

Negative air ions as a source of superoxide

Naum I. Goldstein, Roman N. Goldstein¹, and Mark N. Merzlyak²

Institute of Pathological Physiology, Faculty of Medicine, Humboldt University of Berlin, Schumannstraße 20/21, O-1040, Berlin, FRG

¹ Laboratory of Toxicology, Latvian Medical Academy, 226007 Riga

² Department of Cell Physiology and Immunology, Faculty of Biology, Moscow State University, Moscow B-234, USSR

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Abstract. The physico-chemical characteristics and possible formation mechanisms of negative air ions are considered. It was found that the products of oxygen and nitrogen negative ionization reduce ferricytochrome *c* and nitroblue tetrazolium, and that these reactions were inhibited by superoxide dismutase. The interaction of negatively ionized oxygen with water led to hydrogen peroxide accumulation, which was inhibited by tetranitromethane or catalase. Nitrogen ionization under these conditions caused the formation of the hydrated electron e_{aq}^- and the superoxide anion $O_2^{\cdot-}$. The data obtained indicate that the biological activity of negative air ions may be dependent on superoxide. The generation of reactive oxygen ions in the gas phase and also at a gas/water interface is described. A scheme for superoxide production under oxygen and nitrogen ionization is proposed.

Key words: Negative air ions – Superoxide anion – Formation mechanisms

Introduction

Biological effects of superoxide $O_2^{\cdot-}$ are considered in relation to the reactions leading to cell death and to the metabolism of certain endogenous compounds (for review see Byczkowski and Gessner 1988; Fridovich 1989). At the same time $O_2^{\cdot-}$ production probably takes place under natural conditions as a result of gas ionization in the atmosphere. In spite of a low concentration in air (one ion per 10^{15} – 10^{16} of nonionized molecules) the particles are believed to be important climatological agents with pronounced biological activity, including therapeutic action. Biologically beneficial and therapeutic effects are widely attributed to the negatively charged ions (Tchijevsky 1960; Sulman et al. 1978; Kröling 1985). Although numerous experimental observations

on air ion activities have been reported, little is known about the primary mechanisms of the interaction of the particles with biological objects.

It is noticeable, that chemically described as $O_2^{\cdot-}$ and recognised as a particle responsible for biological activity of negative air ions (Krueger and Reed 1976; Dolezalek 1985), is none other than superoxide anion-radical $O_2^{\cdot-}$. The analysis of physical processes of production of air ions in a corona discharge (Goldstein 1982) indicated a high probability of superoxide anion-radical generation. Moreover many of the known physiological effects of small negatively charged air ions can also be attributed to superoxide participation (reviewed by Goldstein 1988). In the present study, we have evaluated the possibility of superoxide production in aqueous media as a result of the interaction of both oxygen and nitrogen ionization products at a gas/water interface. The occurrence of features characteristic of $O_2^{\cdot-}$ reactions under these conditions may be used to substantiate the hypothesis of the involvement of superoxide radicals of exogenous origin in cell metabolism.

Materials and methods

Reagents. Ferricytochrome *c* was obtained from Boehringer (USA), catalase and bovine Cu,Zn-superoxide dismutase (SOD) from Sigma Chemical Co. (USA), nitroblue tetrazolium (NBT) from Reanal (Hungary) and tetranitromethane from Aldrich (USA). Luminol obtained from Yerevan Chemical Reagent Factory (USSR) was recrystallized twice from hydrochloric acid. The other reagents used were of reagent grade. Water was triply distilled in glass.

Gases. Nitrogen was purified from oxygen by filtering through saturated pyrogallol solution. Medical oxygen and nitrogen monoxide and argon were used without further purification.

Equipment. The absorption spectra were recorded using an M-40 spectrophotometer (Carl Zeiss, Jena, FRG). Chemiluminescence measurements were performed with a self-made chemiluminometer equipped with a photoelectron multiplier PhEU-148 (Melz, Moscow), as described by Goldstein (1986). A special injection cell made of Teflon was designed for generation of negatively charged ions (Fig. 1). Oxygen excitation was performed in a gas stream

flowing through a hollow platinum needle under negative voltage in the range of 2.5–6.0 kV from a stabilized source. Gas excitation products generated in the closed chamber of 2 cm³ were injected either onto the surface of the investigated solution in another 2 cm³ chamber, or onto the surface of a millipore filter (Vladipor, USSR) moistened by the test solution. The electric circuit was earthed via a platinum anode with a surface of 5 mm². The temperature of the solution was 20 ± 1° C; the solution was stirred by a magnetic stirrer.

Ionometry. Negative air ions were generated in a corona discharge under described conditions. Measurements of air ion concentrations were performed using the aspiration condenser method with an air ion counter SAI-TGU-66m (Tartu, Esthonia). Under a needle potential of –3 kV, the conditional concentration of small air ions at a distance of 10 cm from the needle was 10⁶/cm³.

Ferricytochrome *c* reduction. Reduction of ferricytochrome *c* was determined in 0.1 M potassium phosphate buffer, pH 7.8, containing 10^{–4} M EDTA, as described by Fridovich (1986a, b). The initial cytochrome *c* concentration was 300 μM.

Nitroblue tetrazolium reduction. This was measured in 0.05 M potassium phosphate buffer, pH 7.8, in the presence of 10^{–4} M EDTA and 500 nM NBT according to Anclair and Voisin (1986). Experiments showed that produced formazan was quantitatively absorbed by the Vladipor filter. After being dried in air, the filter with the precipitate was dissolved in distilled pyridine and the optical density of the solution was read at 560 nm versus pyridine solution containing a dissolved clear filter of the same size. This procedure avoided any difficulties related to the low solubility of formazan in water.

H₂O₂ assay. The assay for the generation of H₂O₂ was performed with modifications according to the method described by Babko et al. (1966). The reagent contained a mixture of 0.1 M potassium-phosphate-NaOH adjusted to pH 8.0, 500 μM luminol and 50 μM [Co(NH₃)₄(NO₂)₂]Cl as a catalyst. The reagent (1.8 ml) was rapidly mixed with the solution for testing (0.2 ml) and measurements of integrated chemiluminescence were made during the first 10 s. Under these conditions the linear dependence of chemiluminescence on the hydrogen peroxide concentration was found to be in the range of 120–500 nM.

Results

One of the widespread methods for air ion generation is excitation in a silent corona discharge. The injection cell shown in Fig. 1 was used to investigate superoxide formation both in oxygen and under interaction of the nitrogen excitation products with water. The cell is an implementation of the model with a semi-infinite wire section previously considered by Goldstein (1988). The virtue of this approach is the possibility to reproduce the main events occurring in the interaction of air ionization products with biological substrates. In order to select optimal conditions for the operation of the cell, current versus voltage dependences were determined under different rates of nitrogen or oxygen flow (Fig. 2). The results obtained revealed quite different relationships between the current and gas flow rates under conditions of nitrogen and oxygen ionization. Furthermore, higher quantities of charge carriers were produced under conditions of nitrogen compared to oxygen ionization.

Ozone generation is possible during corona discharge from a needle electrode in the presence of oxygen. Al-

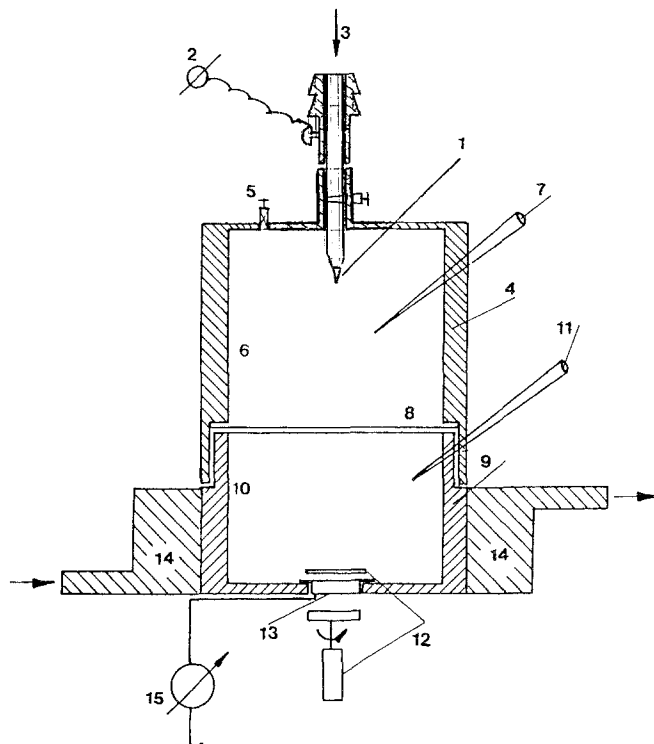


Fig. 1. Construction of injection cell. 1, needle electrode; 2, high-voltage source; 3, gas (O₂ or N₂) input; 4, upper part of injection cell; 5, hole for gas output; 6, space for gas ionization; 7, needle for neutral gas (N₂ or Ar) input; 8, Vladipor filter; 9, body of injection cell; 10, space for solution; 11, needle for obtaining samples; 12, magnetic stirrer; 13, anode; 14, thermostate jacket; 15, instrument for measuring electric current

though the particle does not show properties characteristic for superoxide and does not reduce ferricytochrome *c* and NBT, its oxidizing nature can influence the results. However, it was found that under a negative voltage of the corona electrode of –3.0 ± 0.1 kV, the ozone level did not exceed normal values for ambient gas (approx. 0.03–0.05 ppm).

Analysis of NBT reduction showed that in ionized oxygen medium at 0.1 μM and gas flow rate of 100 ml/min formazan production was linear over at least 10 min and the reduction was inhibited by catalytic quantities of superoxide dismutase (Fig. 3). The inhibition by SOD strongly indicates the presence of O₂^{•–} in the aqueous medium. Superoxide formation in this case occurs as a result of molecular oxygen dissociation with the capture of the electron by a neutral oxygen molecule, as follows:



While diffusing into the solution, superoxide reduces NBT on the interface between the gas and water phases. As there are no significant differences between the energies of oxygen and nitrogen ionization (1315 and 1400 kJ/mol respectively), the following overall process is possible in air:



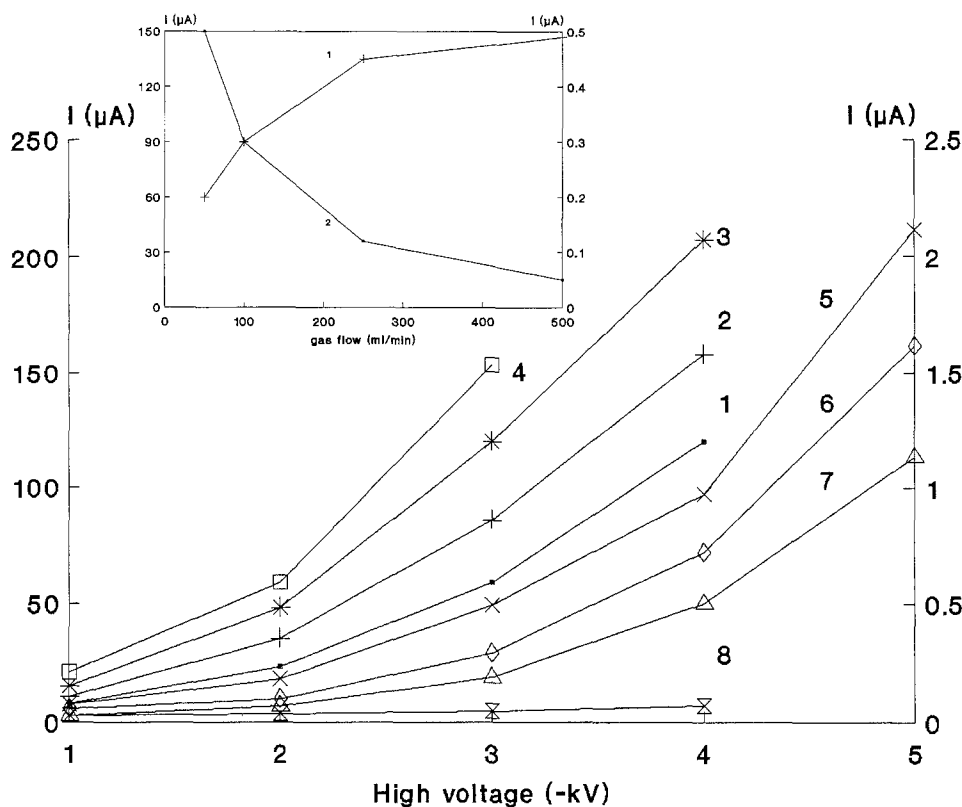


Fig. 2. Voltamperic characteristics of the injection cell as a function of gas flow rate. Ionization of nitrogen (curves 4-1; $y=0-250 \mu\text{A}$) and oxygen (curves 5-8; $y=0-2.5 \mu\text{A}$) are shown at flow rates of 50, 100, 250 and 500 ml/min respectively. *Insert*: Dependence of current on nitrogen (curve 1) and oxygen (curve 2) flow rates at electrode potential = -3 kV

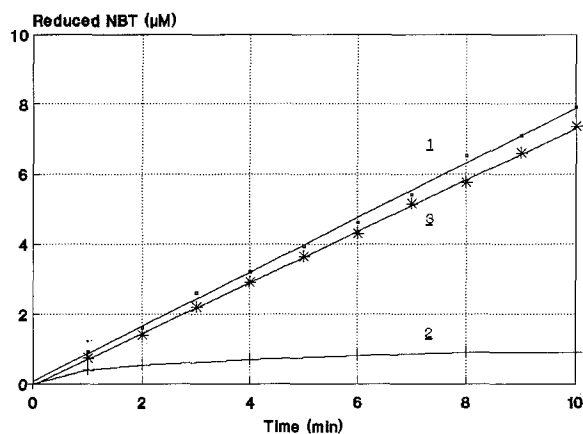


Fig. 3. Time-course of nitroblue tetrazolium (NBT) reduction by negatively ionized oxygen: without additions ($\square-\square$, 1), in the presence of $0.1 \mu\text{M}$, SOD ($+\text{---}+$, 2) and with thermoinactivated SOD ($*-\text{---}*$, 3)

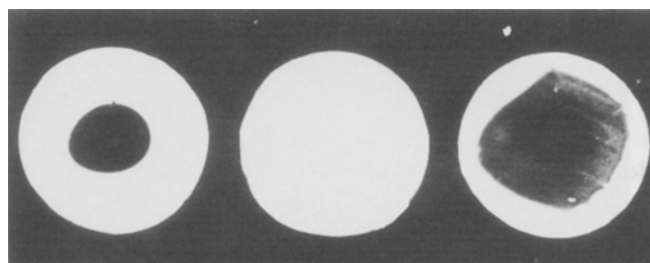
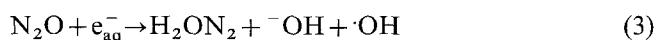


Fig. 4. Formazan accumulation on Vladipor filters caused by: Expt. 1, negatively ionized nitrogen; Expt. 2, after addition of N_2O ; and Expt. 3, addition of nonionized N_2 (3)

There is the possibility of a hydrated electron e_{aq}^- formation in the injection cell as a result of injection of nitrogen products into the solution. This electron is capable of both reduction of complex molecules (for example NBT), and of forming O_2^- in water. NBT reduction observed during nitrogen ionization under a current of $160 \mu\text{A}$ and gas flow rate of 500 ml/min may be due to O_2^- production mediated by e_{aq}^- . Considering that e_{aq}^- has a half-life of ca. $230 \mu\text{s}$ in neutral medium (Streffer 1969), the supposed participation of the particles in the processes described above was evaluated indi-

rectly using the following reaction between e_{aq}^- and N_2O (Seki et al. 1976):



It was assumed that nitrogen monoxide would inhibit NBT reduction caused by both e_{aq}^- and O_2^- . Indeed, simultaneous injection of nitrogen monoxide and excited nitrogen onto the surface of the filter inhibited NBT reduction. Analogous injection of argon or nonionized nitrogen (instead of N_2O) did not alter the overall quantity of formazan production, although the pattern of

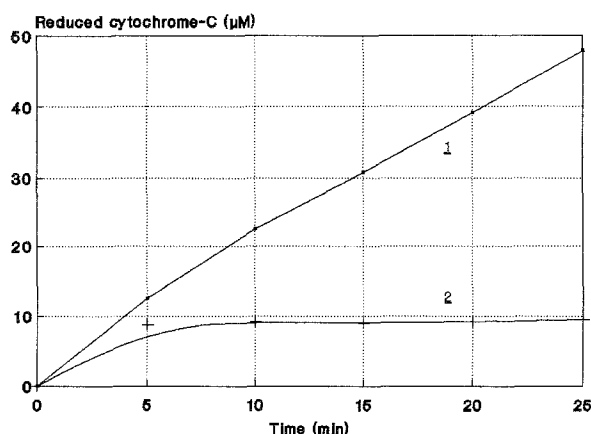


Fig. 5. Time-course of ferricytochrome *c* reduction by negatively ionized oxygen without SOD (■—■, 1) and in the presence of 1 μM SOD (+—+, 2)

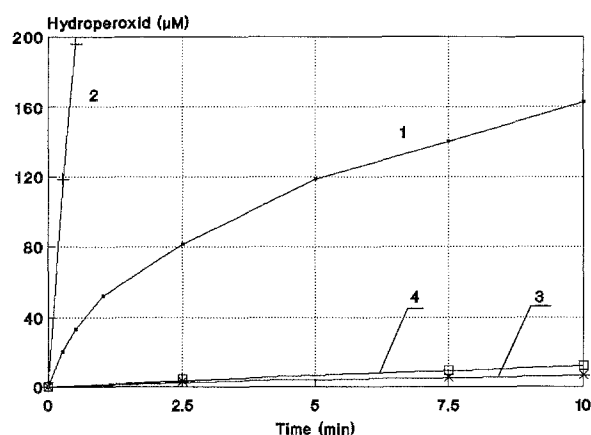


Fig. 6. Time-course of H_2O_2 accumulation in solution caused by negatively ionized oxygen (■—■, 1) and in presence of 30 nM SOD (+—+, 2), 1 μM catalase (×—×, 3) or 1 μM tetranitromethane (□—□, 4)

the product distribution over the surface of the filter was altered (Fig. 4). This phenomenon probably occurs due to changes in gas flow focusing. Taking into account the fact that NBT reduction on the surface of the filter was inhibited by SOD (Fig. 3) the presented reaction scheme may be quite possible. Thus, we demonstrated the possibility of superoxide formation during the interaction of the ionization products of the principal atmospheric gases with water.

The evidence of $\text{O}_2^{\cdot -}$ formation in corona discharge in oxygen was obtained while investigating ferricytochrome *c* reduction and hydrogen peroxide formation. Accumulation of these products was studied directly in solution without a millipore filter. Quantitative compensation of the loss of solution due to the evaporation and recovery of a primary mass in these experiments was achieved by adding water. As can be seen from the data presented in Fig. 5, oxygen ionization products reduced ferricytochrome *c* and this process was also inhibited by SOD. In proton-containing media, superoxide is known to undergo spontaneous dismutation, accompanied by hydrogen peroxide formation as a stable product (see Fridovich 1983):



Thus, hydrogen peroxide production could also provide evidence of superoxide formation. Using luminol-dependent chemiluminescence, it was found that negative oxygen ionization induced H_2O_2 production at an initial rate of ca. 50 μM/min and this value was increased considerably upon addition of 30 nM SOD. Addition of the superoxide scavenger tetranitromethane or catalase into the system without SOD completely prevented the accumulation of hydrogen peroxide (Fig. 6).

The results obtained may serve as evidence that a product having the main properties of superoxide is formed as a result of oxygen and nitrogen ionization in corona discharge. Calculations performed for NBT reduction and hydrogen peroxide production showed that the steady-state rate of generation of the product, which we identify as $\text{O}_2^{\cdot -}$, is ca. $1.5\text{--}2.0 \times 10^{-6}$ M/min.

Discussion

Under natural conditions of atmospheric air ionization, superoxide production may take place both due to direct oxygen ionization and as result of nitrogen ionization during the interaction of ionized products with oxygen-containing aqueous media. Free electrons which appear at the early stages of gas ionization may have different energies. If the electron energy exceeds 360 kJ/mol, the following reactions are assumed to take place, according to Pethig (1984):



Superoxide formation was also shown to be probable via interaction of ionized gases with water in the following reactions:



The scheme shown in Fig. 7 postulates several pathways of $\text{O}_2^{\cdot -}$ generation in gaseous medium and at an air/water interface. The limited lifetime of superoxide in aqueous medium prevents the diffusion of this product. Due to this fact its biological activity can manifest itself only at the boundary surface. These features probably explain the well-known observations (see review of Krueger and Reed 1976) that air ions have stronger, more lethal effects upon microorganisms on the surface of a liquid culture compared to those cells in deeper layers. The observations of Kellogg et al. (1979; see also Rosenthal and Ben-Hur 1980) of the protective effect of SOD on *Staphylococcus albus* treated with negative air ions may be attributed to the elimination of superoxide.

In contrast to superoxide generated by radiolysis or in the processes of cell metabolism, the lifetime of air ions in a gaseous medium may reach dozens of seconds. The disappearance of negative ions under these conditions is caused evidently not only by ion recombination in the gas phase, but also by $\text{O}_2^{\cdot -}$ dismutation in the presence of water vapour. This may explain a well-

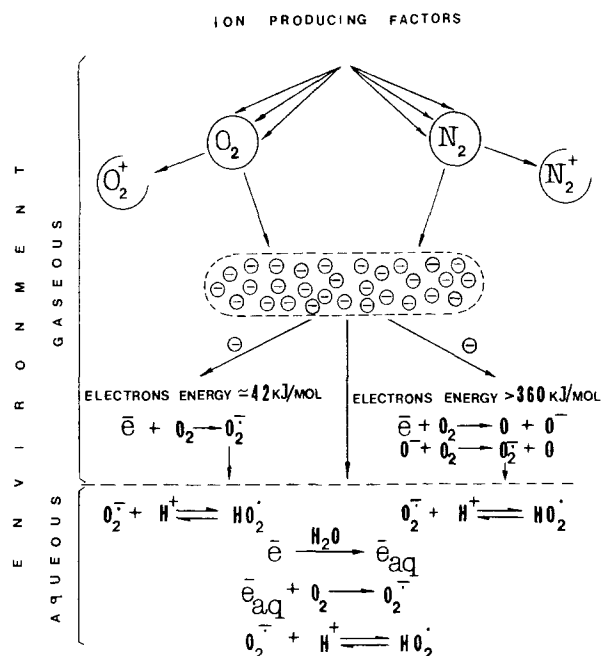


Fig. 7. Proposed general scheme for superoxide formation in gaseous and in aqueous phases as a result of oxygen and nitrogen negative ionization

known influence of air humidity on the physiological effects of negative air ions, as described by Tchijevsky (1960), Krueger and Reed (1976) and others.

The results obtained in this study support suggestions of Krueger et al. (1975) and Krueger and Reed (1976) on the probable participation of free radicals in the biological activity of air ions. It must be emphasized that the biological effects of negative ions are not related solely to superoxide, but also to hydrogen peroxide, hydroxyl radicals and other activated oxygen species (for review see Byczkowski and Gessner 1988; Fridovich 1989).

Thus, the biological activity of negative air ions can be conditioned by several active products and, evidently, are primarily due to the superoxide anion. Keeping in mind that negative air ions are an integral component of environment. A prominent ecological and evolutionary role of exogenous superoxide is suggested. One can also propose that the biological effects of the radical must depend on the level of organization of living matter: while microorganisms and microscopic fungi are killed or lose their viability, the interaction of this exogenous superoxide with tissue receptors of mammals (mucous membrane of the nose and the airways, skin, etc.) apparently serves as a basis for regulation of various physiological functions. Thus there may exist several mechanisms of tolerance towards this factor. The possible role of exogenous O_2^- as an adaptogen towards the

effectors of the agents that induce their damaging effects via participation of activated oxygen species is suggested.

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