. This assignment, which is based on literature data from silicas ground under controlled atmosphere [20, 21] and on the evolution of the spectrum upon chemical and thermal treatments, is discussed in detail elsewhere [19].

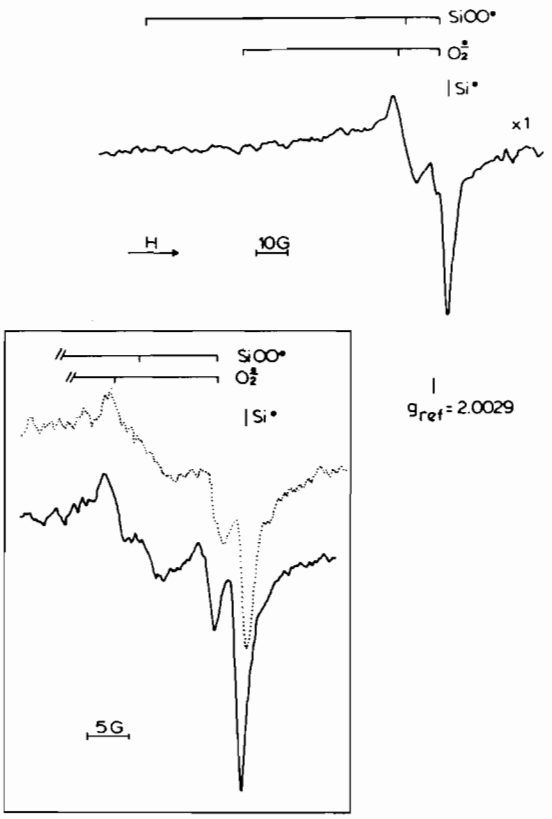

Fig. 1. EPR spectrum of a pure quartz crystal (99.999%) ground in air in an agatha ball mill. Inset: solid curve, central part of the spectrum on an expanded scale; dotted curve, same sample after treatment in buffered SOD (bovine superoxidismutase). Spectra were recorded *in vacuo* at 77 K; g values of components arising from $Si^{\bullet}$, $SiO_2^{\bullet}$ and $O_2^{-\bullet}$ are indicated.

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The composition of the grinding atmosphere markedly influences the population of the radicals produced [19]. In the present work we investigate the effect of grinding in liquid media in order to mimic the reactivity of the freshly ground particle, just inhaled, particularly in contact with oxidizing agents (e.g. H_2O_2) and superoxide scavengers (e.g. SOD superoxidismutase).

If grinding is performed in water (Fig. 2, inset b), the formation of oxygen radicals is largely depressed, whereas, when ground in hydrogen peroxide aqueous solution (Fig. 2, inset a), their formation is enhanced and the spectrum is even more intense than that observed after grinding in air (Fig. 1). The prevailing features, in this case, are those of the superoxide anion. The formation of superoxide from H_2O_2, already found with asbestos [22], is an unusual reaction: in our opinion this is clear proof that the freshly crushed quartz surface controls redox reactions involving oxygen.

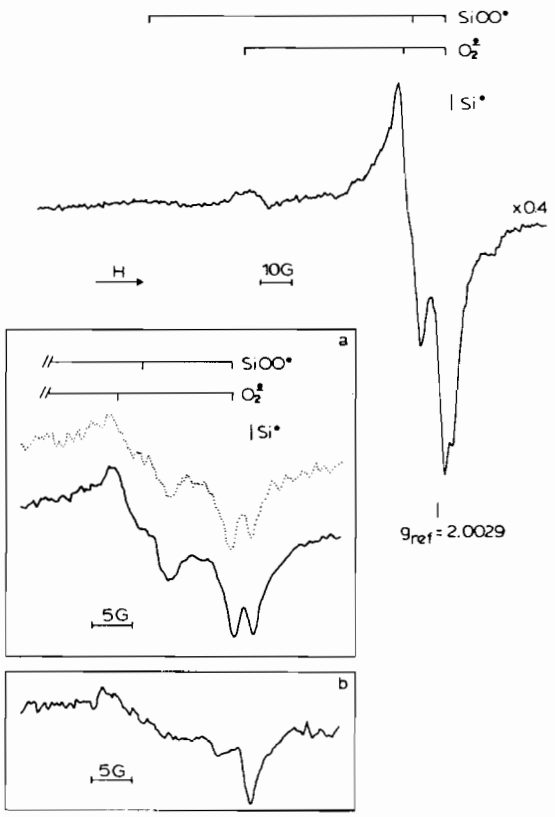

Fig. 2. EPR spectrum of a pure quartz crystal (99.999%) ground in aqueous hydrogen peroxide solution in an agatha ball mill. Inset (a): solid curve, central part of the spectrum on an expanded scale; dotted curve, same sample after treatment in buffered SOD (bovine superoxidismutase). Inset (b): central part of the spectrum of the same crystal ground in water. Spectra were recorded *in vacuo* at 77K; *g* values of components arising from $Si^•$, $SiO_2^•$ and $O_2^{-•}$ are indicated.

In order to test the 'biological availability' of the $O_2^{-•}$ at the surface, the samples of Figs. 1 and 2 were left in a buffered (phosphate) aqueous solution of SOD for *c.* 1 h and then the EPR spectra (dotted spectra in the insets of Figs. 1 and 2) were taken on the washed and dried solids, under the same conditions as the starting materials. A modification caused by this treatment is visible, particularly in the central part of the spectra. Similar modifications were detected by treating the standard powdered quartz in the same way. The changes are not very pronounced but, taking into account the small dimensions of the active site of the enzyme, with respect to crystallites, it is clear that very few protruding $O_2^{-•}$, situated at the sharp edges or corners, may interact with the enzyme. It has been reported that the phosphate ion may interfere with some sites on quartz, as treatment of quartz dusts with phosphoric acid reduced cytotoxicity [23]. A blank experiment with the phosphate buffer, however, only showed minor modifications in the spectra with respect to those reported for the reaction with SOD in Figs. 1 and 2.

The fate *in vivo* of the here reported surface oxygen species is an open question. One possibility, already advanced in the case of asbestos [24, 25], is that hydroxyl radical and singlet dioxygen are produced. Indeed, the presence of hydroxyl radicals in quartz aqueous suspensions has been revealed by some of us [26]. The yield is enhanced in presence of H_2O_2 [26, 27]. As to the singlet oxygen, we note that, on the one hand, this is the form into which $O_2^{-•}$ evolves from the quartz surface, e.g. upon heating [28]; on the other hand, it has been reported that singlet oxygen is formed, from hydrogen peroxide and hypoclorite ion, during phagocytosis [29].

The hypothesis advanced in the present paper, i.e. that oxygen radical species at the surface of freshly ground quartz may act as triggering agents of the fibrogenic process, is in agreement with some well-known facts in silicosis:

(i) It is usually believed that only crystalline modifications of SiO_2 are pathogenic: chemically prepared amorphous silicas are in fact inert. Amorphous silica glasses, however, also obtained in the divided form by grinding, are fibrogenic [2]: the way the surface is created, i.e. grinding, is thus the determining factor and not the crystallinity.

(ii) The hydroxyl population may not be the only factor in determining quartz toxicity because, although with different distribution, any silica exposed to moist air tends to be covered by silanols.

(iii) The presence of active radicals within the macrophage may account for the abnormal production of the fibrogenic growth factor during phagocytosis.

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