Biological and biochemical effects of air pollutants: synergistic effects of sulphite

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Abstract

Air pollution has become a major public and political concern since the beginning of industrialization, particularly motor exhaust over the past three decades. Epidemiological studies, together with clinical trials and experiments in exposition chambers (including biochemical model reactions), have contributed to our knowledge of potential dangers and increased our understanding of the corresponding mechanisms and dose-response effects. Comparison of the threatening reports that appear almost daily in the press with the digest of over 800 scientific publications allows the statement that the impact of ozone and nitric oxide on the health and performance of plants and animals is widely overestimated and appears to be used as a political instrument. In contrast, the combination of SO₂ with soot or asbestos particles may represent an underestimated toxic potential.

In this communication, we shall concentrate on basic redox mechanisms involving SO₂ and important target molecules, as well as looking at the cooperative effects of sulphite and soot particles.

Introduction

Air pollution research has tremendously increased in the past two decades, accompanied by the publication of a wealth of data. To mention just a few titles: ‘Responses of Plants to Air Pollutants’ [1], ‘Introduction to Environmental Toxicology’ [2], ‘Air Pollution and Forests’ [3], ‘Effects of Gaseous Air Pollution in Agriculture and Horticulture’ [4], ‘Gaseous Air Pollutants and Plant Metabolism’ [5], ‘Air Pollution by Photochemical Oxidants’ [6] and ‘Air Pollution and Plant Metabolism’ [7]. Several reviews emphasizing the importance of oxygen radicals in air pollution have also appeared recently [8–10].
The role of oxidative processes in air pollution has already been reported in the last century when the production of acid rain was recognized as an oxidation by atmospheric hydrogen peroxide of sulphur dioxide stemming from coal combustion [11].

SO₂ emissions are of special interest as the main component of the acid (London-type) smog and as a monocausal trigger of forest decline (‘Waldsterben’) in SO₂-polluted areas.

Certain trace gases stemming from industry, traffic and agriculture are a growing threat, especially for the industrialized countries all over the world. In the last few years especially, automobile exhaust has advanced to become a focus of public and political discussions and decisions. Airborne soot particles stemming both from industrial incinerators and diesel engines are discussed as potential carcinogens, allergens and/or inducers of respiratory diseases. The combinations of SO₂ and soot from coal burning have been epidemiologically shown to be responsible for thousands of deaths during several severe smog episodes both in London and in New York not too long ago. Several reports have appeared concentrating on biochemical mechanisms underlying these synergisms [10,12–14].

Significant health-threatening effects brought about by gases or air-borne particles may in the first line be seen in the cooperation of catalytically active particles with SO₂. This will be outlined in more detail below.

**Reactions of SO₂ with biomolecules**

SO₂ is produced during combustion of organic materials, especially coal. SO₂ is a highly water-soluble gas which is rapidly hydrated forming sulphite (SO₃²⁻) [15]. Principally, SO₂ or HSO₃⁻ preferentially reacts with the following types of molecules: (a) aldehydes and ketones, where hydroxysulphonates are formed, which in turn are inhibitors of several enzymes; (b) olefines, where sulphonic acids are formed and the double bond is lost; (c) pyrimidines under formation of dihydrosulphonates, which may have mutagenic effects; (d) disulphides during formation of S-sulphonates, where the S–S bridge is split; and (e) superoxide (O₂⁻⁻) under formation of the very reactive bisulphite radical:

\[
O₂⁻⁻ + SO₃²⁻ + 2H⁺ ⇌ SO₃⁻⁻ + H₂O₂
\]

In this reaction, superoxide may be substituted by transition metal ions:

\[
M^{n+} + SO₃²⁻ → M^{(n-1)+} + SO₃⁻⁻
\]

SO₃⁻⁻ is a powerful initiator of several radical chain processes such as lipid peroxidation (see below). Sulphite modifies the cellular energy metabolism in mammalian tissues by decreasing the cellular ATP pool; this may be due to changing the function of pyridine and flavin nucleotides [16]. Sulphite oxidase, an enzyme absent in human lung tissue, oxidizes sulphite. Thus, if sulphite is not metabolized, it may be oxidized via radical chain mechanisms.

**Plant damage by SO₂**

Plant damage, caused by SO₂, is oxygen and light dependent. Typical SO₂ effects are the rapid inhibition of photosynthesis before direct symptoms such as
bleaching of chlorophyll can be observed. The bleaching of chlorophyll is the most prominent effect of SO$_2$, SO$_3$, or its aqueous solution sulphite, can be oxidized in the presence of superoxide (O$_2^-$) [15]. Limited NAD$^+$ availability in the stroma of chloroplasts results in the formation of superoxide via 'over'-reduction of the electron transport system and subsequent electron channelling to oxygen. Free radicals generated during sulphite autoxidation attack cellular membranes via lipid (L) peroxidation and subsequent chlorophyll (Chl) bleaching:

$$\text{SO}_3^{2-} + \text{O}_2^- + 2\text{H}^+ \rightarrow \text{SO}_3^{3-} + \text{H}_2\text{O}_2$$

$$\text{SO}_3^{3-} + \text{LH} \rightarrow \text{HSO}_5 + \text{L}^-$$

$$\text{L}^- + \text{O}_2 \rightarrow \text{LOO}^-$$

$$\text{LOO}^- + \text{LH} \rightarrow \text{LOOH} + \text{L}^-$$

$$\text{LOOH} \rightarrow \text{LO}^- + \text{OH}^-$$

$$\text{LO}^- + \text{Chl}_{\text{red}} \rightarrow \text{LOH} + \text{Chl}_{\text{ox}}$$

The bleaching of chlorophyll by lipid peroxidation is only observed if SO$_3^{2-}$ is simultaneously present. Neither LOOH nor SO$_3^{2-}$ alone can destroy the green colour of chlorophyll.

The generated H$_2$O$_2$ is thought to be responsible for the inactivation of ascorbate peroxidase, glutathione reductase and enzymes of the Calvin cycle, such as phosphatases [17,18]. Uncoupling of oxidative phosphorylation results in insufficient reoxidation of NADPH and thus causes inhibition of photosynthesis in the light due to a lack of ATP.

**Cooperative effects of sulphite and soot particles**

The discussion on the potential toxicity of diesel exhaust is of growing importance, especially due to the increasing truck traffic in Middle Europe since the opening of the East. Much of the threat of Otto-engine exhaust has been taken away by the introduction of the three-way catalytic converter. Such a redox converter is not applicable for diesel engines due to the different incineration concept: only oxidative reactions are relevant for the diesel catalyst. Of special importance, however, are the particulate emissions which are 30–70 times higher than those from catalyst-equipped spark ignition engines [19,20], rising to approximately 1 g/km [19]. The soot particles are small in size (less than 0.5 µm), easily respirable, and have carbon cores with a very large surface area on to which a variety of organic compounds (polycyclic aromatic compounds, nitro aromatics and quinones) are adsorbed. The number and structure of these compounds depend on the type of engine and the mode of its operation, and may thus be extremely variable [21]. The adsorbed compounds of diesel soot are subject to atmospheric conversions, which make them even more toxic [22–27].

Soot particles have been shown to exhibit mutagenic and carcinogenic properties in certain cell and animal models [28–35]. This is also evident from epidemiological studies concerning indoor coal burning in China [36].
There is also growing evidence that respiratory diseases and allergic reactions may be induced and/or enhanced by particle inhalation. Model experiments have shown that diesel soot particles (DPs), enhanced by aqueous SO₄ solutions (bisulphite), exhibit a considerable destructive potential concerning vital biomolecules such as SH compounds, polyenes, linolenic acid and certain enzymic properties [12]. The cooperative mechanism observed in the presence of DPs and sulphite indicates monovalent oxygen reduction and subsequent lipid peroxidation in the presence of an unsaturated fatty acid. In this process, sulphite is supposed to function both as electron donor and radical propagating agent:

\[
\begin{align*}
SO_3^{2-} + O_2^{DP} & \rightarrow SO_3^{-} + O_2^{2-} \\
SO_3^{-} + O_2 + H_2O & \rightarrow SO_4^{2-} + O_2^{2-} + 2H^+ \\
O_2^{2-} + DP & \rightarrow DP^{+} + O_2 \\
DP^{+} + SO_3^{2-} & \rightarrow DP^{-} + SO_3^{2-} \\
DP^{+} + O_2 & \rightarrow DP^{+} + O_2^{2-} \\
2DP^{+} & \rightarrow DP^{+} + DP^{-} \\
DP^{+} + O_2^{2-} + H^+ & \text{SOD} \rightarrow DPH + O_2 \\
O_2^{2-} + O_2^{2-} + 2H^+ & \text{SOD} \rightarrow H_2O_2 + O_2
\end{align*}
\]

As activating principles of DPs in reaction (a) both nitroaromatics and naphthoquinones have to be considered, since both classes of compounds have been shown to undergo redox cycling, thus driving oxidative destruction in the presence of appropriate electron donor molecules. Reactions (b)-(e) may operate as propagators of the synergistic radical chain reaction observed in the presence of both DPs and sulphite. Superoxide plays an important role as mediator between the DP-redox factors and different sulphur oxidation states. Disproportionation of DPs (f) and superoxide dismutase (SOD)-catalysed dismutations [(g) and (h)] represent chain-terminating events, where reaction (g) would be in agreement with the function of SOD as a superoxide-semiquinone-oxidoreductase [37].

Beside nitroaromatics and naphthoquinones iron may play an important role as an activating principle, as is well documented for asbestos fibres [38,39]. Asbestos fibres are able to generate OH' radicals and O₂' radicals from H₂O₂ via the catalysis of iron as an integral part of the asbestos complex. Pulmonary epithelial cell injury is mediated by H₂O₂ release from asbestos-activated polymorphonuclear neutrophils (PMNs) [40]. Asbestos alone has less cytotoxic effect on epithelial cells in leucocyte-free media; however, asbestos in combination with PMNs causes significant damage dependent on asbestos dose. As summarized by Mossman et al. [41] several studies support 'the concept of a cause and effect relation between activated oxygen species and the development of asbestosis'. Therefore asbestos fibres, in addition to their mechanical properties, may act as immobilized catalysts for Fenton- or Haber–Weiss-type reactions.

Using simple biochemical model reactions, simulating activated PMNs, the iron-mediated formation of strong oxidants in the presence of asbestos could be demonstrated. The free radical indicator ketomethylthiobutyrate (KMB) is
fragmented in the presence of certain strong oxidants such as the OH' radical yielding ethylene, which can be sensitively monitored by gas chromatography.

The oxidoreductase of PMNs, responsible for the respiratory burst occurring after activation of these phagocytes, was simulated using a diaphorase (from pig heart), which is able to reduce molecular oxygen at the expense of NADH. As shown in Fig. 1 crocidolite stimulates ethylene release from KMB, triggered by the NADH/diaphorase-system. The chelator ethylenedinitrilotetra-acetic acid (EDTA) strongly enhances certain oxidative processes by facilitating Fe³⁺ reduction as well as electron transfer from Fe²⁺ to H₂O₂, thus allowing the formation of OH' radicals according to the Haber–Weiss sequence. Addition of EDTA to the asbestos-stimulated NADH/diaphorase system results, as expected, in a strong increase of ethylene release. Both SOD and catalase inhibit the reaction of the model system in the presence of asbestos and EDTA. Desferrioxamine, a chelator forming an unreactive complex with Fe³⁺ ions, suppresses the crocidolite-stimulated ethylene release of the enzyme system. These results clearly indicate an iron-mediated formation of reactive oxygen species via the Haber–Weiss-type reaction.

Fig. 1. Ethylene release from KMB in the NADH/diaphorase system. Reaction mixtures contained in a total volume of 2 ml: 2.5 mM KMB; 2.2 units of diaphorase; 75 μM NADH; 400 μg crocidolite or DPs; 0.5 mM EDTA or desferrioxamine (Desf); 100 units of SOD or catalase; 0.1 M phosphate buffer. The reactions were done at 37 °C in the dark. Standard deviations represent n = 6.
As also shown in Fig. 1, crocidolite can be replaced by aqueous diesel soot suspensions, underlining the role of iron in diesel soot toxicity.

In addition, aqueous suspensions of soot particles from domestic fuel burners are able to function as catalysts for Fenton-type reactions (Fig. 2). In this respect, different oxidative capacities are observed, allowing a differentiation between diverse types of soot. Of special interest is the distinction of soot particles stemming from mobile (traffic) and immobile (heat systems) sources respectively. As demonstrated in Fig. 2 the soot sample derived from the chimney of a wood-charged boiler causes no increase in the ethylene formation in the NADH/diaphorase system.

Also, addition of EDTA shows no effect. In contrast all soot samples derived from fuel oil (diesel soot, soot from the central heating and soot particles from the room oil stove) enhance the NADH/diaphorase reaction and are stimulated by EDTA. (Increasing ethylene release of the NADH/diaphorase system in the presence of EDTA is due to ubiquitary iron impurities.) Desferrioxamine, as well

![Graph](image_url)

**Fig. 2. The oxidative capacities of different soot types.** Reaction mixtures are as described for Fig. 1. Soot (S.) samples (400 μg): diesel soot; wood soot (from the chimney of a wood-charged boiler with an efficiency about 730 kW); central heating soot (from the chimney of a fuel oil-fitted boiler for central heating, efficiency 35 kW); oil stove soot (from the stove-pipe of a room oil stove, efficiency 8 kW).
as SOD and catalase, inhibits the ethylene release in these model systems (data not shown).

As mentioned above, the combination of (diesel) soot particles and sulphite exhibits a remarkably deleterious potential. In Fig. 3 the effects of sulphite on the NADH/diaphorase system are demonstrated. Sulphite alone causes a strong increase in KMB fragmentation. This reaction is strongly inhibited by SOD, but not by catalase. On the contrary this haem-enzyme stimulates the sulphite-modified ethylene release due to the NADH/diaphorase system. In the presence of both sulphite and crocidolite, diesel soot and wood soot respectively, a comparable 2-fold enhancement of ethylene release can be observed. SOD decreases the KMB fragmentation, whereas catalase shows no effect. In contrast the soot samples derived from immobile fuel oil using burners inhibit the sulphite-stimulated generation of reactive oxygen species due to the NADH/diaphorase system. From this model reaction a clear differentiation between fuel oil-derived soot samples from mobile and immobile sources appears to be possible.

In addition vital functions of human PMNs are influenced by aqueous suspensions of soot particles in combination with sulphite in vitro ([14]; S. Hippeli and E.F. Elstner, unpublished work).

Since both SO₂ and DPs are present in significant concentrations in urban air pollution (smog) the indicated reactions may contribute to certain respiratory disorders discussed in the context of severe air pollution.

Superoxide, the main radical generated during the interaction of sulphite and asbestos, as well as soot particles, is shown to be also involved during influenza infections [42]. In this connection immune defence against infectious lung diseases
in mice is reduced after exposure to diesel exhaust [43]. Thus superoxide-generating systems like soot/sulphite cooperating in the alveolar space, may modify certain immune responses in vivo.

References


